SECTION 1-A5
PROTOCOL APPENDIX 5

WOMEN'S HEALTH INITIATIVE OBSERVATIONAL STUDY
OVERVIEW OF OBJECTIVES AND HYPOTHESES

1-A5.1 Objectives

The objective of the Observational Study (OS) is to provide information complementary to that obtained from the Clinical Trial (CT). Measurement of baseline characteristics, remeasurement after three years, storage of frozen blood specimens, and ascertainment of clinical events in a large cohort of postmenopausal women allow the following specific objectives to be formulated:

1) Prediction of risk of outcome on the basis of:
   - Questionnaires and interview data
   - Physical exam findings
   - Laboratory data

2) Extension of results in the CT to related exposures and regimens

3) Assessment of temporal relationships between risk factors and disease occurrence

4) Documentation of variation in the incidence of cardiovascular disease, cancer, osteoporosis and fracture in postmenopausal women on the basis of geographic region and other demographic characteristics, and an evaluation of the extent to which differences among demographic subgroups in the prevalence of identified risk factors account for such variation. *Table 2-A5.1 - Cumulative Number of Events For 100,000 Women Age 50-79 Years At Baseline* presents estimates of the number of events of various types at 3, 6, and 9 years of follow-up.

1-A5.2 Hypotheses

These hypotheses include those of high priority that have been stated to date. This is not an exhaustive listing and future hypotheses will be added as they are developed. *Table 1-A5.2 - Summary of Exposure/Disease Hypotheses* summarizes the general exposure/disease hypotheses of interest initially.

Disease-Related Hypotheses Classified by Predictive Factors

1) **Diet**

   a) Antioxidant intake (vitamins C and E, carotenoids, selenium, zinc) predicts decreased risk of cancer, coronary heart disease (CHD), and stroke.

   b) Fiber intake is associated with lower risk of colorectal cancer, breast cancer, and other cancers, as well as CHD and stroke.

   c) Alcohol intake predicts decreased risk of cardiovascular disease, and increased risk of breast and colorectal cancer.

   d) Alcohol intake during adolescence increases risk of breast cancer during adulthood.

   e) Intake of vitamins B6, B12, and folate is associated with decreased risk of CHD and stroke. Folate intake predicts reduced risk of colorectal cancer.

   f) Coffee and caffeine are related to increased risk of CHD and stroke, as well as fracture.

g) Coffee and caffeine predict reduced risk of breast and ovarian cancer and increased risk of colorectal cancer.

h) Salt, alcohol, and calcium intake are predictors of hypertension.

i) Intake of vitamin D and calcium predicts lower risk of CHD and stroke, as well as cancer.

j) Dietary fat and fatty acid intake is related to breast, endometrial and other cancers. Different types of dietary fat may have different effects on the risk of breast and other cancers. Dietary fat may also have an effect on breast cancer survival.

k) Dietary fat and subtypes are related to risk of cardiovascular disease. Trans fatty acids increase risk of CHD and stroke; oleic acid may decrease these risks. Fish and omega-3 FA's predict reduced risks. Dietary fat and subtypes also predict total mortality.

l) Excessive intake of alcohol is associated with decreased bone density and increased risk of fractures.

m) Other dietary factors such as intake of excessive carbonated beverages may reduce bone density and increase fracture incidence, possibly secondary to high phosphoric acid content. Similarly, high levels of phosphate in the diet may predispose to bone loss, possibly as a result of increased parathyroid hormone levels.

n) High protein intake may increase bone loss and fracture due to associated increased calcium excretion.

o) Increased dietary fiber, magnesium, potassium, calcium, and antioxidant vitamins, as well as reduced dietary fat, decrease the occurrence of non-insulin-dependent diabetes mellitus (NIDDM).

p) Frequency of eating alters the risk of colorectal cancer.

2) **Physical Activity**

a) Physical activity independent of adiposity predicts lower risk of CHD and stroke.

b) Physical activity predicts increased bone mineral density and decreased risk of fracture.

c) Regular physical activity reduces the incidence of NIDDM.

d) Physical activity decreases the risk of breast and colorectal cancer.

e) Physical activity decreases total mortality.

3) **Body Habitus**

a) Weight, adipose distribution, weight cycling are predictors of CHD, stroke, and cancer.

b) Height is a predictor of cardiovascular disease and cancer.

c) Weight gain since early adulthood (age 18) is related to breast, endometrial and colorectal cancer, as well as CHD and stroke.

d) Body weight is related to breast cancer survival.

e) Lower weight is related to decreased bone density and osteoporosis-related fractures.

f) Body fat distribution and weight change predict risk of NIDDM.

g) Predictors of weight gain in adulthood include decreased physical activity, increased percentage of energy from fat, weight cycling, and obesity in late adolescence.

h) Blood pressure is associated with waist-hip ratio (WHR) and weight gain.

i) Some determinants of the variance in waist-hip ratio are modifiable (physical activity, dietary fat, smoking, alcohol, hormone therapy). These and other variables can also be assessed as predictors of change in WHI at the three-year visit.

j) Higher birth weight is associated with breast cancer.

