

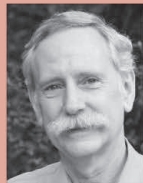


NHS NEWS



DEAR COLLEAGUES,

We are pleased to announce that the large National Institutes of Health grant that funds the Nurses' Health Study (NHS) has been renewed for another five years! The re-funding of this grant, coming 34 years after the NHS was first started, is a true testament to the continued importance of this study.



In this year's in-depth news article, we remind you of the many diverse and important contributions you have made over the course of the study. In addition to completing questionnaires, many of you have provided biological samples, which have greatly broadened the scientific scope of our study. We also provide an update on several recently completed projects, and describe new substudies that are about to be launched. In addition, we are starting enrollment for the Nurses' Health Study III and we are enlisting your assistance to recruit the next generation of nurses. The last year has been unusually busy and productive!

Your ongoing dedication and care allows us to continue learning about how women can maintain their health throughout life. We thank you most sincerely for your participation and look forward to our continued collaboration.

Best regards,

Susan E. Hankinson, Sc.D., R.N.
Principal Investigator,
Nurses' Health Study

Walter C. Willett, M.D., Dr.P.H.
Principal Investigator,
Nurses' Health Study II

Biological Samples

FOR OVER 30 YEARS, NHS AND NHS II PARTICIPANTS have completed biennial questionnaires about their nutrition and lifestyle choices. This information has yielded important discoveries for women's health. Less well-known – but certainly crucial to our research – are the various biological samples that many thousands of NHS and NHS II participants have provided over the years. By gathering such samples, we can evaluate hypotheses about genetic variation, biomarkers in blood, and molecular characteristics of tumors – allowing us to better understand disease biology and improve our ability to predict the risk of diseases such as cancer, cardiovascular disease, and diabetes.

Sample Storage and Handling

The NHS Biomarker Laboratory and Biorepository collects, processes, and stores the biological samples provided by NHS and NHS II participants. Blood, urine, and saliva are put into vials and stored in liquid nitrogen freezers. Tissue blocks (for example, from tumor tissue in women diagnosed with cancer) and slides are stored in secure cabinets. Film mammograms are digitized, the digital images are de-identified, and the original films are returned immediately to the lending radiology departments. These labs also prepare the samples for scientific analyses. (See 'Focus on our research team' for more information about the people who oversee these labs, page 8)



Research assistant Kathryn Doyle removes biological samples from the NHS freezer archive
Photo Credit: Frank Siteman

Sample Types

We collect blood, tissue, urine, toenails, mammograms, and saliva; each type of sample gives us information on a unique exposure or marker. These samples are extremely valuable because participants have been followed for up to 30 years since their initial sample collection. However, they are a finite resource; thus, much effort is put forth to handle the samples carefully and prioritize their use.

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In Brief

Web Submissions

When participants in the NHS II think about our questionnaires, increasingly they envision a mouse and keyboard rather than a paper form and a Number 2 pencil. So far, over 54,000 women have completed their NHS II survey on our website since the current cycle began in June of 2009. (Another 30,000+ have mailed in their paper surveys.) As you can imagine, reducing the number of paper forms we receive, review, scan, and store makes our life much easier, and helps the environment at the same time. In addition, our ability to communicate with the nurses by email allows for rapid, inexpensive updates on our results as well as announcements of new surveys. We have a total of nearly 100,000 nurses who have provided us with their email addresses. For the nurses in the original NHS, we are testing some limited web survey options in 2010. Whether by web or by paper, we value every completed survey as we try to make participation more convenient.

Nurses' Health Study II Second Blood and Urine Collection

We recently received funding from the National Cancer Institute to ask NHS II participants for a second set of blood and urine samples (these same participants gave samples for the first time between 1996 and 1999). This will create a unique resource of blood and urine samples collected twice from the same nurses, about 10 to 15 years apart. Of particular interest is that many participants were premenopausal at the first collection and will be postmenopausal at the second collection – creating the only biorepository in the world with a large number of matched premenopausal and postmenopausal samples. With these samples, we will learn much more about the relationship between hormones and other biomarkers – measured at two different times in life – with risk of breast cancer and other chronic diseases. We will be contacting women to invite them to donate samples throughout the year. We look forward to your participation! 

