DIET AND CANCER — THE EUROPEAN PROSPECTIVE INVESTIGATION INTO CANCER AND NUTRITION

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Diet is thought to be one of the most important contributing factors to cancer risk. The contribution of diet to cancer is linked to genetic factors, and uncovering the details of this linkage requires that very large studies be carried out over long time periods, with a detailed analysis of food intake. For this reason, the European Prospective Investigation into Cancer and Nutrition — a study of over 500,000 people in 10 European countries — was devised, to investigate the relationship between diet, metabolic and genetic factors, and cancer. How will this study be run, and will it be able to avoid some of the problems of measurement error that were previously encountered with other dietary studies?

Worldwide, approximately 10 million people annually are diagnosed with cancer and more than 6 million people die of the disease every year. The most common cancers worldwide (excluding skin cancers other than melanoma) are lung (12.3% of all cancers), breast (10.4%) and colorectal cancer (9.4%)[1]. The chances of developing cancer of the lung, large bowel, breast, prostate and bladder are greatest in developed countries. So, there is a marked overall difference in the total cancer burden between developed and developing countries. For example, the rates of these cancers in northern Europe are about three times greater than in sub-Saharan Africa, where large bowel, breast and lung cancer are virtually absent. Cancers of the cervix, liver, stomach and mouth are the most common cancers in developing countries. There are also 'hot spots' of cancers at certain sites in particular geographical areas; for example, oesophageal cancer in parts of Iran and nasopharyngeal cancer in parts of Southeast Asia.

Taking age factors into account, by using world standardized rates, there are up to 100-fold variations in the incidence of certain cancers in different geographical areas; for example, in the incidence of melanoma of the skin and of the nasopharyngeal cancer. Worldwide, the incidence of cancer of the colon varies 20-fold (highest in the United States, lowest in India), and the incidence of breast cancer varies sevenfold (highest in United States Hawaiians, lowest in Israeli non-Jews)[1]. There are also marked difference in cancer rates in different parts of Europe — cancer rates in Greece are about half those in Germany[2] (FIG. 1).

Evidence that this variation is mainly due to environmental factors and lifestyle, rather than genetic factors, comes from several sources. First, studies of migrants moving from a low- to a high-risk area have shown that the migrants acquire the cancer pattern of the host country within a relatively short period of time — for example, the development of bowel cancer within a single generation[3]. Increasing and decreasing trends over time periods have also been observed, such as the rising incidence of tobacco-associated lung cancer in women in western countries. There has also been a marked increase in the incidence of colon cancer in Japan over the past 40 years[4] (FIG. 2). It is important to note that at ages 55–60 years the incidence of colon cancer in Japanese men is now twice that of men in the United Kingdom, whereas it was extremely rare in Japan only 40 years ago. Clearly, there cannot have been a change in the Japanese gene pool within a generation that could account for this increase, but it is possible that the Japanese had a susceptibility to this cancer that has been unmasked by their rapidly changing diet. In addition, studies show that the

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Summary

- Diet is thought to be one of the most important contributing factors to cancer risk, but is linked to metabolic and genetic factors.
- The European Prospective Investigation into Cancer and Nutrition (EPIC) — a study of over 500,000 people in 10 European countries — was devised to investigate the relationship between diet, metabolic and genetic factors, and cancer.
- In the EPIC study, food consumption has been measured by country-specific questionnaires that were designed to capture local dietary habits and to provide high compliance. At the time of enrolment, weight, height, lifestyle and personal history data were also collected, along with biological samples including plasma, serum, leukocytes and erythrocytes, from approximately 400,000 individuals, which have been stored in liquid nitrogen.
- Over 9 million samples have been stored, making the EPIC centres, collectively, the largest biorepository in the world that is available for genetic, metabolic, biochemical and epidemiological data.
- Subsets of the EPIC study, called 'The Eurogast project' and 'Genair', have been initiated to investigate correlations between tumour-associated genetic mutations, polymorphisms, diet and cancer.
- EPIC study data are already available on the associations between dietary fibre intake and colorectal cancer, and between fat intake and breast cancer.
- Large, prospective studies are the best way to study the interaction between diet and cancer. Information on lifestyle and environmental exposure, especially of diet and biomarkers, can only be accurately determined by studying large populations over prolonged periods of time.

chances of identical twins developing cancer at the same site are generally less than 10%, indicating that the genetic component has a relatively modest impact on risk[5]. Finally, it has been possible to establish strong and consistent links that are likely to be causal; for example, between lung cancer and smoking, using various epidemiological methods[1].

The existence of a relationship between nutrition and cancer at the experimental level was first clearly shown in the 1940s, in a series of classic studies in which severe caloric restriction markedly reduced the occurrence of cancer in rodents. There are also very strong links between the amount of certain dietary items that are consumed in various populations and cancer rates. The relationship between fat consumption and breast cancer is shown in FIG. 3 (REF. 6). The strength of these associations indicates that a large proportion of the variation in cancer incidence is due to differences in dietary practices. On the basis of such comparisons, two studies estimate that, on average, 32–35% of cancers could be attributed to nutrition, although the contribution of diet to specific types of cancer varies from as little as 10% for lung cancer to 80% for cancer of the large bowel[7,8].

