Title: EPIC-Heart: The cardiovascular component of a prospective study of nutritional, lifestyle and biological factors in 520 000 middle-aged participants from 10 European countries

Corresponding Author: Professor John Danesh,

Corresponding Author's Institution: Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom

First Author: John Danesh

Order of Authors: John Danesh; Rodolfo Saracci; Göran Berglund; Edith Feskens; Kim Overvad; Salvatore Panico; Simon Thompson; Agnès Fournier; Francoise Clavel-Chapelon; Marianne Canonico; Rudolf Kaaks; Jakob Linseisen; Heiner Boeing; Tobias Pischon; Cornelia Weikert; Anja Olsen; Anne Tjønneland; Søren Paaske Johnsen; Majken K Jensen; Jose R Quirós; Carlos Alberto Gonzalez Svatetz; Maria-José Sánchez Pérez; Nerea Larrañaga; Carmen Navarro Sanchez; Concepción Moreno Iribas; Sheila Bingham; Kay-Tee Khaw; Timothy Key; Andrew Roddam; Antonia Trichopoulou; Vassiliki Benetou; Dimitrios Trichopoulos; Giovanna Masala; Sabina Sieri; Rosario Tumino; Carlotta Sacerdote; Amalia Mattiello; W. M. Monique Verschuren; H. Bas Bueno-de-Mesquita; Diederick E Grobbee; Yvonne T van der Schouw; Olle Melander; Göran Hallmans; Patrik Wennberg; Eiliv Lund; Merethe Kumle; Guri Skeie; Pietro Ferrari; Nadia Slimani; Teresa Norat; Elio Riboli

Manuscript Region of Origin:

Abstract: EPIC-Heart is the cardiovascular component of the European Prospective Investigation into Cancer and Nutrition (EPIC), a multi-centre prospective cohort study investigating the relationship between

nutrition and major chronic disease outcomes. Its objective is to advance understanding about the separate and combined influences of lifestyle (especially dietary), environmental, metabolic and genetic factors in the development of cardiovascular diseases by making best possible use of the unusually informative database and biological samples in EPIC. Between 1992 and 2000, 519,978 participants (366,521 women and 153,457 men, mostly aged 35-70 years) in 23 centres in 10 European countries commenced follow-up for cause-specific mortality, cancer incidence and major cardiovascular morbidity. Dietary information was collected with quantitative questionnaires or semi-quantitative food frequency questionnaires, including a 24-hour dietary recall sub-study to help calibrate the dietary measurements. Information was collected on physical activity, tobacco smoking, alcohol consumption, occupational history, socio-economic status, and history of previous illnesses. Anthropometric measurements and blood pressure recordings were made in the majority of participants. Blood samples were taken from 385,747 individuals, from which plasma, serum, red cells, and buffy coat fractions were separated and aliquoted for long-term storage. By 2004, an estimated 10,000 incident fatal and non-fatal coronary and stroke events had been recorded. The first cycle of EPIC-Heart analyses will assess associations of coronary mortality with several prominent dietary hypotheses and with established cardiovascular risk factors. Subsequent analyses will extend this approach to non-fatal cardiovascular outcomes and to further dietary, biochemical and genetic factors.

1

EPIC-Heart

The cardiovascular component of a prospective study of nutritional, lifestyle and biological factors in 520 000 middle-aged participants from 10 European countries

John Danesh[1], Rodolfo Saracci[2], Göran Berglund[3], Edith Feskens[4], Kim Overvad[5], Salvatore Panico[6], Simon Thompson[7], Agnès Fournier[8], Françoise Clavel-Chapelon[8], Marianne Canonico[9], Rudolf Kaaks[10], Jakob Linseisen[10], Heiner Boeing[11], Tobias Pischon[11], Cornelia Weikert[11], Anja Olsen[12], Anne Tjønneland[12], Søren Paaske Johnsen[5], Majken Karoline Jensen[5], Jose R Quirós[13], Carlos Alberto Gonzalez Svatetz[14], Maria-José Sánchez Pérez[15], Nerea Larrañaga[16], Carmen Navarro Sanchez[17], Concepción Moreno Iribas[18], Sheila Bingham[19], Kay-Tee Khaw[20], Timothy Key[21], Andrew Roddam[21], Antonia Trichopoulou[22], Vassiliki Benetou[22], Dimitrios Trichopoulos[22], Giovanna Masala[23], Sabina Sieri[24], Rosario Tumino[25], Carlotta Sacerdote[26], Amalia Mattiello[6], W. M. Monique Verschuren[27], H. Bas Bueno-de-Mesquita[28], Diederick E Grobbee[29], Yvonne T van der Schouw[29], Olle Melander[3], Göran Hallmans[30], Patrik Wennberg[31], Eiliv Lund[32], Merethe Kumle[32], Guri Skeie[32], Pietro Ferrari[2], Nadia Slimani[2], Teresa Norat[33], Elio Riboli[33]

Correspondence to: EPIC-Heart Secretariat, Department of Public Health and Primary Care, University of Cambridge, Strangeways Research Laboratory, Worts Causeway, Cambridge, CB1 8RN, UK, Tel: +44 (0)1223 741302, Fax: +44 (0)1223 741339, E-mail: epic-heart@phpc.cam.ac.uk