STUDY UPDATES

Launching the Nurses' Health Study III!

A new phase of the NHS is here! Starting June 1, 2010 we began enrolling a new cohort of young nurses: the NHS III. We will recruit female RNs and LPNs ages 22-45 years old from across the country to join this new cohort, which will be entirely web-based. We wish to examine how recent changes in hormone preparations, dietary patterns, and nursing occupational exposures influence women's health. Prominent new features include a closer look at fertility and pregnancy events, and a greater focus on adolescent diet and breast cancer risk. During our pilot evaluations we learned that no one is better qualified to attest to the rewards of contributing to women's health research than you and your fellow participants in the study. Therefore we would appreciate

it if you would encourage your colleagues to join this new study by visiting www.nhs3.org. With your help we plan to make this new study a success!

Growing Up Today Study (GUTS)



The Growing Up Today Study, a cohort of 16,882 children of participants in the NHS II ages 9 to 14 years at enrollment in 1996, has published ten articles since the last NHS newsletter on diverse topics including eating disorders, sexual orientation, and Human Papilloma Virus. In one study, we found that participants who ate family dinners on most days were less likely to develop eating disorders.

This year we will send questionnaires to both GUTS 1 (in the spring/summer) and GUTS 2 (in the fall). For the first time, the 2010 GUTS 1 questionnaire

will include questions on relationships, pregnancy, and fertility. By asking all GUTS 1 participants about fertility, we hope to gather information that can help us examine the causes of infertility and trends in contraceptive use.


GUTS is one of the few studies in the world that gathers information about the health of young adults – a traditionally difficult group to reach. Your help in keeping the GUTS study updated with your children's email addresses is greatly appreciated, especially in this digital age (almost 80% of the 2007 questionnaires were completed online!) You can update all contact information for GUTS members at any time (see box below). As always, thanks for your help!

SENDING GUTS UPDATES:


 www.nhs2survey.org/nhs2/gutmoms
 617-525-2279

The Lifestyle Validation Study (LVS)

The new Lifestyle Validation Study, funded by the National Institutes of Health, will investigate novel ways to improve our methods of measuring dietary intake and physical activity. This detailed study will be conducted among 750 women selected from the NHS and NHS II. Those who agree to participate will provide detailed information on diet and physical activity using a variety of methods including web-based diet and physical activity recalls as well as meal-by-meal diet recordings. Additionally, participants will provide multiple blood, urine, and saliva samples.

This study will provide invaluable information that will aid the interpretation of previous reports of diet and physical activity collected in the entire NHS over the last 30 years. The LVS will be the largest and most important validation study of methods to measure diet and physical activity ever conducted; its (de-identified) results will be available to other researchers in the United States and worldwide. At the completion of this study, every LVS participant can gain great satisfaction knowing that these findings will significantly impact how we measure diet and physical activity and, subsequently, how we study the role of these lifestyle factors in disease development for the next 20 years. Thank you, LVS participants! 

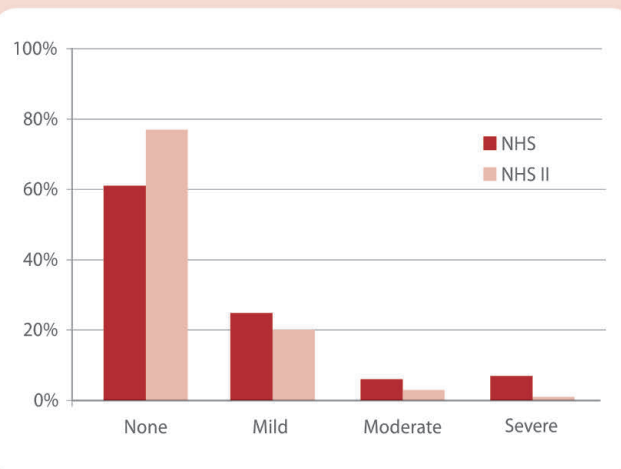
Interested in Updates on Nutrition?

