Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiologies of ulcerative colitis: a nested case–control study within a European prospective cohort study

The IBD in EPIC Study Investigators

ABSTRACT

Objective: Dietary linoleic acid, an n-6 polyunsaturated fatty acid, is metabolised to arachidonic acid, a component of coloconocyte membranes. Metabolites of arachidonic acid have pro-inflammatory properties and are increased in the mucosa of patients with ulcerative colitis. The aim of this investigation was to conduct the first prospective cohort study investigating if a high dietary intake of linoleic acid increases the risk of developing incident ulcerative colitis.

Design and setting: Dietary data from food frequency questionnaires were available for 203 193 men and women aged 30–74 years, resident in the UK, Sweden, Denmark, Germany or Italy and participating in a prospective cohort study, the European Prospective Investigation into Cancer and Nutrition (EPIC). These participants were followed up for the diagnosis of ulcerative colitis. Each case was matched with four controls and the risk of disease calculated by quartile of intake of linoleic acid adjusted for gender, age, smoking, total energy intake and centre.

Results: A total of 126 participants developed ulcerative colitis (47% women) after a median follow-up of 4.0 years (range, 1.7–11.3 years). The highest quartile of intake of linoleic acid was associated with an increased risk of ulcerative colitis (odds ratio (OR) = 2.49, 95% confidence interval (CI) = 1.23 to 5.07, p = 0.01) with a significant trend across quartiles (OR = 1.32 per quartile increase, 95% CI = 1.04 to 1.66, p = 0.02 for trend).

Conclusions: The data support a role for dietary linoleic acid in the aetiology of ulcerative colitis. An estimated 30% of cases could be attributed to having dietary intakes higher than the lowest quartile of linoleic acid intake.

Ulcerative colitis is a chronic condition that produces distressing symptoms, impairs patients’ quality of life and is associated with an increased risk of complications such as colorectal cancer. The causes of ulcerative colitis are unknown although it is plausible that dietary n-6 polyunsaturated fatty acids (n-6 PUFAs) could be involved. The essential n-6 PUFA, linoleic acid, is present in many sources including red meat, various cooking oils and certain margarines. Linoleic acid undergoes metabolic conversion to a further n-6 PUFA, arachidonic acid, which is a component of colonic cell membranes. Arachidonic acid can be released from this phospholipid membrane and metabolised to pro-inflammatory eicosanoids including prostaglandin E2, leukotriene B4 and thromboxane A2. These eicosanoids are present in excess in the mucosa of patients with ulcerative colitis and in animal models of the disease.2–3 The concentrations correlate with the degree of histological inflammation2 and medications containing 5-aminosalicylic acid inhibit their formation.4 Data from epidemiological studies are needed to examine the linoleic acid hypothesis generated from the experimental and clinical observations. Several such investigations have reported a positive association with an increased total polyunsaturated fatty acid intake.4–6 However, only one used a prospective cohort design,7 and just one, a retrospective case–control investigation, specifically investigated linoleic acid consumption.6

The aim of this study was to conduct the first prospective cohort investigation of dietary linoleic acid intake and the risk of developing incident ulcerative colitis. Participants were enrolled in the European Prospective Investigation into Cancer and Nutrition (EPIC) study8 which recruited a total of 519 978 volunteers in 23 centres in 10 countries. Participants provided information on diet and lifestyle and were followed up for health endpoints including ulcerative colitis. The methodological advantages of this prospective cohort design over retrospective case–control work, are that the dietary information is more accurate, as current intake is recalled which is less affected by recall bias. Additionally, the cohort design avoids potential selection biases, as those who develop ulcerative colitis and those who remain disease-free are drawn from the same population.