4) **Reproductive factors**

a) Reproductive factors including increased age at first birth, lower parity, early age at menarche, late menopause, oligomenorrhea, and infertility may be associated with breast, endometrial, ovarian and colorectal cancer.

b) Reproductive factors including age at menopause and parity predict risk of CHD and stroke.

c) Several reproductive variables including parity and lactation are predictors of bone density and osteoporosis-related fractures.

d) Lactation is associated with decreased risk of breast and other cancers. Having been breast fed as an infant may also predict a reduced risk of breast cancer.

e) Tubal ligation and hysterectomy reduce risk of ovarian cancer.

5) **Medications**

a) Non-steroidal anti-inflammatory drugs (NSAIDs) may prevent CHD and stroke events, colorectal cancer, and may decrease dementia in arthritis patients.

b) Antioxidant drugs may prevent tissue damage when an acute coronary or cerebrovascular occlusion occurs.

c) Multivitamin and mineral supplement use may decrease risk of cancer, CHD, stroke, and osteoporotic fractures.

d) Past oral contraceptive use:

1) may be associated with increased risk of breast cancer and decreased risk of ovarian and endometrial cancer (variables of interest would include duration, age at first use, use before first full-term pregnancy).

2) is not associated with increased risk of CHD and stroke.

3) is a predictor of bone density and osteoporosis-related fractures.

e) Past use of diethylstilbestrol (DES) is associated with increased risk of breast cancer.

f) Hormone replacement therapy (HRT) predicts CHD, stroke, cancer, and fracture risk. Dosage, type, duration, and regimen used can be examined.

g) Higher endogenous estrogen levels is related to benefit from HRT with regard to fracture risk.

h) Medications such as thiazide diuretics are predictors of osteoporosis-related fractures. Also of interest are glucocorticoids, lasix, dilantin and tamoxifen. Further, thyroxine replacement therapy, particularly when associated with suppression of thyroid stimulating hormone, may be a determinant of bone density and fracture risk.

i) Antacids with high levels of calcium are related to fracture risk.

j) Class of antihypertensive medication may modify the risk of CHD and stroke.

k) Cimetidine increases breast cancer risk (via effects on estrogen metabolism).

6) **Smoking**

a) Cigarette smoking is a predictor of reduced bone density and osteoporosis-related fractures.

b) Smoking increases risk of CHD, stroke, diabetes, cataracts, colorectal cancer, disability, and total mortality.

c) Smoking is a risk factor for asthma in postmenopausal women.

d) Exposure to passive smoking is a risk factor for CHD, stroke, cancer and fractures.

7) Pathology

a) Mammographic patterns of dysplasia, as well as benign breast disease histologic subtypes are predictors of breast cancer.

8) **Medical History**

a) History of high cholesterol is related to CHD and stroke events.

b) History of high blood pressure is related to CHD and stroke.

c) History of benign breast disease alters breast cancer risk (depending on histologic subtype).

d) History of polyps is associated with risk of colorectal cancer.

e) History of atrial fibrillation is associated with CHD and cerebrovascular events.

f) Breast implants increase risk of breast cancer.

g) Breast implants increase risk of collagen vascular disorders.

9) **Family History**

a) The magnitude of the increase in risk of cancer, CHD, stroke, and fractures is associated with a positive family history. Also any modifying effect of age at diagnosis in family members can be examined.

10) **Behavioral/Psychosocial/Functional**

a) Participants with greater social support, less depression, or fewer life events, will have fewer chronic diseases, fewer hospitalizations, and lower mortality.

b) Moderators of stress predict recurrence of disease.

c) Physical function measures assessed at baseline (hand grip, chair stands, timed gait) predict risk of osteoporosis/fractures, CHD, stroke, disability, and total mortality.

11) **Environmental/Occupational Exposures**

a) Sun exposure (assessed by residential history) is associated with CHD, stroke, cancer and fracture risk.

b) Organochlorine residues from pesticides increase risk of breast cancer.

c) Talc use predicts ovarian cancer.

d) Electric blankets/waterbed use predicts increased risk of breast and other cancers.

e) Work as a cosmetologist increases risk of breast cancer.

12) **Special Populations**

a) Black women have similar fracture rates to other women, after adjusting for leanness.

b) CHD, stroke, cancer and fracture risks are not geographically-related when adjusted for other risk factors.

c) CHD, stroke, cancer and fracture risks are not ethnically-related when adjusted for other risk factors.

