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Symposium on 'Plant foods and public health'

The fibre–folate debate in colo-rectal cancer

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Intervention and prospective studies showing no effect of fibre in protection against colo-rectal cancer have challenged consensus recommendations that population intakes of fibre should be increased to reduce the risk of colo-rectal cancer. The European Prospective Investigation of Cancer and Nutrition (EPIC) of 519 978 individuals aged 25–70 years is the largest prospective study of diet and cancer to date worldwide. It incorporates ten different European countries in order to increase heterogeneity in dietary habits and calibration procedures to reduce measurement error. Data for 1065 reported cases of colo-rectal cancer were reported in 2003. There was a 40 % reduction in risk for the highest quintile *v*. lowest quintile of fibre in food after calibration. It has been suggested that these effects were a result of confounding by folate and other factors. Although there are a number of hypotheses to explain why folate should be protective in colo-rectal cancer, a meta-analysis has shown that folate in food may be protective but there is no effect of total folate (i.e. food plus supplements). In a further analysis of 1826 cases in EPIC, identified in the latest follow-up, the inclusion of an additional 761 cases has confirmed the previously published results, with a strong and significant reduction in colo-rectal cancer of approximately 9 % reduction in risk for each uncalibrated quintile increase in fibre ($P<0{\cdot}001$ for linear trend) compared with an 8 % reduction in the previous report, which had not been adjusted for folate. Inclusion of the other covariates (physical activity, alcohol, smoking and red and processed meat) with folate has confirmed this significant inverse association for colon cancer and strengthened the association with left-sided colon cancer ($P<0{\cdot}001$).

Diet: Colo-rectal cancer: Fibre: Folate

Fibre is one of the most important, if controversial, factors that are thought to prevent colo-rectal cancer, with well-established biological mechanisms underlying the hypothesis. However, in large prospective studies in the USA, Finland and Sweden no protective effects of fibre have been seen (Fuchs *et al*. 1999; Terry *et al*. 2001; Pietinen *et al*. 1999). In addition, large intervention trials have shown that supplements of bran, soluble fibre or vegetables have not reduced recurrence rates of adenomatous colo-rectal polyps in patients (Schatzkin *et al*. 2000; Alberts *et al*. 2000; Bonithon-Kopp *et al*. 2000). Mortality rates for colo-rectal cancer in vegetarians are no different from those of non-vegetarians (Key *et al*. 1998).

These studies showing no effect have challenged consensus recommendations, drawn from a large body of epidemiological and experimental findings, that the population intakes of fibre should be increased to reduce the risk of colo-rectal cancer (World Cancer Research Fund/American Institute for Cancer Research, 1997; Department of Health, 1998). However, all prospective studies so far conducted on diet and cancer have been carried out in single populations for whom dietary habits are relatively homogeneous, so that the extent of measurement error would have obscured any but very large underlying diet–disease associations (Day *et al*. 2001; Kipnis *et al*. 2001). One way of reducing measurement error is to study different populations with diverse dietary practices, thus increasing the between-individual variance in diet and enabling measurement error to be minimised (Day *et al*. 2001). Such was the approach behind the large prospective

Abbreviation: EPIC, European Prospective Investigation of Cancer and Nutrition.

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collaborative project carried out in ten different European countries, the European Prospective Investigation of Cancer and Nutrition (EPIC; Riboli & Kaaks, 1997).

The European Prospective Investigation of Cancer and Nutrition Study

EPIC currently includes 519 978 individuals, and is the largest ever study conducted specifically on the relationship between diet and cancer. Other publications have demonstrated the heterogeneity of dietary intakes of foods supplying dietary fibre in this collaborative cohort. For example, there is a >3-fold range in the average population consumption of total fruit and vegetables (excluding potatoes) between centres in Sweden and in southern Spain (Agudo *et al*. 2002; Wirfalt *et al*. 2002). The eligible study subjects were mostly aged 25–70 years and recruited from the general population residing in a given geographical area, a town or a province. The total EPIC cohort consists of subcohorts recruited in twenty-two centres in ten European countries (Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the UK), allowing comparisons between areas with very different rates of cancer occurrence and distribution of lifestyle and food habits. Food-related questionnaires and lifestyle and personal questionnaires, as well as anthropometric measurements, were collected from all subjects at the time of enrolment in the cohort.