METHODS

Participants were resident in seven regions in five European countries. These EPIC sub-cohorts constituted 203 193 men and women in the age range 30–74 years (table 1). At recruitment, between 1991 and 1998, participants provided information on diet, physical activity, and other lifestyle factors such as smoking and alcohol intake. Diet was measured by country-specific food frequency questionnaires (FFQs) that were designed to capture local dietary habits and to give high compliance. Nutrient intake was calculated by multiplying the frequency of consumption of relevant foods by their fatty acid content as determined from national databases of food content. The dietary fatty acids which were calculated were: linoleic acid (n-6 PUFA), α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid (n-3 PUFAs) and oleic acid (an n-9 monounsaturated fatty acid). In all
centres, the FFQs were validated against 24 h recall questionnaires and most centres also compared their questionnaire data against plasma and urinary biomarkers for specific nutrients including vitamin C, vitamin E and protein. The research protocols were approved by ethics committees in each centre and all participants gave written informed consent.

Incident cases of ulcerative colitis which developed in participants who were initially free of the disease were identified by several methods. These were disease registries of inflammatory bowel disease in Italy, Sweden and Denmark, from follow-up questionnaires in Germany and in the Norfolk, UK, cohort by a combination of follow-up questionnaires and hospital inpatient and pathology databases. For each case, physicians were asked to confirm the diagnosis of ulcerative colitis according to whether there was information available from radiological, endoscopic and histological reports. Information on both the extent of colonic inflammation and the investigations used to confirm disease were collected. Prevalent cases of ulcerative colitis at recruitment were excluded as well as participants who were diagnosed less than 18 months after recruitment into the EPIC study. This helped to ensure that the dietary data reflected participants’ dietary intake prior to the development of symptoms. Incident cases were identified up to the end of June 2004 for most centres.

In the analysis, using a nested case–control method within a prospective cohort study, each case was matched with four randomly selected controls (from the same centre), of the same gender, date of birth (±6 months) and date of recruitment into EPIC (±3 months). Controls were alive on the date when the matched case was diagnosed. The last two criteria ensured that the periods of follow-up for both cases and controls were similar. The dietary intakes of linoleic acid and the other fatty acids were divided into gender-specific quartiles from the distribution across both cases and controls. A multivariate analysis was performed, using unconditional logistic regression, adjusting for age at recruitment, gender, total energy intake, cigarette smoking and centre. Energy adjustment helps to account for variables which affect dietary intake, namely body mass index, metabolic rate and physical activity. Smoking was included in the analysis due to the observed inverse association with the risk of ulcerative colitis. Only one measure of smoking was used, namely that at recruitment. Adjustment for centre helps correct for possible differences in the methodology of the food frequency questionnaires between countries. Finally, the odds ratios for the individual fatty acids were adjusted for each of the four other fatty acids, for the centres for which such complete data was available. The rationale for this was that oleic acid and α-linolenic acid competitively inhibit the metabolism of linoleic acid and reduce the formation of arachidonic acid. Additionally, the n-3 polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid have anti-inflammatory properties. The quoted p values for levels of statistical significance were two-sided. The attributable fraction, namely the percentage of all cases of ulcerative colitis attributed to the higher three intakes of dietary linoleic acid, was calculated using the formula, attributable fraction = (OR – 1/OR) × % cases in that quartile. The data were analysed by A Hart and R Luben using the STATA statistical package.

RESULTS

A total of 126 incident cases of ulcerative colitis were identified of which 47% were women. The age at diagnosis was known for 111 (88%) cases with the median age being 60.0 years (range, 39.6–80.8 years) with a median time between recruitment and diagnosis of 3 months. Controls were alive on the date when the matched case was diagnosed.

