Published in final edited form as:
Epidemiology. 2011 March ; 22(2): 170–179. doi:10.1097/EDE.0b013e31820839bc.

Biomarker-Calibrated Energy and Protein Consumption and Cardiovascular Disease Risk Among Postmenopausal Women

Ross L. Prentice[1], **Ying Huang**[1,2], **Lewis H. Kuller**[3], **Lesley F. Tinker**[1], **Linda Van Horn**[4], **Marcia L. Stefanick**[5], **Gloria Sarto**[6], **Judith Ockene**[7], and **Karen C. Johnson**[8]

[1] Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA

[2] Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY

[3] Department of Medicine, University of Pittsburgh, Pittsburgh, PA

[4] Department of Medicine, Northwestern University, Chicago, IL

[5] Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, CA

[6] Department of Obstetrics and Gynecology, University of Wisconsin, Madison, WI

[7] Division of Preventive & Behavioral Medicine, University of Massachusetts Medical School, Worcester, MA

[8] Department of Preventive Medicine, University of Tennessee, Memphis, TN

Abstract

Background—Nutritional epidemiology cohort studies primarily use food frequency questionnaires (FFQs). In part because FFQs are more reliable for nutrient densities than for absolute nutrient consumption, reports from association studies typically present only nutrient density measures in relation to disease risk.

Methods—We used objective biomarkers to correct FFQ assessments for measurement error, and examined absolute energy and protein consumption in relation to cardiovascular disease incidence. FFQs and subsequent physician-adjudicated cardiovascular disease incidence were assessed for 80,370 postmenopausal women in the age range 50–79 years at enrollment in the comparison group of the Dietary Modification Trial or the prospective Observational Study in the Women's Health Initiative. Urinary recovery biomarkers of energy and protein were obtained from a subsample of 544 women, with concurrent FFQ information.

Results—Following biomarker correction, energy consumption was positively associated with coronary heart disease incidence (hazard ratio = 1.18 [95% confidence interval = 1.04–1.33], for 20% consumption increment) and protein density was inversely associated (0.85 [0.75–0.97]). The positive energy association appeared to be mediated by body fat accumulation. Ischemic stroke incidence was inversely associated with energy and protein consumption, but not with protein density.

Address for correspondence: Ross L. Prentice, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, P.O. Box 19024, Seattle, WA 98109-1024; phone: 206-667-4264; fax: 206-667-4142; rprentic@fhcrc.org.

SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.epidem.com).

Conclusions—A positive association between energy and coronary heart disease risk can be attributed to body mass accumulation. Ischemic stroke risk is inversely associated with energy and protein consumption, possibly due to correlations between consumption and physical activity.

The food frequency questionnaire (FFQ) has been ubiquitous in nutritional epidemiology research for the past 25 years. Its self-administered and machine-readable features make it practical for application to large study cohorts. An early report comparing FFQ consumption estimates to 28 days of food records showed moderate to high correlations for calorie-adjusted nutrient consumption, but generally weak correlations for absolute consumption estimates.[1] More recent evaluations of FFQ measurement properties have, instead, made comparisons with urinary recovery biomarkers.[2] Recovery biomarkers have measurement errors that are plausibly unrelated to corresponding self-report measurement errors or to study subject characteristics such as body mass index (BMI), age, and ethnicity. This measurement-error independence is crucial to the adequacy of measurement-error correction procedures. The National Cancer Institute's OPEN study (Observing Protein & Energy Nutrition) reported[3, 4] a correlation of only 0.098 between log-transformed FFQ and log-transformed biomarker energy consumption as determined using a doubly-labeled water procedure.[5] Utilizing a urinary nitrogen biomarker[6] for protein assessment, the corresponding correlations were 0.298 for protein and 0.346 for protein density (percent of energy from protein). Energy was found to be underreported overall, and there was greater underreporting among overweight and obese persons.

