

## **Overview of Women's Health Study (WHS) Study Design**

The WHS was designed as a randomized, double-blind, placebo-controlled, factorial trial of low-dose aspirin (100 mg every other day), vitamin E (600 IU every other day), and  $\beta$ -carotene (50 mg every other day) in the primary prevention of both cancer and cardiovascular disease (CVD) among 39,876 U.S. female health professionals aged  $\geq 45$  (Refs 1-3; Clinical Trials.gov NCT00000479).

The trial was funded jointly by the National Cancer Institute (NCI) and the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH) from inception in 1991 to its scheduled end on March 31, 2004; the average duration of treatment and follow-up in the WHS trial was 10 years. At the end of the trial, participants were invited to be followed observationally, by completing yearly endpoint and risk factor questionnaires, to evaluate cancer and CVD hypotheses. NCI has funded the WHS Continued Follow-up, which supports the infrastructure for ascertaining and validating both the cancer and CVD endpoints. A total of 33,681 women (88% of those alive) agreed to be followed observationally; those unwilling are tracked only for mortality. Average duration of follow-up is currently almost 28 years. The methodology for both the WHS trial and WHS follow-up as a cancer cohort is detailed below.

### **Trial enrollment and randomization**

Beginning in 1991, 1,757,247 female health professionals were identified from state nursing boards and other organizations of health professionals. From September 1992 to May 1995, each woman received an invitation letter; a questionnaire on demographic data, health habits, and medical history; and an informed consent form. A total of 453,787 women returned their questionnaires, and 194,659 indicated they were willing to participate in the WHS. Of these, 65,169 met eligibility criteria: age  $\geq 45$ ; postmenopausal or with no intention of pregnancy; no history of CHD, cerebrovascular disease, cancer (except nonmelanoma skin cancer), or other serious illness; no history of serious side effects to the study treatments; not taking aspirin, aspirin-containing medications, or nonsteroidal anti-inflammatory drugs  $>$ once/week, or willing to forego the use of these medications; not taking anticoagulants or corticosteroids; and not taking vitamin A, E, or  $\beta$ -carotene supplements  $>$ once/week. Willing and eligible women were entered into a 3-month run-in phase, receiving calendar packs containing placebos and a dietary questionnaire. At the end of the run-in, 39,876 women who indicated continued willingness and eligibility, and reported taking at least two-thirds of their pills and not regularly taking outside aspirin,  $\beta$ -carotene, or vitamin E supplements during the previous month, were randomized into the trial, using a 2x2x2 factorial design, to aspirin, vitamin E, and  $\beta$ -carotene, or placebos.

The percentages of women belonging to each of the minority ethnic/racial groups reflected the race/ethnic distribution of female health care professionals in the US in the early 1990s: 2.3% African American, 1.1% Hispanic, 1.4% Asian/Pacific Islander, 0.3% American Indian/Alaskan Native, and 0.1% more than one race. However, while the percentage of a specific ethnic or racial group such as African-Americans, for example, is only 2.3%, that translates to over 900 African-Americans given the large sample size. In addition, WHS participants have a wide range of education and income levels (Ref 4)

### **Baseline blood collection**

Women were asked at baseline whether they would be willing to provide a venous blood sample by mail. Those who responded affirmatively and who were enrolled into the run-in received a blood collection kit containing instructions and supplies, including EDTA and sodium citrate tubes, a gel-filled freezer pack, and an overnight courier air bill. Women were asked to freeze the freezer pack overnight to serve as a coolant, to have a fasting blood sample drawn the next morning, and to return the samples via overnight courier. Upon receipt at our laboratory,

samples were logged into the computer and were kept chilled until processed. Processing and storage in nitrogen freezers (-170 °C) took place within several hours of specimen receipt (30-36 hours after venipuncture). Samples (plasma, buffy coat, red blood cells) were stored in 12 locations across three nitrogen freezers for security. Strict quality control procedures have proven highly reliable; no specimen has been inadvertently thawed.

A total of 28,345 women (71% of participants) provided a blood sample prior to randomization, and this sub cohort of the WHS is referred to as the Women's Genome Health Study [WGHS], which is under the leadership of Dr. Daniel Chasman (Ref 5). Baseline characteristics among women who did and did not provide blood specimens were largely similar with regard to age, BMI, current use of postmenopausal hormones, ever use of oral contraceptives, aspirin use before the trial, current use of multivitamin supplements, history of benign colon polyps, family history of colorectal cancer, and median alcohol intake. Women who provided blood specimens were more physically active but were less likely to be current smokers.

