Diet, Lifestyle, Biomarkers, Genetic Factors, and Risk of Cardiovascular Disease in the Nurses' Health Studies

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Objectives. To review the contributions of the Nurses' Health Studies (NHSs) to the understanding of cardiovascular disease etiology in women.

Methods. We performed a narrative review of the publications of the NHS and NHS II between 1976 and 2016.

Results. Diets low in trans fat, saturated fat, refined carbohydrates, and sugar-sweetened beverages and rich in fruits and vegetables, whole grains, and sources of unsaturated fats are associated with reduced risk of cardiovascular disease. Healthy lifestyle choices include smoking avoidance, regular physical activity, maintaining a normal body mass index, and moderate alcohol consumption. Adherence to a combination of these healthy diet and lifestyle behaviors may prevent most vascular events. Studies also covered oral contraceptive use, postmenopausal hormone therapy, shift work, sleep duration, psychosocial factors, and various biomarkers and genetic factors. Findings, such as the association of trans fat with cardiovascular disease, have helped shaped medical guidelines and government policies.

Conclusions. The NHS has provided compelling evidence that the majority of vascular events may be prevented by avoiding smoking, participating in regular physical activity, maintaining normal body mass index, and eating a healthy diet. (*Am J Public Health.* 2016; 106:1616–1623. doi:10.2105/AJPH.2016.303316)

Cardiovascular disease (CVD) has remained the leading cause of death in the United States for more than 8 decades.[1] Since their inceptions in 1976 and 1989, the Nurses' Health Studies (NHS) I and II, respectively, have contributed much new knowledge with the goal of reducing CVD incidence and mortality. A search on PubMed for the "Nurses' Health Study," "cardiovascular disease," "myocardial infarction," "heart disease," and "stroke" yields more than 300 primary articles. Many more have been published related to the risk factors of CVD, such as hypertension.

The breadth of exposures examined is great: dietary variables include macronutrients, micronutrients, nonnutrient dietary constituents, foods, beverages, and dietary patterns; lifestyle and other factors such as smoking, physical activity, adiposity, body fat distribution, sleep-related and psychosocial exposures characterize earlier studies from the NHS. The NHS research on exogenous hormone use, including oral contraceptives and postmenopausal hormone therapy, is addressed in Bhupathiratju et al. in this issue. More recently, biomarkers in plasma and red blood cells, as well as genetic factors, have been investigated. These articles have been included in many systematic reviews and have greatly contributed to clinical guidelines, public health campaigns, and government policies.

DIETARY FACTORS

Table 1 details the major findings of dietary factors and CVD in the NHS.

CVD was once thought to be an inevitable consequence of aging and related risk factors,[2] but evidence from cross-country comparisons by Keys suggested that diet was an important determinant of CVD risk.[3] However, this ecological analysis was limited by intractable confounding and limited dietary data. A theoretical framework for modern nutritional epidemiology research initially arose largely out of work from the NHS, and these techniques have become the basis for a large body of research relating diet to CVD.[4] See Hu et al. in this issue (p1567) for additional discussion on the development of these techniques.

Macro- and Micronutrients

Initial dietary advice for the prevention of coronary heart disease (CHD), largely on the basis of small controlled feeding studies with serum cholesterol as the outcome, emphasized replacing saturated fat with polyunsaturated fat.[4] It is likely that the early decline in CHD mortality in the United States was largely owing to the resulting replacement of saturated fat with polyunsaturated fat. However, beginning in the early 1980s, dietary guidelines emphasized that Americans should limit their total fat intake, including both saturated and unsaturated fats. Today, we have a clearer picture of the role of types

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TABLE 1—Major Findings From the Nurses' Health Studies Regarding Diet and Cardiovascular Disease: United States, 1976–2016