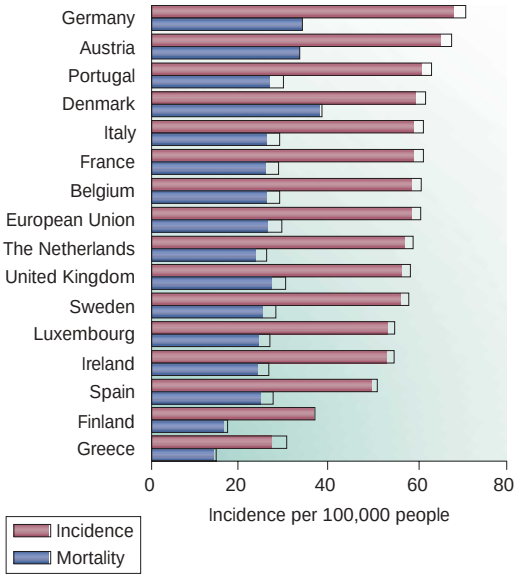


Figure 1 | **Colorectal cancer incidence in males in the European Union.** Rates of colorectal cancer by incidence, per 100,000 people, and mortality during 1996. Data were collected from Eucan — a service that provides data on the incidence and mortality of 24 key cancers in 15 member states of the European Union[2].

Nevertheless, despite several decades of epidemiological research that report links between diet and cancer, comparatively few specific nutrition-related factors have been unequivocally shown to contribute to pathogenesis. Agreement reached so far by various international expert committees is limited to factors such as obesity and alcohol consumption for cancer causation, and fruit and vegetable consumption for protective effects. This could be because the problems of measuring diet in individuals have been underestimated. All analytical methods are associated with measurement error, which, in epidemiology, attenuates estimates of disease risk and reduces statistical power, so that a correlation between the measured factor and disease might be obscured. In dietary studies, this is traditionally corrected with factors that are derived after comparing results from one method (for example, a questionnaire) with those from another method that is assumed to be more accurate. However, it has recently been shown that errors between both the methods used for measuring diet can be correlated, so that results from the reference method are not independent of those that are derived from the test method. So, the extent of measurement error might be underestimated. Biomarkers of diet have been developed in order that accurate factors for correction can be obtained[9]. In addition, few studies have linked diet, biological risk markers (such as plasma hormone levels) and cancer risk.

PROSPECTIVE STUDY
A study in which the individuals are identified and then followed over time.

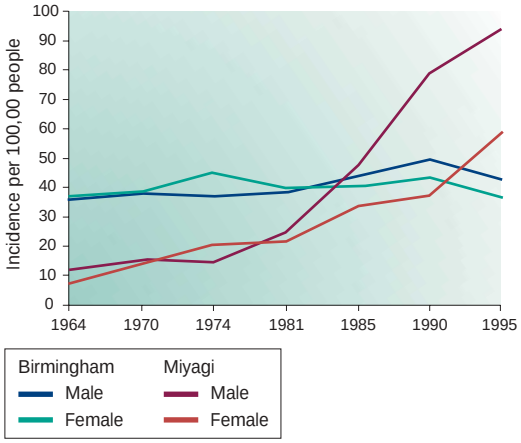


Figure 2 | **Thirty-year trends in colon cancer rates for the United Kingdom and Japan.** Thirty-year trends (*x* axis) for age-specific colon cancer incidence, reported from the Birmingham and Myagi cancer registries in the United Kingdom and Japan, respectively[3]. The *y* axis indicates colon cancer incidence per 100,000 people at ages 55–60. Although rates have remained relatively constant in the United Kingdom, they have risen sharply for males in Japan, from relatively low rates in 1960 to double the rate of males in United Kingdom by 1990.

Furthermore, cancer arises from a complex interaction between genetic factors, individual metabolic characteristics and diet. By themselves, common polymorphisms in genes that regulate the metabolism of food constituents are unlikely to confer large cancer risks, but they might do so in individuals who smoke, drink or have a particular dietary pattern. The association between dietary factors and genetic damage in somatic tumour cells remains to be established. However, the effects of genetic variation on cancer risk depend on individual environmental exposures, so it seems unlikely that even the most detailed characterization of an individual's genome will provide a good predictor of risk in the absence of detailed information about dietary exposure[10].

Another challenge to studies of lifestyle and subtle variations in environmental exposure is that dietary assessments should be made in PROSPECTIVE STUDIES, so that data can be collected from healthy participants, knowing that some will develop cancer at a later stage. This avoids bias in reporting that is introduced by the presence or absence of disease (also known as 'recall bias'). Observations should instead be made over a long time period, ideally at least 10 years before participants develop cancer. This prospective type of study (sometimes called a cohort study) requires a large number of study participants in order for a sufficient number of patients to accrue for comparative analyses.

So far, prospective studies of the effects of gene–environment interactions in cancer risk have been relatively small ones, in which significant effects, if any were observed, could have arisen by chance. Statistical estimates of the sample size that is needed to show such an interaction generally indicate that at least 1,000 patients with a particular cancer are needed to accurately identify the joint effect of two relatively frequent risk factors (for example, a gene variant and a lifestyle factor), which in combination increase or decrease cancer risk by 50%. Even greater sample sizes are needed if the risk factors are rare or if there is any error in the assessment of either diet or other environmental exposures, or in the characterization of the relevant genetic variants[11]. So, for a study designed to last 10 years, and assuming current incidence rates, 500,000 healthy people would need to be recruited, investigated and followed up to acquire a sufficient number of patients for analysis.