Much of what we now understand about diet and health comes from the Nurses' Health Studies, and we make a point of communicating through this newsletter the most important findings as they emerge. However, if you are interested in additional information on nutrition and health, we invite you to visit the website maintained by the Department of Nutrition at Harvard School of Public Health called The Nutrition Source: www.hsph.harvard.edu/nutritionsource. In addition to research from the Nurses' Health Studies, this site includes findings from other studies around the world, including our cohort of men, the Health Professionals Follow-Up Study. The website also contains reviews on controversial topics in nutrition and helpful articles on how to put newfound knowledge into practice, such as recent features on healthful beverages ("How Sweet Is It?") and salt reduction. We also provide healthful recipes for foods served in our food service at Harvard, including those developed by the famous cookbook writer, Mollie Katzen. In reading this website, we hope you will feel good that much of the information would not be available without your many contributions as a member of the Nurses' Health Studies. 

Hearing Loss & Tinnitus

HEARING LOSS AND TINNITUS (noise or ringing in the ears) are very common, affecting tens of millions of individuals in the United States – making the identification of modifiable risk factors a vital public health issue. Given this, it is surprising that so little is known about common and potentially modifiable risk factors for these disabling conditions. In addition to noise, factors potentially associated with risk of hearing loss or tinnitus include analgesics, vitamins, alcohol, menopausal status, and postmenopausal hormone use. We have begun examining such risk factors in the Nurses' Health Studies. Some participants have already provided information about their symptoms and soon we will be asking others as well. If we obtain funding, we will invite some participants to undergo a formal hearing test with a local audiologist.

The figure below shows how common hearing loss is in the Nurses' Health Studies.



Severity of hearing loss reported on the NHS 2006 and NHS II 2009 questionnaires.

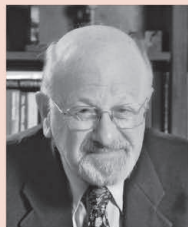


Photo Credit: Frank Siteman

Update on Frank Speizer

We often hear from nurses asking about Frank Speizer, MD, the Founding Principal

Investigator of the NHS. He is still active with the study and, like those of you who have been with us for many years, is especially interested in studying lifestyle factors that affect healthy aging. He also enjoys watching many of his former trainees and colleagues become academically successful by their own scientific achievements using the NHS data. For more information on Dr. Speizer and his many contributions, visit www.brighamandwomens.org/development/supportnhs.aspx

World War II United States Cadet Nurse Corps

In previous newsletters, we reported on a bill that would grant veteran status and benefits to women who served in the Cadet Nurse Corps during World War II. The current version of the legislation (HR 1522), the tenth attempt to pass this bill, is still pending with 21 co-sponsors in the House of Representatives. To support this bill, contact your local US Representatives and ask them to co-sponsor HR 1522. For more information contact: Shirley Harrow at saharrow@verizon.net or 65 Gladstone St. Quincy, MA 02171.

More Than One?

Are you routinely receiving two simultaneous copies of mailings from the study? It's normal to receive letters from us a few months apart, but if you are getting two identical letters at once, something is amiss. If this is happening to you, give us a call at 617-525-2279 so that we can resolve this issue.

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Examples of what we examine using the biological samples provided by NHS and NHS II participants.

