THE NURSES' HEALTH STUDY: LIFESTYLE AND HEALTH AMONG WOMEN

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Abstract | The Nurses' Health Study has grown from a simple questionnaire-based study initiated in 1976 to a rich resource of information collected over 29 years. Important details about lifestyle have been collected throughout the study and, as the study has progressed, blood samples and DNA from buccal cells have been collected and stored. Tumour samples have also been collected from participants who developed cancer. Through analyses that integrate information from these various sources we are advancing our understanding of the causes of cancer and the potential for prevention.

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To avoid potential biases in retrospective studies and to establish a base for the evaluation of both the risks and benefits of oral contraceptives after they became widely used, Frank Speizer, of Harvard Medical School, initiated the Nurses' Health Study (NHS) in 1976 (see TIMELINE). Speizer and colleagues enrolled 121,700 women aged 30–55 with the primary goal of investigating the long-term health consequences of oral-contraceptive use. Modelled after the British Doctors Study of health consequences of smoking — the first study to establish a causal relationship between smoking and a range of cancers, as well as heart disease among men[1] — the NHS aimed to address growing concerns regarding the long-term health effects of oral contraceptives. These drugs had been introduced to the United States (US) market in the 1960s and became widely used, and several side effects had already been documented. The researchers were particularly interested in assessing the association between oral-contraceptive use and breast cancer, because an increased risk was observed with the use of oral contraceptives in retrospective, case–control studies[2]. Uniquely, the NHS cohort of women were asked to complete questionnaires every 2 years (FIG. 1), which allowed participants to update information on oral-contraceptive use and reproductive variables that are related to breast cancer risk. This very large study of women was the first of this size to include updated data collection on lifestyle to relate to cancer risk.

Background on the Nurses' Health Study

The researchers initially attempted to study the association between oral contraceptives and cancer in pilot studies that involved the wives of physicians. These had unsatisfactory response rates, but subsequent pilot tests among registered nurses proved sufficiently successful to justify a full study. Nurses were also chosen as a study population because of their broad health knowledge, which increased the accuracy of self-reported information such as oral-contraceptive use and medical conditions. Because of the need to confirm all self-reported cancers, for example, the initial accuracy of the self-report has a major impact on the efficiency of the study. When the first study was designed, there were no state tumour registries, so each self-report had to be confirmed by a review of medical records. This involved contacting the patient to obtain permission and the hospital to obtain copies of the pertinent records, before the records could be reviewed by physician investigators who were collaborating on the study.

Because oral contraceptives were a relatively recent innovation, the study population was restricted to women under the age of 55 to ensure that participants had had the opportunity to take

Summary

- The Nurses' Health Study is the first large prospective cohort study of women with updated exposure assessment for a broad range of lifestyle factors, endogenous hormones and DNA, in relation to risk of cancer.
- The Nurses' Health Study can assess overall risks and benefits of lifestyle factors by evaluating them in relation to a range of cancers and other chronic conditions, and in terms of total mortality.
- Using diet data, which was first collected in 1980, we showed that alcohol intake is related to an increased risk of breast cancer independent of other dietary factors. Adult dietary-fat intake was not related to increased breast cancer risk in this and subsequent prospective studies.
- The long-term use of multivitamins containing folate is associated with a reduced risk of colon cancer. This association was confirmed by findings from several studies, including the discovery that a functional polymorphism in the methylene-tetrahydrofolate reductase gene was associated with increased colon cancer risk and that plasma levels of folate were inversely related to colon cancer risk.
- Both oestrogen and testosterone levels in postmenopausal women are related to an increased risk of breast cancer. Because of this, plasma oestrogen and testosterone levels are now being added to risk-prediction models for breast cancer.

the drugs. Furthermore, because, at this time, oral contraceptives could only be prescribed to married women, the study was limited to women who had indicated they were married as of 1973.

The original NHS questionnaire inquired about standard reproductive risk factors for breast cancer, history of benign breast disease and family history of breast cancer. Questions about oral-contraceptive use were also asked, including the start and end date for each interval of use. The questionnaire also inquired about the use of postmenopausal hormones, along with the history of hair-dye use and cigarette smoking (FIG 1). The first questionnaire was returned by 73% of the women who were invited to participate. They reflect the racial composition of women trained as registered nurses at that time; 97% were Caucasian[3].

