

Diet and overall survival in elderly people

Antonia Trichopoulou, Antigone Kouris-Blazos, Mark L Wahlqvist, Charalambos Gnardellis, Pagona Lagiou, Evangelos Polychronopoulos, Tonia Vassilakou, Loren Lipworth, Dimitrios Trichopoulos

Abstract

Objective—To assess the influence of a specific dietary pattern on overall survival.

Design—Cohort study.

Setting—Three rural Greek villages, the data from which were collected as part of an international cross cultural study of food habits in later life.

Subjects—182 elderly residents of the three villages.

Main outcome measure—Overall mortality.

Results—Diet was assessed with a validated extensive semiquantitative questionnaire on food intake. A one unit increase in diet score, devised a priori on the basis of eight component characteristics of the traditional common diet in the Mediterranean region, was associated with a significant 17% reduction in overall mortality (95% confidence interval 1% to 31%).

Conclusion—A diet meeting currently understood health criteria does predict survival among people.

Introduction

There is extensive scientific literature on the relation between diet and incidence of or mortality from coronary heart disease, various types of cancer, and several other diseases.¹ Case-control studies of the nutritional epidemiology of certain chronic diseases also have been undertaken in Greece, with results generally similar to those previously or subsequently reported by other investigators from other parts of the world.² No analytical epidemiological study, however, has ever properly documented an association between a precisely defined dietary pattern and overall survival. Data collected in three Greek villages as part of an international cross cultural study of food habits in later life provided us with the opportunity to evaluate prospectively the role of diet in the survival of elderly Greeks. The cross cultural study was undertaken under the auspices of the International Union of Nutritional Sciences and was coordinated by one of us (MLW). The prospective component of the present study was initiated by the Greek investigators to evaluate the hypothesis that the traditional Mediterranean diet, which is still widely followed in the rural parts of the country, has beneficial effects on health and survival.

Subjects and methods

Between October 1988 and June 1990 we recorded the dietary habits over a period of one year of 91 men and 91 women more than 70 years old resident in three Greek villages by using a validated extensive (190 food items or beverages) semiquantitative questionnaire on food frequency.³ We also established whether the

subjects were current smokers (including the few who had stopped smoking within five years) or non-smokers (including the few who had not smoked for more than five years). Between April 1993 and January 1994 we revisited the three villages, ascertained the exact date of death of the 53 subjects who died, and confirmed the survival of the 129 remaining. Although all death certificates were available, cause of death was not specifically studied as the relevant information was not always adequately substantiated.

For analysis the frequency of consumption of different food items was quantified approximately in terms of the number of times a month the food was consumed, as done by Graham *et al.*⁴ and Katsouyanni *et al.*⁵ Thus, daily consumption was multiplied by 30 and weekly consumption by 4, a value of 0 was assigned to food items rarely or never consumed. Food items were considered in groups as recommended by Davidson and Passmore⁶ and used by Graham *et al.*,⁴ Dales *et al.*,⁷ and Trichopoulou *et al.*⁸ Food frequencies were translated into food quantities in grams per day on the basis of standard portion size estimations, and they were further adjusted to daily intakes of 2500 kcal for men and 2000 kcal for women. Nutrient intakes for individual people were estimated by multiplying the nutrient contents of a selected typical portion for each specified food item by the frequency that the food item was eaten a month and adding these estimates for all food items. Data on the nutrient composition of Greek foods and recipes were based on a nutrient database developed in Greece by the department of nutrition and biochemistry, National School of Public Health.⁹ The estimation of portion size was based on the results from previous validation studies.^{3,10,11}

Composite scores are often used to describe total diet; these scores are necessary for the evaluation of epidemiological associations,^{11,12} although they require some operational definitions. We used the food groups recommended by Davidson and Passmore⁶ in devising a score except that we combined starchy roots with cereals and did not consider sugars and syrups for which no systemic health implications have been documented over and beyond their contribution to net energy intake. The traditional Mediterranean diet is also defined in terms of these food groups with the addition of moderate intake of ethanol^{13,14} and therefore can be reasonably scored in terms of eight component characteristics: high monounsaturated:saturated fat ratio; moderate ethanol consumption (there were no men who drank more than seven glasses of wine a day and no women who drank more than two glasses of wine a day so that no study subject could be considered a heavy drinker); high consumption of legumes; high consumption of cereals (including bread and potatoes); high consumption of fruits; high consumption of vegetables; low consumption of meat and meat products; and low consumption of milk and dairy products. We