BLOOD	TISSUE	URINE	TOENAIL	MAMMOGRAM	SALIVA
Hormones Dietary Biomarkers Infections Telomere Length Genotype	Protein Expression RNA Expression Infections Epigenetic Modifications	Estrogen Melatonin Calcium Creatinine Albumin Sodium	Selenium Nicotine Fluoride	Breast Density	Genotype

Blood

From 1989 to 1990, 32,826 NHS participants provided blood samples; similarly, from 1996 to 1999, 29,611 NHS II participants provided blood. These samples enable us to measure levels of blood biomarkers in participants who went on to develop disease and compare them to levels in participants who did not. In the NHS, from 2000 to 2001, many of the same participants provided a second blood sample ten years after their first sample. We are also currently collecting a second blood sample from women in the NHS II. Blood samples at two time-points will allow us to examine changes in biomarker levels, and the timing of exposure in relation to disease.

Tissue

For women diagnosed with cancer, we request permission to collect tissue blocks and slides. To date, 5,574 NHS participants and 6,818 NHS II participants have provided tissue samples. We have collected blocks for most cancers including breast, ovarian, colorectal, brain, renal, bladder, pancreas, melanoma, and non-Hodgkin's lymphoma. In addition, we have collected samples of benign tissue including colon polyps, benign breast disease, and Barrett's esophagus. Most cancers are molecularly diverse – possibly reflecting differences in cancer development – and may be associated with varied

prognosis and survival. Examining these molecular differences will provide insight into ways to prevent certain cancers, and improve survival.

Urine

From 2000 to 2001, 18,743 NHS participants who gave a blood sample in 1989-1990 provided a first morning spot urine sample. In the NHS II, 29,611 participants provided a first morning urine sample. A number of biomarkers, such as hormones, can be measured in urine.

Toenail

From 1982 to 1984, 62,641 NHS participants provided toenail clippings. Because toenails grow slowly (approximately 1cm every 9-12 months), they can better reflect long term exposure than blood or urine samples. These samples have been primarily used to evaluate exposure to trace elements such as selenium and fluoride.

Mammogram

Breast density is a predictor of breast cancer risk. For some women, we request permission to review film mammograms. To date, 5,679 NHS participants and 1,215 NHS II participants have provided mammograms. After collection, we use computer software to measure the amount of breast density in the mammogram. We are investigating which lifestyle factors increase breast density, as well as the biological pathways involved.

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Saliva

From 2002 to 2004, 33,040 NHS participants provided a saliva sample; similarly, from 2004 to 2006, 29,859 NHS II participants provided saliva. These samples are a source from which we can obtain cheek cell DNA for genetic analyses.

What We Have Learned: Two Examples Estradiol and Breast Cancer


Twenty years ago there was laboratory evidence to support a role for circulating levels of estrogen in breast cancer, but few prospective human studies had been conducted. We evaluated and confirmed blood estradiol levels as a strong risk factor of breast cancer in postmenopausal women. This added substantial rationale to evaluate drugs such as Tamoxifen to prevent breast cancer and revealed the potential of circulating estradiol levels as a biomarker for identifying women at high risk of breast cancer. (Hankinson SE, et al. *J Natl Cancer Inst.* 1998;90(17):1292-9)

Aspirin, COX-2, and Colorectal Cancer

Regular use of aspirin reduces the risk of colorectal cancer, but we did not understand why this occurred. Using colon cancer tissue blocks, we were able to classify tumors by the amount of cyclooxygenase-2 (COX-2) activity, a protein marker of inflammation and pain. We found that aspirin reduced the risk of developing tumors with increased COX-2 activity, but not other tumor types. This study showed the critical role of COX-2 in the link between aspirin and colorectal cancer and suggested the potential use of COX-2 as a molecular marker for tailoring cancer prevention in participants with a history of colorectal cancer. These findings were only possible by having both the tissue sample and the lifestyle data collected in the questionnaires. (Chan AT, et al. *N Engl J Med.* 2007;356(21):2131-42)

In addition to these examples, numerous manuscripts have been published using data from biological samples obtained through the Nurses' Health Studies.