13) **Biological Markers**

"Nested" case-control or case-cohort analyses can be performed to assess prediagnostic blood measurements as predictors of subsequent disease. These hypotheses are summarized in in *Table 1-A5.3 - Biomarker Hypotheses and Plasma/Serum Volume Required*.

a) Endogenous sex hormones (estradiol, estrone, prolactin, progesterone, androgens) are predictors of cardiovascular disease, cancer, and osteoporosis.

1) Serum total estradiol, percent free estradiol, percent bioavailable estradiol, estrone, and estrone sulfate are associated with increased risk of breast cancer and decreased risk of CHD/stroke/fractures.

2) Serum progesterone is associated with increased risk of breast cancer.

3) Androgens such as androstenedione, testosterone, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEA-S) are associated with increased risk of breast cancer and CHD/stroke and decreased risk of fractures.

4) Peptides such as prolactin are associated with increased risk of breast cancer.

b) Plasma lipids (total cholesterol and subfractions, apo B, Lp(a), plasma omega-3 fatty acids, and trans fatty acids) are predictors of cardiovascular disease in postmenopausal women. Lp(a) may predict acute MI, sudden death and stroke. The role of plasma lipids as predictors of cancer and total mortality can also be examined.

c) Insulin has powerful growth-promoting properties and may increase bone density and reduce risk of fracture. Growth hormone secretion, and its consequent metabolic functions, decrease with age and replacement hormone has been used in elders to promote muscle mass and physical function. In addition, the declining production of adrenal steroids dehydroepiandrosterone (DHEA) and 11B-hydroxyandrosterone has been related to lower bone density and offers promise as predictors of bone loss and fracture.

d) Fasting hyperinsulinemia is a predictor of future occurrence of NIDDM in nondiabetic women and of increased risk of CHD/stroke in both nondiabetic women and diabetic women without prior hypoglycemic therapy. Potentially modifiable determinants of fasting hyperinsulinemia, including physical activity level, body mass index, diet composition, postmenopausal hormone therapy, smoking, and other variables, could also be explored. Further, the role of glycemic control (as measured by serum fructosamine) could be examined as a predictor of CHD/stroke events in both nondiabetic and diabetic women.

e) Endogenous estrogen levels are associated with dietary fat intake.

f) Plasma antioxidants (vitamin C, vitamin E, carotenoids, ubiquinol, zinc, selenium) are associated with risk of breast cancer, ovarian cancer, endometrial cancer, colorectal cancer, CHD, and stroke. Levels of antioxidants will be affected by smoking.

g) Plasma retinol and cholecalciferol are associated with reduced risk of breast and other cancers.

h) Blood levels of organochlorine residues are associated with increased breast cancer risk.

i) Hemostatic factors (TPA, PAI, fibrinogen, Factor II) are predictors of CHD and stroke and venous thromboembolic disease. Factor VII levels are associated with levels of Lp(a).

j) Serum levels of 25-hydroxyvitamin D_3 are associated with higher levels of HDL.

k) Other markers such as homocysteine, folate, iron/ferritin, vitamins B6 and B12, calcium, magnesium, anti-cardiolipin antibodies, sialic acid, ceruloplasmin level may be related to CHD and stroke.

14) **Genetic markers**

White blood cell DNA can be used to explore genetic markers for the prediction of cancer, CHD, stroke, diabetes, and osteoporosis.

Examples:

a) Estrogen-receptor gene

b) Vitamin D receptor gene

c) Colorectal cancer genes

d) P53

e) Glycogen synthase gene

Quality of Life Hypotheses

a) The influence of several baseline variables, including physical activity level, diet, body habitus, smoking and co-morbid conditions, can be examined in relation to quality of life in the cohort.

b) Use of hormone replacement therapy can be assessed in relation to quality of life.

c) Participants with greater social support who develop chronic diseases can be assessed in relation to quality of life.

Outcome Research Hypotheses

Functional Outcomes of Chronic Illness

This would require baseline and periodic testing for physical and cognitive functions; simple self-report and performance testing protocols are available. Social variables would include impact on women's employment, insurance availability, social networks and support, care-giving activities, family structure, and personal and family assets. This may be valuable for community-based health and social planning.

Risk Factors for Functional Severity and Impact of Chronic Conditions

The goal here is to determine whether "standard" vascular risk factors (e.g., diabetes, hypertension, smoking habits) and other social and hygienic behaviors predict whether illnesses are fatal vs. non-fatal, and among survivors, predict disease severity in terms of functional impact and use of medical services. This could be done for incident diabetes mellitus, myocardial infarction, stroke, hip and spine fracture and also for various common neoplasms and neurologic illnesses.