National Centre for Nutrition, National School of Public Health, Leoforos Alexandras 196, Athens 115-21, Greece
Antonia Trichopoulou, professor
Charalambos Gnardellis, research fellow
Pagona Lagiou, research fellow
Evangelos Polychronopoulos, lecturer
Tonia Vassilakou, research fellow

Department of Medicine, Monash Medical Centre, Clayton, Melbourne, Victoria 3168, Australia
Antigone Kouris-Blazos, lecturer
Mark L Wahlqvist, professor

Department of Epidemiology and Harvard Center for Cancer Prevention, Harvard School of Public Health, Boston, MA 02115, United States
Loren Lipworth, doctoral candidate
Dimitrios Trichopoulos, professor

Correspondence to: Professor Trichopoulos.

BMJ 1995;311:1457-60

used as a cut off point for all characteristics the corresponding median values specific for each sex. We a priori hypothesised that a diet with more of these components has beneficial health effects whereas a diet with fewer of these components would be less healthy. These considerations are based on the collective epidemiological and biological evidence as summarised in the report of the National Academy of Science¹ and a recent critical overview.¹⁵ In our study sample only 34 subjects (or 19% of the total) were found to have two or fewer of the eight desirable dietary components, whereas 104 subjects (57%) were found to have four or more of the eight desirable components, a reasonable pattern given the attachment of elderly rural Greeks to their traditional diet.

The statistical analysis was undertaken by modelling the data through Cox's proportional hazards regression.¹⁶ This approach takes into account not only the event of death but also the time until its occurrence. An assumption in the model is that the rate ratio is constant over follow up time. Initially, eight Cox's models were developed; these controlled for age at enrolment (in three month intervals), sex (0=female, 1=male), and current smoking status (0=non-smoker, 1=smoker) and evaluated alternatively the eight individual components of the diet score adjusted for energy. An additional Cox's model was developed that controlled for age at enrolment, sex, and current smoking status and evaluated the total diet score as a predictor of the hazard of death. Survival curves were plotted by using the Kaplan-Meier method.

Results

Fifty three of the study subjects died during the follow up period; 30 (57%) were men, 17 (32%) were current smokers, and their mean age at enrolment was 78.5 years. Among the 129 survivors, 61 (47%) were men, 30 (23%) were current smokers, and their mean age at enrolment was 75.4 years.

Table 1 shows mean (SD) daily consumption in grams of the components of the diet score adjusted to 2500 kcal for men and 2000 kcal for women as well as daily energy intake. These data are not directly interpretable because of confounding and variability in survival and censoring time. Table 2 shows the median daily consumption (in grams) adjusted for energy for the individual components of the diet score according to sex.

Table 3 presents the results of the Cox's proportional hazards models with age, sex, current smoking status, and total diet score as predictor variables. Table 4 presents similar data but with the total diet score replaced by each one of the eight individual components adjusted for energy. The eight individual models yielded no significant results for any of the eight components with the exception of dairy products, which were associated with a 4% increase of the hazard

Table 1—Mean (SD) daily consumption in grams adjusted for energy* for eight components of diet score

| Consumption | Dead (n=53) | Survivors (n=129) |
|-------------------------------------|-------------|-------------------|
| Vegetables | 264 (149) | 308 (152) |
| Legumes | 53 (34) | 68 (51) |
| Fruits and nuts | 272 (211) | 256 (165) |
| Dairy products | 254 (210) | 238 (173) |
| Cereals | 306 (126) | 270 (105) |
| Meat and meat products | 118 (72) | 107 (51) |
| Ethanol | 12 (16) | 8 (14) |
| Monounsaturated:saturated fat ratio | 1.7 (0.4) | 1.8 (0.5) |
| Energy (kcal) | 1979 (609) | 2093 (694) |

*To 2500 kcal for men and 2000 kcal for women.

Table 2—Median daily consumption in grams adjusted for energy* for eight components of diet score

| Consumption | Men (n=91) | Women (n=91) |
|-------------------------------------|------------|--------------|
| Vegetables | 303 | 248 |
| Legumes | 60 | 49 |
| Fruits and nuts | 249 | 216 |
| Dairy products | 201 | 194 |
| Cereals | 291 | 248 |
| Meat and meat products | 109 | 91 |
| Ethanol | 10 | 0 |
| Monounsaturated:saturated fat ratio | 1.6 | 1.6 |
| Energy (kcal) | 2206 | 1760 |

*To 2500 kcal for men and 2000 kcal for women.