Conclusion


The samples NHS participants have so generously provided allow us to evaluate hypotheses related to genetic variation, circulating biomarkers, and tumor markers. These samples are particularly valuable because participants have been followed for up to 30 years since collection and they are accompanied by a wealth of data on medical histories and health-related behaviors. The goal of the biorepositories is to maximize research use of biological samples in a cost-effective, ethical, state-of-the-art manner. Thus every effort is made to ensure high-quality specimen processing, tracking, and assay methodology. In addition, we conduct and evaluate pilot studies of new methods or assays as technology changes to ensure that only valid, reproducible, and up-to-date methods are used. These biological samples are an important part of the research we are doing today, and will continue to be so in the future. 

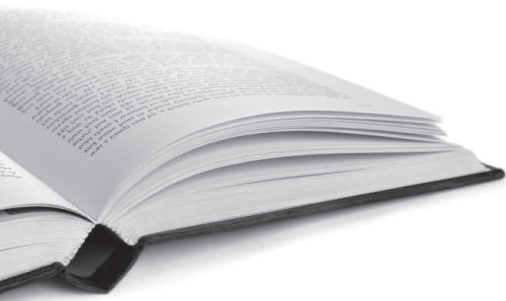
We thank all NHS participants for their continued dedication to the study. Your ongoing support makes this study uniquely able to evaluate important hypotheses and study numerous diseases in women.

Our Approach to Data Sharing

All recipients of NIH funding have been asked by the U.S. Congress to do a better job of sharing our data with other scientists. Our primary approach has always been to collaborate with scientists who have high standards and who are pursuing issues that we agree are important. Together we analyze any shared data and prepare joint reports for publication. Notably, we never allow these scientists to access personally identifiable information: confidentiality is of the utmost importance to us, and we only work with scientists who value your privacy as much as we do.

Due to the value of pooling data from multiple studies, the NIH has mandated that we submit genotype data to the database of Genotypes and Phenotypes (dbGaP), which archives and distributes the results of genetic studies – while maintaining strict oversight and investigator accountability for the data. Any data we send to this database is completely devoid of personal identifiers (e.g., your name, year of birth, address, or zipcode). To learn more about dbGaP, please visit www.ncbi.nlm.nih.gov/gap

Over the past five years, we have participated in more than 50 collaborative projects and are proud that NHS data have helped to advance science in so many ways. As NIH continues to develop its data-sharing recommendations, we will keep you abreast of any changes in how we approach our collaborative efforts. 



Recent Findings

Aspirin Intake and Survival After Breast Cancer

We measured aspirin intake after diagnosis of early stage breast cancer in over 4000 participants in the NHS. Women who took aspirin were approximately 50% less likely to die from breast cancer than women who did not take aspirin. To our knowledge, this is the first study to specifically show that, among women who have breast cancer, aspirin intake is associated with a lower overall risk of dying from this disease. Aspirin's effect on breast cancer may be attributed to its anti-inflammatory properties, but side effects such as gastrointestinal bleeding can be detrimental. A clinical trial is required to confirm these findings. However, women with breast cancer who are already advised by their doctors to take aspirin for another reason (for instance, heart disease prevention) can take comfort knowing that this regimen may also be preventing them from dying from breast cancer. (Holmes MD, et al. *J Clin Oncology*. 2010;28(9):1467-72)

Prevention of Hypertension

High blood pressure affects over 60 million individuals in the United States and is one of the leading causes of preventable death in women. To determine what percentage of new onset hypertension in the NHS II could have been prevented through lifestyle modification, we studied

the risk of developing hypertension associated with six healthy lifestyle factors: having a normal body mass index (under 25); daily physical exercise; eating a diet rich in fruits, vegetables and low-fat dairy products but low in meats, refined grains, and sweets; moderate alcohol intake (up to an average of 1 drink per day); avoidance of over-the-counter analgesics; and use of supplemental folic acid (at least 400 µg/day). Although less than 0.5% of women followed all six of these healthy lifestyle factors, we estimated that nearly 80% of all hypertension development among women in the NHS II could have been prevented if all women had followed all six factors. Even achieving a handful of these six factors was still associated with lower risk: if all women in the NHS II had a healthy weight, exercised daily, and ate a healthy diet, more than half of all cases of hypertension may have been prevented. (Forman J, et al. *JAMA*. 2009;302:401)

ABO Blood Type and Cancer

Pancreatic cancer is the fourth leading cause of cancer death in the United States. However, we know little about the inherited factors that contribute to the development of this disease. In 77,360 women who reported their blood type on the 1996 NHS questionnaire, we found that nurses with blood types A, B, and AB were more likely to develop pancreatic cancer than those with blood type O. The mechanisms underlying this difference in risk are not yet clear, but may be related to susceptibility to chronic inflammation or infections.

