

EXTENDED REPORT

Vitamin C and the risk of developing inflammatory polyarthritis: prospective nested case-control study

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Objective: To investigate whether, there is an association between consumption of fruit and vegetables and dietary antioxidants and the risk of developing inflammatory polyarthritis (IP).

Methods: In a prospective, population based, nested case-control study of residents of Norfolk, UK, men and women aged 45–74 years were recruited, between 1993 and 1997 through general practice age-sex registers to the Norfolk arm of the European Prospective Investigation of Cancer (EPIC-Norfolk). Dietary intake was assessed at baseline using 7 day diet diaries. Seventy three participants who went on to develop IP between 1993 and 2001 and were registered by the Norfolk Arthritis Register (NOAR) were identified. Incident cases of IP, assessed by general practitioners, fulfilled the criteria of two or more swollen joints, persisting for a minimum of 4 weeks. Each case of IP was matched for age and sex with two controls free of IP.

Results: Lower intakes of fruit and vegetables, and vitamin C were associated with an increased risk of developing IP. Those in the lowest category of vitamin C intake, compared with the highest, increased their risk of developing IP more than threefold, adjusted odds ratio (OR) with 95% confidence intervals (CI) 3.3 (95% CI 1.4 to 7.9). Weak inverse associations between vitamin E and β -carotene intake and IP risk were found.

Conclusion: Patients with IP (cases) consumed less fruit and vitamin C than matched controls, which appeared to increase their risk of developing IP. The mechanism for this effect is uncertain. Thus similar studies are necessary to confirm these results.

Rheumatoid arthritis (RA) is a complex disease whose aetiology is not fully understood but is almost certainly due to a combination of genetic factors and unknown environmental agents. Diet has been increasingly implicated in the pathogenesis of other chronic diseases like cardiovascular disease^{1,2} and some cancers,³ with particular interest in the possibly protective role of fruit and vegetables and their high antioxidant nutrient content. Cardiovascular disease and RA share very similar inflammatory and immunological responses,⁴ with increased production of reactive free oxygen radicals, and proinflammatory cytokines.⁵ Products of free radical oxidation have in fact been identified in the synovial fluid of inflamed rheumatoid joints,^{6,7} which supports the theory that inflammation is mediated by free radical activity. A major role of the exogenous antioxidants, vitamins C and E (α -tocopherol), β -carotene, and selenium, is to act as efficient scavengers of reactive oxygen radicals, thereby protecting against oxidative damage. Not surprisingly, the role of antioxidant micronutrients in the study of chronic, inflammatory disease has generated much interest.

Low plasma levels of antioxidants have also been found in patients with adult⁸ and juvenile RA⁹ compared with healthy controls, although it is unclear in established disease whether this state exists before disease onset or as a result of the inflammatory process. In samples collected before the onset of symptoms, lower levels of α -tocopherol, β -carotene, and selenium have been identified in the serum of people who subsequently developed RA compared with those who did not.^{10–12}

Epidemiological studies in RA need to be able to distinguish factors that may be involved in the initiation of the disease as opposed to those which influence its course (that is, severity). Therefore, it is essential to collect dietary information before the onset of symptoms of inflammatory

arthritis (IP). Our study aimed at investigating the association between fruit and vegetable and dietary antioxidant intake and the onset of undifferentiated IP in a population in whom diet had been measured before the onset of symptoms.

METHODS

Study design

This was a prospective, population based, nested case-control study made possible by the coincidental investigation of the same population by two independent studies: the Norfolk arm of the European Prospective Investigation of Cancer (EPIC-Norfolk) and the Norfolk Arthritis Register (NOAR). Details of both studies have been published elsewhere.^{13,14} The dietary intake of all participants was assessed at baseline using a 7 day diet diary, thereby ensuring that diet was measured before the onset of symptoms in new cases of IP. Diets of cases were compared with diets of a referent group who had not developed IP.