Table 3—Rate ratio estimates (95% confidence intervals) derived from Cox's proportional hazards model for diet score as predictor* of survival time

| Predictor variable (category or unit) | P value (two tailed) | Rate ratio* (95% confidence interval) |
|-----------------------------------------|----------------------|---------------------------------------|
| Age (1 year) | < 10 ⁻⁴ | 1.12 (1.06 to 1.17) |
| Sex (female 0, male 1) | 0.87 | 1.06 (0.55 to 2.03) |
| Smoking status (non-smoker 0, smoker 1) | 0.16 | 1.67 (0.82 to 3.41) |
| Diet score (1 unit) | 0.04 | 0.83 (0.69 to 0.99) |

*From model including terms for age, sex, smoking status, and total diet score; components of the score were adjusted to 2500 kcal for men and 2000 kcal for women.

Table 4—Rate ratio estimates (95% confidence intervals) derived from alternative Cox's models with each of eight components* replacing diet score

| Component (category or unit) | P value (two tailed) | Rate ratio† (95% confidence interval) |
|----------------------------------------------|----------------------|---------------------------------------|
| Vegetable intake (20 g) | 0.20 | 0.97 (0.93 to 1.02) |
| Legume intake (20 g) | 0.13 | 0.90 (0.78 to 1.03) |
| Fruit and nut intake (20 g) | 0.75 | 1.01 (0.97 to 1.04) |
| Cereal intake (20 g) | 0.49 | 1.02 (0.97 to 1.07) |
| Dairy intake (20 g) | 0.01 | 1.04 (1.01 to 1.07) |
| Meat intake (20 g) | 0.65 | 1.02 (0.93 to 1.12) |
| Monounsaturated:saturated fat ratio (1 unit) | 0.14 | 0.60 (0.31 to 1.18) |
| Ethanol intake (10 g) | 0.47 | 1.07 (0.89 to 1.28) |

*Adjusted to 2500 kcal for men and 2000 kcal for women.

†From model including terms for age, sex, and smoking status.

of death for every 20 g increase in daily consumption adjusted for energy. From the model including the combined diet score, older age was, as expected, a highly significant predictor of the hazard of death, whereas current smoking and male sex were not significantly associated with this hazard. A higher diet score was significantly associated with a sharply reduced risk of death, by 17% per one unit increase and by more than 50% per four unit increase. Exclusion of deaths that occurred during the first year of follow up did not alter the regression coefficients related to tobacco and diet. The figure presents the Kaplan-Meier survival curves for individual people with diet score values up to 3 and 4 or more.

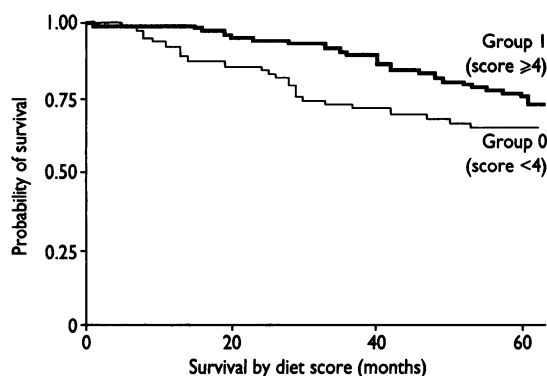
Discussion

The Greek variant of the traditional Mediterranean diet is low in saturated fat, high in monounsaturated fat (mainly from olive oil), high in complex carbohydrates

(from grains and legumes), and high in fibre (mostly from vegetables and fruits). Total fat may be high (around 40% of total energy intake), but the mono-unsaturated:saturated fat ratio is high (around 2 or more). Actual foods and dishes include large quantities of whole grain bread, and cooked meals, soups, and salads rich in olive oil in which legumes and vegetables are consumed in large amounts. Intake of milk is rather low, but consumption of cheese and, to a lesser extent, yogurt is high; feta cheese is regularly added to most salads and vegetable stews. Until recently meat was expensive whereas fish consumption was a function of proximity to the sea. The high content in the diet of vegetables, fresh fruits, and cereals and the liberal use of olive oil guarantee a high intake of β carotene, vitamin C, tocopherols, various important minerals, and several possibly beneficial non-nutrient substances like polyphenols and anthocyanines. Wine is consumed in moderation and almost always during meals.^{13 14 17 18}