We confirmed this important finding in collaboration with colleagues at the National Institutes of Health and from around the world. In total, we analyzed DNA from nearly 4,000 patients with pancreatic cancer and 4,000 people without the disease, including DNA from nearly 200 NHS participants. Although these findings are not ready to be used in the clinic, they provide a new avenue of exploration to better understand why particular people develop this difficult disease. In addition, we are currently investigating whether blood type may be an important risk factor for other cancers. (Wolpin BM, et al. *J Natl Cancer Inst*. 2009;101(6):424-431; Amundadottir L, et al. *Nat Genet*. 2009;41(9):986-90; Wolpin BM, et al. *Cancer Res*. 2010;70(3):1015-23)

Physical Activity and "Successful" Survival

We examined how physical activity impacted "successful" survival, which we defined as living past age 70 years with no chronic diseases and no major issues regarding physical function, cognitive function, or mental health. Compared to women whose activity levels were in the lowest quintile, women with higher levels of activity were up to twice as likely to be successful survivors. Increasing levels of walking were also associated with a similar elevation in odds of successful survival. Overall, higher levels of midlife physical activity were associated with exceptional health status among women who survive to older ages. (Sun Q, Townsend MK, et al. *Arch Intern Med*. 2010;170:194-201)

Genetic Update

OVER THE LAST THREE YEARS, genome-wide association studies (GWAS) have identified hundreds of genetic markers associated with dozens of diseases and traits – a pace of discovery unprecedented in human genetics. The NHS has been at the forefront of this research. Over 5,000 nurses have participated in GWAS of breast and pancreatic cancers, type 2 diabetes, coronary heart disease, and kidney function. Additional studies of endometrial, colon, and brain cancers, among other diseases, are planned.


Moreover, thanks to the detailed questionnaires and biological samples provided by participants, NHS researchers have studied a wide range of disease-related traits and have published novel genetic associations with height, ages at menarche and menopause, and circulating levels of vitamins B12 and D, among others.

GWAS leverage recent technological developments that allow researchers to quickly and with reasonable cost measure up to 1 million markers across the entire genome. These markers can serve as proxies for as many as 10 million common variants in the human genome – distinct bits of DNA at a particular location shared by more than 10% of people. These recent successes have opened up new avenues for research and raised important questions.

First, by design, instead of identifying specific causal genetic variants, GWAS highlight genomic regions that harbor these variants. By discovering novel regions associated with disease risk, GWAS results generate new hypotheses for additional investigation. This may ultimately improve our knowledge of disease biology.

Second, the new markers each have small effects on disease risk. They cannot entirely explain the increase in risk observed for close relatives of people with disease – suggesting that there are more variants yet to be found. Some of these variants may be less common or rare, which are difficult to detect using GWAS. One alternative design better suited to identifying rare variants is a sequencing study, which painstakingly measures each letter of DNA code in a region in many individuals. We plan to begin several sequencing studies this year.


Finally, it may be possible to use these markers to estimate individualized disease risks. However, because their effects are small, additional research is needed to examine the application of these new genetic markers in a clinical setting. NHS researchers are currently exploring the clinical utility of disease risk models that combine these genetic risk markers with widely-used non-genetic risk factors.