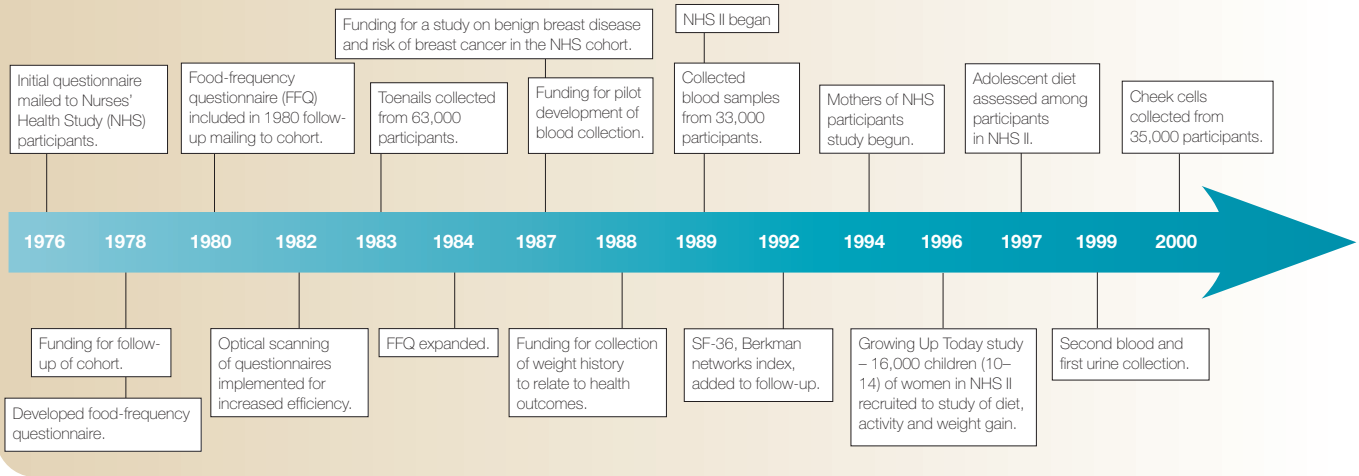
Updated information was obtained from these original participants by biennial mailed questionnaires. And, 29 years after the study was initiated, it has maintained at least a 90% follow-up rate for questionnaire information from the women who were originally recruited to the study. Furthermore, using the US National Death Index (a centralized, computerized index of death-record information for the USA) to search for deaths among study participants, the study has achieved over a 98% follow-up rate for mortality[4]. In 1980, the scope of the NHS was greatly expanded to include questions about diet and physical activity, and, subsequently, this information has been updated every 4 years.

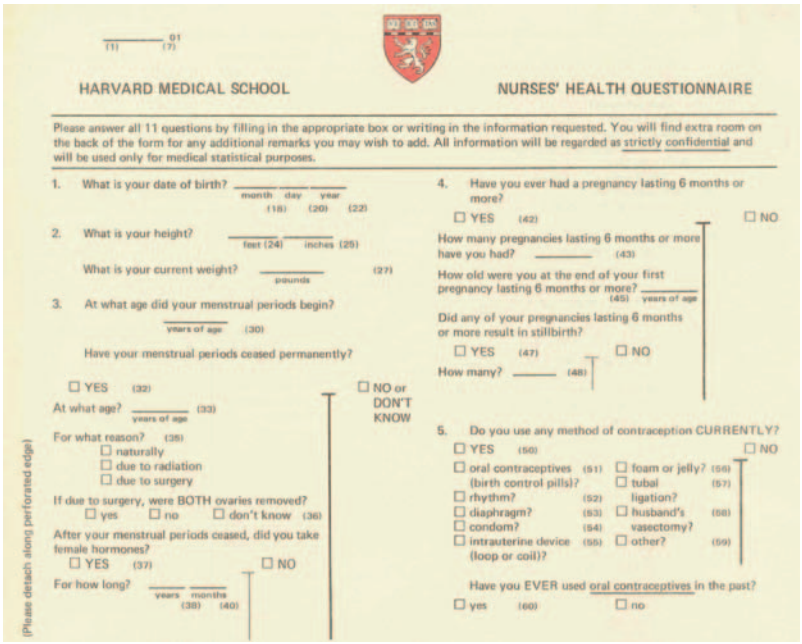
There have been continuing efforts to validate questionnaire-based exposure measures used in the study (BOX 1). We have also worked to improve the methods for measuring the environmental exposures of study participants. For example, in 1983, we collected toenail samples from over 65,000 women to assess selenium status. In 1989, blood samples were collected from 32,826 cohort members and stored in plasma, white-cell and red-cell aliquots for future NESTED CASE–CONTROL STUDIES. In 1999, we collected a second blood sample and a first urine sample from 18,743 of these women. During 2002–2004, we obtained BUCCAL SAMPLES from over 35,000 participants who had not previously provided a blood sample. As a result of these efforts, the NHS has, over time, contributed greatly to knowledge regarding the determinants of cancer and other chronic diseases among women.