The results of this study provide evidence that an a priori defined nutritional pattern which closely reflects the Greek version of the Mediterranean diet favourably affects life expectancy among elderly people. As most components of the diet score in the present study have established or possibly beneficial influences with respect to the principal causes of overall mortality,^{15 19-23} it may be argued that the results should not be surprising. The individual components of the diet score, however, had weak and generally non-significant associations with survival, whereas the overall score had a substantial and significant effect. A complex statistical model with appropriate interaction terms could perhaps harvest the same information, but the approach we have used has the advantages of being simple and derived from collective clinical and epidemiological evidence rather than from a best fitting process based on an individual dataset.

As the focus of our study was survival beyond age 70 it may be argued that our results are strictly applicable to this "surviving cohort" of elderly people. All studies in humans, however, are studies of "survivors" beyond the age of enrolment, and there is no evidence in the epidemiological literature for interactive effects of diet and age with respect to survival. The present study has the strength of a prospective cohort study with complete follow up and an indisputable outcome, thereby minimising selection bias and outcome related information bias. The small study size is compensated for by the inherently high mortality among subjects, allowing the generation of significant results with respect to diet score. Exposure misclassification is of course possible but in this context most likely to have been non-differential with attenuating effects. Residual confounding by smoking or age is unlikely as neither of these variables was strongly related to diet score and age was controlled for in sufficiently narrow intervals. Confounding by variables that were not



Kaplan-Meier survival curves for individual subjects with diet score up to 3 and 4 or more

Key messages

- A Mediterranean diet is beneficial to health
- The longevity of Mediterraneans can be explained by their diet
- The overall dietary pattern is more important for health and longevity than individual nutritional components

introduced into the model is possible but seems unlikely. Energy intake and expenditure are predictors of mortality and are associated with the composition of food intake, but nutritional data were energy adjusted.^{23 24} Finally, neither biochemical measurements nor blood pressure were modelled in the analysis, but these variables are thought to be intermediate factors rather than confounders. We did not attempt to investigate cause-specific mortality differentials by degree of adherence to the studied dietary pattern; cause of death is not always substantiated among elderly people in rural Greece.

Mortality statistics from the World Health Organisation²⁵ have documented the long survival of people in the European Mediterranean countries despite the high prevalence of smoking in these populations and gaps in the health services available to them. Our findings provide a plausible explanation for what is widely considered a paradox and strengthen the hypotheses advanced by Keys and his colleagues²⁶ on the basis of indirect evidence of ecological nature.

We have identified only two other reports in the international literature which have studied overall survival in relation to diet.^{11 27} In the Oslo study of a randomised trial in healthy men¹¹ a non-significant 32% reduction of overall mortality was noted in the intervention group, based on a total of 40 deaths in both groups. In the Zutphen study a significant decrease in overall mortality was observed, based on 107 deaths, in relation to higher intake of dietary fibre; stepwise regression, however, was used to assess confounding by other nutritional factors on the basis of significance, a procedure which is now considered inadequate because it leaves room for substantial aggregate residual confounding by individually non-significant variables.^{28 29}

Funding: In Greece, by the ministry of health (grant No E139/94), and in Boston by a grant to Harvard University from Theodore and Gianna Angelopoulos.

Conflict of interest: None.