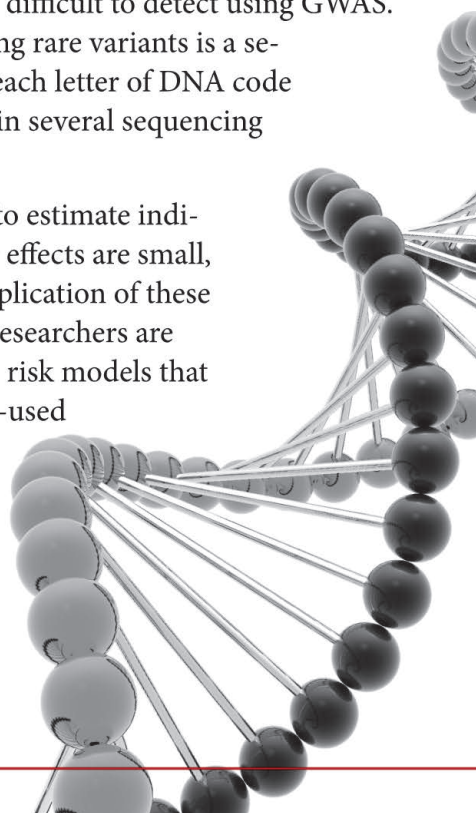
For more information regarding genetic analyses, please read “Genetic risk prediction – are we there yet?” by NHS researchers Peter Kraft and David Hunter, available at <http://content.nejm.org> 

Recent Findings

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Oral Contraceptives and Urinary Incontinence

We previously found that post-menopausal hormone therapy is related to increased risk of urinary incontinence; yet little is known about the effects of taking oral contraceptives in younger women. In the NHS II, we found that women who used oral contraceptive pills were 27% more likely to experience urinary incontinence at least weekly compared with those who never used oral contraceptive pills. In women with 10 or more years of use, this increased to 48% greater odds. This suggests that use of oral contraceptive pills may be associated with a modest increase in urinary incontinence among premenopausal women. However, this is one of the first reports of such an association, and further research is needed to confirm our findings and investigate the possible mechanisms behind this relationship. (Townsend MK, et al. *J Urol*. 2009;181:2170-5) 




Focus On Our Research Team

Janine Neville-Golden & Greg Kirkner

JANINE NEVILLE-GOLDEN AND GREG KIRKNER are the senior lab managers for the NHS and NHS II biorepositories. They shepherd investigators' projects through the sample collection and access process. They constantly work to optimize the use of precious biological donations. Last year alone, they oversaw the completion of 75 projects that examined the relationship between various biomarkers and chronic disease.

With over 25 years of experience in cancer biology and neurobiology research, **Janine Neville-Golden** manages the collection of blood, urine, and cheek cell samples and oversees daily Blood Lab operations. Additionally, she has made numerous improvements to the collections process while assisting with grants and expanding the freezer farms. A few of Janine's outside interests include history, fine arts, architecture, and international travel.

Since the spring of 2002, **Greg Kirkner** has worked with the Channing Blood Lab and overseen all aspects of the tissue and mammography collections. He is involved in identifying study eligibility, tracking and organizing specimens, and developing strategies to improve collection success rates from hospitals. In his free time, Greg likes to snowboard, run and swim, and more recently he and his wife have begun to travel across the country to participate in triathlons. 



Janine Neville-Golden



Greg Kirkner

Funding the Nurses' Health Studies

We are strongly committed to the Nurses' Health Studies and are doing everything we can to assure their long-term stability. In addition to seeking grants from major foundations and government institutes, we also solicit support from private donors in our effort to sustain the continued operation of the Nurses' Health Studies. As the largest ongoing studies of women's health that include repeated measures of diet, physical activity, and other lifestyle factors related to a broad range of chronic conditions, we are confident we will meet our goal of diversifying the funding base for the NHS.

If you or someone you know is interested in contributing to the study, please contact Dr. Hankinson, Dr. Speizer, or Dr. Willett at (617)525-2258, or visit the web site www.nurseshealthstudy.org and click the "Donate" link.

Nurses' Health Study

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To report name or address changes,
visit www.NursesHealthStudy.org

Letters & feedback are welcome.