Clearly, only very large prospective studies that include accurate dietary, biochemical and genetic analyses hold the promise of overcoming the limitations of previous attempts to associate diet with cancer. For this reason, the European Prospective Investigation into Cancer and Nutrition (EPIC) was undertaken. EPIC is the largest study ever to specifically investigate the relationship between diet, metabolic and genetic factors and specific types of cancer. The association between diet and histological/molecular subtypes of common cancers will also be investigated. The international setting for the study, covering a wide variety of food habits across Europe, is expected to mitigate some of the problems of measurement error previously encountered by dietary studies that were carried out within single populations with relatively homogeneous dietary habits[12].

The EPIC study

The EPIC study, which began in 1992, includes 519,978 volunteers — 366,521 women and 153,457 men (TABLE 1). These volunteers are divided into sub-cohorts, which have been recruited to 23 regional or national centres in 10 European countries (Denmark, France, Germany, Greece, Italy, Netherlands, Norway, Spain, Sweden and the United Kingdom; BOX 1). In general, individuals who were eligible for the study were selected from the general population of a specific geographical area, a town or a province. Exceptions include the French cohort, which was based on members of the health insurance system for state-school employees, and the Utrecht cohort, which was based on women who underwent breast cancer screening[13,14].

Food consumption was measured by country-specific questionnaires that were designed to capture local dietary habits and to provide high compliance. They were tested against biomarkers before the start of the study[15]. Trained personnel collected personal lifestyle data, as well as height, weight, waist and hip-circumference information, from all individuals at the time of enrolment. In addition, a detailed dietary assessment, made during the course of one day, was collected for a randomly selected 7% of the cohort (38,000 individuals). This assessment involved the use of a computerized, highly standardized 24-hour diet-recall method. By comparison with biomarkers, this second method was found to accurately identify each centre within the cohort[15]. Furthermore, this second set of information allowed for statistical methods to be developed to correct for bias in relative-risk estimates, which are caused by both random and systematic measurement errors in

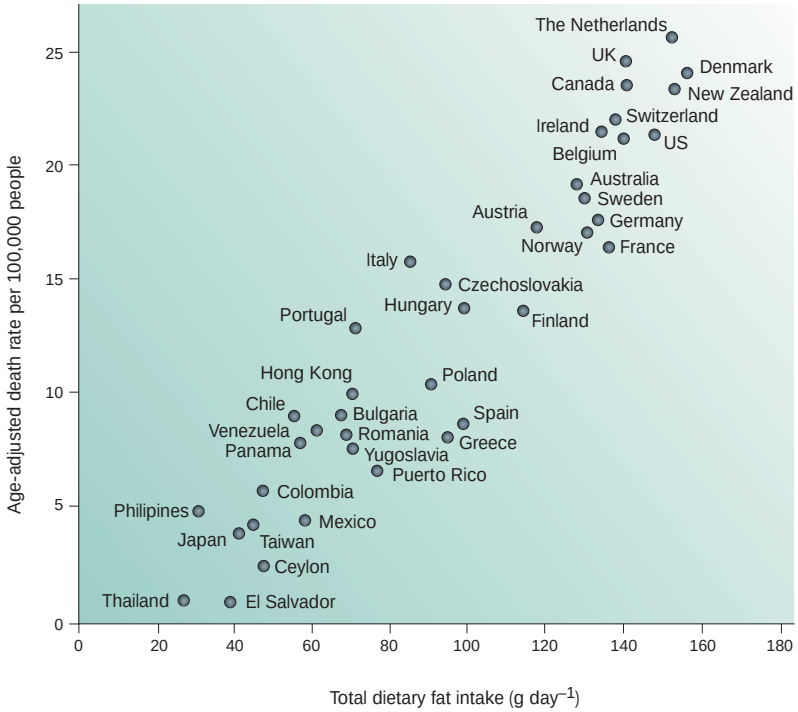


Figure 3 | **Association between fat intake and breast cancer.** Comparison of age-standardized death rates of breast cancer with fat intake, from national food-consumption data for various countries. The *x* axis indicates the total dietary fat intake (g/day), and the *y* axis indicates the age-adjusted death rate, per 100,000 people. Breast cancer incidence was strongly correlated with average dietary fat intake. Data from Ref. 6.

the baseline questionnaire. This makes the cohort-specific estimates more comparable between study centres[16].

Lifestyle and personal history data were also collected using multiple-choice questionnaires. These included questions on education, socio-economic status and employment. Participants were asked about current and past occupation in industrial settings that might have led to exposure to carcinogens, life status of parents and siblings, and their cause of death. Questions also covered lifetime history of consumption of tobacco and alcoholic beverages; physical activity (in relation to, for example, occupation, walking, cycling, gardening, housework, physical exercise and climbing stairs); sexual maturation; contraception and reproduction; history of previous and current diseases; medical and surgical treatment; and hospitalization.

Biological samples including plasma, serum, leukocytes and erythrocytes were collected from approximately 400,000 individuals at the time of enrolment and have been stored in liquid nitrogen. Together, the EPIC biorepositories (BOX 2) at the International Agency for Research on Cancer (IARC; see online links box) and in the 23 EPIC collaborating centres (BOX 1), which host a total of over 9 million aliquots, constitute the largest biorepository in the world that is available for genetic, metabolic, biochemical and epidemiological investigations of cancer pathogenesis. Some collaborating centres, such as the Norfolk EPIC centre in the United Kingdom (known as the 'EPIC-Norfolk' centre), also measured additional factors such as blood pressure, body fat, blood lipid levels, plasma vitamin C levels, and collected information on lung function, heel-bone ultrasound data and urine samples. The EPIC-Norfolk centre also collected data on diet using three different methods and collected another set of blood samples and dietary data four years after the study began[17–19].