### Table 1 Participating centres and characteristics of the cohorts

<table>
<thead>
<tr>
<th>Centre and country</th>
<th>Size of cohort</th>
<th>Nature of cohort</th>
<th>No of participants identified with incident ulcerative colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aarhus and Copenhagen, Denmark</td>
<td>57053</td>
<td>Population-based cohort of men and women aged 50–64 years. Recruited between 1993 and 1997. Cases identified from national databases of inflammatory bowel disease</td>
<td>40</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>203193</strong></td>
<td><strong>Population-based cohort of men and women aged 45–74 years. Recruited between 1993 and 1997. Cases identified from follow-up questionnaires, in-patient admission data and histopathology records</strong></td>
<td><strong>126</strong></td>
</tr>
</tbody>
</table>
diagnosis of 4.0 years (range, 1.7–11.3 years). For the cases, 79% had the extent of their disease assessed by colonoscopy or barium enema, with 68% of these having left-sided disease up to the splenic flexure. The cases were age- and gender-matched with 504 controls from the centre in which the case was diagnosed. The completeness of the dietary data for the individual fatty acids was 100% for controls and 99% for cases. In the cohort, no patients were vegetarians and only three (0.6%) controls were. In this cohort, cigarette smoking at recruitment did not affect the risk of incident ulcerative colitis (OR = 1.38, 95% CI = 0.90 to 2.12, p = 0.14). All seven centres supplied data on the dietary intake of linoleic, α-linolenic and oleic acids. Information for eicosapentaenoic and docosahexaenoic acid was available for six centres, as the food frequency questionnaires have not been computed for these in one centre (Italy).

The highest quartile of intake of linoleic acid was associated with an increased risk of ulcerative colitis (OR = 2.49, 95% CI = 1.25 to 5.07, p = 0.01) when adjusted for age at recruitment, gender, centre, energy intake and cigarette smoking (table 2). Similarly, there was a statistically significant trend across quartiles of linoleic acid intake (OR = 1.52 per quartile increase, 95% CI = 1.04 to 1.66, p = 0.02). Furthermore, the effect of linoleic acid intake adjusted for aspirin use, for which data were available for 78.1% of subjects, still maintained the statistically significant effect. Similarly, adjusting for educational level did not affect the results for linoleic acid. The highest quartile of linoleic acid increased the risk of ulcerative colitis in both genders, which was statistically significant in women (OR = 3.47, 95% CI = 1.15 to 10.62, p = 0.05), but not men (OR = 2.05, 95% CI = 0.80 to 5.26, p = 0.13). The results were similar if only cases were analysed, for whom details of the confirmatory investigations were supplied (87% of cases), (OR trend = 1.41, 95% CI = 1.11 to 1.80, p = 0.005). The attributable fraction for the highest three quartiles of intake of dietary linoleic acid was 30%; ie, nearly a third of cases of ulcerative colitis could be attributed to the intake of that in the highest three quartiles. The effect of linoleic acid intake, adjusted for other fatty acids, for which complete data was available from six of the seven centres, showed a similar effect size to the previous analyses, although it was of borderline statistical significance (trend OR = 1.28, 95% CI = 0.97 to 1.68, p = 0.08).

The only other fatty acid for which an association was found with the development of ulcerative colitis was α-linolenic acid, which can be released from cell membranes and metabolised to prostaglandins, leukotrienes and thromboxanes. These have immuno-stimulatory effects14–16 and may predispose to the development of ulcerative colitis. The metabolites of arachidonic acid, including prostaglandin E2, leukotriene B4 and thromboxane A2, have pro-inflammatory effects including aggregation, chemotaxis and release of lysosomal enzymes from neutrophils.14–16 Thromboxanes have many actions including activation of neutrophils,27 production of leukotriene B429 and modulation of T cell function.28 A high dietary intake of n-6 PUFA and their metabolites, prostaglandin E2, leukotriene B4 and thromboxane B2, are found in the colonic mucosa of patients with ulcerative colitis.17 The degree of inflammatory cell infiltrate also correlates with the mucosal arachidonic acid concentration (r = 0.59, p<0.05) in humans.29 Finally, the first-line drugs used to treat ulcerative colitis, namely 5-amino salicylic compounds, suppress prostaglandin E2 levels in a dose-dependent manner.4 The positive association with linoleic acid was unlikely to be a confounding effect of the other PUFA, as adjustment for these, did not alter the magnitude of the odds ratio. However, the p value changed from a significant (p = 0.02) to a borderline significant effect (p = 0.08) which may be due to a reduced sample size for this particular analysis, as data on certain n-3 PUFA were not available from one centre. Furthermore, the intake of linoleic acid is likely to be lower in this southern European centre than northern regions, making any true differences more difficult to detect.