Table 1-A5.1
Cumulative Number of Events For 100,000 Women Age 50-79 Years At Baseline

Average Years of Follow-Up	3	6	9
Total Deaths	5,000	11,100	18,200
CHD	1,900	4,200	6,700
CVD	4,000	8,500	13,800
Breast Cancer	1,000	2,000	3,100
Colorectal Cancer	500	1,100	1,900
Composite Fracture	3,300	7,000	11,200
Diabetes	1,500	3,330	5,460

Table 1-A5.2
Summary of Exposure/Disease Hypotheses

	CHD	Stroke	Breast Cancer	Colorectal Cancer	Fractures	Diabetes
Diet	X	X	X	X	X	X
Physical Activity	X	X	X	X	X	X
Body Habitus	X	X	X	X	X	X
Reproductive	X	X	X	X	X	
Medications	X	X	X	X	X	
Smoking	X	X		X	X	X
Pathology	X		X			
Medical History	X	X	X	X		
Family History	X	X	X	X	X	
Behavioral/ Psychosocial	X	X	X	X	X	X
Environmental	X	X	X	X	X	
Special Populations	X	X	X	X	X	X
Biological Markers	X	X	X	X	X	X

Table 1-A5.3
Biomarker Hypotheses and Plasma/Serum Volume Required

	Endpoints					
Biomarkers	Breast Cancer*	Colorectal Cancer	CHD/ Stroke	Fractures	Diabetes	Volume of Plasma/Serum Required
Endogenous estrogen levels (total estradiol,% bioavailable estradiol estrone, estrone-sulfate)	↑		↓	↓		2.5 ml
Endogenous androgens (androstenedione, testosterone, free testos, DHT, DHEA, DHEA-S)	↑		↑	↓		1.0 ml
Prolactin	↑					0.25 ml
Progesterone	↑		↑	↓		0.5 ml
Sex-hormone binding globulin	↓		↓			0.125 ml
Antioxidant vitamins (beta-carotene, other carotenoids, retinol, tocopherols, vitamin C)	↓	↓	↓	↓	↓	1.0 ml
Cholecalciferol	↓	↓	↓	↓		1.0 ml
Organochlorine residues	↑					1.0 ml
Genetic markers	↑ or ↓	↑ or ↓	↑ or ↓	↑ or ↓	↑ or ↓	
Lipids and lipoproteins (cholesterol, LDL, subtypes, HDL-2,HDL-3, VLDL, apolipoproteins)	↑ or ↓		↑ or ↓			0.5 ml
Fatty acids (poly-unsaturated and mono-unsaturated FA's)	↓	↓	↓			0.5 ml
Trans fatty acids			↑			0.5 ml
Marine oils (omega-3 FA's [EPA & DHA])	↓	↓	↓			0.5 ml
Lp(a) and isoforms			↑			0.5 ml
Oxidized LDL			↑			0.5 ml
Saturated FA's	↑	↑	↑		↑	0.5 ml
Homocysteine			↑			0.5 ml
Folate, vitamin B6, vitamin B12	↓	↓	↓			0.5 ml
Selenium, zinc, ubiquinol	↓	↓	↓	↓	↓	1.0 ml
Ferritin	↑	↑	↑		↑	0.5 ml
Calcium, magnesium	↓	↓	↓	↓	↓	0.5 ml
Fasting insulin level	↑	↑	↑	↓	↑	0.5 ml
C-peptide/pro-insulin			↑		↑	1.0 ml
Fibrinogen			↑			0.5 ml

Table 1-A5.3 (Continued)

	Endpoints (continued)					
Biomarkers	Breast Cancer*	Colorectal Cancer	CHD/ Stroke	Fractures	Diabetes	Volume of Plasma/Serum Required
Tissue plasminogen activator (TPA) and PAI-1			↑			0.5 ml
Factors II and VII			↑			0.5 ml
Anticardiolipin antibodies			↑			0.5 ml
Serum fructosamine			↑		↑	0.5 ml
Ceruloplasmin			↑			0.5 ml
C-reactive protein			↑			0.5 ml
Sialic acid			↑			0.5 ml
Chlamydia antibody titer			↑			0.5 ml
Herpes Simplex Virus Types 1 and 2 antibody			↑			0.5 ml
Cytomegalovirus antibody titer			↑			0.5 ml
Thyroid stimulating hormone (TSH)			↑ or ↓	↑		0.5 ml
Parathyroid hormone (PTH)				↑		0.2 ml
Bone-specific alkaline phosphatase (BsAP)				↑		0.2 ml
Osteocalcin				↑		0.2 ml
IGF - 1 IGF - BP3, and IGF II				↓		0.25 ml

* The above hypotheses can also be tested for endometrial and ovarian cancer.

Section 1-A5
Protocol Appendix 5
Women's Health Initiative Observational Study
Overview of Objectives and Hypotheses

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