Since the trial was initiated, researchers at collaborating centres have regularly obtained information from trial participants regarding the occurrence of cancer and survival times. Data on cancer diagnoses are collected from population cancer registries in seven of the participating countries (Denmark, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom) and through a combination of methods that include analysis of individual follow-up records, health-insurance records, as well as cancer and pathology registries, in three other countries — France, Germany and Greece. By the end of 2002, a complete set of data had been reported to the IARC for the period up to 31 December 2000. Individual data collected in the study have been stored centrally at the IARC in a database called 'Oracle8 Enterprise Edition Release 8.05', and are made available to study collaborators for more specific studies. So far, over 20,000 new cancer cases have been reported to the IARC coordinating centre. These include 6,169 cases of breast cancer, 1,913 cases of colorectal cancer, 1,515 cases of prostate cancer and 1,309 cases of lung cancer (TABLE 2). Working groups have been formed to analyse and publish these results.

Table 1 | Sample numbers in the main EPIC-Europe cohort

Country	Number of questionnaires	Number of blood samples collected	Individuals that developed cancer
Spain	41,440	39,579	1,560
Italy	47,749	47,725	1,609
United Kingdom	87,940	43,138	4,358
The Netherlands	40,072	36,318	1,814
France	72,996	20,725	5,180
Germany	53,094	50,679	2,011
Greece	28,572	28,500	337
Sweden	53,830	53,755	3,850
Denmark	57,054	56,131	2,965
Norway	37,231	9,197	511
Total	519,978	385,747	24,195

EPIC, European Prospective Investigation into Cancer and Nutrition. Sample numbers are from October 2003.

Recent findings on diet and biomarkers

One of the key features of the EPIC study is its wide geographical coverage — from northern to southern regions of Europe. Diet heterogeneity is a key component of the study, and allows researchers to overcome some of the problems of measurement error in diet mentioned above[12]. Some variations are shown in TABLE 3. This feature should help to overcome the limitations of some cohort studies conducted in North American populations, which are characterized by relatively uniform dietary habits.

Studies of biomarkers of diet in the EPIC blood samples have provided independent evidence that this reported variation is reflected in physiological effects[20]. For example, levels of lycopene — a carotenoid that is present in tomatoes — has been associated with reduced prostate cancer incidence[21]. Intake levels of this carotenoid (and others) vary fourfold across the EPIC centres, with highest levels in southern European areas and lowest in northern Europe, and varies substantially between individuals within each geographical area. Features such as these provide powerful conditions for investigating the association between dietary factors and cancer risk (FIG. 4).

EPIC investigators have established uniform methods to analyse dietary biomarkers and cancer risk in the stored samples. Examples of ongoing studies include analysis of 26 fatty acids — from short-chain saturated fatty acids such as C:12:0, to long-chain n-3 and n-6 fatty acids — vitamins C, E, B6, B12 and folic acid, and some phytochemicals, including carotenoids and phytoestrogens. One concern has been that it is thought that blood has to be stabilized with the

Box 1 | EPIC study principal investigators and collaborating centres

International Agency for Research on Cancer/ World Health Organization

- Elio Riboli (European Prospective Investigation into Cancer and Nutrition (EPIC) Coordinator), Nadia Slimani, Teresa Norat, Pietro Ferrari, Gwyneth Davey, Marlin Friesen, Federico Canzian, Rudolf Kaaks and Rodolfo Saracci.

Denmark

- Anne Tjønneland, Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen.
- Kim Overvad, Institute of Epidemiology & Social Medicine, University of Aarhus.

France

- Françoise Clavel-Chapelon, INSERM, U. 521, Institut Gustave Roussy, Villejuif.

Germany

- Paolo Boffeta, Division of Clinical Epidemiology, DKFZ, Heidelberg.
- Heiner Boeing, Department of Epidemiology, German Institute of Human Nutrition, Potsdam.

Greece

- Antonia Trichopoulou, Department of Hygiene & Epidemiology, University of Athens.

Italy

- Franco Berrino, Department of Epidemiology, National Cancer Institute, Milan.
- Domenico Palli, Cancer Research and Prevention Centre-CSPO, Florence.
- Salvatore Panico, Department of Clinical and Experimental Medicine, University of Naples.
- Rosario Tumino, Cancer Registry, Italian League Against Cancer, Ragusa.
- Paolo Vineis, Department of Cancer Epidemiology, University of Turin.