---

[1] Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom
[2] International Agency for Research on Cancer – WHO, Lyon, France
[3] Department of Clinical Sciences, Malmö, University of Lund, Lund, Sweden.
[4] Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands
[5] Department of Clinical Epidemiology, Aalborg Hospital, Aarhus University Hospital, Aarhus, Denmark.
[6] Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy.
[7] MRC Biostatistics Unit, Institute of Public Health, University of Cambridge, Cambridge, United Kingdom
[8] Inserm ERI20, Institut Gustave Roussy, Villejuif ,France
[9] Inserm U780, Villejuif ,France
[10] Division of Clinical Epidemiology, German Cancer Research Centre, Heidelberg, Germany.
[11] Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany.
[12] Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark
[13] Public Health and Planning Directorate, Asturias, Spain.
[14] Department of Epidemiology and Cancer Registry, Catalan Institute of Oncology, Barcelona, Spain.
[15] The Andalusian School of Public Health, Granada, Spain.
[16] Public Health Department of Gipuzcoa, San Sebastian, Spain.
[17] Department of Epidemiology, Murcia Health Council, Murcia, Spain
[18] Public Health Institute of Navarra, Pamplona, Spain.
[19] MRC Centre for Nutritional Epidemiology in Cancer Prevention and Survival, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom
[20] Clinical Gerontology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom
[21] Cancer Research UK Epidemiology Unit, University of Oxford, Oxford, United Kingdom.
[22] Department of Hygiene and Epidemiology, University of Athens Medical School, Athens, Greece
[23] Molecular and Nutritional Epidemiology Unit, CSPO, Scientific Institute of Tuscany, Florence, Italy
[24] Nutritional Epidemiology Unit, Italian National Cancer Institute, Milan, Italy.
[25] Cancer Registry, Azienda Ospedaliera "Civile M.P. Arezzo", Ragusa, Italy.
[26] Department of Biomedical Science and Human Oncology, CPO-Piemonte, Torino, Italy
[27] Centre for Prevention and health Services Research, National Institute for Public Health and the Environment, Bilthoven, The Netherlands.
[28] Centre for Nutrition and Health, National Institute for Public Health and the Environment, Bilthoven, The Netherlands.
[29] Julius Centre for Health Sciences and Primary care, University Medical Centre Utrecht, Utrecht, The Netherlands.
[30] Department of Public Health and Clinical Medicine, Nutrition Research, Umeå University, Sweden.
[31] Department of Medicine, Skellefteå Hospital, Skellefteå, Sweden.
[32] Institute of Community Medicine, University of Tromsø, Tromsø, Norway.
[33] Department of Epidemiology and Public Health, Imperial College London, London, United Kingdom.

Final manuscript

7 December 2006

2500 words

6 tables, 1 figure

ABSTRACT

EPIC-Heart is the cardiovascular component of the European Prospective Investigation into Cancer and Nutrition (EPIC), a multi-centre prospective cohort study investigating the relationship between nutrition and major chronic disease outcomes. Its objective is to advance understanding about the separate and combined influences of lifestyle (especially dietary), environmental, metabolic and genetic factors in the development of cardiovascular diseases by making best possible use of the unusually informative database and biological samples in EPIC. Between 1992 and 2000, 519,978 participants (366,521 women and 153,457 men, mostly aged 35-70 years) in 23 centres in 10 European countries commenced follow-up for cause-specific mortality, cancer incidence and major cardiovascular morbidity. Dietary information was collected with quantitative questionnaires or semi-quantitative food frequency questionnaires, including a 24-hour dietary recall sub-study to help calibrate the dietary measurements. Information was collected on physical activity, tobacco smoking, alcohol consumption, occupational history, socio-economic status, and history of previous illnesses. Anthropometric measurements and blood pressure recordings were made in the majority of participants. Blood samples were taken from 385,747 individuals, from which plasma, serum, red cells, and buffy coat fractions were separated and aliquoted for long-term storage. By 2004, an estimated 10,000 incident fatal and non-fatal coronary and stroke events had been recorded. The first cycle of EPIC-Heart analyses will assess associations of coronary mortality with several prominent dietary hypotheses and with established cardiovascular risk factors. Subsequent analyses will extend this approach to non-fatal cardiovascular outcomes and to further dietary, biochemical and genetic factors.

INTRODUCTION

EPIC-Heart is the cardiovascular component of the European Investigation into Cancer and Nutrition (EPIC), a multi-centre prospective cohort study designed to investigate the relationships of nutrition with cancer and other major chronic disease outcomes.(1;2) The overall objective of EPIC-Heart is to make best possible use of the data and biological samples available in EPIC in order to advance understanding about the separate and combined influences of lifestyle (especially dietary), environmental, metabolic and genetic factors in the development of cardiovascular diseases, including coronary disease, stroke and other vascular diseases.

The EPIC-Heart resource differs from many previous epidemiological investigations of cardiovascular diseases in several ways that should increase its scientific value: (i) it is large, involving data on 519 978 adults, mostly aged 35-70 years thereby enabling precise estimates of the impact of suspected risk factors; (ii) it is geographically diverse, with participants recruited from 23 centres in 10 European countries, providing an opportunity to investigate the impact of contrasts in various dietary and other exposures (as well as in cardiovascular disease rates); (iii) it involves 366 521 female participants, thereby enabling detailed analyses in women; (iv) it involves prospective monitoring of participants, the great majority of whom reported no history of cardiovascular or other recorded diseases at the initial examination, thereby minimizing the influence of disease itself on the characteristics recorded at baseline (such as on dietary habits and biochemical factors); (v) it contains information on a range of lifestyle and other exposures (notably dietary intake), as well as, in about three-quarters of participants, stored biological samples, enabling biochemical and genetic analyses; and (vi) it monitors participants, after the initial examination, for cause-specific mortality and, in a large subset, for major cardiovascular morbidity. This combination of features will enable EPIC-Heart to make a substantial contribution to the prevention and understanding of cardiovascular disease by elucidating its determinants.

The present report provides a description of the aims and resources of EPIC-Heart in order to bring it to the attention of the wider scientific community, to outline certain planned analyses, and to simplify references to it in further papers reporting empirical or methodological findings. Previous reports have provided a detailed account of the individual EPIC study cohorts, their source populations, and information on samples collected at the baseline examination with particular reference to cancer outcomes.(3;4) The present report complements those accounts by focusing chiefly on aspects relevant to EPIC-Heart.