- 1 National Research Council, Commission on Life Sciences, Food and Nutrition Board, Committee on Diet and Health. *Diet and health. Implications for reducing chronic disease risk*. Washington DC: National Academy Press, 1989.
- 2 Trichopoulos D, Tzonou A, Katsouyanni K, Trichopoulou A. Diet and cancer: methodological complexities and case-control studies in Greece. In: Mettlin CJ, Aoki K, eds. *Recent progress in research on nutrition and cancer*. New York: Wiley-Liss, 1990.
- 3 Gnardellis C, Trichopoulou A, Katsouyanni K, Polychronopoulos E, Rimm EB, Trichopoulos D. Reproducibility and validity of an extensive semi-quantitative food frequency questionnaire among Greek school teachers. *Epidemiology* 1995;6:74-7.
- 4 Graham S, Dayal S, Swanson M, Mittelman A, Wilkinson G. Diet in the epidemiology of cancer of the colon and rectum. *J Natl Cancer Inst* 1978;61:709-14.
- 5 Katsouyanni K, Skalkidis Y, Petridou E, Polychronopoulou-Trichopoulou A, Willett W, Trichopoulos D. Diet and peripheral arterial occlusive disease: the role of poly-, mono-, and saturated fatty acids. *Am J Epidemiol* 1991;133:24-31.
- 6 Davidson SS, Passmore R. *Human nutrition and dietetics*. Edinburgh: Churchill Livingstone, 1979.
- 7 Dales LG, Friedman GD, Ury HK, Grossman S, Williams SR. A case-control study of relationships of diet and other traits of colorectal cancer in American blacks. *Am J Epidemiol* 1979;109:132-44.
- 8 Trichopoulou A, Katsouyanni K, Stuver S, Tzala L, Gnardellis C, Rimm E, et al. Consumption of olive oil and specific food groups in relation to breast cancer risk in Greece. *N Natl Cancer Inst* 1995;87:110-6.
- 9 Trichopoulou A. *Composition of Greek foods and dishes* (in Greek and English). Athens: Athens School of Public Health, 1992.
- 10 Katsouyanni K, Trichopoulou A, Trichopoulos D, Willett W. *Dietary variability in Greece. A report to the Secretaria of Research and Technology*.

- Ministry of Industry, Research and Technology, 1991:1-64. (In Greek. Available from DT.)
- 11 Hjermann I, Velve Byre K, Holme I, Leren P. Effect of diet and smoking intervention on the incidence of coronary heart disease. Report from the Oslo study group of a randomised trial in healthy men. *Lancet* 1981;ii:1303-10.
 - 12 Manousos O, Day N, Trichopoulos D, Garovassilis F, Tzonou A, Polychronopoulou A. Diet and colorectal cancer: a case-control study in Greece. *Int J Cancer* 1983;32:1-5.
 - 13 Helsing E, Trichopoulou A, eds. The Mediterranean diet and food culture: a symposium. *Eur J Clin Nutr* 1989;43(suppl 2):1-92.
 - 14 Trichopoulou A, Lagiou P, Trichopoulos D. Traditional Greek diet and coronary heart disease. *J Cardiovascular Risk* 1994;1:9-15.
 - 15 Willett WC. Diet and health: what should we eat? *Science* 1994;264:532-7.
 - 16 Breslow NE, Day NE. *Statistical methods in cancer research. Vol II. The design and analysis of cohort studies.* Lyons: International Agency for Research on Cancer, 1987. (IARC Scientific Publication No 82.)
 - 17 Trichopoulou A, Katsouyanni K, Gnardellis CH. The traditional Greek diet. *Eur J Clin Nutr* 1993;47(suppl):76-81.
 - 18 Trichopoulou A, Toupadaki N, Tzonou A, Katsouyanni K, Manousos O, Kada E, et al. The macronutrient composition of the Greek diet: estimates derived from six case-control studies. *Eur J Clin Nutr* 1993;47:549-58.
 - 19 Sacks FM, Willett WC. Chewing the fat: how much and what kind. *N Engl J Med* 1991;324:121-3.
 - 20 Shekelle RB, Shroock AM, Paul O, Lepper M, Stamler J, Liu S, et al. Diet, serum cholesterol and death from coronary heart disease: the Western Electric Study. *N Engl J Med* 1981;304:65-70.
 - 21 Rimm EB, Giovannucci EL, Willett WC, Colditz GA, Ascherio A, Rosner B, et al. Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet* 1991;331:464-8.
 - 22 Menotti A, Keys A, Aravanis C, Blackburn H, Dostas A, Fidanza F, et al. The seven countries study. First 20-year mortality data in 12 cohorts of six countries. *Ann Med* 1989;21:175-9.
 - 23 Willett WC. *Nutritional epidemiology.* New York: Oxford University Press, 1990.
 - 24 Willett WC, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* 1986;124:17-27.
 - 25 World Health Organisation. *World health statistics annual.* Geneva: World Health Organisation, 1992.
 - 26 Keys A. *Seven countries: a multivariate analysis of death and coronary heart disease.* Cambridge: Harvard University Press, 1980.
 - 27 Kromhout D, Bosschieter EB, De Lezenne Coulander C. Dietary fibre and 10-year mortality from coronary heart disease, cancer, and all causes. *Lancet* 1982;ii:518-22.
 - 28 Thompson WD. Statistical analysis of case-control studies. *Epidemiol Rev* 1994;16:33-50.
 - 29 Dales LG, Ury HK. An improper use of statistical significance testing in studying covariables. *Int J Epidemiol* 1978;7:373-5.