The Netherlands

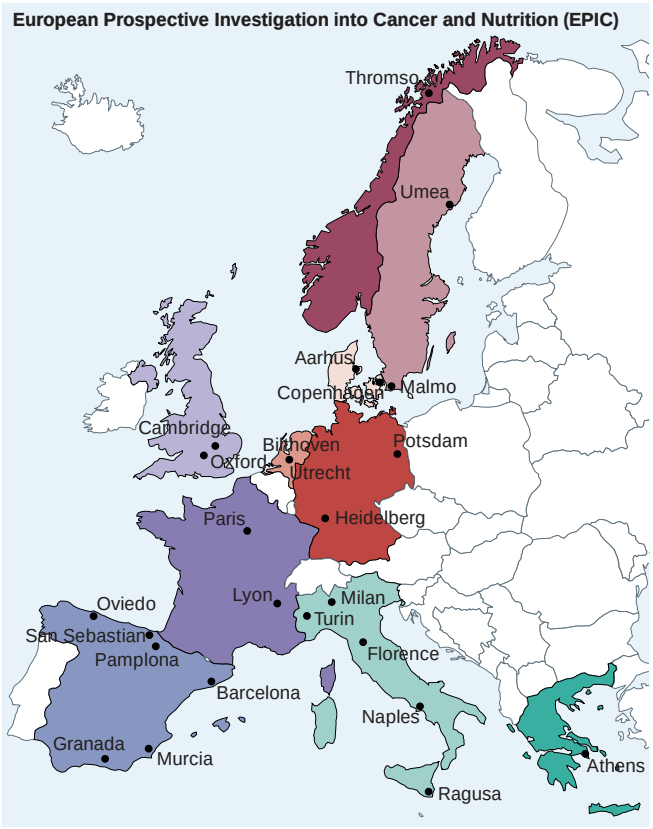
- Bas Bueno de Mesquita, National Institute of Public Health, Bilthoven.
- Petra Peeters, Julius Center for Health Sciences and Primary Care, University Medical Centre, Utrecht.

Norway

- Eiliv Lund, Institute of Community Medicine, University of Tromsø.

Spain

- Carlos A. González, Department of Epidemiology, Catalan Institute of Oncology, Barcelona.
- Aurelio Barricarte, Department of Epidemiology, Institute of Public Health of Navarra, Pamplona.
- Miren Dorronsoro, Public Health Office of Guipuzcoa, San Sebastian.



- Carmen Navarro, Department of Epidemiology, Health Council of Murcia.
- Carmen Martínez, Andalusian School of Public Health, Granada.
- J. Ramon Quirós, Department of Health Information, Regional Public Health Office, Oviedo.

Sweden

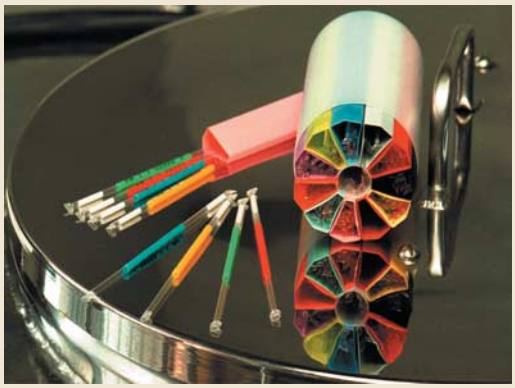
- Göran Berglund, Malmö Diet and Cancer Study, Lund University, Malmö.
- Göran Hallmans, Department of Nutritional Research, University of Umeå.

United Kingdom

- Nicholas E. Day, Strangeways Research Laboratory, University of Cambridge.
- Kay-Tee Khaw, Clinical Gerontology Unit, University of Cambridge.
- Sheila Bingham, MRC Dunn Human Nutrition Unit, Cambridge.
- Nicolas Wareham, MRC Epidemiology Unit, Cambridge.
- Timothy J. Key, Cancer Epidemiology Unit, Cancer Research UK, University of Oxford.

Box 2 | Sample storage

The logistics of how to store such a large number of samples at such low temperatures over very long periods took some time to solve. Using specially devised machinery, blood was separated into 28 small aliquots (in plastic straws of 500 microliters) of plasma (12 aliquots), serum (8), leucocytes (4) and erythrocytes (4). The sample identification was stamped directly onto a coloured sleeve to avoid loss of labelling, and each colour was specific to the type of sample stored (see image). For historical reasons (the collection was initiated several years before the common European Prospective Investigation into Cancer and Nutrition protocol) samples from Sweden and Denmark were stored in larger tubes at either –80 °C (standard freezers, in Sweden) or liquid-nitrogen vapour (–120 °C, in Denmark). For each subject, half of the blood aliquots (14) are stored in the regional collaborating centres and half have been shipped to the International Agency for Research on Cancer for central storage.



antioxidant metaphosphoric acid (MPA) at the time of collection if plasma vitamin C is to be measured. Levels of vitamin C — a marker of antioxidant status — are likely to be associated with many different types of cancer. Only one centre, EPIC-Norfolk, stabilized all blood samples with MPA and then analysed them within one week of sampling[18]. To test whether samples that are held in the IARC biorepository could be used for analysis of plasma vitamin C levels, random samples from EPIC-Norfolk participants were withdrawn from liquid nitrogen after up to 10 years of storage. When compared with the plasma vitamin C levels in the original sample, the results from the stored sample compared surprisingly well with the original.

The complex relationships between diet, weight, height, waist/hip ratio, physical activity and (for women) sexual-maturation/reproductive history are also being studied by analysing blood levels of steroid sex hormones, oestrogens, androgens and their transport globulin (sex-hormone-binding globulin). Levels of growth factors such as insulin-like growth factors (IGFs), growth hormone and IGF-binding proteins will also be analysed.

Linkage with genetic factors

In the multistep model of cancer development, alterations in several genes are required for generation of a carcinoma, but what causes these mutations? Identifying the presence of somatic changes in genes in tumour samples would provide powerful evidence of a causal link, if an interaction with dietary factors and polymorphisms in genes that control their function was shown. A subset of the EPIC study called 'The Eurogast project' has been initiated to investigate associations. In this project, samples of gastric cancer tissue are being collected from EPIC participants and will be analysed for somatic changes in cancer-associated genes such as *TP53* and the gene that encodes E-cadherin. Next, the association between these changes and putative cancer risk factors, such as consumption of fruit, vegetables, salt-preserved foods, serum vitamin C and infection with *Helicobacter*, as well as genetic polymorphisms in genes that are involved in inflammation and antioxidants, will be investigated.