Following the results of several methodological studies conducted in the early 1990s diet was measured by country-specific questionnaires designed to capture local dietary habits and to provide high compliance (Riboli *et al*. 2002). For calibration a second dietary measurement was taken from an 8% random sample (36 000 individuals) of the cohort using a computerised 24 h diet-recall method (Slimani *et al*. 2002). Lifestyle questionnaires included questions on history of consumption of tobacco and alcoholic beverages, and physical activity. The follow up was based on population cancer registries or by active follow-up. All incident cases of colo-rectal cancer (International Classification of Disease ICD-0–2 C 18; C19; C20) with dietary data for the period of complete follow-up were included, but prevalent cases were excluded. Methods have been reported in full by Riboli *et al*. (2002).

The first report on fibre from EPIC (Bingham *et al*. 2003), in which the results were calibrated against the 24 h recall method, shows a relative risk of 0·58 (95% CI 0·41, 0·85) for colo-rectal cancer incidence at 35 g dietary fibre (the mean of the highest quintile) compared with the baseline mean fibre intake of 15 g in the lowest quintile (Fig. 1). Although a borderline significant ($P<0·06$) reduction in risk associated with cereal fibre was found, the trends for fruit, legume and vegetable sources of fibre were not found to be significant, nor were they significantly different from each other. Analyses were also conducted for fibre from cakes and biscuits, potatoes, tomato pastes and soya products but trends in these items were not found to be significant.

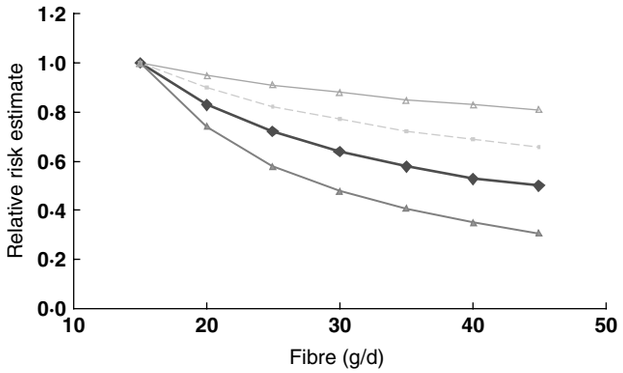

Fig. 1. Relative risk for colo-rectal cancer according to dietary fibre intake for the European Prospective Investigation of Cancer cohort (*n* 1065), calculated from Cox regression using age, weight, height, gender, non-fat energy and energy from fat. (▫), Original estimates calculated from the hazard ratio for each quintile increase in energy-adjusted fibre; (◆), corrected estimates and upper (△) and lower (▲) 95% CI; Corrected estimates were calculated by calibration with a standardised 24 h recall (From Bingham *et al*. 2003; reproduced with permission from Elsevier.).

Mechanisms

The effects of fibre on entering the large bowel are well established. Fibre increases stool weight, reduces transit time and dilutes colonic contents, and stimulates bacterial anaerobic fermentation, which reduces contact between the intestinal contents and mucosa, and lead to the production of SCFA, acetate, propionate and butyrate, thus reducing pH and the conversion of primary bile acids to secondary bile acids (Bingham, 1990). Butyrate is a major source of energy for the distal colon and in cell lines it reduces cell proliferation and induces apoptosis, factors that are associated with inhibition of the transformation of the colonic epithelium to carcinoma (Boffa *et al*. 1992; Chai *et al*. 2000; Domon-Dell *et al*. 2002). However, sources of fibre in food are also sources of folate and it has been suggested that folate is equally, if not more, important in protecting against colo-rectal cancer, via its role in DNA synthesis, stability, repair and hypomethylation (Kim, 2004).