We conducted a similar Nutrient Biomarker Study among 544 postmenopausal women enrolled in the Women's Health Initiative (WHI) Dietary Modification Trial during 2004–2005.[7] In addition to overall energy underreporting, we found various sources of systematic bias in the FFQ energy and protein assessments. Groups who underreported to a greater extent included women with higher body mass index, younger women, and racial/ethnic minorities.[8]

By simple linear regression of log-transformed biomarker values on log-FFQ assessments and other subject characteristics, we developed calibration equations that yield "calibrated" consumption estimates thatinclude corrections for these systematic biases) for energy, protein, and protein density.[8] The FFQ assessments in conjunction with data on study subject characteristics explained a substantial fraction of the variation among women in the log-biomarker measurements, supporting study of the association between calibrated consumption estimates and clinical outcomes of interest.

There are few epidemiologic data relating energy consumption to cardiovascular disease risk. The joint WHO/FAQ expert consultation that summarized the world literature on diet, nutrition, and the prevention of chronic diseases[9] does not list energy consumption among the factors that are convincingly, probably, or possibly associated with cardiovascular disease risk. Rather, overweight is described as convincingly associated with increased risk, and regular physical activity is convincingly associated with reduced risk. Similarly, an expert panel reviewing the pertinent literature on food, nutrition, and the prevention of cancer writes[10] that, "In the view of the panel, the effect of energy intake on cancer is best assessed by examining the data on related factors: rate of growth, body mass, and physical activity." Hence, it seems that energy consumption per se has not been carefully studied in relation to cardiovascular disease risk, presumably because of uncertainties concerning self-reported energy consumption estimates. Note that log-transformed FFQ energy was not clearly associated with body mass index in our Nutrient Biomarker Study (correlation 0.07), whereas corresponding biomarker-derived log-energy consumption was strongly correlated with BMI (correlation 0.81).[8] Reliable information on energy consumption and such major cardiovascular diseases as coronary heart disease (CHD) and stroke is needed to inform

dietary recommendation and guidelines, in the context of our national and international obesity epidemic. Here, we report analyses to relate biomarker-calibrated energy consumption to these diseases in WHI cohorts.

Epidemiologic reports on protein consumption in relation to cardiovascular disease almost exclusively focus on protein density, or more generally on protein consumption with some form of total energy adjustment. For example, energy-adjusted protein has been reported to be inversely associated with CHD risk in women,[11, 12] whereas no association was found with stroke risk in men.[13] In addition to examining the association of absolute protein consumption with these diseases, we provide analyses of biomarker-calibrated protein density in relation to cardiovascular disease incidence in WHI cohorts, for comparison with these earlier reports.

METHODS

The methods employed here are similar to those used in our recent report on calibrated energy and protein in relation to cancer risk,[14] and hence will be summarized only briefly.

Study Cohorts

Between 1993 and 1998, 48 835 eligible women were randomized in the Dietary Modification trial to either a diet modification intervention group (40%) or a usual-diet comparison group (60%). Between 1994 and 1998, 93,676 eligible women were enrolled in the prospective WHI Observational Study. The calibration equations mentioned above were applied to FFQ data collected at one year following randomization in the Dietary Modification trial comparison group, and at three years following enrollment in the Observational Study. We used these data, rather than baseline dietary data, to avoid assessment biases due to the use of the FFQ for eligibility screening in the Dietary Modification trial.[14] Observational Study enrollees who had a diagnosis of myocardial infarction, stroke, or transient ischemic attack within six months prior to enrollment were excluded to match the eligibility criteria for the Dietary Modification trial. A total of 26,595 (91%) women in the Dietary Modification trial comparison group and 67,492 (88%) women in the Observational Study provided the requisite FFQ assessments and were without a cardiovascular disease diagnosis during cohort follow-up, prior to Year 1 in the Dietary Modification trial or to Year 3 in the Observational Study cohort. Of these, 21,573 (81%) of Dietary Modification trial comparison women and 59,157 (88%) of Observational Study women provided all data that were used for energy and protein calibration, and for confounding control in the analysis of the cardiovascular outcomes. These 80,730 women constitute the analytic cohort for this prospective study.