Additional federal and non-federal funding has allowed extensive plasma biomarker and genetic analyses. DNA has been extracted and whole-genome scans have been completed on >25,000 samples, using the standard Illumina 317K chip with ~60,000 additional single-nucleotide polymorphisms (SNP; Duo Plus) chosen to provide added coverage for genes related to inflammation, hemostasis, and thrombosis. Exome Chip genotyping was successful for 22,618 of these WGHS participants. In addition, the >28,000 plasma samples have been assayed for total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density cholesterol (HDL-C), triglycerides, lipoprotein(a) [Lp(a)], apolipoprotein (apo) A-1, apolipoprotein (apo) B-100, high-sensitivity C-reactive protein (hsCRP), fibrinogen, soluble intercellular adhesion molecule type-1 (sICAM-1), homocysteine, glycosolated hemoglobin (HbA1c), and creatinine.

### **Dietary assessment**

A self-administered food frequency questionnaire (FFQ) developed by Willett et al. was mailed to each participant during the run-in. Of randomized participants, 39,345 (98.7%) completed and returned the FFQ. A second FFQ was collected from 32,880 participants at the trial's conclusion in 2004. The estimates of dietary intakes derived from this FFQ have been shown to reflect long-term intakes in women.

### **Randomized treatment and follow-up**

At baseline, 6 months, 12 months, and then annually, participants received a supply of randomized pills in monthly calendar packs, accompanied by a questionnaire that included items on pill-taking compliance, nonstudy medication use, occurrence of major illnesses or adverse effects, and other risk factor information (see [Matrix of Variables](#) collected at baseline and on trial follow-up questionnaires). For the small number of women who did not provide information by mail or phone, vital status was ascertained. A very high percentage of participants provided annual follow-up information, ranging from 99% in the early phases of the trial to 97% ten years later at the end of the trial. Vital status was known for >99% of the cohort; by trial's end, 1251 of the participants had died.

### **Endpoint ascertainment in the trial**

Nonfatal endpoints were based on self-reports from follow-up questionnaires, letters, or telephone calls. For each reported cancer or CVD endpoint, we requested permission from the participant to obtain relevant medical records. For fatal endpoints, permission was requested from next of kin to obtain medical records, and a copy of the death certificate was obtained from the next of kin or from the vital records bureau of the state in which the participant died. Additional records, such as autopsy reports, were requested as needed. A National Death Index (NDI) search was conducted midway through the trial, and at the end of the trial period, for those who were lost to follow-up.

An Endpoints Committee of physicians with expertise in oncology, cardiology, and neurology reviewed the medical records to confirm reported diagnoses of cancer and CVD, blinded to the participants' randomized treatment status and using a defined protocol. In brief, a cancer diagnosis was confirmed with histologic or cytologic evidence. In the absence of such data, strong clinical evidence accompanied by radiologic evidence or laboratory markers (such as CA 125) was used to confirm cancer occurrence. Pathology slides were obtained for all reported lung cancers and were reviewed by a lung pathologist. In addition, the Endpoints Committee documented additional details from the medical record – e.g., for cancer, characteristics such as site, histology, grade, metastases, and tumor characteristics. Myocardial infarction was confirmed by using Joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Federation (ESC/ACCF/AHA/WHF) Task force for the Redefinition of Myocardial Infarction criteria. Stroke is confirmed and categorized according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.

Self-reported cases were classified as confirmed if they fulfilled the validation criteria. When consent was not provided or records could not be obtained, the reported endpoint was not considered confirmed. Only confirmed endpoints are included in final reports. The confirmation rates in WHS were 82% for cancer, 61% for myocardial infarction, and 68% for stroke; of those not confirmed, most had a related diagnosis, such as in situ breast cancer instead of invasive breast cancer, or transient ischemic attack instead of stroke.

### **WHS observational follow-up population**

In May 2005, after the end of the trial, a cover letter explaining the aims of the continued follow-up and the first observational follow-up questionnaire were mailed to 37,772 of the 39,876 randomized trial participants. The remaining women were not contacted because they had died (n=1484), previously elected not to participate, or were lost to follow-up. A total of 33,682 women, or 89% of the surviving participants, were willing to continue to be followed observationally. Participants in the continued follow-up have been mailed a questionnaire every year, requesting information on medical history and lifestyle risk factors (see [Matrix of Variables](#) collected on the observational follow-up questionnaires). After over 20 years, the retention rate remains high: as of the last completed full observational follow-up cycle, 89.6% of those who did not opt out of the extended follow-up were still returning their latest follow-up questionnaire, and mortality follow-up remained virtually 100% complete. Procedures for endpoint confirmation for the observational follow-up are the same as those used during the trial.