Exposure	Year of First Publication	General Findings and Associations
Trans fat	1993	Higher risk of CHD, independent of other dietary factors
Saturated fat	1997	Higher risk compared with unsaturated fats and whole grains
Unsaturated fat	1997	Lower risk with PUFAs and MUFAs compared with SFAs
Carbohydrates and glycemic load	1999	Total carbohydrates were not associated with risk, but higher glycemic load was associated with higher risk of CHD and ischemic stroke, especially among overweight women
Alcohol	1988	Lower risk with moderate consumption (about 1 drink/d)
Coffee	1996	Modest reduction in risk
Sugar-sweetened beverages	2009	Significantly higher risk
Nuts and legumes	1998	Lower risk
Eggs	1999	Higher risk of CHD in patients with diabetes; no association in those without diabetes
Whole grains	1999	Modestly lower risk
Fruits and vegetables	2001	Lower risk
Red meat	2007	Higher risk, especially compared with other protein sources
Dairy	2007	No or higher risk
Dietary patterns	2001	Reduced risk with adherence to Mediterranean dietary pattern, DASH, or AHEI; higher risk with Western dietary pattern
Folate and vitamin B_6	1998	Lower risk of CHD, especially among alcohol consumers
Vitamin C	2003	Lower risk of CHD, but only from supplement sources
Vitamin E	1993	Lower risk of CHD, but mostly from supplement sources
Calcium	1999	No association with CHD, but lower for stroke, particularly when from dairy sources
Potassium	1999	Modestly lower risk of stroke
Magnesium	1999	Lower risk of SCD; no association with stroke
Carotenoids	2003	Lower risk of CHD
Flavonoids	2007	Flavanones, anthocyanidins, and certain foods associated with lower risk of CHD but not stroke mortality

Note. AHEI = Alternative Healthy Eating Index; CHD = coronary heart disease; DASH = Dietary Approaches to Stop Hypertension; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SCD = sudden cardiac death; SFA = saturated fatty acids.

of fats and carbohydrates and their relationships to disease risk.

Although ideally these questions would be settled by large randomized trials, problems, such as high rates of attrition, nonadherence, incomplete blinding, short follow-up time, and unethicality of studying harmful interventions, have largely prevented such trials from yielding informative results.[4] Conversely, prospective cohort studies allow the study of a wide range of exposures in free-living populations. Thus, a combination of replicated long-term prospective studies, such as the NHS, combined with controlled feeding studies with intermediate risk factors as outcomes, will usually provide the best evidence for causal effects of dietary factors.

One of the major achievements of nutritional epidemiology research is the near elimination of industrial trans-fatty acids from diets of the United States and many other countries. In 1993, the first study of trans-fatty acids in a large prospective cohort showed that intake was strongly associated with CHD risk,[5] and this was confirmed in subsequent analyses in the NHS and other cohorts.[6,7] With findings that trans-fatty acids are even worse than are saturated fatty acids because they lower high-density lipoproteins (HDL) and elevate low-density lipoproteins,[8] the US Department of Agriculture Dietary Guidelines in 2000 recommended a reduction in trans fat intake,[9] which has been emphasized in subsequent dietary guidelines in the United States and elsewhere.

The role of saturated fatty acid intake in the etiology of CHD has been a point of contention, with recent meta-analyses concluding that saturated fatty acid intake has no role in CHD risk. The most important reason for the null results is that contributing studies typically did not specify the reference macronutrient in an isocaloric model. Because about half of total energy in most Western diets consists of carbohydrates (mostly refined starch and sugar), saturated fat was compared mainly with these unhealthy forms of carbohydrates by default. In a recent analysis, the intake of saturated fat was compared with the same number of calories from unsaturated fats and from different sources of carbohydrates in relation to CHD risk in the NHS and the Health Professionals' Follow-up Study (HPFS).[10] Replacing saturated fat with equivalent energy from polyunsaturated fats, monounsaturated fats, or carbohydrates from whole grains was associated with a significant reduction in CHD risk, whereas replacing saturated fat with carbohydrates from refined starches or added sugars was not associated with CHD risk. This finding, together with findings on the effects of these fats on blood lipids, indicates that unsaturated fats, especially polyunsaturated fats, or high-quality carbohydrates can be used to replace saturated fats to reduce CHD risk. However, as reported in an earlier pooled analysis of cohort studies, replacing saturated fat with overall carbohydrates is not associated with a lower risk of CHD.