Baseline population survey in EPIC-Norfolk

Briefly, EPIC-Norfolk is a continuing, population based, prospective study investigating dietary and other risk factors for cancer in men and women. Participants aged between 45 and 74 years at baseline were recruited from general practice age-sex registers.¹³ Baseline investigations included dietary assessment, a clinical examination, with height (in metres) and weight (in kg) measured, and used to calculate body mass index (BMI) (kg/m^2), and a comprehensive health and

Abbreviations: ACR, American College of Rheumatology; BMI, body mass index; EPIC-Norfolk, Norfolk arm of the European Prospective Investigation of Cancer; IP, inflammatory polyarthritis; NOAR, Norfolk Arthritis Register; RA, rheumatoid arthritis; RNI, UK Reference Nutrient Intake

Table 1 Median (IQR) daily food and nutrient intakes between cases and controls

	Cases (n = 73)	Controls (n = 146)	p Value*
Energy (MJ)	7.4 (6.1–8.8)	7.7 (6.4–8.9)	0.28
Fruit (g/day)	100.0 (44.4–229.1)	132.9 (75.0–216.7)	0.07
Vegetables (g/day)	81.3 (42.5–117.4)	93.1 (54.7–124.3)	0.42
Fruit and vegetables (g/day)	187.4 (110.0–312.5)	228.7 (162.5–326.2)	0.09
Vitamin C (mg/day)	63.4 (41.8–105.8)	78.2 (52.5–107.9)	0.04
Vitamin E (mg/day)	6.3 (4.1–8.0)	6.4 (4.8–8.7)	0.10
β-Carotene (μg/day)	1753.7 (1092.8–2586.9)	1719.7 (1216.7–2661.0)	0.60
Retinol (μg/day)	282.9 (182.8–488.0)	288.7 (193.0–450.9)	0.67
Selenium (μg/day)	52.5 (41.2–65.1)	52.0 (40.0–65.3)	0.87

*Wilcoxon signed rank test.

lifestyle questionnaire. The questionnaire provided information on medical and social histories, including a detailed smoking history from which pack-years of smoking were estimated. Each participant was assigned a Townsend score (an index of social deprivation) based on their residential postcode.¹⁵ Between 1993 and 1997, 25 663 people had attended for clinical examination of whom 23 654 completed a 7 day food diary.¹³

Dietary assessment

A trained nurse gave participants detailed instructions on how to complete the diet diary at the baseline clinic visit. Participants were asked to estimate the quantity of food and drink consumed using household measures¹⁶ and food portion photographs (printed in the diary) that illustrated small, medium, and large portions of various foods.

Ascertainment of cases and controls

Cases were participants in EPIC-Norfolk who went on to develop IP and were referred by their general practitioner (GP) to NOAR, a prospective, primary care based, inception study to identify and follow up incident cases of IP in Norfolk. Patients are referred to NOAR if they are over 16 years of age and present with a history of two or more swollen joints lasting for 4 weeks or more, with the onset of symptoms after 1 January 1990.¹⁴ The NOAR database was run against EPIC-Norfolk records to identify IP cases already participating in EPIC at the time of their symptom onset. Two EPIC-Norfolk subjects, free of IP at baseline, acted as controls for each case. Cases and controls were matched for sex, age (to within 3 years), and date of baseline dietary assessment to within 3 months.

Statistical analysis

Data from the food diaries for all cases and controls were entered onto computer by one of us who did not know the case-control status (DJP). Data were analysed for the daily intake of the foods and nutrients of interest using a specially developed programme: DINER (Data Into Nutrients for Epidemiological Research),¹⁷ based on UK standard food composition tables.¹⁸

Dietary intake of foods and nutrients is expressed as the median and interquartile range (IQR) (table 1). We used the Wilcoxon sign rank test to compare medians between the two groups whilst maintaining the matched sets, and the χ^2 test was used to compare the proportion of cases and controls who were smokers and obese (BMI >30). Foods and nutrients were categorised according to tertiles of intake based on the intake of the whole group. The highest tertile was used as the referent group and odds ratios (OR) and 95% confidence intervals (CI) were estimated for the lowest and middle tertiles of intake using conditional logistic regression, again to maintain matching. We also created a dichotomous variable for vitamin C using the current UK Reference Nutrient Intake (RNI) of 40 mg/day as the cut off value.¹⁹ Pack-years of smoking, Townsend deprivation score, and total energy intake were considered as potential confounding factors and entered as continuous variables into the regression model. Vitamin C was also considered as a confounder when neither it nor fruit was the variable being investigated. BMI was categorised as obese or not and entered as a categorical variable. All analysis was performed using STATA 7.²⁰