In addition to the primary focus on cancer aetiology, several substudies have been conducted to address specific questions about cancer progression. For example, in 1987, a nested case–control study of women who had undergone a previous biopsy for benign breast disease was initiated to assess the relationship between the histological features of the benign lesion and the subsequent risk of breast cancer. This study showed that proliferative changes and atypical hyperplasia of the breast are strong predictors of subsequent breast cancer[5], and also identified microscopic RADIAL SCARS as a new, independent histological risk factor[6].

NESTED CASE–CONTROL STUDY
A study that is nested within a cohort study, whereby all cases and a small sample of non-cases are included — a design used frequently when genetic or other biomarker analyses are being conducted. It maintains the strengths of the prospective cohort (with exposure information collected before disease diagnosis) but is more efficient and economical because laboratory assays are conducted on only a small subset of the cohort.

Timeline | **Evolution of the Nurses' Health Study**





HARVARD MEDICAL SCHOOL

NURSES' HEALTH QUESTIONNAIRE

Please answer all 11 questions by filling in the appropriate box or writing in the information requested. You will find extra room on the back of the form for any additional remarks you may wish to add. All information will be regarded as strictly confidential and will be used only for medical statistical purposes.

1. What is your date of birth? month day year (18) (20) (22)

2. What is your height? feet (24) inches (25)

What is your current weight? pounds (27)

3. At what age did your menstrual periods begin? years of age (30)

Have your menstrual periods ceased permanently?

☐ YES (32) ☐ NO or DON'T KNOW

At what age? years of age (33)

For what reason? (35)
☐ naturally
☐ due to radiation
☐ due to surgery

If due to surgery, were BOTH ovaries removed?
☐ yes ☐ no ☐ don't know (36)

After your menstrual periods ceased, did you take female hormones?
☐ YES (37) ☐ NO

For how long? years months (39) (40)

4. Have you ever had a pregnancy lasting 6 months or more?
☐ YES (42) ☐ NO

How many pregnancies lasting 6 months or more have you had? (43)

How old were you at the end of your first pregnancy lasting 6 months or more? (45) years of age

Did any of your pregnancies lasting 6 months or more result in stillbirth?
☐ YES (47) ☐ NO

How many? (48)

5. Do you use any method of contraception CURRENTLY?
☐ YES (50) ☐ NO

☐ oral contraceptives (birth control pills)? (51)
☐ rhythm? (52)
☐ diaphragm? (53)
☐ condom? (54)
☐ intrauterine device (loop or coil)? (55)
☐ foam or jelly? (56)
☐ tubal ligation? (57)
☐ husband's vasectomy? (58)
☐ other? (59)

Have you EVER used oral contraceptives in the past?
☐ yes (60) ☐ no

(Please detach along perforated edge)

Figure 1 | **Original questionnaire used in Nurses Health Study.** The first page of the 1976 Nurses' Health Study questionnaire that was completed by the study participants. Information was collected on oral-contraceptive use and reproductive variables that relate to breast cancer risk. Image provided by Alan Park, from the Channing Laboratory, Boston, USA.

At present, questionnaires inquire about lifestyle factors, including diet, physical activity and use of aspirin and other non-steroidal anti-inflammatory drugs. These data are also used to evaluate predictors of survival after diagnosis with breast and colon cancers. For example, we have found that higher protein intake is associated with improved survival after breast cancer treatment[7], whereas weight gain increases the recurrence and mortality of the disease[8].

The NHS has also evaluated the association between a range of genetic factors, for example, genes that are involved in hormone and/or carcinogen metabolism and cancer risk. Breast and colon cancer genetic studies also have evaluated the potential interaction between variants in these genes and environmental factors such as obesity and hormone use[9,10]. The National Cancer Institute's (NCI's) Breast and Prostate Cancer and Hormone-Related Gene Variants Cohort Consortium is also combining data from several cohorts, including the NHS, to study gene–environment interactions and to use consistent approaches to evaluate genetic variation.

In addition, the NHS is designed to obtain information about the effects of diet and lifestyle on the development of a wide range of chronic diseases including heart disease, stroke, diabetes, rheumatological conditions and osteoporotic fractures. Previous reviews summarize these findings[11,12] as well as the methodological contributions of the NHS to the conduct of cohort studies[13].