Traditionally, carbohydrates have been classified as simple or complex on the basis of chemical structures. However, many complex carbohydrates or starchy foods, such as baked potatoes, corn, and white bread, produce even higher glycemic responses

than do simple sugars. Thus, the concept of glycemic index was introduced to represent the quality of carbohydrate-containing foods on the basis of their ability to raise postprandial blood sugar. Glycemic load, the product of the glycemic index value of a food and its carbohydrate content, first described in an NHS report, has been developed to represent the quality and quantity of carbohydrates consumed.[11] In the NHS, a higher dietary glycemic load was associated with an elevated risk of CHD and ischemic stroke, possibly via an HDL cholesterol lowering and fasting triacylglycerol-raising effect of high glycemic load. The increased risk was more pronounced among overweight and obese women, suggesting that the adverse effects of a high glycemic load diet are exacerbated by underlying insulin resistance. A diet rich in fiber, especially cereal fiber, was associated with a lower CHD risk in the NHS.[12]

In addition to macronutrients, various micronutrients have been the subject of investigation in the NHS. Both vitamin E[13] and vitamin C[14] intake, mainly from supplementation sources, were associated with reduced incidence of CHD in mostly healthy participants. However, these results have not been replicated in randomized trials of high-risk patients, perhaps because of the dissimilarity of participants with regard to baseline risk and because most primary prevention trials had shorter durations of exposure and follow-up times. Higher folate and vitamin B_6 intake was associated with lower risk of CHD, and the inverse association was particularly strong among women who regularly drank alcohol. Calcium was reported to predict lower rates of stroke but not CHD.

Conversely, 2 separate analyses indicated that dietary magnesium intake was associated with lower rates of sudden cardiac death but not stroke or CHD, although plasma magnesium appeared to be inversely associated with stroke. Finally, studies of plant-derived compounds found that both carotenoid[15] and flavonoid[16] intake was associated with lower CVD risk. Specifically, flavonones (derived primarily from citrus fruits) and anthocyanidins (derived primarily from berries) showed the strongest inverse associations with CHD mortality, although no significant relationships were observed with stroke mortality.[16]

Foods and Beverages

Before the NHS, analyses of the association of individual foods and beverages with the risk of CVD were limited. The gradual shift from small case–control studies to large prospective cohort studies using validated food frequency questionnaires allowed these scientific questions to be investigated with less concern about reverse causation, selection bias, and recall bias.

One of the early reports from the NHS was published in 1988; it showed that moderate alcohol intake was related to a reduced risk of stroke.[17] Follow-up articles confirmed inverse associations of alcohol intake with CHD and ischemic stroke, and more recent publications corroborate the relationship between light to moderate alcohol intake and risk of myocardial infarction, sudden cardiac death, and hypertension.[18] Using a Mendelian randomization approach, studies of variants of alcohol dehydrogenase and cholesteryl ester transfer protein indicate that genetically associated moderate alcohol intake was related to higher HDL levels and a substantially decreased risk of CVD, providing causal evidence for this relationship. See Mostofsky et al. in this issue for additional discussion on research involving alcohol.

Results from the NHS have provided strong evidence to counter earlier concerns that coffee consumption may increase the risk of CHD. A meta-analysis of case–control and cohort studies in 1994 suggested a moderate increase in risk when drinking 5 cups versus none, whereas a publication from the NHS found a null association.[19] The picture became clearer by the late 2000s, with newer studies indicating no increased risk of CHD with regular consumption of coffee and even a modest reduction in stroke risk. An updated analysis of the NHS found that moderate coffee consumption (3–5 cups per day) is associated with a significantly lower risk of total and CVD mortality.[20] Earlier findings linking coffee to increased CVD risk may be owing to recall bias, which is common in case–control studies, and confounding by smoking.