Ethical approval for both studies was obtained from the Norwich District Health Authority Research Ethics Committee, and all participants gave informed consent.^{13 14}

Table 2 Demographic and anthropometric characteristics of matched cases and controls. Values are medians (IQR) unless otherwise stated

	Cases (n = 73)	Controls (n = 146)	p Value*
Women, No (%)	51 (70)	102 (70)	
Age in years (range)	60 (52–68)	61 (52–68)	
Height (m)	164.4 (159.6–170.5)	161.8 (157.8–169.5)	0.03
Weight (kg)	72.6 (67.4–82.4)	70.8 (63.0–80.2)	0.09
BMI (kg/m ²)	27.1 (24.9–30.0)	26.3 (24.3–29.2)	0.42
Obesity: BMI >30, No (%)	18 (25)	27 (18)	0.29†
Smoking, No (%)			
Current	15 (21)	19 (13)	
Former	28 (39)	57 (40)	0.28†
Never	28 (39)	68 (47)	
Pack-years of smoking	25.0 (9.0–34.0)	12.6 (5.3–26.5)	0.03
Townsend deprivation score‡	–2.61 (–3.48 to –1.11)	–2.51 (–3.6 to –1.21)	0.71

*Wilcoxon signed rank test; † χ^2 test; ‡Townsend deprivation score: larger numbers indicate higher level of deprivation.

Table 3 Association between tertiles of fruit, vegetables, and fruit plus vegetable intake, and IP. Lowest and middle tertiles of intake are compared with the highest tertile

	Cases No (%)	Controls No (%)	Crude OR (95% CI)	Adjusted OR* (95% CI)	p Value for trend
Fruit (g/day)					
1, <78	33 (45)	40 (27)	1.8 (0.9 to 3.6)	1.6 (0.8 to 3.3)	0.3
2, >78–176	16 (22)	57 (39)	0.6 (0.3 to 1.3)	0.6 (0.3 to 1.3)	
3, >176 (referent)	24 (33)	49 (34)	1.0	1.0	
Vegetables (g/day)					
1, <64.6	25 (34)	48 (33)	1.2 (0.6 to 2.5)	1.3 (0.6 to 2.7)	0.5
2, 64.6–113.0	26 (36)	47 (32)	1.3 (0.6 to 2.6)	1.2 (0.6 to 2.5)	
3, >113.0 (referent)	22 (30)	51 (35)	1.0	1.0	
Fruit plus vegetables (g/day)					
1, <167	33 (45)	40 (27)	2.0 (1.0 to 4.1)	1.9 (1.0,4.0)	0.08
2, 167–275	18 (25)	55 (38)	0.8 (0.4 to 1.6)	0.8 (0.4 to 1.7)	
3, >275 (referent)	22 (30)	51 (35)	1.0	1.0	

n, number; OR, odds ratio; CI, confidence interval.

*Adjusted for obesity, smoking (pack-years), total energy intake.

RESULTS

Up to 31 December 2001, 74 incident cases of IP, who had participated in EPIC-Norfolk and completed the 7 day diet diary, were identified from the NOAR database. One case was subsequently found not to meet the entry criteria for NOAR and was not included in the final analysis. At the NOAR baseline assessment, 29 (40%) cases satisfied the American College of Rheumatology (ACR) criteria for RA,²¹ and 27 of the 69 with available sera (39%) were rheumatoid factor positive. Experience shows that by 5 years approximately 66% satisfy these criteria.²² This group has not yet completed 5 years of follow up, but by 2 years' follow up 60% had satisfied the ACR criteria cumulatively. The median duration between dietary assessment and symptom onset was 2.1 years (IQR 0.9–2.9).