A similar project called 'Genair', which involves using EPIC dietary data and analysis of polymorphisms in genes that control carcinogen metabolism, is underway. The goal of this study is to determine the role of environmental factors other than smoking — particularly air pollution and nutrition — in cancers of the lung, larynx, pharynx and bladder in non-smokers. Other trials are being planned to study the effect of nutrition, polymorphisms in genes that control nutrient and hormonal metabolism, and other factors in the development of breast and bowel cancer.

Recent results from EPIC-Europe

There have been numerous publications on non-cancer end points from each of the associated EPIC centres since the outset of the study (for example, see the links to EPIC-Europe and EPIC-Norfolk in the online links box, and REF. 14). Because of the long time that is necessary to accumulate sufficient cases of cancer, analyses for the main EPIC-Europe study have only just begun.

Table 2 | EPIC-study cancers

Cancer type	Number
Upper gastrointestinal tract	372
Stomach	368
Colorectal	1,913
Pancreatic	336
Lung	1,309
Breast	6,169
Cervical	676
Ovarian	659
Uterine	785
Prostate	1,515
Kidney	368
Bladder	700
Other	9,025

EPIC, European Prospective Investigation into Cancer and Nutrition.

Table 3 | Dietary patterns associated with participating countries

Country	Foods consumed at ≥ 150% mean overall intake worldwide
Italy	Vegetables, fruits, cereal products, vegetable oils, sauces
Greece	Vegetables, legumes, vegetable oils
Spain	Vegetables, fruits, legumes, vegetable oils, milk, eggs, fresh meat, fish
France	Sugars, butter, dairy products
Germany	Butter, processed meat, coffee, juices
The Netherlands	Potatoes, margarines, dairy products, processed meat, tea, coffee
United Kingdom	Potatoes, cakes, sugar, margarine, butter, tea, soft drinks
Denmark	Sugars, margarines, tea, coffee, soft drinks, alcohol
Sweden, Norway	Potatoes, cakes, sugars, margarine, dairy products, coffee, soft drinks

Data from Ref. 14.

Fibre and colorectal cancer. The first analysis of the EPIC study data examined the effects of dietary fibre intake on colorectal cancer risk. An analysis of all studies worldwide completed up to the late 1990s indicated that fibre intake reduced colorectal cancer risk, and scientists therefore recommended an increase in fibre intake[22,23]. This consensus view had been challenged by later studies that showed no protective effect of fibre intake in either prospective or intervention studies. Data from the EPIC study collected since 1992 included data on fibre intake. During the first five-year follow-up period, 1,056 individuals developed colorectal cancer among the initial cohort of 521,000 subjects[16]. The study showed that dietary-fibre intake was significantly and inversely related to colorectal cancer incidence and colon cancer incidence, but not to rectal cancer incidence[16]. After correction of the risk estimates with the more detailed dietary data carried out on the sub-sample of EPIC participants, the study showed that an approximate doubling of fibre intake was associated with a 40% reduction in colorectal cancer incidence.

These findings indicate that doubling total fibre intake from current average levels in most populations (about 20 g/day) might halve the risk of colorectal cancer — particularly colon cancer. This would represent a substantial change in dietary habits for most people to obtain the maximum effect. About eight portions (rather than just five) of fruits and vegetables would need to be eaten per day, as well as the equivalent of five slices of wholemeal bread. Previous studies might have missed these effects because both the average and the range of fibre intake in the populations studied were much lower than those in EPIC participants — particularly of cereal fibre.

Breast cancer and fat intake. The strong geographical association between fat and breast cancer shown in FIG. 3 has prompted many epidemiologists to investigate whether or not individual links could be shown. The resulting lack of clear evidence for or against a causal association has generated much controversy over whether fat intake is a risk factor for breast cancer[22–24]. Fat is one of the most difficult nutrients to measure. It has been proposed that the simple questionnaire methods that are used to assess fat intake might not be able to detect an association in populations that have a small variation in dietary habits.

The wide range of diets among participants in the EPIC-Europe trial should make it possible to circumvent this problem, but in single populations, it is probable that more accurate assessments will be needed to show effects. Evidence for this comes from the EPIC-Norfolk centre, where, as a result of previous studies to assess accuracy as judged by biomarkers, three methods were used in study participants between 1993–1997, as mentioned above[9,19]. In 2003, two methods — a questionnaire and a detailed seven-day diary of food and drink consumption — were analysed for 168 participants who developed breast cancer, and four healthy controls were matched to each case (840 participants in total). The risk of breast cancer was strongly associated with the amount of saturated fat consumed, according to the food diary. Women who consumed over 35 g/day of saturated fat had a more than twofold increased risk of developing breast cancer, compared with those who ate a low amounts of saturated fat — 10 g/day or less. However, no relationship between fat intake and cancer could be detected using the information given in the questionnaire[25].