METHODS

Participants

The procedures used in the recruitment of participants into the EPIC study have been described in detail elsewhere.(5) The seven initial EPIC countries (France, Germany, Greece, Italy, the Netherlands, Spain, and the United Kingdom) used a common protocol, and they were later joined by centres in Denmark, Norway, Sweden and one centre in Italy (Naples) that were conducting broadly similar prospective studies (Figure 1 and Table 1). Between 1992 and 2000, 519 978 participants (366 521 women and 153 457 men, mostly aged 35-70 years) were recruited in 23 centres located in 10 European countries. The majority of centres invited participants (typically identified according to age, sex, and, optionally, other criteria: Table 1) from population-based registers (Denmark, Germany, certain Italian centres, the Netherlands, Norway, Sweden, the UK). In other centres, various sampling frameworks were used, including: blood donors (Spain and Turin and Ragusa in Italy); screening clinic attendees (Florence in Italy and Utrecht in the Netherlands); people in health insurance programmes (France); and health-conscious individuals (Oxford, UK). Participant eligibility within each cohort was mainly based on geographical or administrative boundaries. In France, Norway, Utrecht in the Netherlands and Naples in Italy, however, only women were recruited. As a rule, participants were invited to participate either by mail or in person or both (Table 1).

Dietary intake assessment

Dietary intake was assessed using a number of different instruments that were developed and validated in various populations participating in EPIC, as described previously.(6-10) Three dietary assessment approaches were used: (i) extensive quantitative dietary questionnaires, containing at least a few hundred food items and estimating individual average portions (used in France, Germany, Italy [except in Naples], the Netherlands and Spain), involving self-administration except in Spain, Greece and Ragusa in Italy where interviewers were used; (ii) semi-quantitative food-frequency questionnaires, with the same standard portion(s) assigned to all participants (used in Denmark, Norway, Naples, and Umea in Sweden); and (iii) combined dietary methods in the UK (where centres used a semi-quantitative food-frequency questionnaire and a 7-day food diary) and in Malmo in Sweden (where a non-quantitative food-frequency questionnaire was used together with a 14-day record on lunches and dinners). To facilitate combined analyses given the use of different dietary assessment methods across centres, additional data were collected by computer-assisted 24-hour dietary recall in 36 900 EPIC participants, representing approximately 5-12% sub-samples in each centre (or about 1.5% for the UK centres). This calibration study has been described elsewhere(11-13) and a

common standardized food composition database for several nutrients has been developed.(14;15)

Other questionnaire-based data

For the seven initial EPIC countries, a common set of questions and possible answers was agreed upon and translated into national questionnaires to assess factors related to lifetime history of tobacco and alcohol consumption, occupational history, socio-economic status, physical activity, and history of previous illnesses (Table 2), typically involving self-completion postal questionnaires. A comprehensive system of re-coding has been developed to maximize the comparability of data from these initial centres with those which joined EPIC at a later stage (i.e., Denmark, Sweden and Norway and the Naples centre in Italy) and which had used different questionnaires to record information on a similar set of factors. Of particular relevance to EPIC-Heart, baseline questionnaires in most centres recorded self-reported histories of myocardial infarction, angina, stroke and hypertension (generally including the reported age at onset of such diagnoses: Table 2). In Spain, validation studies demonstrated moderately good agreement between self-reported history of hypertension and medical records about this condition(16).

Anthropometry and blood pressure

Height, weight, and waist and hip circumference were measured on all subjects using similar protocols (in Umea, only weight and height were measured), except in France, the Oxford centre and Norway. In France and Oxford, weight, height and waist and hip circumference were measured in only a subset of participants, but self-reported weight and height were obtained from all individuals (and, in Oxford, self-reported measurements also included waist and hip circumference). For the "health-conscious' group based in Oxford (UK), linear regression models were used to predict sex and age-specific values from participants with either measured or self-reported body measures, as previously described(17). In Norway only self-reported height and weight are available. Systolic and diastolic blood pressure recordings were made only in Denmark, Italy, Germany, Greece, in subsets of participants in three of five centres in Spain (comprising 14% of Spanish participants), Sweden, the Netherlands and in UK centres, in a total of 322 869 individuals (or in about 62% of baseline participants). Blood pressure measurements were obtained using a variety of devices and methods, as described in detail elsewhere(18). In most centres the measurements were taken in duplicate, from the right arm, in a sitting position, using either a mercury manometer or an oscillometric device(19-24).

Biological samples

Biological samples – including plasma, serum, white blood cells and erythrocytes – have been collected, separated and aliquoted for long-term storage from 385 747 of the EPIC study

participants (Table 1). In the seven initial EPIC countries and in Naples, blood samples have been aliquoted into 28 plastic straws containing 0.5ml each (12 plasma with sodium citrate, eight serum, four erythrocytes, four buffy coat for DNA). To ensure standardization, the same materials (syringes, straws, etc) were purchased centrally and distributed to the centres. The samples were then split into two halves of 14 aliquots each, one stored locally and one transported to the International Agency for Research on Cancer (IARC) in Lyon, France, to be stored in liquid nitrogen (at -196°C) in a central repository with computerized sample tracking capabilities. Samples in Norway were collected into twenty 0.5ml plastic straws, a subset of which was shipped to IARC for storage. In Sweden and Denmark, blood samples were collected into tubes and have been kept solely in local freezers at -80°C and -150°C, respectively.

Biochemical and genetic analytes

Measurements of established biochemical risk factors and emerging biomarkers have so far been done only in subsets of participants in some EPIC centres, typically involving local sample aliquots and sometimes using different assay methods. For example, a number of reports have emerged from individual EPIC centres in relation to associations of coronary disease or stroke (or carotid thickening) with established cardiovascular risk factors, dietary factors, novel lipid markers, glycated haemoglobin, growth factors, haemostatic factors, and markers of insulin resistance, oxidation status and iron metabolism(25-40). EPIC-Heart intends to measure a range of established risk factors (e.g., concentrations of serum lipids and their sub-fractions) and various emerging risk markers (e.g., concentrations of novel lipid and apolipoprotein fractions, markers of inflammation and haemostasis and metabolic characteristics) in subsets of the entire collection (e.g., on a "nested" case-control or case-cohort basis), using standardized assays according to a common protocol. A few of the individual EPIC centres have reported on genetic factors in relation to cardiovascular diseases(41-43). EPIC-Heart plans to conduct genotyping studies on subsets of the entire collection, particularly in relation to hypotheses related to lipid, inflammatory, haemostatic and metabolic pathways.