All WHI women were postmenopausal, in the age range 50–79 years, and with no medical condition at recruitment associated with less than three years[1] predicted survival.[7] All completed core questionnaires at baseline on medical history, reproductive history, family history, personal habits, and psychosocial attributes, and provided a fasting blood sample.

WHI Food Frequency Questionnaire

FFQs were collected in conjunction with visits to the 40 participating clinical centers, where completeness and quality control checks were applied. The self-administered FFQ included 122 line items for individual foods/food groups and 19 adjustment items regarding fat intake, as well as summary questions.[15] We used the Nutrition Data System (Version 2005, University of Minnesota) to compute daily average nutrient consumption estimates.[16]

Nutrient Biomarker Study

The Nutrient Biomarker Study was conducted in 2004–2005.[8] A total of 544 weight-stable women from the Dietary Modification trial cohort were enrolled (n = 276 comparison group; n=268 intervention group). Participants were recruited at 12 of the 40 WHI clinical centers with the intent of obtaining a subsample that was representative of the entire trial cohort in terms of age, BMI, and race/ethnicity, with about 50% of the subsample from each of the comparison and intervention groups. Study participants turned out to be generally representative, although slightly younger and less obese.[8] Women were excluded if they had any medical condition precluding participation, weight instability, or plans to travel during the study period. Of the 1456 women invited, 46% declined, 15% were ineligible, and 38% agreed to participate. Those who agreed provided informed consent and 98% completed study procedures.

In weight-stable persons, total energy intake is approximately equal to total energy expenditure. Thus, if total energy expenditure is accurately measured, it provides an objective assessment of energy intake. The method of doubly-labeled water[5] administered orally and recovered in urine provides a precise measure of short-term total energy expenditure. Similarly, in weight-stable persons who are neither anabolic or catabolic, recovery of nitrogen from a 24-hour urine specimen can be used to estimate nitrogen consumption, from which an objective measure of protein consumption can be obtained as 6.25 (urinary nitrogen)/0.81. The participating 544 women each completed a doubly-labeled water protocol to estimate daily total energy expenditure over a two-week period, and a urinary nitrogen protocol to estimate protein consumption over a 24-hour period. All provided a concurrent FFQ and data describing individual characteristics. Twenty percent (n = 111) repeated the entire protocol an average of six months later, to provide repeatability information. Calibration equations were developed for energy, protein, and protein density by linear regression of log-biomarker estimates on corresponding log-FFQ estimates, BMI, age, ethnicity, and other factors.[8] The fact that women in the Nutrient Biomarker Study are slightly younger and less obese should not bias calibrated consumption estimates, because age and BMI are among the variables included in each calibration equation. A flow diagram showing pertinent WHI cohorts and the Nutrient Biomarker Study subsample is presented as an eFigure (http://links.lww.com).

Ascertainment of Outcomes

Ascertainment of outcomes and adjudication methods in the WHI have been described.[17] Initial self-reports of disease events and hospitalizations were obtained semi-annually in the Dietary Modification trial and annually in the Observational Study. Potential outcomes were adjudicated by trained physicians at each clinical center, based on review of pertinent medical records. A fraction of CHD events and a fraction of strokes, were further adjudicated by central reviewers. Confirmation rates were 90% for myocardial infarction, 97% for deaths due to coronary heart disease, and 95% for stroke.

Statistical Methods

Hazard ratios (HRs) and 95% confidence intervals (CIs) for cardiovascular risk in relation to nutrient consumption, adjusted for other risk predictors, were estimated using Cox regression.[18] Follow-up times began with the Year 1 visit for women in Dietary Modification comparison group or the Year 3 visit for women in Observational Study and continued to the earliest of any of the cardiovascular outcomes studied here, death, loss to follow-up, or 31 March 2005 (when the “intervention phase” of the WHI clinical trial ended). The baseline hazard ratios of the Cox model were stratified by age (Year 1 age for Dietary Modification trial comparison group and Year 3 age for Observational Study) in 5-year categories. For women in the Dietary Modification trial comparison cohort, the hazard