The Nurses' Health Study II

By 1989, the nurses who were enrolled in the original NHS were 43–68 years of age, and as oral contraceptives were introduced into common use in the early 1960s, few of these women had the opportunity to use them for prolonged periods before their first pregnancy. In addition, the prevalence of use of oral contraceptives had continued to rise, with over 80 percent of women using these contraceptives at some point in their life. So a new cohort of registered nurses — some 116,000 women of 25–42 years of

Box 1 | **Validation of diet and other exposure measures**

Semiquantitative food-frequency questionnaire

Assessment of long-term diet is necessary to relate nutrient intake to the risk of chronic diseases. For large population studies this is best accomplished through the use of a food-frequency questionnaire. The Nurses' Health Study (NHS) investigators have devoted great attention to the development, evaluation and refinement of food-frequency questionnaires for epidemiological applications. This process and the results of validation studies are described in detail in the textbook *Nutritional Epidemiology*[71].

Physical-activity validation

A detailed validation study of the physical-activity questionnaire was conducted among 325 participants in the NHS II (241 participants represented a random sample from the whole cohort, and 84 participants represented a random sample of African-American participants)[72]. Participants completed four 1-week activity recalls and four 7-day activity diaries over 1 year and then repeated the NHS II activity questionnaire. For the total activity score, the correlations of the last activity questionnaire with the diaries was 0.64 for the total cohort sample and 0.59 for the African-American sample. Also, the measure of late-adolescent activity correlates negatively, as expected, with age at menarche, body mass index (BMI) at age 18, and weight gain between age 18 and age in 1989, whereas the measure of recent vigorous activity correlates negatively with current BMI and weight gain between age 18 and age in 1989. Furthermore, the same measure of recent activity is predictive of colon cancer[17] and is inversely related to the risk of coronary heart disease[73], stroke[74] and type 2 diabetes[75], whereas sedentary behaviours increase the risk of colon cancer[76].

Weight

Self-reported weight was validated in 1980 and 1986 among NHS participants. The mean self-reported weights were about 1.5 kg lighter than the measured values, which is compatible with the difference between a random casual weight in clothing and a nude, post-void morning weight. The correlations between technician-measured and self-reported weight were $r = 0.96$ in 1980 and $r = 0.97$ in 1986, and did not differ by level of BMI. Correlations between self-reported and technician-made measurements were ($r = 0.89$) for the waist and ($r = 0.84$) for the hips[77].

BUCCAL SAMPLES
Cheek-cell samples used as a source of DNA and collected by a swish-and-spit method using mouthwash, or by using a buccal brush.

RADIAL SCARS
Benign lesions with a fibroelastic core from which ducts and lobules radiate.

age — were enrolled by Walter Willett in the Nurses' Health Study II (NHS II).

The dose of oestrogen and progesterone in oral contraceptives had changed over time, as had the pattern of hormone administration over the 28-day menstrual cycle. This led to concerns being raised in case–control studies that newer combinations of oral contraceptives might increase the risk of breast cancer and lack the protection against ovarian cancer that was seen with the original formulations. So, these women were sent a detailed baseline questionnaire to collect a thorough history of the specific brand of oral contraceptive used. Subsequent follow-up of this younger cohort has mirrored the pattern and methods used in the original NHS study, including comparable questions on diet, other lifestyle factors and disease outcomes. This study was designed to identify factors, particularly exposures occurring earlier in life, that might be associated with premenopausal breast cancer.

Factors considered in these studies

The size and breadth of the NHS and NHS II has allowed the evaluation of the risks and benefits of a range of lifestyle factors, which are often summarized through their association with total mortality. In fact, one of the main strengths of these studies is that they can be used to evaluate, for any given exposure, the association with a wide range of disease outcomes. This provides a much better understanding of the overall risks and benefits of that exposure.

Incidence rates and risk factors. In a 20-year follow-up of nurses who were enrolled in the first NHS (1976–1996), the incidence of breast cancer was 97% of the expected number of cases as predicted by the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database. The SEER database is a federally funded programme that collects and publishes cancer statistics from a geographically defined sample of the US population. Age-specific incidence rates were also very similar to those predicted by the SEER data, indicating that results from the NHS can be generalized to US white women.

Oral contraceptives. Building on the primary hypothesis relating oral-contraceptive use to risk of breast cancer, the NHS revealed in 1986 that there was no long-term adverse effect among past oral-contraceptive users, but noted that current users had a modest increase in risk[14]. This finding was subsequently confirmed by the combined re-analysis of over 50 separate epidemiological studies, including the NHS[15]. Given the very low incidence of breast cancer in the age groups of women using oral contraceptives, this adverse effect has only a minor influence on the total burden of breast cancer. Analysis of oral contraceptives in relation to other cancers showed that long-term use lowers the risk of ovarian cancer and also of colon cancer. The protective benefit against ovarian cancer of taking oral contraceptives seems to persist for many years after the cessation of use of the drug[16]. At least in part, this benefit is probably due to the suppression of ovulation that occurs while taking oral contraceptives, which reduces proliferation and repair of the ovary epithelium, thereby reducing cancer risk. For colon cancer, women who had used oral contraceptives for 8 years or more had a 40% reduction in colon cancer risk compared with those who had never used these drugs[17]. The NHS was therefore one of the first studies to report that contraceptive use showed no overall increase in total mortality — although these drugs increase the risk of breast cancer mortality, this was balanced by a reduced risk of ovarian and colon cancer mortality[18].