Cardiovascular outcomes

Data on cause-specific mortality, including cardiovascular causes, have been collected in all EPIC study centres through mortality registries or active follow-up and death-record collection. By April 2004, a total of 15 619 deaths had been recorded, of which 3663 were attributed to circulatory causes (including approximately 1544 coronary deaths: Table 3). Additionally, a further approximately 5000 non-fatal myocardial infarctions and a further approximately 3700 non-fatal strokes have been recorded by April 2004 across all centres (data available on request). Different centres have used different methods to ascertain incident non-fatal coronary and stroke events, depending on the follow-up procedures used (e.g., active versus passive

follow-up). These methods have included self-report questionnaires, queries of medical records, linkage with hospital morbidity registers, MONICA registries or combinations of these methods (Tables 4 and 5). For example, in relation to non-fatal coronary outcomes, record linkage systems have been used in Cambridge in the UK, Denmark, Italy, the Netherlands, Spain and in Swedish centres (including the Umea centre, which involves a MONICA registry for myocardial infarction and stroke); in the case of the Italian centres, record linkage is routinely supplemented by information from medical records. In Potsdam in Germany, in Greece and in Spain, self-report questionnaires about cardiovascular outcomes are checked against medical records by physicians and/or hospital discharge information. In Norway and in Oxford in the UK, so far only self-report questionnaires have been used to record coronary outcomes.

This variation in ascertainment methods has resulted in some differences in definitions used (and in the detail of classification available) for non-fatal myocardial infarction and for non-fatal stroke events. To help judge the validity and comparability of such diagnoses, validation studies for non-fatal coronary outcomes have been conducted, are in progress or planned in ten centres (Table 6). Such studies assess in sub-samples of participants the concordance of diagnoses based on a centre's routine ascertainment method(s) with those based on an internationally agreed criteria (such as MONICA criteria). Overall, 1658 of 1894 (87.5%) of suspected non-fatal coronary outcomes were confirmed against MONICA (or similar) criteria, with the successful validation rate varying from 82% in Denmark (which employed particularly broad diagnoses at ascertainment) to in excess of 95% in Cambridge in the UK, Italy, and Navarra and Murcia in Spain. These findings suggest that the non-fatal coronary outcomes already recorded in most EPIC centres should be sufficiently accurate for use in epidemiological studies. Nonetheless, EPIC-Heart is planning further validation studies of non-fatal cardiovascular outcomes to optimize comparability of outcomes across centres (and an update will soon take place for cause-specific mortality recorded after April, 2004, in the central database) in order to enhance the numbers of fatal and non-fatal cardiovascular outcomes available for analyses.

Statistical approach

A methodology guideline statement has been developed to achieve a harmonized approach to data analyses (http://phpc.cam.ac.uk/EPIC-Heart/). Data will be analysed using Cox's proportional hazards models, with age as the primary time variable, and stratified by sex, centre and age at baseline. Main exposure variables will, where possible, be treated as continuous variables (following logarithmic or other transformation, if required) or as indicator variables (e.g., quantiles). Calibration of dietary exposures using information from the 24-hour dietary recall substudy will be performed where appropriate. Tests for linear trend will involve data on the

continuous scale (or, where appropriate, use of a score variable for categories of exposure). Sub-group analyses will be done sparingly for pre-specified hypotheses (which will be specific to exposures).

Study organization

Since 2005 the activities of EPIC-Heart have been organized by a secretariat (coordinated from the University of Cambridge) comprised largely of members from several different EPIC centres. In addition to leading the drafting of the present report, the secretariat has prepared a strategy document to help the planning of the EPIC-Heart working group and a methodology guideline statement including statistical and epidemiological approaches that can be used in publications that will emerge from EPIC-Heart (http://phpc.cam.ac.uk/EPIC-Heart/). Like all EPIC working groups, EPIC-Heart is directed by and responsible to the EPIC steering committee (which is comprised of principal investigators from all ten participating countries and led by Professor Elio Riboli at Imperial College, London).

Concluding remarks

EPIC-Heart involves almost 520 000 middle-aged participants from 10 European countries and has already recorded an estimated 10 000 incident fatal and non-fatal cardiovascular outcomes. It will become increasingly informative as the numbers of cardiovascular outcomes recorded continue to increase and as further efforts are made to improve the characterization of outcomes. Although the EPIC study was initially set up to investigate nutrition and cancer, its data and biological resources provide an excellent opportunity to study the determinants of cardiovascular diseases in order to help advance scientific understanding and disease prevention strategies. The first cycle of EPIC-Heart analyses will assess associations between coronary mortality and several prominent dietary hypotheses (e.g., fish, meat, nut, fruit and vegetable consumption) and established cardiovascular risk factors (e.g., anthropometric indices, blood pressure). Subsequent analyses will extend this approach to non-fatal cardiovascular outcomes as well as to further dietary, biochemical and genetic factors.

Acknowledgements

References

(1) Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002 Dec;5(6B):1113-24.

(2) Bingham S, Riboli E. Diet and cancer--the European Prospective Investigation into Cancer and Nutrition. Nat Rev Cancer 2004 Mar;4(3):206-15.

(3) Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002 Dec;5(6B):1113-24.

(4) Bingham S, Riboli E. Diet and cancer--the European Prospective Investigation into Cancer and Nutrition. Nat Rev Cancer 2004 Mar;4(3):206-15.

(5) Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002 Dec;5(6B):1113-24.

(6) Overvad K, Tjonneland A, Haraldsdottir J, Bang S, Ewertz M, Moller-Jensen O. Development of a semi quantitative food frequency questionnaire to assess food, energy and nutrient intake in Denmark. Int J Epidemiol 1991;20:906-12.