ratios were also stratified on their participation in the hormone therapy trial[7] (active estrogen; estrogen placebo; active estrogen plus progestin; estrogen plus progestin placebo; not randomized). Analyses that combine the Dietary Modification trial comparison and Observational Study cohorts also stratify on cohort. To control for confounding, we adjusted the effects of nutrient consumption on risk of each cardiovascular outcome by a standard set of cardiovascular disease risk factors, including ethnicity, education, history of cardiovascular disease, family history of premature cardiovascular disease, smoking status, hypertension, treated diabetes, statin use, aspirin use, prior hormone use, and an estimate of recreational physical activity. Women who were missing one or more confounding factors were excluded from analysis.

The calibrated consumption hazard ratio estimates derive from a classical measurement model

$$W=Z+u$$

for a biomarker estimate W of a nutrient consumption Z (e.g., log-energy consumption), and a more flexible model

$$Q=a_0+a_1Z+a^T_2V+(r+e)$$

for a FFQ estimate Q of Z, where V is a vector of study subject characteristics to characterize the systematic bias of Q, r is a random effect (person-specific bias), and a_0, a_1, and $a^T_2 = (a_{21}, a_{22}, \ldots)$ are coefficients to be estimated. All random variables on the right side of the equations for W and Q are assumed to be statistically independent given V. A joint normality assumption for Z and (r + e) given V leads to a linear model for the expectation of Z given (Q, V), and hence for W given (Q, V), under the crucial assumption that the measurement error u for W is statistically independent of that for the self-report assessment Q. We refer to u and (r + e) as the "random" aspects of the measurement errors for W and Q, respectively. The calibration equations provide estimates of the coefficients in a linear model for the expectation of W given Q and V.

Nutrient consumption estimates were based on these equations for all women except the 276 Nutrient Biomarker Study participants in the Dietary Modification trial comparison group, for whom biomarker values (W) were used. We used a "bootstrap" resampling procedure, with 500 bootstrap samples, to estimate standard errors for calibrated consumption log HRs. HR estimates based on calibrated nutrient intake turned out to have considerably wider confidence intervals than corresponding HRs based on uncalibrated nutrient consumption. This is due, in part, to the fact that the calibration equations were based on data from only 544 women in the Nutrient Biomarker Study, and also to the attenuation of uncalibrated HRs and confidence intervals resulting from the random error (r + e) in the log-transformed FFQ assessments.

Hazard ratio estimates given here target the consumption estimated by the nutrient biomarker (Z in above measurement model), and hence apply to short-term nutrient consumption. The Nutrient Biomarker Study reliability subsample did not yield evidence of systematic consumption changes over the approximately six months between biomarker collections. Hence, one can reasonably think of Z as reflective of consumption over a period of, say, a few weeks or months, while the biomarker error (u) incorporates temporal

consumption variation over such a time period, along with any technical error in biomarker assessment during the two-week protocol period.

Log-hazard ratios were modeled as a linear function of log-nutrient consumption, so that the HR for a fractional increase in nutrient consumption is independent of consumption level. Here, in order to be specific, we present HRs for a 20% increment in nutrient consumption. For a woman with median consumption, a 20% increment corresponds to about 412 kcal of energy, 15.2 grams of protein, or 2.9 units in percent of energy from protein.

For uncalibrated nutrient consumption, we estimated standard errors for regression coefficients using standard Cox model[18] procedures. For calibrated nutrient consumption, uncertainty in the calibration coefficient estimates needed to be acknowledged. Hence, a re-sampling procedure with 500 "bootstrap" samples was used to obtain standard error estimates, with bootstrap sampling stratified on Dietary Modification trial comparison group or Observational Study cohort, membership in the Nutrient Biomarker Study, and in its reliability subset. A bootstrap procedure with 500 samples was also used to test equality of HRs between the Dietary Modification trial comparison group and Observational Study cohorts. Note that even with a calibration subsample as large as 544 a noteworthy fraction of the variance in hazard ratio or odds ratio estimates as a function of calibrated consumption may be due to uncertainty in calibration equation coefficient estimates.[20] These analyses were carried out using the R software package without the need for specialized software. Illustrative R-code is given in the eAppendix (http://links.lww.com).