The consumption of sugar-sweetened beverages has been implicated in weight gain and type 2 diabetes in the NHS,[21] and recent studies have found a deleterious effect of sugar-sweetened beverages on heart health. Two articles found positive relationships of sugar-sweetened beverage intake with CHD and stroke. These reports add to the growing body of evidence that sugar-sweetened beverage consumption is an important driver of the obesity epidemic and its cardiometabolic complications, laying the foundation for current public health recommendations and policies to reduce sugar-sweetened beverages.

The NHS group has examined other individual foods in relation to CVD. For example, regular consumption of nuts was associated with lower CHD incidence and CVD mortality[22]; egg consumption was not associated with risk except in those with diabetes, signifying that dietary cholesterol in and of itself may not predict disease incidence; and increased whole grain consumption was associated with a lower risk of CVD. In addition, higher consumption of fruits and vegetables was associated with a lower risk of CVD incidence and mortality.[23] An updated analysis in NHS and HPFS found that the total amount of fruits and vegetables consumed was a more important determinant of CHD than was the variety of fruits and vegetables. Plausible biological mediators include potassium, which reduces blood pressure, and phytochemicals such as carotenoids[15] and flavonoids,[16] which have both been linked to lower CHD risk in the NHS.

Red meat has been a recent target of investigation. Four articles from the NHS point to heightened risks of CHD incidence and CVD mortality as well as unfavorable plasma concentrations of inflammatory biomarkers with a higher consumption of red meat, especially processed meat, than of other major protein sources, such as poultry, fish, nuts, and legumes. The high levels of both saturated fatty acids and heme iron in red meats have been implicated in this relationship. Dairy intake has been inconsistently associated with CVD outcomes, depending on the types of dairy and the types of CVD endpoints,[24] and additional investigation is needed.

Dietary patterns, specifically the Mediterranean diet, have garnered significant interest in the scientific community. In the NHS, a significant inverse association was observed between adherence to a Mediterranean diet pattern and the risk of CHD and stroke.[25] In addition, a study using principal component analysis reported that

a Western diet, which is characterized by a high intake of processed meats, refined grains, and French fries, was independently associated with a higher risk of CHD, whereas adherence to the prudent diet characterized by a high intake of fruits, vegetables, legumes, fish, poultry, and whole grains was related to a lower risk.[26]

Similarly, adherence to a Dietary Approaches to Stop Hypertension diet predicted lower CHD and stroke incidence. Finally, the NHS group developed the Alternative Healthy Eating Index-2010, which is a tool grounded on 11 of the most important components of a healthy diet within the cohort. This new index, designed to overcome the limitations of the US Dietary Guidelines for Americans, was associated with substantially lower CVD incidence and mortality in the NHS, the HPFS, the NIH-AARP Diet and Health Study, the Whitehall cohort, and the Women's Health Initiative.

NONDIETARY LIFESTYLE FACTORS

Table 2 details the major findings for nondietary lifestyle factors and the risk of CVD in the NHS.

Aspects of lifestyle in addition to diet have been examined in the NHS in relation to risk of CVD. Although perhaps surprising to some readers, the relationship between smoking and CHD in women was a matter of controversy in the early 1970s. This question was the subject of an early publication from the NHS in 1987,[27] which found a clear dose–response relationship between the number of cigarettes smoked daily and the risk of nonfatal myocardial infarction and fatal CHD. A follow-up article showed a similar positive relationship with stroke and provided convincing evidence that smoking was associated with CVD in women. Later analyses concluded that smoking cessation was associated with a lower risk of stroke and CHD and that passive smoking was related to CHD. Although smoking cessation is reported to be associated with weight gain, the net effect is in favor of cessation to lower CVD risk.