(7) Bingham SA, Gill C, Welch A, Day K, Cassidy A, Khaw KT, et al. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. Br J Nutr 1994 Oct;72(4):619-43.

(8) Margetts BM, Pietinen P. European Prospective Investigation into Cancer and Nutrition: validity studies on dietary assessment methods. Int J Epidemiol 1997;26 Suppl 1:S1-S5.

(9) Riboli E, Elmstahl S, Saracci R, Gullberg B, Lindgarde F. The Malmo Food Study: validity of two dietary assessment methods for measuring nutrient intake. Int J Epidemiol 1997;26 Suppl 1:S161-S173.

(10) Kroke A, Klipstein-Grobusch K, Voss S, Moseneder J, Thielecke F, Noack R, et al. Validation of a self-administered food-frequency questionnaire administered in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study: comparison of energy, protein, and macronutrient intakes estimated with the doubly labeled water, urinary nitrogen, and repeated 24-h dietary recall methods. Am J Clin Nutr 1999 Oct;70(4):439-47.

(11) Slimani N, Deharveng G, Charrondiere RU, van Kappel AL, Ocke MC, Welch A, et al. Structure of the standardized computerized 24-h diet recall interview used as reference method in the 22 centers participating in the EPIC project. European Prospective Investigation into Cancer and Nutrition. Comput Methods Programs Biomed 1999 Mar;58(3):251-66.

(12) Slimani N, Ferrari P, Ocke M, Welch A, Boeing H, Liere M, et al. Standardization of the 24-hour diet recall calibration method used in the european prospective investigation into cancer and nutrition (EPIC): general concepts and preliminary results. Eur J Clin Nutr 2000 Dec;54(12):900-17.

(13) Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. Public Health Nutr 2002 Dec;5(6B):1125-45.

(14) Charrondiere R, Vignat J, Moller A, Ireland J, Becker W, Church S, et al. The European Nutrient Database (ENDB) for nutritional epidemiology. J Food Comp Anal 2002;15:435-51.

(15) Slimani N, Charrondiere R, Van Staveren WA, Riboli E. Standardisation of food composition databases for the European Prospective Investigaion into Cancer and Nutrition (EPIC); general theoretical concept. J Food Comp Anal 2000;13:567-84.

(16) Tormo MJ, Navarro C, Chirlaque MD, Barber X. Validation of self diagnosis of high blood pressure in a sample of the Spanish EPIC cohort: overall agreement and predictive values. EPIC Group of Spain. J Epidemiol Community Health 2000 Mar;54(3):221-6.

(17) Haftenberger M, Lahmann PH, Panico S, Gonzalez CA, Seidell JC, Boeing H, et al. Overweight, obesity and fat distribution in 50- to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 2002 Dec;5(6B):1147-62.

(18) Schulze MB, Kroke A, Saracci R, Boeing H. The effect of differences in measurement procedure on the comparability of blood pressure estimates in multi-centre studies. Blood Press Monit 2002 Apr;7(2):95-104.

(19) Appleby PN, Davey GK, Key TJ. Hypertension and blood pressure among meat eaters, fish eaters, vegetarians and vegans in EPIC-Oxford. Public Health Nutr 2002 Oct;5(5):645-54.

(20) Canoy D, Luben R, Welch A, Bingham S, Wareham N, Day N, et al. Fat distribution, body mass index and blood pressure in 22,090 men and women in the Norfolk cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk) study. J Hypertens 2004 Nov;22(11):2067-74.

(21) Khaw KT, Bingham S, Welch A, Luben R, O'Brien E, Wareham N, et al. Blood pressure and urinary sodium in men and women: the Norfolk Cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk). Am J Clin Nutr 2004 Nov;80(5):1397-403.

(22) Psaltopoulou T, Orfanos P, Naska A, Lenas D, Trichopoulos D, Trichopoulou A. Prevalence, awareness, treatment and control of hypertension in a general population sample of 26,913 adults in the Greek EPIC study. Int J Epidemiol 2004 Dec;33(6):1345-52.

(23) Schulze MB, Kroke A, Bergmann MM, Boeing H. Differences of blood pressure estimates between consecutive measurements on one occasion: implications for inter-study comparability of epidemiologic studies. Eur J Epidemiol 2000;16(10):891-8.

(24) Tormo MJ, Navarro C, Chirlaque MD, Barber X. Is there a different dietetic pattern depending on self-knowledge of high blood pressure? Eur J Epidemiol 2000;16(10):963-71.

(25) Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet and Cancer Study. Design and feasibility. J Intern Med 1993 Jan;233(1):45-51.

(26) Boekholdt SM, Kuivenhoven JA, Wareham NJ, Peters RJ, Jukema JW, Luben R, et al. Plasma levels of cholesteryl ester transfer protein and the risk of future coronary artery disease in apparently healthy men and women: the prospective EPIC (European Prospective Investigation into Cancer and nutrition)-Norfolk population study. Circulation 2004 Sep 14;110(11):1418-23.

12

(27) Hedblad B, Nilsson P, Janzon L, Berglund G. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a cross-sectional study in Malmo, Sweden. Diabet Med 2000 Apr;17(4):299-307.

(28) Hoffmann K, Heidemann C, Weikert C, Schulze MB, Boeing H. Estimating the proportion of disease due to classes of sufficient causes. Am J Epidemiol 2006 Jan 1;163(1):76-83.

(29) Jansson JH, Olofsson BO, Nilsson TK. Predictive value of tissue plasminogen activator mass concentration on long-term mortality in patients with coronary artery disease. A 7-year follow-up. Circulation 1993 Nov;88(5 Pt 1):2030-4.

(30) Johnsen SP, Hundborg HH, Sorensen HT, Orskov H, Tjonneland A, Overvad K, et al. Insulin-like growth factor (IGF) I, -II, and IGF binding protein-3 and risk of ischemic stroke. J Clin Endocrinol Metab 2005 Nov;90(11):5937-41.