Because an elevated body mass is an expected consequence of a sustained high energy diet, including body mass index in the regression analysis is likely to involve "overcorrection" to the energy and cardiovascular disease associations. Hence, we present analyses for calibrated energy consumption with and without control for BMI in the disease risk model.

RESULTS

Table 1 shows the distribution of some baseline characteristics for women included in the present analyses. The majority of women in both cohorts were over 60 years of age, either overweight or obese, and white. Women in the Dietary Modification trial tended to be somewhat more overweight and less likely to engage in frequent recreational physical activity.

There were a total of 3917 cardiovascular disease events. Table 2 presents incidence rates per 1000 person-years of follow-up, and shows the number of women who experienced cardiovascular events during cohort follow-up. Incidence rates were fairly similar for the two cohorts.

Table 3 shows the geometric mean for consumption of energy, protein, and protein density, with and without biomarker calibration, for the women included in these analyses. The distribution of calibrated nutrients is similar for the two cohorts.

There was little evidence of hazard ratio differences between the two cohorts; hence we present HRs for each nutrient based on the combined Dietary Modification trial comparison group and Observational Study cohorts. HR estimates and their 95% CIs are displayed in Table 4–6. Uncalibrated energy consumption (Table 4) is not associated with total or specific cardiovascular disease risk. However, calibrated energy is positively associated with a risk of total coronary heart disease (HR = 1.18 [95% CI = 1.04–1.33], for a 20% consumption increment), coronary death (1.29 [1.07–1.56]), and coronary revascularization (1.18 [1.08–1.29]). These associations are not evident after including BMI in the disease risk model. There is also a inverse association of calibrated energy with stroke risk (0.86 [0.75–

1.00]), which is stronger after including BMI in the risk model. HRs for uncalibrated energy were little changed by adding BMI to the risk model, and are not shown.

Table 5 shows corresponding HRs for protein consumption. While there is no evidence of association between uncalibrated protein and risk of any type of cardiovascular disease, there is an inverse association between calibrated protein intake and risk of stroke (0.89 [0.82–0.98]), which remains after including BMI in the risk model.

Table 6 shows that calibrated protein density is inversely associated with the risk of total coronary heart disease (0.85 [0.75–0.97]), coronary death (0.74 [0.59–0.93]), and total cardiovascular disease (0.89 [0.81–0.98]), each of which is little changed by adjustment for BMI in the disease incidence model. Stroke risk is not associated with protein density. FFQ associations without calibration are in the same direction as the calibrated results, but weaker.

The analyses of Tables 4–6 were repeated excluding the small fraction (Table 1) of women having a cardiovascular disease event prior to WHI enrollment. The HR patterns were changed very little by these exclusions, although the inverse association for protein density became more apparent for nonfatal myocardial infarction, and for total CVD including revascularization.

DISCUSSION

FFQ assessments of energy and protein include systematic biases related to BMI, age, ethnicity, and other factors.[8] These biases can substantially distort estimated disease associations. Utilizing data from a biomarker substudy of 544 women, we developed consumption estimates in WHI cohorts that make a correction for such systematic biases, as well for the attenuation that arises from the random components of FFQ measurement error. The resulting calibrated consumption estimates proved to be sufficient to demonstrate cardiovascular disease associations of public health importance.

After these corrections for measurement error, higher energy consumption is associated with increased risk of CHD, coronary revacularization, and a combined CVD outcome that includes coronary revascularization. However, at a given BMI, a higher short-term energy consumption does not have a clear association with risk. Note also that BMI is positively associated with these outcomes in analyses that exclude energy from the risk model (not shown). A plausible interpretation is that a sustained high-energy diet leads to elevated disease risk that is substantially mediated by body-fat deposition and a temporal increase in BMI. Simultaneously, a comparatively high-energy consumption for a woman at a specified BMI may reflect a greater activity-related energy expenditure with associated cardiovascular effects that may be favorable.