This was a comparatively small study and the results, which indicate that effects of fat intake on breast cancer risk can only be shown using very accurate methods, needs confirmation. Analyses of fat intake are underway in the EPIC-Europe study, and these are also likely to reveal the association between breast cancer and fat intake, despite the use of questionnaires and their associated measurement error. This is because of the wider variation in dietary habits in the trial participants in EPIC-Europe, which makes it easier to distinguish individual dietary habits within a population — even with less accurate questionnaires[12].

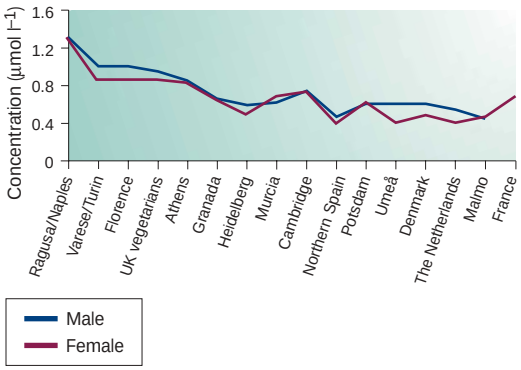
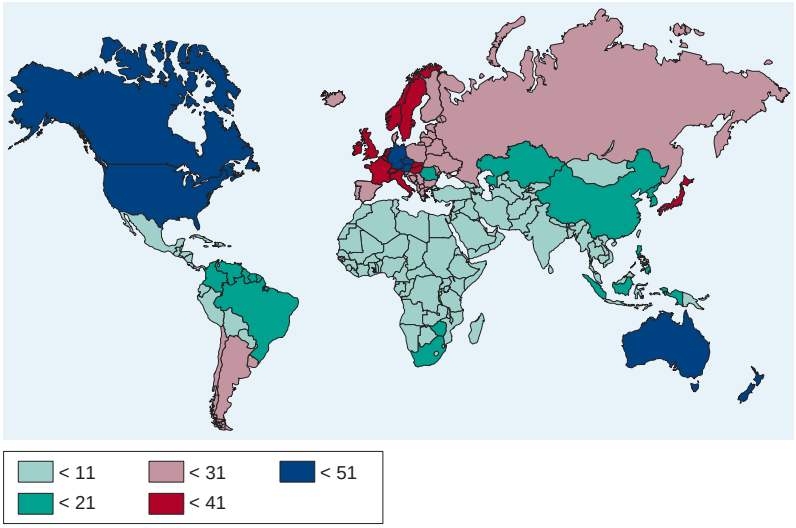


Figure 4 | **Plasma lycopene concentrations in 16 regions of the European Prospective Investigation of Cancer study.** Plasma lycopene levels (*y* axis), in µmol/litre, for men and women in different European Union nations (*x* axis). Data were collected from 1,485 men and 1,581 women. The study involved 50 individuals (25 men and 25 women) in each of four age strata (45–49; 50–54; 55–59; 60–64 years). Data from Ref. 20.

Future directions

The EPIC study is clearly going to yield an enormous amount of data over the forthcoming years as follow-up time increases, and sufficient cases for the analysis of rarer cancers, such as those of the pancreas, brain, kidney and oesophagus, arise. More detailed analyses of the common cancers, according to sub-site, sub-type (for example oestrogen-receptor-positive breast tumours), or family history will also be possible. Sub-studies of the effect of diet on the survival of patients with cancer will be carried out. Furthermore, three main types of analyses will be conducted.

a **Incidence rates of colorectal cancer**



b **Estimated red-meat consumption (grammes/day)**

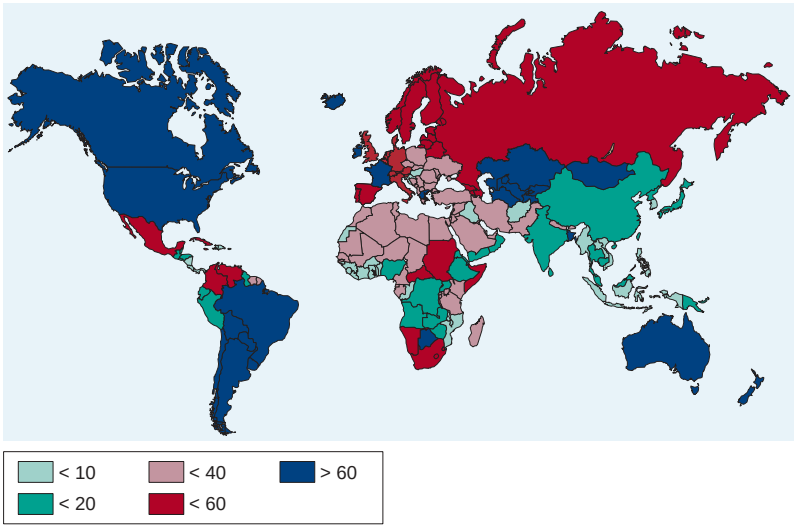


Figure 5 | **Colorectal cancer incidence and red-meat consumption worldwide in men.** **a** | Countries with a high incidence of colon cancer (cases per 100,000 people) are indicated with blue (North America, Australia); countries with moderate levels in pink or red; and countries with low incidence in green (Asia, Africa). Colon cancer incidence is correlated with red-meat intake. **b** | Countries that consume the most red meat, in g/day, are indicated in blue (North and South America, Australia); countries with moderate levels of consumption in pink or red; and countries with the lowest levels of red-meat intake in green (Africa, Asia). Figure adapted with permission from Ref. 1 © (2003) IARC Press.