Diet. The NHS has helped to uncover several important dietary factors that determine cancer risk. The first major dietary finding, published in 1987, showed that moderate alcohol intake increased the risk of breast cancer[19] — even after statistical control for other components of diet, which had not been addressed in previous studies. This increase in risk has now been confirmed in many studies, and alcohol intake remains the best-established dietary risk factor for breast cancer. Alcohol intake is also related to an increased risk of colon cancer in this study, as it has been in others[20].

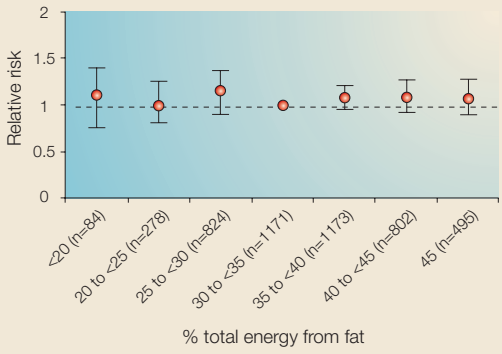
Folate is involved in ONE-CARBON METABOLISM and is crucial for DNA synthesis and methylation. Evaluation of folate levels and colon cancer incidence showed that long-term use of multivitamins, an important source of folate for many adults, was associated with a substantial reduction in the risk of colon cancer[21]. Findings from studies of the plasma levels of folate as well as of functional polymorphisms in methylenetetrahydrofolate reductase (*MTHFR*), a gene involved in folate metabolism, support a causal role for folate deficiency in colon carcinogenesis[22]. Subsequent investigation also showed an inverse association between folate intake and breast cancer, which was most evident among women who were considered to be 'higher risk' due to them having an alcohol consumption of greater than 15g — about one drink — per day[23]. Similarly, studies of plasma levels of folate revealed a significant reduction in breast cancer risk particularly among women who consumed alcohol[24]. Cumulatively, these data indicate an important role for folate in the development of colon and breast cancer, and perhaps other cancers, although these data are more limited. An adequate folate intake can be ensured by regularly taking a multivitamin that contains folate.

Initially, no association between fat intake during adult years and risk of breast cancer was observed among participants of the NHS, after 4 years follow-up[25] (published in 1987) or after 12 years of follow-up[26] (published in 1999). These findings were surprising, because international ecological studies, case–control studies in humans, and studies in animal models had reported a correlation between fat intake and breast cancer risk. But this lack of association has since been confirmed in subsequent prospective studies that

ONE-CARBON METABOLISM
The chemical reactions and physical changes involving compounds that contain a single carbon atom. One-carbon metabolism is crucial for nucleotide biosynthesis and for methylation reactions. Several dietary factors, including folate and vitamin B6, are important cofactors in this process.

Box 2 | **Pooling project of prospective studies of diet and cancer**

The Pooling Project of Prospective Studies of Diet and Cancer is a collaborative project that involves investigators from various international cohort studies. The goal of the project is to analyse diet and cancer associations using standardized criteria across studies. So far, the pooled data show no association between intake of fat and risk of breast cancer. Based on 4,980 cases of breast cancer, and using ENERGY-ADJUSTED data, women with the highest levels of fat intake had a similar risk of developing disease as women with the lowest levels of fat intake (see figure). Furthermore, when intake of saturated fat, mono- and polyunsaturated fat, and cholesterol was considered individually, none of them showed an association with increased breast cancer risk[78]. This relationship did not vary when considered alongside any of the established non-dietary risk factors for breast cancer[79]. Graph modified with permission from REF. 78 © (1996) the Massachusetts Medical Society.



were conducted in other countries[27]. The results of these studies have been combined with those from the NHS through a consortium that conducts combined re-analysis of prospective studies (BOX 2). The NHS also reported no evidence that levels of meat or fish consumption during mid-life or later were associated with an increased risk of breast cancer[28]. Other dietary factors were studied in 1998 as part of the NHS II. In one of the first ever evaluations of diet during adolescence, women who reported a higher intake of vegetable fat during high-school years had a lower risk of developing breast cancer in later years[29] and also of developing proliferative benign breast disease[30]. In contrast to these findings for breast cancer, we observed a positive association between animal-fat intake, red-meat consumption and colon cancer risk[31]. However, we observed no association between fat intake and ovarian cancer[32].