Table 2 shows the distribution of demographic and anthropometric characteristics presented according to case or control status. With the exception of height and smoking history, there were few important differences in baseline characteristics between cases and controls. However, sex-specific differences in height were not significant (median (IQR) height of male cases 174.0 (170.8–177.4) v controls 173.2 (167.5–177.4), Wilcoxon test $p = 0.3$; and female cases 161.4 (157.8–164.9) v controls 160.5 (155.9–164.5), Wilcoxon test $p = 0.058$), and cases were more likely to be obese than controls. A higher proportion of cases had "ever smoked" (61% v 53%, respectively; OR 1.3 (95% CI 0.7 to 2.5)) and were heavier smokers than controls. There was no significant departure from linearity in the association between smoking and IP ($p = 0.3$). As smoking is associated with a lower consumption of fruit and vegetables²³ and with an increased risk of RA, and obesity has previously been shown to be a risk

factor for RA in a different group of NOAR cases and matched controls,²⁴ pack-years of smoking and obesity were treated as potential confounding covariates and entered into the analysis.

Fruit and vegetables

Cases reported a lower, but not statistically significant, median daily fruit and vegetable consumption than controls (table 1). We investigated tertiles of fruit and vegetable intake individually and as a composite variable of fruit plus vegetables for an association with IP. Lower intakes of fruit and the composite variable were associated with an increased risk of IP, but less so for vegetables alone (table 3).

Antioxidant nutrients

There was a significant difference in the dietary intake of vitamin C between cases and controls but not for other antioxidants (table 1). Table 4 shows the crude and adjusted ORs of IP risk, based on tertiles of dietary vitamin C intake. Subjects in the lowest tertile of daily vitamin C intake had a more than threefold chance of being a case than those in the referent tertile, after adjusting for smoking, obesity, and total energy intake. Twenty one per cent of cases and 7% of controls reported daily dietary vitamin C intakes below the UK RNI of 40 mg/day.¹⁹ For those with an intake of vitamin C below 40 mg/day the risk of developing IP was nearly four times greater than for those with intakes above 40 mg/day (table 4).

Of the other antioxidants, lower intakes of vitamin E and β -carotene were weakly associated with an increased risk of IP (table 5), but there was no association with retinol or selenium.

Table 4 Association between dietary vitamin C (tertiles and UK RNI¹⁹) and IP. Lowest and middle tertiles of intake are compared with the highest tertile

	Cases No (%)	Controls No (%)	Crude OR (95% CI)	Adjusted OR* (95% CI)	p Value for trend
Tertiles of vitamin C (mg/day)					
1, <55.7	33 (45)	40 (27)	2.6 (1.2 to 5.8)	3.3 (1.4 to 7.9)	0.01
2, 55.7–94.9	19 (26)	54 (37)	0.9 (0.4 to 1.8)	0.8 (0.4 to 1.7)	
3, >94.9 (referent)	21 (29)	52 (36)	1.0	1.0	
Vitamin C (mg) by RNI					
<40 mg v \geq 40 mg	15 (21)†	10 (7)†	3.4 (1.4 to 8.2)	3.9 (1.5 to 9.7)	

n, number; OR, odds ratio; CI, confidence interval.

*Adjusted for obesity, smoking and total energy intake; †cases and controls with vitamin C intake <40 mg/day.

Table 5 Association between tertiles of daily intake of antioxidants and developing IP. Lowest and middle tertiles of intake are compared with the highest tertile