This investigation also found a statistically significant negative association with an increasing dietary intake of docosahexaenoic acid, when adjusted for the other fatty acids which can affect its metabolism. The highest dietary intake of this n-3 PUFA was associated with a 77% (95% CI = 5% to 94%) reduction in the chances of developing ulcerative colitis. There are several plausible biological mechanisms to support how, through its anti-inflammatory properties, docosahexaenoic may prevent colonic inflammation. These include promoting the release of phospholipases D from membranes, which leads to the activation of its anti-proliferative effects in lymphoid cells.30,31 The nutrients also inhibit protein kinase C, which decreases the levels of secondary messengers involved in inflammation. Finally, they may have a direct action on gene expression for inflammatory mediators, as fish oil has such effects on several tissues, including spleen lymphocytes.32 The independent effects of linoleic and docosahexaenoic make confounding by social class less likely, as both may be associated with higher social classes.

The effect of total dietary polyunsaturated fatty acids on the risk of developing incident ulcerative colitis has been investigated in only a few epidemiological studies.6–8 In the only prospective investigation, again in this EPIC cohort, a marginally significant positive association was reported with an increasing percentage intake of energy from the total of all polyunsaturated fatty acids (trend across quartiles OR = 1.19 (95% CI = 0.99 to 1.45) p = 0.067).3 Three case-control studies from Israel, Holland and Japan have investigated total PUFA, although by the nature of this study design, recall bias may exist. The investigation from Israel of 54 patients with ulcerative colitis, most of whom were diagnosed 3–4 months before interview, reported that a high intake of total polyunsaturated fatty acids increased the risk of ulcerative colitis by 6.54 times (95% CI = 1.45 to 29.68).3 Similar results were
Table 2  Odds ratios for developing ulcerative colitis by quartile of intake of specific polyunsaturated fatty acids (PUFAs)