### **Assessment of physical activity in the WHS**

The WHS baseline and follow-up questionnaires have included and updated information on a range of potential risk factors for cancer and CVD over the last decades, but physical activity has been, and will continue to be, a major focus of the WHS research portfolio, under the leadership of Dr. I-Min Lee, one of the Principal Investigators of the WHS. Dr. Lee is particularly well qualified to provide leadership in this area: she has served on panels worldwide to develop expert reports and physical activity guidelines, including the 2008 US Department of Health and Human Services Physical Activity Guidelines Advisory Committee, the 2010 WHO Global Recommendations on Physical Activity for Health, and the 2013 AHA/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Risk.

The combination of self-reported data spanning decades and more recent data from device assessments, makes the WHS an extremely valuable physical activity resource. Physical activity was assessed at baseline (1992-1995) by self-report on a mailed questionnaire, and has been updated every 2-3 years. Between 2011 and 2015, the WHS used the Actigraph GT3X+ device, which houses a triaxial accelerometer capable of measuring accelerations in 3 planes, to measure physical activity and sedentary behavior over 7 days, during waking hours, among

17,708 women living throughout the United States. WHS is one of the largest cohorts in the world with device measured physical activity and sedentary behavior, and who are being followed for health outcomes. The WHS is currently preparing to deposit and share detailed, individual-level accelerometer data from the WHS through an NCI-controlled database. A link to this will be provided in the future when available.

## Endpoints

The primary endpoints of the WHS trial and observational follow-up period are total cancer and a composite endpoint of total cardiovascular disease (nonfatal myocardial infarction, total stroke, and fatal cardiovascular disease). Event rates are increasing substantially over time with the aging of the cohort. The numbers of the primary endpoints and their individual components are included as of 12/31/21 at [WHS Cancer and Cardiovascular Outcomes](#).

## Data sharing

WHS has a policy of data sharing that actively promotes new research collaborations which will make use of the comprehensive questionnaire, biomarker, genetic, and objectively measured physical activity data that have already been and will continue to be collected during the trial and observational follow-up.

The WHS has had great success in data sharing. We will continue to encourage the submission of ancillary studies, grants, career development awards, and analyses that include investigators from other departments and institutions. To date, there have been over 600 publications using WHS data since the beginning of the study ([WHS Bibliography](#)); over 20 NCI Cohort Consortium projects in which the WHS has and is currently participating ([WHS Consortium Projects](#)), and over 100 funded WHS ancillary grants ([WHS Ancillary Grants](#)).

In brief, the WHS data sharing protocol is as follows: any investigator interested in using WHS data contacts one of the two PI's with their research question. The investigator is then asked to prepare a brief proposal. Drs. Buring and Lee discuss the proposal together at the WHS Oversight Committee, then bring this proposal to a regularly scheduled meeting of the WHS Steering Committee for approval. The Steering Committee will give final approval to requests for use of WHS data, after considering the importance of the hypothesis, the priority compared to other ongoing or requested analyses, the burden on the WHS participants and staff, and the amount of WHS plasma or DNA resources that would be required. For outside investigators, the committee also decides on the choice of the appropriate WHS investigator who will serve as the official collaborator on the particular study. All manuscripts containing WHS data are checked by the study analysts prior to submission to a journal. Fuller information and the required forms are included in [Guidelines and Procedures for Collaboration](#) and [Forms to Request Collaboration](#). Investigators may be asked to provide financial support to complete the proposed project.

## WHS grant numbers for affiliations

Because of the long duration of the WHS, there are a number of NIH grant numbers that must be acknowledged in any manuscript. Specifically,

1. For any paper using only data up to the end of the randomized trial period, include CA047988 and HL043851.
2. For any paper using the randomized period data plus any subsequent observational follow-up data or any whole genome scan data, include the two numbers above **plus** HL080467.
3. For any paper that includes risk factor or endpoint data from the observational follow-up periods of 2009 or beyond, include the three numbers above **plus** HL099355.

4. For any paper using data from the 2014 questionnaire or beyond, include the four grant numbers above **plus** CA182913.

Thus any paper that includes data from the beginning of WHS to the present time will need five NIH grant numbers to be included.

## REFERENCES

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3. Ridker PM, Cook NR, Lee I-M, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. *New Engl J Med* 2005;352:1293-304.
4. Rexrode KM, Lee I-M, Cook NR, Hennekens CH, Buring JE. Baseline characteristics of participants in the Women's Health Study. *J Women's Health Gend Based Med* 2000;9:19-27.
5. Ridker PM, Chasman DI, Zee RY, et al. Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25,000 initially healthy American women. *Clin Chem* 2008;54:249-55.