Dietary effects. Virtually every item in the diet is being investigated for its possible link with cancer risk. This is done using sophisticated databases of standardized food classifications and chemical composition. Initial analyses are nearing completion for all types of meat (red, white and processed) and fish consumption and folic acid intake, in relation to colorectal cancer risk. The role of fruits, vegetables, potatoes and legumes in colorectal cancer and lung cancer risk is also being analysed, as well as the effects of fruits, vegetables and food groups such as tomatoes on prostate cancer risk. The analyses of saturated-, monounsaturated- and polyunsaturated-fat intake and breast cancer risk (in the main EPIC cohort) are nearing completion. Dietary associations can be confounded by other factors, however, such as medications, vitamin supplements and smoking — these are included in the statistical analyses.

Diet biomarker associations. The EPIC biorepository will yield much definitive data on the effects of diet for which there are biomarkers in blood and, in cases where they are collected, urine samples. Biomarkers might be subject to less measurement error than dietary questionnaires and can confirm associations for which there is only tentative evidence. For example, although there is some evidence that the lycopene in tomatoes is associated with reduced risk of prostate cancer, as is vitamin E, not all the evidence is consistent[21–23]. EPIC researchers will therefore be able to analyse a wide variety of nutritional markers from blood samples, and will be able to make adjustments for confounding factors such as fibre or alcohol consumption.

One important aspect of the study will be to examine the hypothesis that lack of the B vitamin folic acid, which is involved in DNA methylation, is a key factor in the development of colorectal cancer. This hypothesis has been based on reports that people who take supplements of folic acid have a decreased risk of developing colorectal cancer[26]. The association between vitamin C and stomach cancer risk, and between fatty acids (such as the anti-inflammatory long-chain n-3 fatty acids) and cancer can also be investigated. Levels of phyto-oestrogens, which are thought to act as anti-oestrogens and so reduce risk of breast and other hormone-related cancer, are very low, but can now be measured in blood or urine using new, highly sensitive mass spectrometry methods[27]. The biorepository is also being used to measure other 'risk markers' such as plasma levels of hormones and DNA adducts. This will eventually lead to information such as whether the type of DNA damage that occurs in tumour cells can be related to diet and other environmental exposures.

Gene–nutrient interactions. A complex question is whether or not an individual's risk of cancer, resulting from a particular dietary habit, is altered by inherited polymorphisms. It is known that red meat is associated with increased colorectal cancer risk in populations (FIG. 5). An example of this 'risky gene, risky diet' combination is an individual with a diet that is high in meat intake who inherits a polymorphism that confers the

fast-acting form of an enzyme that converts a carcinogen (for example, in overcooked meat) into the active form[28]. This individual would be particularly susceptible to cancer, but only if he or she indulged in the high-risk behaviour (red-meat eating).

It has been suggested that the need to analyse the interactions between diet (and other environmental exposures) and genetic factors in prospective studies could be avoided if we simply focus our research on the genetic polymorphisms that affect tumorigenic pathways. As genetic polymorphisms are not affected by the disease itself, the prospective design would not be necessary, and research costs could be greatly reduced by simply investigating only the patients (cases) and matched healthy controls[28]. For example, the results of one large case–control study indicated that a group of carcinogens called heterocyclic amines, which are formed in cooked meat, are not important in the development of colorectal cancer, because no associations with the 'fast' polymorphisms that are responsible for converting these compounds into the active material have been found[29]. In another example of this approach, a variant of the gene that encodes methylene tetrahydrofolate reductase confers low blood levels of folic acid in carriers. The fact that this variant has been associated with reduced risks of colorectal cancer would indicate that the hypothesis that folic acid supplements are associated with low risk of colorectal cancer is incorrect[26]. However, as this is such a complex area of research, abandoning attempts to measure effects of joint exposure and genotyping is inappropriate. In most instances, the detailed mechanisms of how a particular nutrient affects cancer risk are not known, and when genes are studied exclusively, effects in subgroups of patients cannot be studied[30]. Numerous polymorphisms exist in genes, different groups of carcinogens can be included in a single food, and several genes are involved in the regulation of particular enzymes. All of these elements could have different effects on the development of different types of cancer (for example, bowel versus bladder cancer). In the case of large-bowel cancer, bacterial, not mammalian, genetics might be important[16].

Although cancer is a disease of genes, there is overwhelming evidence that environmental and lifestyle factors are the predominant cause of somatic genetic alterations that lead to most sporadic cancers. It is unlikely that the characterization of individual genomes will provide the best estimate of cancer risk in the absence of detailed information on environmental exposure. Information on lifestyle and environmental exposure, especially of diet and biomarkers, requires painstaking work on large populations over prolonged periods of time.

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Competing interests statement

The authors declare that they have no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:

Cancer.gov: http://cancer.gov/

bladder cancer | breast cancer | cervical cancer | colorectal cancer | liver cancer | lung cancer | mouth cancer | prostate cancer | skin cancer | stomach cancer

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/

E-cadherin | *TP53*

FURTHER INFORMATION

EPIC-Copenhagen centre: http://www.kost.cancer.dk
EPIC-Europe centre: http://www.iarc.fr/epic/
EPIC-Florence centre: http://www. cspo. it/
EPIC-France centre: http://www.e3n.net/
EPIC-Greece centre: http://www.nut.uoa.gr/english/epic/EpicEN.htm
EPIC-Heidelberg centre: http://www.dkfz-heidelberg.de/epi/Home_e/Programme/Workgroups/Diet/Ernaeh_e.htm
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