The data from the NHS have demonstrated the critical role of physical activity in preventing CVD. In middle-aged and older women, even moderate intensity physical activity such as brisk walking was associated with a lower risk of CHD.[28] Likewise, higher physical activity levels were associated with a lower risk of total and ischemic stroke. A brisk or striding walking pace was related to a lower risk of both CHD and stroke compared with an average or casual pace.

The range of optimal body weight has been a long-standing subject of NHS investigations, in part because of the 1990 Dietary Guideline conclusion that optimal body weight increased with age and was above a body mass index (BMI; defined as weight in kilograms divided by the square of height in meters) of 25.0 for those older than 35 years. In a 1990 report, BMI was monotonically related to a higher incidence of nonfatal and fatal CHD.[29] At the time, obesity was not an established risk factor for CHD despite strong links with diabetes, hypertension, and dyslipidemia. An analysis conducted in response to the 1990 US weight guidelines concluded that the positive association existed even within the recommended "normal" (at the time): a BMI range of 21.0 to 27.0.[2,30] This study helped shift the definition of a normal BMI range to the 18.5–24.9 we know today.

Later studies linked elevated BMI with stroke and high blood pressure[31] and increased abdominal adiposity with CHD. An article in 2001 warned against being overweight or even in the upper end of the

TABLE 2—Major Findings From the Nurses' Health Studies Regarding Lifestyle and Cardiovascular Disease: United States, 1976–2016

Exposure	Year of First Publication	General Findings and Associations
Smoking	1981	Higher risk with active and passive smoking; lower risk with smoking cessation
Physical activity	1999	Lower risk with moderate intensity activity, such as brisk walking
BMI and fat distribution	1990	Risk increases monotonically with BMI and fat distribution as measured by waist circumference or waist to height ratio; moderate weight gain since young adulthood increases risk
Shift work	1995	Higher risk of CVD and CVD mortality with longer time doing shift work
Sleep-related exposures	2000	Reductions in HDL and elevations in CRP associated with long or short sleep durations; snoring associated with higher risk of CVD
Phobic anxiety	2005	Higher risk of CHD and SCD
Caregiving	2003	Higher risk of CHD
Job strain	2002	No association with CHD
Job insecurity	2004	Higher risk of short-term MI
Depressive symptoms	2009	Higher risk of CHD and SCD
Oral contraceptives	1980	Higher risk of CHD and MI with current use
Postmenopausal hormone use	1981	Lower risk of CHD in women initiating hormone therapy in early, but not late, menopause; higher risk of stroke in all age groups
Aspirin use	1999	1–6 aspirin/wk associated with lower risk of stroke but >15 may increase risk

Note. BMI = body mass index; CHD = coronary heart disease; CRP = C-reactive protein; CVD = cardiovascular disease; HDL = high-density lipoprotein; MI = myocardial infarction; SCD = sudden cardiac death.

new normal weight range, because this was linked with a significantly increased risk of total CVD and stroke. In 2008, the first analysis on waist circumference implicated high waist circumference in excess CVD mortality.[32] Several analyses from the NHS showed that even moderate weight gain since young adulthood (aged 18 years) was associated with a subsequent risk of CHD incidence and CVD mortality.[30] In addition, being physically active did not completely mitigate the deleterious effects of being overweight or obese on CVD risk. Furthermore, obesity and physical inactivity independently contributed to CHD risk in the NHS, underscoring the importance of both maintaining a healthy weight and engaging in regular physical activity in preventing CHD.[33]

The complex relationship between exogenous hormone use (oral contraceptives and postmenopausal hormone therapy) and CVD outcomes has been extensively studied in the NHS and NHS II. These findings are discussed in detail in Bhupathiraju et al. in this issue (p1631).