(Accepted 20 September 1995)

Cost effectiveness of antenatal screening for cystic fibrosis

H S Cuckle, G A Richardson, T A Sheldon, P Quirke

Abstract

Objective—To estimate the cost effectiveness of different antenatal screening programmes for cystic fibrosis.

Setting—Antenatal clinics and general practices in the United Kingdom.

Design—Four components of the screening process were identified: information giving, DNA testing, genetic counselling, and prenatal diagnosis. The component costs were derived from the literature and from a pilot screening study in Yorkshire. The cost of a given screening programme was then obtained by summing the components according to the specific screening strategy adopted (sequential and couple), the proportion of carriers detected by the DNA test, and the uptake of screening. Baseline assumptions were made about the proportion with missing information on carrier status from previous pregnancies (20%), the proportion changing partners between pregnancies (20%), and the uptake of prenatal diagnosis (100%). Sensitivity analysis was performed by varying these assumptions.

Main outcome measure—Cost per affected pregnancy detected.

Results—Under the baseline assumptions sequential screening costs between £40 000 and £90 000 per affected pregnancy detected, depending on the carrier detection rate and uptake. Couple screening was more expensive, ranging from £46 000 to £104 000. From the sensitivity analysis a 10% change in the assumed proportion with missing information from a previous pregnancy alters the cost by £4000; a 10% change in the proportion with new partners has a similar effect but only for couple screening; and cost will change directly in proportion to the uptake of prenatal diagnosis.

Conclusions—While economic analysis cannot determine screening policy, the paper provides the NHS with the information on cost effectiveness needed to inform decisions on the introduction of a screening service for cystic fibrosis.

Introduction

Cystic fibrosis is the most common recessive condition in the United Kingdom, with a birth prevalence of 1 in 2500,¹ implying a carrier frequency of 1 in 25. Since the discovery of the principal genetic mutations

involved,^{2,4} antenatal screening has become feasible, and pilot studies show that it is generally acceptable in the United Kingdom.⁵⁻⁹ Each health authority now needs to decide whether to introduce a service. One consideration will be cost effectiveness, and in this paper we estimate this for different screening strategies and under a range of assumptions.

Methods

The aim was to estimate the cost per affected pregnancy detected. This is dependent on the screening strategy adopted, the proportion of carriers detected by the DNA test, and the uptake rate.

SCREENING STRATEGIES

The aim of screening is to identify women and their partners who are both carriers. There is then a 1 in 4 chance that the infant has cystic fibrosis, and the couple are referred for genetic counselling about having invasive prenatal diagnosis. Those offered screening require basic information about cystic fibrosis, carrier testing, prenatal diagnosis, and consequent options available to them. Carrier couples can be identified by using two different strategies.

Sequential carrier testing is offered to mothers, and a sample is requested from the partner only if the mother is found to be a carrier. Basic information is given initially to all women and subsequently to the partner of each carrier.

Couple carrier testing is offered to couples, and samples are obtained from both parents at the outset. The DNA testing, however, is done exactly as in sequential screening so that only a small percentage of samples from fathers are actually tested. The result is reported as "positive" for carrier couples, otherwise as "negative."¹⁰ This strategy removes the period of anxiety while the partner's result is awaited.

The results of a test in the first pregnancy may suffice for subsequent pregnancies unless the couple cannot remember their carrier status and it is not in the antenatal notes or there is a new partner. In our economic analysis we assume that all women have two pregnancies, the projected average family size in the United Kingdom.¹¹ In the absence of published data we made the baseline assumptions that carrier couples remember their status but otherwise 20% have missing information and that of the remainder, 20% change

Centre for Reproduction,
Growth and Development,
Research School of
Medicine, University of
Leeds, Leeds LS2 9LN
H S Cuckle, professor of
reproductive epidemiology

Centre for Health
Economics, University of
York, York YO1 5DD
G A Richardson, research
fellow

NHS Centre for Reviews
and Dissemination,
University of York,
York YO1 5DD
T A Sheldon, director

Molecular Oncology,
Centre for Cancer
Research, University of
Leeds, Leeds LS2 9JT
P Quirke, reader

Correspondence to:
Professor Cuckle.

BMJ 1995;311:1460-4