(31) Key TJ, Appleby PN, Davey GK, Allen NE, Spencer EA, Travis RC. Mortality in British vegetarians: review and preliminary results from EPIC-Oxford. Am J Clin Nutr 2003 Sep;78(3 Suppl):533S-8S.

(32) Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. Ann Intern Med 2004 Sep 21;141(6):413-20.

(33) Kok HS, van Asselt KM, van der Schouw YT, van dT, I, Peeters PH, Wilson PW, et al. Heart disease risk determines menopausal age rather than the reverse. J Am Coll Cardiol 2006 May 16;47(10):1976-83.

(34) Mann JI, Appleby PN, Key TJ, Thorogood M. Dietary determinants of ischaemic heart disease in health conscious individuals. Heart 1997 Nov;78(5):450-5.

(35) Panico S, Celentano E, Galasso R, Mattiello A, Masala G, Palli D, et al. Dietary Pattern and Ischemic heart disease incidents in female population of a mediterranean country; findings from EPIC-Italy Collaboration. Circulation 108[Supp IV], 737. 2003.
Ref Type: Journal (Full)

(36) Rubba P, Panico S, Bond MG, Covetti G, Celentano E, Iannuzzi A, et al. Site-specific atherosclerotic plaques in the carotid arteries of middle-aged women from southern Italy: associations with traditional risk factors and oxidation markers. Stroke 2001 Sep;32(9):1953-9.

(37) Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation 1998 Nov 24;98(21):2241-7.

(38) Tolstrup J, Jensen MK, Tjonneland A, Overvad K, Mukamal KJ, Gronbaek M. Prospective study of alcohol drinking patterns and coronary heart disease in women and men. BMJ 2006 May 27;332(7552):1244-8.

(39) van der AD, Marx JJ, Grobbee DE, Kamphuis MH, Georgiou NA, Kats-Renaud JH, et al. Non-transferrin-bound iron and risk of coronary heart disease in postmenopausal women. Circulation 2006 Apr 25;113(16):1942-9.

(40) Weikert C, Hoffmann K, Dierkes J, Zyriax BC, Klipstein-Grobusch K, Schulze MB, et al. A homocysteine metabolism-related dietary pattern and the risk of coronary heart disease in two independent German study populations. J Nutr 2005 Aug;135(8):1981-8.

(41) Vaessen SF, Schaap FG, Kuivenhoven JA, Groen AK, Hutten BA, Boekholdt SM, et al. Apolipoprotein A-V, triglycerides and risk of coronary artery disease: the prospective Epic-Norfolk Population Study. J Lipid Res 2006 Sep;47(9):2064-70.

(42) Boekholdt SM, Sandhu MS, Wareham NJ, Luben R, Reitsma PH, Khaw KT. Fibrinogen plasma levels modify the association between the factor XIII Val34Leu variant and risk of coronary artery disease: the EPIC-Norfolk prospective population study. J Thromb Haemost 2006 Oct;4(10):2204-9.

(43) van der AD, Peeters PH, Grobbee DE, Roest M, Marx JJ, Voorbij HM, et al. HFE mutations and risk of coronary heart disease in middle-aged women. Eur J Clin Invest 2006 Oct;36(10):682-90.

Figure 1: Countries and centres involved in the EPIC study

TABLE 1: Recruitment information and source populations

Country	Geographical /political area (centre)	Source population* and description	Target eligibility criteria	Initial contact	Enumeration of those invited	Total no. recruited	No. of females	Years of recruitment	No. with blood samples
Core EPIC cohorts									
Greece	Nationwide	Active recruitment of the general population	Apparently healthy men and women aged 25-82	In person and by mail	No	28572	16618	1994-1999	28526
Spain	Granada: province	Blood donors, general population (recruited through census, health centres)	Residents: men aged 40-64, women aged 35-64	In person and by mail	No	7879	6083	1992-1996	6892
	Murcia: region	Blood donors and their partners (67% of cohort), general population of 2 towns (23%), civil servants (5%), employees of 2 companies (3%), participants in a cardiovascular risk study (2%)	Residents: men aged 40-65, women aged 35-65	In person and by mail	No	8516	5831	1992-1996	8146
	Navarra: region	Blood donors	Residents: men aged 40-65, women aged 35-65	In person and by mail	Yes	8084	4176	1992-1995	7799
	San Sebastian: city and Gipuzcoa province	Blood donors, employees of selected enterprises (recruited through census of selected enterprises)	Residents: men aged 40-65, women aged 35-65	In person and by mail	Yes	8417	4259	1992-1995	8325
	Asturias:region	Blood donors, regional civil servants and general population	Men aged 40-64, women aged 35-64	Mail	Yes	8544	5459	1992-1995	8417
Italy	Ragusa: province	Local blood donors association, population-based recruitment in 4 towns (Monterosso, Girralana, Ispica and Chiaramonte), local teachers union and other sources	Residents: men aged 35-65, women aged 35-75	In person	Yes	6403	3350	1993-1997	6396
	Florence: province	Breast cancer screening participants, men and women from the general population	Residents: men aged 35-64, women aged 35-64 without prevalent cancer	In person and by mail	No	13597	10083	1992-1998	13597
	Turin: city	Blood donors, employees, volunteers, medical test users at national health service	Residents: men aged 40-74, women aged 35-74 without prevalent cancer	In person	No	10604	4557	1993-1998	10604
	Varese: province	Volunteers from resident general population, mostly an extension of an ongoing study	Men aged 35-65, women aged 35-75	In person	Yes	12083	9526	1993-1997	12073
France	Nation-wide	Nationwide health insurance programme; teachers and school workers enrolled in an ongoing study prior to EPIC	Women aged 40-65 in 1990 with informed consent to obtain MGEN info on non-respondents	Mail	Yes	72996	72996	1993-1997	20725
Germany	Heidelberg and surrounding areas	General population	Residents: men aged 40-65, women aged 35-65, completed questionnaires and examination	Mail	Yes	25546	13617	1994-1998	24235
	Potsdam and surrounding areas	General population	Residents: men aged 40-65, women aged 35-65, completed questionnaires and examination	Mail	Yes	27548	16644	1994-1998	26444
Netherlands	Bilthoven: Amsterdam, Doetinchem and Maastricht (3 cities)	Population-based age and sex-stratified samples of the general population	Residents: men and women aged 20-59 in Amsterdam and Maastricht and aged 20-65 in Doetinchem	Mail	Yes	22715	12435	1993-1997	19388
	Utrecht: district	Population-based breast cancer screening participants	Residents: women aged 49-70	Mail	Yes	17357	17357	1993-1997	16930
United Kingdom	Cambridge: Norfolk	Population-based patients of general practitioners	Listed by general practitioners: men and women aged 45-74	Mail	Yes	30442	16744	1993-1998	24035
	Oxford: (1) local counties; (2) "health conscious" individuals from England, Wales, Scotland & N. Ireland	(1) Population-based in collaboration with general practitioners (2) Vegetarians, vegans and other health-conscious individuals in collaboration with vegetarian societies and magazines	(1) Listed by general practitioners: men and women aged 40-65; (2) men and women aged 20+ but targeted at those aged 35+	Mail	No	57498	44284	1993-2000	19103
Associated EPIC cohorts									
Italy	Naples	Female volunteers from resident general population	Women aged 30-69	In person and by mail	No	5062	5062	1993-1997	5055
Denmark	Aarhus	Population-based	Born in Denmark: men and women aged 50-64 without prevalent cancer	Mail	Yes	17154	8721	1995-1997	17094
	Copenhagen	Population-based	Born in Denmark: men and women aged 50-64 without prevalent cancer	Mail	Yes	39900	21154	1993-1997	39037
Sweden	Malmö: city	Population-based	Residents: men aged 50-72, women aged 46-72	Mail	Yes	28098	17035	1991-1996	28023
	Umeå: the Vasterbotten county	Population-based	Residents: men and women aged 30, 40, 50 or 60	Mail	Yes	25732	13299	1992-1996	25732
Norway	Tromsø: national sample	Population-based	Women born in Norway between 1943 and 1957	Mail	Yes	37231	37231	1998-1998	9197
						519978	**366521**		**385747**