The nature of the nursing occupation has allowed our researchers to conduct unique analyses regarding sleep and shift work. In 1995, we conducted the largest investigation of shift work and CVD and found that performing shift work for 6 or more years was associated with a 51% increased risk of CHD.[34] This conclusion was confirmed for stroke. With regard to sleep duration, findings point to a U-shaped relationship, with an optimal length of 8 hours of sleep a day. Additionally, snoring was associated with a modest but significantly increased risk of CVD in women, independent of age, smoking, BMI, and other cardiovascular risk factors. Later analyses demonstrated that longer sleep duration was associated with higher concentrations of circulating C-reactive protein and that a short or longer sleep duration was associated with lower levels of HDL, providing plausible biological mechanisms for these relationships.

With respect to psychosocial factors, high levels of phobic anxiety were associated with an increased risk of fatal CHD and sudden cardiac death, perhaps owing to elevated levels of leptin and inflammatory markers. In 1992, participants were asked about caregiving, employment characteristics, and depressive symptoms. High levels of caregiving responsibilities and depression but not job strain were associated with higher CHD incidence.

Dietary and lifestyle factors together have a more powerful effect on CVD risk than does any single factor alone. In the NHS, the incidence of CHD was 80% lower among women who did not smoke, were not overweight, maintained a healthful diet (high in cereal fiber, fish, folate, and polyunsaturated fats and low in saturated fatty acids, trans-fatty acids, and glycemic load), exercised moderately or vigorously for 30 minutes on most days, and consumed alcohol moderately (half a drink per day or more) compared with the rest of the cohort.[35]

Similar findings were recently reported for younger women in the NHS II.[36]

TABLE 3—Major Findings From the Nurses' Health Studies for Biomarkers and Cardiovascular Disease: United States, 1976–2016

Biomarker	Year	General Findings and Associations
CRP, IL-6, TNFR I and II	2004	Higher risk of CHD
HDL- and HDL-related ratios	2004	Lower risk of CHD
Homocysteine	2004	Higher risk of CHD
Lipoprotein(a)	2004	Higher risk of CHD among diabetes patients
oxLDL	2006	No association with CHD after adjustment for lipid markers
15:0 and *trans* 16:1n–7	2007	Markers of dairy intake associated with higher risk of ischemic heart disease
Long chain *n*-3 fatty acids	2008	Lower risk of nonfatal MI
DHEAS	2008/2013	Increases risk of MI but lowers risk of stroke
IGF-1 and IGFBP-3	2008	No association with MI
sTfR:ferritin ratio and ferritin	2008	No association with CHD
Vitamin B_6	2009	Lower risk of MI
Toenail nicotine	2008	Higher risk of CHD even after adjustment for smoking
Placental growth factor	2009	Higher risk of CHD
NT-proBNP	2009	Higher risk of SCD
Lp-PLA2	2011	Higher risk of CHD
Adiponectin	2011	Lower risk of CHD
Magnesium	2011/2014	Lower risk of SCD and stroke
Toenail mercury	2011	No association with any CVD subtype
25-hydroxyvitamin D	2012	Lower risk of stroke
ApoC-III subtypes of HDL	2012	HDL with ApoC-III: higher risk of CHD; HDL without ApoC-III: lower risk
Hemoglobin A1c	2013	Higher risk of CHD
OxPL/apoB	2013	Higher risk of peripheral artery disease
Retinol-binding protein 4	2013	Higher risk of CHD
Telomere length	2013	No association with ischemic stroke
Fetuin-A	2014	No association with ischemic stroke
15:0 and *trans* 16:1n–7	2014	Markers of dairy intake were not associated with stroke
Very long chain saturated fats	2015	Lower risk of CHD

Note. CHD = coronary heart disease; CRP = C-reactive protein; CVD = cardiovascular disease; DHEAS = dehydroepiandrosterone sulfate; HDL = high-density lipoprotein; IGF-1 = insulin-like growth factor 1; IGFBP-3 = insulin-like growth factor binding protein 3; IL-6 = interleukin-6; Lp-PLA2 = lipoprotein-associated phospholipase A2; MI = myocardial infarction; NT-proBNP = *N*-terminal pro-B-type natriuretic peptide; oxLDL = oxidized low-density lipoprotein; OxPL/apoB = oxidized phospholipids on apolipoprotein B; sTfR = soluble transferrin receptor; TNFR = tumor necrosis factor receptor.