Adjustment for folate

A challenge to the EPIC findings has been made by a re-analysis of two large US cohorts, the Nurses' Health Study and the Health Professionals Study (Michels *et al*. 2005). Data from 1596 cases have been combined and examined using the same statistical procedures as in the EPIC study. A significant reduction in colo-rectal cancer incidence has been shown particularly for men ($P<0·001$). The reduction is less significant in women ($P<0·02$) and there are no effects for particular sources of fibre. However, these effects disappear when approximately fifteen other factors (including folate) are added in to the analysis. The authors state that 'the data do not indicate an important association between fibre intake and colo-rectal cancer' and, furthermore, that 'it seems particularly important to control for folate because intake of folate and fibre will generally be

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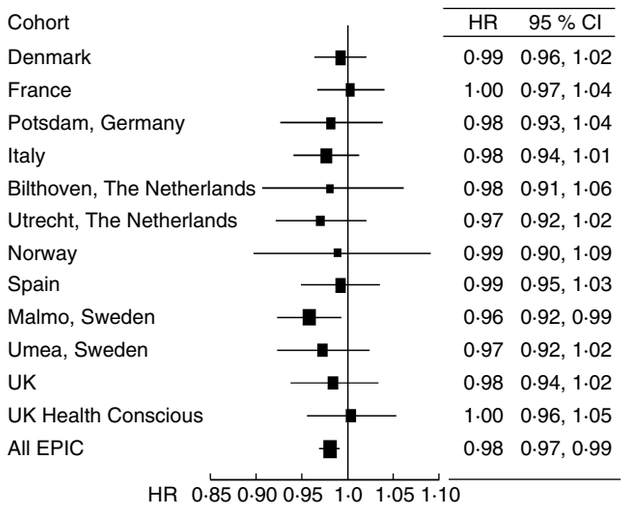

Fig. 2. Risk (multivariate hazard ratio; HR) for colo-rectal cancer per g increase in dietary fibre for the European Prospective Investigation of Cancer (EPIC) cohorts (*n* 1721; Cox regression in the fully-adjusted model). Values are HR and 95% CI. $P=0{\cdot}77$ for heterogeneity (From Bingham *et al.* 2005; reproduced with permission.).

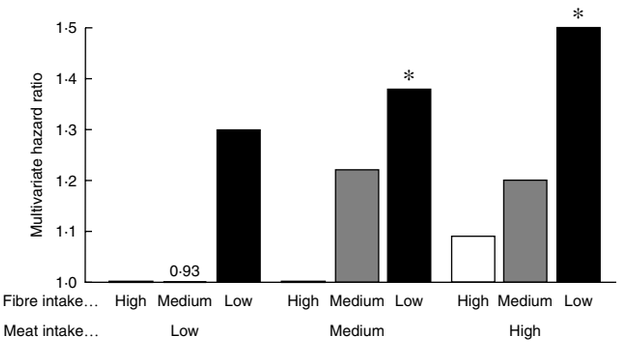

Fig. 3. Risk (multivariate hazard ratio) for colo-rectal cancer with intake of red and processed meat and fibre for the European Prospective Investigation of Cancer cohort (*n* 1721). Cut-off points for fibre (g/d): low <17 (□); medium 17–28 (▩); high >26 (■). Cut-off points for red and processed meat (g/d): low: men <30; women 13; medium: men 30–129; women 13–85, high: men >129, women 85. Red and processed meat increases the risk of colo-rectal cancer particularly in individuals who eat little fibre (<17 g/d) *$P<0{\cdot}05$. (From Norat *et al.* 2005.).

from similar foods, namely fruits, vegetables and whole grains'. In addition, it argued that studies in European populations are more prone to confounding by folate intake because folic acid fortification of cereals is not mandatory (Papas *et al.* 2004).

Re-analysis of the EPIC data

A second report from EPIC (Bingham *et al.* 2005) indicates that 1826 cases of colo-rectal cancer had accumulated (761 more cases than in the earlier report of Bingham *et al.* 2003): 1178 tumours located in the colon (523 located on the right side of the colon and 476 located on the left side of the colon); 648 rectal tumours. The data show that: age is positively associated with fibre intake in men and inversely in women; BMI is inversely related to fibre intake only in men; physical activity is positively related to fibre intake; smoking, alcohol and red meat intakes are inversely related to fibre intake. Trends for folate by quintile of dietary fibre were found to be significant, because of a significant correlation between the two (Spearman partial correlation coefficient adjusted for age, energy intake and centre: 0·35 men, 0·28 women). Partial correlation coefficients between fibre from vegetables and folate intake were also shown to be positive (0·55 men, 0·61 women), as were fibre from fruits (0·25 men, 0·27 women) and from legumes (0·21 men, 0·34 women). The correlation for cereal fibre was shown to be heterogeneous (overall EPIC 0·09 men, −0·21 women, negative or close to zero in France, Italy and Spain, and of similar value to the correlation with fibre from fruits and legumes in the remaining countries).