<table>
<thead>
<tr>
<th>Macro-nutrient</th>
<th>Quartile of increasing intake</th>
<th>Unadjusted ORs (plus 95% CIs) for quartiles of intake*</th>
<th>Adjusted ORs (plus 95% CIs) for quartiles of intake†</th>
<th>Adjusted ORs (plus 95% CIs) for quartiles of intake‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Linoleic acid (n-6 PUFA) (g/day)</td>
<td>1 (27, 131)</td>
<td>1.9–6.1</td>
<td>3.1–7.8</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>2 (31, 126)</td>
<td>6.2–8.8</td>
<td>7.9–11.3</td>
<td>1.21 (0.68 to 2.17)</td>
</tr>
<tr>
<td></td>
<td>3 (26, 132)</td>
<td>8.9–12.5</td>
<td>11.4–14.8</td>
<td>1.00 (0.54 to 1.85)</td>
</tr>
<tr>
<td></td>
<td>4 (41, 115)</td>
<td>12.6–37.5</td>
<td>14.9–35.4</td>
<td>1.85 (1.03 to 3.32)</td>
</tr>
<tr>
<td>α-Linolenic acid (n-3 PUFA) (g/day)</td>
<td>1 (33, 125)</td>
<td>0.4–0.9</td>
<td>0.5–1.0</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>2 (24, 133)</td>
<td>1.0–1.2</td>
<td>1.1–1.5</td>
<td>0.68 (0.38 to 1.23)</td>
</tr>
<tr>
<td></td>
<td>3 (33, 125)</td>
<td>1.3–1.6</td>
<td>1.6–2.0</td>
<td>1.00 (0.57 to 1.75)</td>
</tr>
<tr>
<td></td>
<td>4 (35, 121)</td>
<td>1.7–3.7</td>
<td>2.1–5.0</td>
<td>1.11 (0.62 to 1.99)</td>
</tr>
<tr>
<td>Oleic acid (n-9 MUFA) (g/day)</td>
<td>1 (31, 127)</td>
<td>5.0–15.5</td>
<td>7.4–19.6</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>2 (28, 128)</td>
<td>15.6–20.7</td>
<td>19.7–26.2</td>
<td>0.88 (0.50 to 1.57)</td>
</tr>
<tr>
<td></td>
<td>3 (35, 123)</td>
<td>20.8–28.2</td>
<td>26.3–33.4</td>
<td>1.16 (0.63 to 2.03)</td>
</tr>
<tr>
<td></td>
<td>4 (31, 125)</td>
<td>28.3–69.8</td>
<td>33.5–78.4</td>
<td>1.02 (0.55 to 1.89)</td>
</tr>
<tr>
<td>Docosahexaenoic acid (n-3 PUFA) (g/day)</td>
<td>1 (34, 114)</td>
<td>0.0–0.1</td>
<td>0.00–0.11</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>2 (30, 117)</td>
<td>0.11–0.18</td>
<td>0.12–0.21</td>
<td>0.79 (0.43 to 1.44)</td>
</tr>
<tr>
<td></td>
<td>3 (24, 123)</td>
<td>0.19–0.32</td>
<td>0.22–0.40</td>
<td>0.57 (0.29 to 1.10)</td>
</tr>
<tr>
<td></td>
<td>4 (29, 118)</td>
<td>0.33–1.35</td>
<td>0.41–2.00</td>
<td>0.70 (0.3 to 1.37)</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (n-3 PUFA) (g/day)</td>
<td>1 (30, 118)</td>
<td>0.0–0.04</td>
<td>0.00–0.05</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>2 (30, 117)</td>
<td>0.05–0.09</td>
<td>0.06–0.11</td>
<td>1.00 (0.54 to 1.86)</td>
</tr>
<tr>
<td></td>
<td>3 (27, 120)</td>
<td>0.10–0.16</td>
<td>0.12–0.18</td>
<td>0.87 (0.46 to 1.67)</td>
</tr>
<tr>
<td></td>
<td>4 (30, 117)</td>
<td>0.17–0.73</td>
<td>0.19–1.05</td>
<td>1.00 (0.52 to 1.91)</td>
</tr>
</tbody>
</table>

*Odds ratios (ORs) adjusted for age at recruitment into the European Prospective Investigation into Cancer and Nutrition (EPIC), gender and centre.  
†Odds ratios adjusted for age at recruitment into EPIC, gender, centre, cigarette smoking and total energy intake.  
‡Odds ratios adjusted for age at recruitment into EPIC, gender, centre, cigarette smoking, total energy intake and the other listed fatty acids.  
Results in parentheses are number of cases and number of controls.  
CI, confidence interval; MUFA, mono-unsaturated fatty acid.

reported in a case–control study of 45 patients from Holland, all diagnosed within 5 months of recruitment, which detected an odds ratio of 5.1 (95% CI 1.0 to 26.7) for the highest versus lowest tertile of intake. The Dutch study was the only one to specifically assess linoleic acid intake and reported that the two higher tertiles doubled the risk of disease, although these did not reach statistical significance. In both these investigations, the recall of pre-symptomatic diet was likely to be accurate, as the time interval between diagnosis and recruitment was short. The Japanese case–control study found no statistically significant differences for total PUFAs and n-6 PUFAs, although it is possible that the dietary information was less accurate as patients had to remember their pre-symptomatic diet up to 5 years previously. There is also support for the fatty acid hypothesis from clinical studies in patients with established ulcerative colitis. The arachidonic acid composition of phospholipids in the colonic mucosa was found to be higher in patients than in healthy controls (12.5 mol% vs 6.8 mol%, p<0.001). Furthermore, the amount of inflammatory exudate correlated with the arachidonic acid composition of phospholipids in the colonic mucosa (r = 0.89, p<0.005). Other clinical studies have reported significantly higher concentrations of arachidonic and linoleic acid in the plasma and neutrophil phospholipids in patients with ulcerative colitis than controls. Data collected from this EPIC cohort have previously been assessed for the effect of other general nutrients and certain vitamins and minerals on the risk of developing ulcerative colitis and no associations were reported.