A healthy diet and lifestyle pattern was also associated with a 54% lower risk of ischemic stroke, a 40% lower risk of total stroke,[37] and an 81% lower risk of sudden cardiac death.[38] These data indicate that diet and lifestyle modification could prevent most CVD events. An online risk calculator was developed using these results to help individuals track their own risk and target those areas that need the greatest improvement.

BIOMARKERS AND GENETIC FACTORS

Tables 3 and 4, respectively, detail the major findings of biomarker and genetic studies and CVD in the NHS.

In additional to general diet and lifestyle research, biomarker and genetic data from the NHS has helped enrich the fields of clinical, genetic, and basic biological research regarding CVD. The first NHS analyses on biomarker data in relation to CVD risk linked inflammatory markers, such as C-reactive protein, interleukin-6, and tumor necrosis factor receptors I and II to CHD incidence and found that these markers were predictive of coronary events.[39,40] In addition, low levels of HDL and high levels of homocysteine and lipoprotein(a) were associated with elevated CHD risk. Also, higher levels of plasma long chain *n*-3 fatty acids were associated with a lower risk of nonfatal myocardial infarction. Dehydroepiandrosterone sulfate, a precursor to androgen and estrogen, was associated with a heightened risk of myocardial infarction but a lower risk of stroke, warranting further investigation.

Plasma insulin-like growth factor 1 and binding protein 3 were not related to myocardial infarction risk, although the results were limited by low statistical power. Furthermore, fasting levels of plasma vitamin B_6 were inversely related to myocardial infarction incidence. Other analyses concluded that toenail nicotine, placental growth factor, *N*-terminal pro-B-type natriuretic peptide, lipoprotein-associated phospholipase A2, HDL with apoC-III, hemoglobin A1c, oxidized phospholipids on apolipoprotein B, and retinol-binding protein 4 were associated with an elevated risk of CVD; greater plasma high molecular weight adiponectin, magnesium,[41,42] and 25-hydroxyvitamin D were associated with a lower CVD risk; dairy biomarkers, oxidized low-density lipoprotein, ferritin, toenail mercury, telomere length, and fetuin-A had null relationships with CVD risk.

TABLE 4—Major Findings From the Nurses' Health Studies for Genetic Studies and Cardiovascular Disease: United States, 1976–2016

Gene	Year	General Findings and Associations
Parental history of MI	1986	Higher risk of CHD
ADH3	2001	Moderate alcohol intake associated with lower risk of MI
CCR2 and CCR5	2005	Significantly predictive of CRP levels
PPARG2	2005	No association with CHD
Adiponectin	2006	Significantly predictive of adiponectin levels and CVD risk in women with diabetes
aP2	2006	Significantly predictive of CHD
Lymphotoxin-α	2007	No association with CHD
Complement factor H	2007	Significantly predictive of CHD
ABCA1	2007	Significantly predictive of CHD
CETP	2007	Significantly predictive of CHD
CRP	2008	Significantly predictive of CRP levels but not CHD risk
Cardiac sodium channels	2008	Significantly predictive of SCD
Endothelial lipase	2009	No association with CHD
Lipoprotein lipase	2009	Significantly predictive of CHD
Common variant at 9p21	2009	Significantly predictive of SCD
Genetic risk score for BMI	2010	Higher risk of CVD in women with diabetes
CHD susceptibility loci	2011	More than a 2-fold risk difference in CHD for high vs low genetic risk score in women with diabetes
NFκB1	2011	Significantly predictive of CHD
ADRB1	2011	Integration of GWAS data with protein–protein interaction data are more powerful than is single-gene genome-wide association analysis
Blood type	2012	Those with type O have lower risk of CHD than do other groups
Haptoglobin	2013/2015	Significantly predictive of CHD, but only among those with HbA1c ≥ 6.5%
GWAS	2013	A variant for glutamate metabolism; significantly predictive of CHD, but only among patients with diabetes
GWAS	2013	A systems biology approach successfully identifies loci associated with HDL, LDL, apoB, and triglycerides