Overall, the findings for diet and cancer have led to changes in dietary guidelines such as those of the American Cancer Society[33] emphasizing a limit to alcohol and red-meat intake to reduce cancer risk. Furthermore, the lack of an association between fat intake and breast cancer has moved the emphasis away from this proposed cause to other lifestyle factors, such as maintaining a healthy weight and being physically active.

Postmenopausal hormone therapy. Postmenopausal hormones have been prescribed for many years for the treatment of menopausal symptoms such as hot flushes and insomnia. In the 1980s and 1990s, these hormones (either an oestrogen alone or later with a progestin added) were increasingly prescribed to reduce the risk of chronic diseases such as osteoporosis. An important finding of the NHS that was published in 1995 separated current and past users, reporting that the duration of postmenopausal hormone use was associated with breast cancer risk. Biennially updated information on menopausal status, the use of hormones and the type of hormone used allowed a detailed evaluation that highlighted the adverse affect of current long-term use. Among current users, for 5 years of use, the RELATIVE RISK was 1.2 for oestrogen alone and 1.7 for oestrogen plus progestin[34]. The adverse effect of combining oestrogen and progestin was subsequently confirmed in other epidemiological studies, and, most recently (2002), in the Women's Health Initiative randomized trial[35].

The NHS detected a higher incidence of breast cancer in postmenopausal hormone users compared to women who had never used these drugs — perhaps because the study had very accurate dates of menopause for most participants. Our study showed that age at menopause (in single years) had to be carefully accounted for in the analysis to determine accurate risk estimates for postmenopausal hormone use[36]. Additionally, the increase in risk associated with hormone use was most apparent for oestrogen-receptor-positive tumours[37]. The findings for postmenopausal hormones are also consistent with the study's observed association between plasma oestrogen levels and the risk of breast cancer in postmenopausal women. For example, the relative risk — comparing highest to lowest quartile of plasma oestradiol levels — was 2.1 for all breast cancers combined, and 3.3 for oestrogen-receptor-positive tumours specifically[38]. Cumulatively, these findings indicate that postmenopausal hormones should only be used for short durations, if at all, because of their influence on breast cancer risk.

Endogenous hormones. By analysing blood samples that were provided by over 30,000 participants from 1989–1990, the NHS was able to evaluate endogenous hormone levels and to associate them with breast cancer risk. For all of these assessments, plasma hormone levels that were considered to be within the normal range were compared. Insulin-like growth factor 1 (IGF1), a mitogenic and anti-apoptotic protein, can increase the proliferation of breast epithelial cells, and substantial *in vitro* and animal data indicate that it has an important role in breast carcinogenesis[39]. In the first prospective evaluation of circulating IGF levels and subsequent risk of breast cancer, a positive association was observed among premenopausal women, but no association was seen among postmenopausal women[40]. This finding has been replicated in several other cohorts[41,42] and further evaluation is ongoing in other large studies. Prolactin, a hormone that is produced primarily by the pituitary, has also been proposed to increase breast cancer risk by increasing cell proliferation and survival and by promoting cell motility[43]. Among postmenopausal women, prolactin concentrations[44,45] were positively associated with the risk of breast cancer. Similarly, as mentioned previously, high oestrogen levels also significantly increased breast cancer risk[46].

ENERGY ADJUSTMENT
A statistical technique to account for the influence of energy (calorie) intake when studying the association between a specific nutrient (for example, dietary fat) in relation to disease risk.

RELATIVE RISK
A quantitative measure that is used to describe the increase (or decrease) in risk associated with a specific risk factor. A relative risk is the ratio of two absolute risks: the numerator is the absolute risk among those with the risk factor, while the denominator is the absolute risk among those without the risk factor.

Box 3 | Models of breast cancer incidence

Using data obtained from the Nurses' Health Study, it has been possible to build new statistical models of the relationship between reproductive risk factors and breast cancer incidence, and extend these to a model of breast-tissue ageing as proposed by Malcolm Pike and colleagues in 1983 (REF. 80). In this model, the timing of first birth is associated with both a short-term increase (5–10 years following first birth) and a long-term decrease in breast cancer risk. In addition, the timing of subsequent births modifies risk — the closer additional births are to the first, the lower the ultimate risk of breast cancer[81]. Extending this mathematical model to include additional breast cancer risk factors and to facilitate ease of interpretation (giving results on the relative risk and ABSOLUTE RISK scales)[82], we concluded that the reproductive factors are weaker predictors of risk among women who have a family history of breast cancer[83]. The cumulative risk of developing breast cancer, up to the age of 70, has been determined for various patient profiles, and confidence intervals for these cumulative risks have been estimated. For example, history of benign breast disease, family history of breast cancer, and use of oestrogen plus progestin therapies for 10 years or more all provide comparable increases in breast cancer risk, up to the age of 70 (REF. 84). Evaluation of this model on an independent set of cases arising with additional follow-up of the cohort showed good performance characteristics[85]. This analytical approach has subsequently been applied to evaluate risk factors for tumours that were classified according to oestrogen-receptor and progesterone-receptor status, and shows that risk factors vary. For example, higher parity is protective against developing oestrogen-receptor-positive tumours but not oestrogen-receptor-negative tumours, whereas family history of disease equally increases the risk of developing any tumour type[86].