*Under source population, the term "population-based" implies that participants were invited as a random sample of their population, while the term "general population" implies that volunteers were invited from the general population.

Table 2: Information about variables collected at the baseline assessment

Characteristic	Variables collected
Socio-economic status	Marital status, occupational history, school leaving age, highest school level achieved
Anthropometry (measured or self-reported)	Height, weight, waist and hip circumference, weight at age 20
Blood collection	Timing, fasting status, date of last smoking, drugs consumed, pulse rate, systolic and diastolic blood pressure, blood pressure measuring technique
Previous illnesses and age at diagnosis of each	Myocardial infarction, angina, stroke, circulatory problems in brain, hypertension, hypertension treatment, hyperlipidaemia, hyperlipidaemia treatment, elevated cholesterol, type 2 diabetes, insulin treatment for diabetes, gallstones, gallstones treatment, polyps of large bowel, cancer, hysterectomy, removal of one or both ovaries, breast surgery
Female reproductive history	Age at first menstrual period, age periods stopped, hormones for menopause treatment, number of live born children, age at first delivery, breast-feeding, oral contraceptive
Physical activity	Walking, cycling, gardening, Do-It-Yourself, housework, physical exercise according to season, vigorous physical activity, number of stairs climbed daily, occupational activity
Tobacco smoking	Number per day at certain ages (with/without filter), whether inhaled, age started smoking, age gave up smoking, total years not smoked, smoked occasionally during life, parents smoked in respondent's childhood, someone smokes at home/work
Alcohol consumption	Quantity and frequency of consumption of wine, beer/cider, fortified wine, spirit/liquor at certain ages

TABLE 3: Approximate numbers of fatal coronary heart disease cases reported to the coordinating centre by the latest available follow-up (April 2004), grouped by age and sex

Age at recruitment (years)	Sex		
	Men	Women	Both
<55	200	47	247
55-69	166	47	213
60-64	273	87	360
65-69	192	93	285
70-74	191	95	286
75-80	71	33	104
80+	26	23	49
All ages	1119	425	1544

TABLE 4: Non-fatal coronary endpoints: definition and classification

Country	Region(s)	Ascertainment method	Definition of endpoints			Classification of endpoints			
			Clinical Features	ECG	Cardiac Enzymes	Definite	Possible	Silent	Unspecified
Core EPIC cohorts									
Greece	Nationwide	Self-report questionnaire followed by validation through hospital discharge data and medical records	■	■	■	■	■	□	□
Spain	Granada	Self-report questionnaires + record linkage followed by validation with hospital notes	■	■	■	■	■	■	■
	Murcia	As above							
	Navarra	As above							
	San Sebastian	As above							
	Asturias	As above							
Italy	Ragusa	Record linkage followed by validation with hospital notes	■	■	■	■	■	■	□
	Florence	As above	■	■	■	■	■	■	□
	Turin	As above	■	■	■	■	■	■	■
	Varese	Record linkage followed by validation with hospital notes	■	■	■	■	■	■	□
France	Nationwide	Contact with GPs and hospitals after self-report	■	■	■	■*	■*	■*	■*
Germany	Heidelberg	Self-report questionnaires, partly record linkage and review of medical records	□	□	□	□	□	□	□
	Potsdam	Self-report questionnaire followed by validation by treating physician or clinics,	■**	■**	■**	■	□	□	□
Netherlands	Bilthoven: Amsterdam, Doetinchem, Maastricht	Record linkage, self-report questionnaire	□	□	□	■	■	■	■
	Utrecht	As above	□	□	□	■	■	■	■
UK	Cambridge: Norfolk	Record linkage	■	■	■	□	□	□	■
	Oxford: (1) local counties; (2) "health conscious" from UK	Self-report questionnaire	□	□	□	□	□	□	□
Associated EPIC cohorts									
Italy	Naples	Record linkage followed by validation with hospital notes	■	■	■	■	■	■	□
Denmark	Aarhus Copenhagen	Record linkage	■	■	■	■	■	■	□
Sweden	Malmö	Record linkage (by epidemiologic centre with the National Board of Health and Welfare)	■	■	■	■	□	□	□
	Umeå	Record linkage (by registration with the MONICA centre)	■	■	■	■	■	□	□
Norway	Tromsø								