Note. ABCA1 = ATP-binding cassette transporter 1; ADH3 = alcohol dehydrogenase 3; ADRB1 = adrenergic receptor beta 1; aP2 = fatty acid-binding protein; apoB = apolipoprotein B; BMI = body mass index; CCR = C-C chemokine receptor; CETP = cholesteryl ester transfer protein; CHD = coronary heart disease; CRP = C-reactive protein; CVD = cardiovascular disease; GWAS = genome-wide association study; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MI = myocardial infarction; NFKB1 = nuclear factor κ-light-chain-enhancer of activated B cells 1; *PPARG2* = peroxisome proliferator activated receptor γ 2; SCD = sudden cardiac death.

In the NHS, parental history of myocardial infarction was associated with an increased risk of CHD.[43] Within the NHS, the first investigation of a single gene and CVD risk was for alcohol dehydrogenase, as mentioned by Hines et al.[44] Later candidate gene or gene score analyses characterized CVD risks for variants in the genes for adiponectin, aP2, CCR2 and CCR5,

PPARG2, lymphotoxin-α, complement factor H, ABCA1, C-reactive protein, cardiac sodium channels, endothelial lipase, lipoprotein lipase, a common variant at chromosome 9p21, a genetic risk score for BMI, several CHD susceptibility loci, NFKB1, ADRB1, blood type, and haptoglobin.

Using a genome-wide association study in the NHS, a variant for glutamate metabolism was found to predict CHD incidence only among patients with diabetes. A genome-wide association study analysis from the NHS and HPFS demonstrated that an integrative systems biology approach could successfully replicate previous findings of loci associated with low-density lipoproteins, HDL, apoB, and triglycerides.[45] Gene–environment interactions have also been explored, with the finding that a polymorphism in the CETP gene modifies the effect of alcohol on HDL cholesterol. Altogether, these publications add to previous reports by allowing further insight into the biochemical and genetic basis of CVD.

CONCLUSIONS

Compelling evidence from the NHS suggests that the incidence of CVD is strongly influenced by dietary and lifestyle factors. Robust data from the cohort have identified unhealthy diet, smoking, obesity, physical inactivity, and unhealthy sleep patterns as important determinants of CVD in women. These results have been confirmed in men and in other cohorts. Consistent with results from dietary intervention studies, the NHS findings support the notion that the types of fats and carbohydrates are more important than the total amounts in determining the risk of CVD. Data from the NHS provide strong evidence that dietary patterns rich in fruits, vegetables, whole grains, nuts, and seafood and low in red and processed meats, sugar-sweetened beverages, and refined grains reduce the risk of CVD. The NHS data indicate that approximately 80% of CHD incidence could be prevented by avoiding smoking, consuming a healthful diet, engaging in moderate to vigorous physical activity for at least 30 minutes most days, and consuming alcohol moderately (half a drink to 1 drink per day).

The large volume of evidence generated from the NHS in the past 4 decades has contributed not just to science but also to public health recommendations and polices in developing guidelines regarding diet, obesity, and physical activity (see Hu et al. [p1567] and Colditz et al. [p1540] in this issue). As we continue to investigate various aspects of diet, lifestyle, and environmental exposures, we have unique opportunities to examine novel biomarkers, genetics, gene–environment interactions, epigenetics, metabolomics, and the gut microbiome in relation to the risk of CVD. These new investigations will afford us additional insight to elucidate CVD pathways and to develop innovative approaches to reduce vascular risks. AJPH

CONTRIBUTORS

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Institutional review board approval was not needed for this work because no human participants were involved.

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EDITOR'S NOTE

Because of space restrictions and the large volume of references relevant to the Nurses' Health Study, additional references are provided in a supplement to the online version of this article at http://www.ajph.org.