The inclusion of the additional 761 cases confirms the previously published results of Bingham *et al.* (2003), with a strong and significant reduction in colo-rectal cancer risk of approximately 9% for each quintile increase of fibre ($P<0{\cdot}001$ for linear trend; Bingham *et al.* 2005) compared with an 8% reduction in the previous report. As in the earlier report, the reduction in risk is apparent at the third quintile of fibre intake of approximately 20 g fibre/d compared with 12 g/d. Additional adjustment for folate does not alter materially the results for colon cancer but the inverse association with left-sided colon cancer is slightly strengthened. The results for right-sided colon cancer are not significant, and are therefore in accordance with the earlier report. Adjustment for folate does not materially affect results for rectal cancer. Results are not changed when use of educational levels (five categories) or multivitamins (yes/no) is also included; for example, the hazard ratio for colon cancer for the highest quintile *v.* lowest quintile of fibre is 0·74 (95% CI 0·56, 0·98). Results are consistent across countries ($P=0{\cdot}72$ for heterogeneity; Fig. 2).

In the maximally-adjusted model inclusion of physical activity, alcohol, smoking and red and processed meat with folate strengthen the results for left-sided colon cancer ($P<0{\cdot}001$). After maximum adjustment the association between fibre and rectal cancer is not significant, as was found in the previous analysis. With more cases the hazard ratios have remained essentially the same for all types of fibre, as in the previous report (Bingham *et al.* 2003), although the trends have become significant for fibre from cereals ($P=0{\cdot}01$) and from fruit ($P=0{\cdot}04$). Hence, contrary to the suggestion that results for colo-rectal cancer would be confounded by folate intake in this European population, adjustment for folate does not modify the findings. The protective effect of fibre against colon cancer is observed in both less- and more-adjusted models. Also, further investigation of the use of multivitamin tablets in this European population has not modified the conclusions. The former results for rectal cancer are weaker than those for colon cancer results, and in the second report when fully adjusted are substantially weakened (Bingham *et al.*

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2005). Fig. 3 shows that fibre is particularly protective against colo-rectal cancer in high consumers of meat (Norat *et al*. 2005).

Hence, the suggestion that the protective effect of fibre in bowel cancer is related to folate is not supported by re-analysis of the EPIC data. A recent meta-analysis has shown that folate in food may be protective but that total folate, i.e. food folate plus folate supplements, has no effect on colo-rectal cancer risk (Sanjoaquin *et al*. 2005). Furthermore, the C677T variant of 5,10-methylene-tetrahydrofolate reductase (MTHFR) is associated with a reduced risk of colo-rectal cancer, although studies are ongoing to investigate the possible reduced incorporation of uracil into DNA leading to decreased DNA damage. Results are awaited from intervention trials with folate and adenoma recurrence (Little *et al*. 2003).

An editorial on the finding of a null association of fruits and vegetables with cancer risk in two cohort studies (Schatzkin & Kipnis, 2004) raises the problem of multivariate modelling in the presence of measurement error and weak associations. It is suggested that although prospective epidemiological evidence to date does not provide strong support for a protective association between fruit and vegetable intake and cancer, '... it is important to be alert to the possibility that findings emerging from new, large cohort studies could shift the preponderance of the evidence, as may be occurring with the dietary fibre–colorectal cancer association' (Schatzkin & Kipnis, 2004). As stated in the editorial, efforts should be made to study diet and cancer in populations with a wide range of dietary intake, since it is the inter-individual variation : intra-individual measurement error that determines the magnitude of relative risk distortion. Such was the approach behind EPIC.

Conclusion

Data from EPIC, the largest prospective study of diet and cancer to date worldwide, has shown a 40% reduction in risk for the highest quintile *v*. the lowest quintile of fibre in food after calibration. Although a meta-analysis has shown that folate in food may be protective, such a finding may be confounded by fibre because of the correlation between these two factors. In the EPIC study the association with fibre remains after adjusting for folate so that of the two factors fibre is more strongly associated with colo-rectal cancer risk.

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