□ = Criterion not used
■ = Criterion specifically used
*Determined by ad hoc committee
** Whole range of diagnostic tools used

TABLE 5: Non-fatal stroke endpoints: definition and classification

Country	Region(s)	Ascertainment method	Definition of endpoints		Classification of endpoints		
			Clinical Features	CT/MRI imaging	Ischaemic vs. haemorrhagic	Transient ischaemic attacks	Unspecified
Core EPIC cohorts							
Greece	Nationwide	Self-report questionnaire followed by validation through hospital discharge data and medical records	■	■	■	■	■
Spain	Granada	Self-report questionnaire	□	□	□	□	□
	Murcia	Self-report questionnaire + hospital discharge record	□	□	□	□	□
	Navarra	Self-report questionnaire + record linkage followed by validation with hospital notes	■	■	■	■	■
	San Sebastian	As above	■	■	■	■	■
	Asturias	Self-report questionnaire	□	□	□	□	□
Italy	Ragusa	Record linkage followed by validation with hospital notes	■	■	■	□	□
	Florence	As above			■	□	□
	Turin	As above	■	■	■	□	■
	Varese	As above	■	■	■	□	□
France	Nationwide	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable
Germany	Heidelberg	Self-report questionnaires, partly record linkage and review of medical records	□	□	■		
	Potsdam	Self-reported questionnaire followed by validation by treating physician or clinics	■**	■**	■	□	□
Netherlands	Bilthoven: Amsterdam, Doetinchem, Maastricht	Record linkage, self-report questionnaire	■	■	■	■	
	Utrecht	As above	■	■	■	■	□
UK	Cambridge: Norfolk	Record linkage	■	■	□	□	■
	Oxford: (1) local counties; (2) "health conscious" from UK	Self-report questionnaire	□	□	□	□	□
Associated EPIC cohorts							
Italy	Naples	Record linkage followed by validation with hospital notes	■	■	■	□	□
Denmark	Aarhus Copenhagen	Record linkage	■	■	■	■	■
Sweden	Malmö	Record linkage with STROMA (Stroke Register of Malmö)	■	■	■	□	□
	Umeå	Record linkage (by registration with the MONICA centre)	■	■	■	Unavailable	Unavailable
Norway	Tromsø						

□ = Criterion not used
■ = Criterion specifically used
*Determined by ad hoc committee
** Whole range of diagnostic tools used

TABLE 6: Description of non-fatal coronary disease validation sub-studies already completed or in progress in EPIC

Centre	Method of coronary disease case ascertainment	Method of case validation / criteria	No. successfully validated/No. ascertained
Cambridge, UK	Hospital discharge records involving ICD-9 codes 410-414	Review of medical records / MONICA criteria	38/39 (97%)
Denmark	Hospital discharge records involving incident ICD-8 codes 410 and ICD-10 codes I21	Review of medical records / AHA, WHF, ESC, CDC, and NHLBI criteria	878/1074 (81.8%)[1]
Greece	Hospital discharge data and in patient medical records involving ICD-10 codes I20-I25	Review of medical records/MONICA criteria and/or treating physician diagnosis	Ongoing
Italy	Hospital discharge records involving ICD-9 codes 410 and 412	Review of medical records / MONICA criteria	88/92 (95%)[2]
Malmö, Sweden	Hospital discharge records of ICD-9 code 410	Review of medical records / WHO criteria	275/293 (93%)
Umea, Sweden	MONICA criteria	Review by MONICA registration group	Highest quality MONICA registration score achieved
Bilthoven (Maastricht only), the Netherlands	Hospital discharge records involving ICD-9 codes 410-414	Validation case ascertainment through systematic review of medical records	Ongoing
San Sebastian, Spain	Telephone questionnaires 3 years after recruitment, supplemented by postal questionnaire and hospital discharge records involving ICD-9 codes 410-414	Review of medical records / MONICA criteria	128/143 (90%)[3]
Navarra, Spain	Telephone questionnaires 3 years after recruitment, supplemented by postal questionnaire and hospital discharge records involving ICD-9 codes 410-414 during 1996-2004	Review of medical records / MONICA criteria	153/153 (100%)
Murcia, Spain	Telephone questionnaire 3 years after recruitment, supplemented by postal questionnaire and hospital discharge records involving ICD-9 codes 410-414 during 1996-2004	Review of medical records / MONICA criteria	98/100 (98%)

ICD = International Classification of Disease, MONICA = Multinational MONItoring of trends and determinants in CArdiovascular disease Project, AHA = American Heart Association, WHF = World Heart Federation, ESC = European Society of Cardiology, CDC = Centres for Disease Control and Prevention, NHLBI = National Heart Lung and Blood Institute, WHO = World Health Organization.

[1] The validation rate in the Danish centres may be somewhat lower than those in other EPIC centres owing to the pragmatic approach used in identifying patients in hospital discharge registries, i.e., all patients with the discharge diagnoses listed in the table were included, irrespective of where the diagnosis had been made (emergency room and outpatient clinics were also included) and whether or not it was a primary or secondary diagnosis. A paper is in preparation that discusses the Danish validation study in more detail.

[2] These four "non-validated" cases actually had a previous history of myocardial infarction prior to the study's baseline examination, and, therefore, did not fulfill the validation study's criterion of first myocardial infarction.

[3] The validation rate in San Sebastian may be somewhat lower since all patients with the discharge diagnosis listed in the table were included irrespective of whether it was a primary or secondary diagnosis

There were no suggested changes to the manuscript.