In 2002, in collaboration with investigators from the other studies, we combined the data from all the prospective studies of plasma hormone levels among postmenopausal women that were available worldwide to determine the effects of oestradiol levels, and levels of other sex hormones, on breast cancer risk. This analysis combined data from 663 case subjects and 1,765 control subjects. The NHS contributed the largest data set to this combined analysis. Overall, analysis of the combined data showed an association of both oestrogen and testosterone levels with the risk of breast cancer[47]. In addition, breast cancer risk increased with increasing body-mass index (BMI), and this increase in risk was substantially reduced by adjustment for serum oestrogen concentrations, showing that the higher oestrogen levels among heavier women could account for the association between BMI and breast cancer risk[48]. Because of these findings, plasma oestrogen and testosterone levels are now being added to risk-prediction models for breast cancer (BOX 3) to determine how helpful they are in defining a woman's risk of developing this disease.

Genetic markers. Using blood samples that were collected between 1989–1990, a range of potential genetic markers has since been examined in NHS samples, including polymorphisms in genes that encode proteins involved in hormone metabolism[49,50], hormone-receptor signalling[51,52], carcinogen metabolism[53–55], and IGF1 signalling[56,57]. For example, in 2002, our group analysed samples from the NHS and identified a polymorphism (+331 G/A) in the promoter regions of a gene encoding the progesterone receptor (HPR) that was associated with a twofold increase in endometrial cancer risk[58] and a significant increase in breast cancer risk[52]. This polymorphism increases transcription of the HPR-B isoform[58]. It is now being evaluated in the NCI cohort consortium to quantify gene–environment interactions.

Several polymorphisms in genes that encode products that regulate the IGF signalling pathway have been evaluated in relation to breast cancer risk[56,57]. For example, a polymorphism in the promoter region of the gene encoding IGF-binding protein 3 (IGFBP3; nucleotide –202) was assessed. IGFBP3, one of six IGF-binding proteins, both decreases IGF1 bioavailability and might have independent effects on the growth of malignant cells[39]. Although circulating levels of IGFBP3 were significantly higher among women with the 'AA' genotype, compared to women with the 'CA' and 'CC' genotypes, no association was observed between this *IGFBP3* polymorphism and breast cancer risk[56]. Consideration of other variants in this gene and other genes in the IGF pathway simultaneously might be important to further understand their impact on cancer risk.

To extend these findings, the NHS is part of the NCI-funded Breast and Prostate Cancer and Hormone-Related Gene Variants Cohort Consortium, a large international collaboration of PROSPECTIVE COHORT STUDIES that are conducting a comprehensive evaluation of polymorphisms in the steroid-hormone and IGF1 pathways in relation to breast cancer risk. Because of the number of variants per gene and the number of related genes in a single metabolic pathway, it has become clear that very large sample sizes are necessary for a comprehensive assessment of genetic variation, and in particular gene–environment interactions, with cancer risk. Although this effort has just recently begun (in 2003), because of the clear advantages of such a collaboration it is expected to expand in the future in terms of both the genes and the diseases under study.

Physical activity. In the last analysis of physical activity and breast cancer risk in the NHS, we used measurements of physical activity that had been averaged and updated from 1980 up until 1996 (REF. 59). We compared women reporting moderate or vigorous physical activity for 7 or more hours per week to those who reported less than 1 hour per week. Those reporting 7 or more hours of activity had a 20% reduction in their risk of developing breast cancer. This association was similar in both pre- and postmenopausal women. Among postmenopausal women, physical activity might lower breast cancer risk by reducing fat stores, which convert androstenedione to oestrone[60,61]. Physical activity might also increase the levels of sex-hormone-binding globulin, which would reduce the bioavailable levels of oestrogens[62]. Increased physical activity also reduces insulin resistance and hyperinsulinaemia[63], which has been proposed to be related to breast cancer[64]. The association of physical activity with an increased risk of disease was weaker for breast cancer than for colon cancer, in which the same level of activity reduced risk by approximately 40%[17]. The insulin pathway is thought to be the most likely mechanism for the observed protection against colon cancer that is provided by physical activity[65].