	Cases No (%)	Controls No (%)	Crude OR (95% CI)	Adjusted OR* (95% CI)	p Value for trend
Vitamin E (mg)					
1, <5.3	26 (36)	47 (32)	1.6 (0.7 to 3.3)	1.3 (0.6 to 3.2)	0.5
2, 5.3–7.7	27 (37)	46 (32)	1.6 (0.8 to 3.3)	1.6 (0.7 to 3.4)	
3, >7.7 (referent)	20 (27)	53 (36)	1.0	1.0	
β-carotene (μg)					
1, <1305	27 (37)	46 (32)	1.4 (0.7 to 2.8)	1.3 (0.6 to 2.7)	0.6
2, 1305–2340	24 (33)	49 (33)	1.1 (0.6 to 2.3)	1.0 (0.5 to 2.1)	
3, >2340 (referent)	22 (30)	51 (35)	1.0	1.0	
Retinol (μg)					
1, <216	28 (38)	45 (31)	1.2 (0.6 to 2.2)	1.1 (0.5 to 2.3)	0.6
2, 216–385	20 (28)	53 (36)	0.7 (0.3 to 1.4)	0.7 (0.3 to 1.6)	
3, >385 (referent)	25 (34)	48 (33)	1.0	1.0	
Selenium (μg)					
1, <45.8	24 (33)	49 (33.5)	1.0 (0.5 to 2.0)	0.9 (0.4 to 1.9)	0.8
2, 45.8–60	25 (34)	48 (33)	1.1 (0.5 to 2.1)	1.0 (0.5 to 2.1)	
3, >60 (referent)	24 (33)	49 (33.5)	1.0	1.0	

*Adjusted for smoking, total energy, vitamin C.

DISCUSSION

Fruit and vegetable consumption and, in particular, dietary vitamin C may have an important role in the development of IP. Although a lower intake of fruit and vegetables was associated with a doubling in the risk of developing IP, vitamin C alone demonstrated the strongest association. People who consumed less than 56 mg of vitamin C daily (above the current UK RNI of 40 mg/day) had a more than threefold increase in the risk of IP compared with those who consumed more.

Strengths

This was a prospective, population based study with dietary and clinical assessments undertaken before the onset of IP. Incident cases of IP were easily identified from the NOAR database, and all fulfilled the same entry criteria. Height and weight were measured at the baseline EPIC clinical examination by a standard procedure, thus providing an accurate assessment of BMI rather than self reported values. A detailed smoking history was also obtained and the effects of smoking controlled for in our subsequent analyses. Diet diaries were completed on the day that food was consumed, reducing the likelihood of recall bias, and furthermore, as records were completed before the onset of IP, eating behaviour would not have been influenced by the presence of disease. Although we are not aware of any other publications of associations between dietary vitamin C and the risk of inflammatory arthritis, higher intakes of cooked vegetables,²⁵ cruciferous vegetables, and fruit²⁶ have been reported to protect against the onset of RA in two studies of very different designs, which used food frequency questionnaires to assess diet rather than diet diaries. The 7 day “estimated” food record method of dietary assessment has previously been validated against weighed food records, 24 hour urine collections, and serum biomarkers²⁷ and when compared with other methods, including food frequency questionnaires, found to have greater accuracy in assessing individual dietary intake.

Limitations

This was a relatively small study, but we matched two controls with each case in order to increase the statistical power to detect significant differences in dietary intake between cases and controls. The cases and controls came

from a large population based sample and the number of new cases of IP that we identified is representative of the known incidence of IP.²² EPIC-Norfolk participants have been shown to be similar to UK population samples with respect to anthropometry but contain a lower proportion of smokers.¹³ Therefore, the generalisability of our results may be limited. These data do not include vitamin C intake from dietary supplements, but from the information we have examined so far, supplements likely to contain vitamin C were used by 14% of controls compared with 6% of cases, which supports the association we observed with dietary vitamin C.

Implications

Our findings are biologically plausible, given the antioxidant activity of vitamin C, although it was surprising that only weak associations were found between the other antioxidants and IP risk. However, fruit and vegetables contain many important antioxidant micronutrients, like carotenoids, for which vitamin C may be acting as a marker. Possibly then, a low dietary intake of fruit and vegetables and vitamin C may be increasing the risk of developing IP through a deficiency in powerful antioxidant micronutrients not accounted for in our analyses. A poor diet is also associated with other harmful behaviour, such as smoking,²³ a known risk factor for RA. We have taken smoking into account in the analyses so it is unlikely that this is responsible for our findings.

In conclusion, this study found that a low intake of fruit and, particularly dietary vitamin C, is associated with an increased risk of developing IP. These results may have implications for the primary prevention of inflammatory polyarthritis.

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