The principal advantage of this prospective cohort investigation over previous case–control studies was the minimisation of recall biases, as the dietary information was recorded prior to the diagnosis of the disease. Also, this design reduced the selection biases inherent in retrospective case–control studies, as cases and controls were prospectively ascertained from the same, and a large, baseline population. Random measurement error in the food frequency questionnaire will result in an under-
compared the percentage of linoleic in plasma between patients
acids and the quantitative amounts. Clinical studies which have
findings are surprising as it was expected that linoleate, through
the diet high in linoleate resulted in a higher remission rate than
men, but in women the incidence declined with increasing age.29
showed that the age at diagnosis was similar across all ages in
patients with ulcerative colitis, treated in centres across Europe,
presenting in earlier life. However, a large incidence study of
patients with ulcerative colitis, treated in centres across Europe,
showed that the age at diagnosis was similar across all ages in
men, but in women the incidence declined with increasing age.29
Therefore, our study had an under-representation of younger
women with ulcerative colitis. The disease distribution was
similar in this cohort compared with the European incidence
study (52% vs 21% for disease proximal to the splenic flexure).29
Finally, data from a UK population showed the dietary intake of
n-6 PUFAs is similar across adult age ranges.31 Although we did
not detect a negative association with cigarette smoking at
recruitment, this may be because healthier volunteers are more
likely to participate in a cohort study who are less likely to
smoke.

The findings from aetiological studies may help identify
potential new treatments for patients with inflammatory bowel
disease. There are no dietary modifications of benefit in patients
with ulcerative colitis, although, based on this study’s findings,
a diet low in linoleic acid may merit investigation. In patients
with Crohn’s disease, enteral diets can induce remission and, in
these, the fat content may be important. In one trial, 62
patients with active Crohn’s disease were randomised to receive
either a diet high in oleate and low in linoleate, a diet high in
linoleate and low in oleate, or oral prednisolone.32 At 4 weeks,
the diet high in linoleate resulted in a higher remission rate than
that with the lower content (63% vs 27%, p = 0.008). These
findings are surprising as it was expected that linoleate, through
its conversion to pro-inflammatory eicosanoids, would stimu-
late inflammation. The reasons for this finding are unknown,
although possibilities are the relative proportions of other fatty
acids and the quantitative amounts. Clinical studies which have
compared the percentage of linoleic in plasma between patients
with Crohn’s disease and controls have either reported no
difference28 30 34 or lower amounts.35

In summary, this prospective cohort study found that a high
dietary intake of the n-6 PUFA linoleic acid more than doubled the
risk of developing incident ulcerative colitis. Measurement error
in the food frequency questionnaire means the size of this reported
effect is probably an under-estimate of the true effect. The
positive association may reflect a causal association because of
both a plausible biological mechanism and supportive evidence
from other epidemiological studies. The association needs to be
further investigated in other aetiological work in different
populations to assess consistency. If the positive association is
causal, then there is substantial potential for reducing the
incidence of ulcerative colitis through dietary modification.
Inflammatory bowel disease

NE Day (Emeritus Professor of Epidemiology), R Luben (Head of Bioinformatics), and A Welch (Former Head Research Nutritionist), Stratwernays Research Laboratory, Cambridge, UK
K-T Khaw (Professor of Clinical Gerontology), Clinical Gerontology Unit, Addenbrooke’s Hospital, Cambridge, UK
S Bingham (Group Leader, Diet and Cancer), MRC Dunn Human Nutrition Unit, Cambridge, UK
E Riboli (Head, Division of Epidemiology, Public Health and Primary Care), Faculty of Medicine, Imperial College London, London, UK
H Kennedy (Consultant Gastroenterologist), Norfolk & Norwich University Hospital NHS Trust, Norwich, UK
A Hart (Senior Lecturer in Gastroenterology), University of East Anglia, Norwich, UK

Provenance and peer review: Not commissioned; externally peer reviewed.

REFERENCES