ABSOLUTE RISK
A person's chance of developing a specific disease over a specified time period. The absolute risk of disease is estimated by examining a large number of people who are similar in some respects (in terms of age, for example) and counting the number of individuals in this group who develop the disease over the specified time period.

PROSPECTIVE COHORT STUDY
In prospective cohort studies, exposure information is collected from large groups of participants who are then followed over time to assess their health outcomes, and to determine how these outcomes are related to the previously collected exposure data.

Box 4 | **Implications for prevention**

A range of basic lifestyle choices, based on findings from the Nurses' Health Studies and other epidemiological investigations, indicate that more than 50% of cancer cases can be prevented by adopting a healthy lifestyle. In addition to lowering cancer risk, these lifestyle habits enhance overall health. There are six key lifestyle choices that can be made to reduce cancer risk:

- **not smoking**
- **being more physically active**
- **maintaining a healthy weight and avoiding weight gain as an adult**
- **eating a diet rich in fruits, vegetables, whole grains and fibre, and low in saturated and trans-fats**
- **taking a multivitamin every day**
- **avoiding long-term use of postmenopausal hormone therapy.**

Mammography. Mammographic density, one of the most influencial and most consistent risk factors for breast cancer, is assessed during routine screening mammograms[66]. Breast density on a mammogram represents the epithelial and stromal components of the breast. Women with dense breasts have a significantly increased risk of breast cancer compared with those with low breast density. However, the mechanisms by which breast density relates to cancer risk are not clearly defined.

In 1997, a cross-sectional analysis was therefore initiated, using NHS participants, to determine if plasma levels of IGF1 and IGFBP3, which have important roles in cellular proliferation, might be associated with mammographic density[67]. This was the first study to examine this hypothesis and included both premenopausal and postmenopausal controls from the study of IGF levels and breast cancer risk[40]. The ratio of IGF1 to IGFBP3 was used as an indirect measure of the levels of 'bioavailable' IGF1, which was proposed to be more relevant to breast cancer risk than total IGF1 levels. A higher ratio of IGF1 to IGFBP1 was associated with increased mammographic density in the premenopausal women, while no association was observed for the postmenopausal women[67]. These results parallel the findings for circulating IGF1 and breast cancer risk (as described above), and indicate a possible biological mechanism underlying the association between mammographic density and breast cancer. Although this is a novel and potentially important observation, the sample size of the study was relatively small, and confirmation of these findings is ongoing, both in the NHS and in other cohorts.

Summarizing risks and benefits

As mentioned above, a key strength of the cohort design is the potential to evaluate risks and benefits of lifestyle, drugs, diet and other exposures that cannot always be evaluated through intervention studies. Accordingly, the NHS also has evaluated total mortality in relation to numerous exposures. Studies of smokers in the NHS documented the overall increase in mortality due to smoking and the benefits of quitting among women[68]. Subsequent evaluations have addressed alcohol intake[69], obesity and physical activity[70] in relation to total mortality.

Over the past 30 years, data from the NHS have provided valuable information about how factors such as these affect the risk of cancer and other diseases. These results have helped formulate cancer-prevention messages for women (BOX 4). This is remarkable for a study that began as a simple questionnaire study, and expanded to include biological samples for genetic and biochemical analysis. The study is notable for its high follow-up rates over time, the ability to collect a range of biological samples and the overall low cost per participant (approximately US$14 dollars per participant per year to follow-up, process questionnaire responses, and confirm cancers and deaths among participants). This is due to the dedication and commitment of its participants, as well as their level of medical training, which leads to highly accurate reporting of health conditions.

As we refine our understanding of lifestyle and genetic contributions to disease aetiology, we continue to participate in collaborative efforts to bring the lifestyle and genetic data from over 65,000 participants in the NHS together with other cohorts to generate large enough sample sizes to more rigorously evaluate pathways for disease aetiology. Furthermore, collaborations with pathologists who analyse biopsy samples from malignant and premalignant tumours and the use of gene-expression analyses have allowed us to evaluate markers of exposure and uncover pathways to malignancy across a range of common cancers.

In addition to these collaborative efforts, the NHS has served as a model for a number of other important investigations including the Iowa Women's Cohort (studying diet and cancer in postmenopausal women), the California Teachers Study, the American Cancer Society Cancer Prevention Study II follow-up of diet and cancer (see Cancer Prevention Study Overviews in Online links box) , and the Black Women's Health Study, which all evaluate risk factors for breast and other cancers, and which, when combined, will allow the evaluation of potential associations between lifestyle factors, including diet, and rarer cancers among women.

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Competing interests statement
The authors declare no competing financial interests.

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