Additional longitudinal data on energy consumption, physical activity including objective assessments, and body mass changes may help to more fully interpret these associations. Given that body mass accumulation is likely a mediator of the effects of a high-energy diet, we believe that the hazard ratios that exclude BMI from the disease risk model in Tables 4–6 are the most relevant in interpreting corresponding dietary associations.

The physical activity measure available in WHI (and included in the disease risk model in these analyses) aims to estimate energy expenditure from leisure physical activity only. Such activity is a fairly minor component of total activity-related energy expenditure, and the extent to which this leisure activity measure also incorporates systematic or random measurement error is unknown.

A positive association of CHD risk with energy consumption may be compared with a previously-reported association with energy-adjusted saturated fat consumption in women.[21] Likewise, an inverse association of energy consumption with stroke risk may be compared with a corresponding inverse association of stroke with percent of energy from fat in men.[22] An energy-dense diet is associated with higher energy intake and weight gain over time.[22–24] Hence, there is a need to elucidate whether these associations are attributable to the fat content of the diet or to total energy consumption, or to both. As illustrated by Table 4, these energy associations may not be apparent without biomarker correction of energy consumption estimates. The mechanisms for an inverse association between energy and stroke risk are unclear. This could reflect greater energy consumption by women who are more physically active. Alternatively, Gillman et al[22] speculate that a high consumption of certain types of fat may simultaneously predispose to large vessel (e.g., coronary artery) atherosclerosis and to maintenance of smaller intracranial vessels, the anatomic site of most ischemic strokes.

Absolute protein consumption was not positively associated with coronary disease. Rather, an inverse association was suggested after including BMI in the disease risk model. An inverse association of protein consumption with ischemic stroke also emerged from these analyses. Calibrated protein was positively related to both energy and BMI (correlation of 0.46 for log-calibrated protein with BMI), and these associations could largely reflect energy associations. However, calibrated protein density, which was only weakly associated with energy and BMI (correlation of 0.12 for log-protein density with BMI), was inversely associated with CHD risk but not stroke. These findings are consistent with previous cohort study reports of inverse energy-adjusted protein associations with CHD in women,[11, 12] and a lack of association between protein density and stroke in men.[13] A recent cohort study report[25] from the Netherlands found a possible U-shaped association between cardiovascular disease and protein consumption per kilogram of body weight, with protein measured in 24-hour urine samples. However, results were not presented separately for CHD and stroke. The inverse association of protein density with CHD risk suggests that fat plus carbohydrate consumption tends to be responsible for body fat accumulation and for the positive association of energy consumption with risk.

While our calibrated protein-density estimates benefit somewhat from the inclusion of BMI and age in the calibration equation,[8] the calibration procedure primarily corrects the “noise” aspect of measurement error for this ratio measure. Accordingly, FFQ protein density associations (Table 6) are generally consistent with those for calibrated protein density, but weaker due to measurement error attenuation.

These analyses complement our recent report on calibrated energy and protein in relation to cancer risk,[14] which showed a positive association between energy consumption and total invasive cancer, as well as certain site-specific cancers. However, calibrated energy consumption was not associated with any cancer when BMI was included in the disease risk model. These analyses suggest that positive associations of energy consumption with both cancer and coronary disease may be substantially attributable to body fat accumulation through energy imbalance.

It is perhaps worth noting that the calibrated energy estimates are developed without consideration of the cardiovascular disease outcome data. Also, under our measurement model,[19, 20] hazard ratios for calibrated energy, with or without BMI adjustment, will target the same HR as would be the case if biomarker determinations were carried out for each woman in the study cohort. Hence, these analyses can be expected to give results that are consistent with (if less precise than) results based on biomarker data from all 80,730 women.

The strengths of this study are the large well-characterized WHI cohorts with quality cardiovascular disease ascertainment, a sizeable nutrient biomarker substudy, and the novel use of biomarkers for studying absolute nutrient consumption associations in cardiovascular epidemiology.

Study limitations include the fact that the Nutrient Biomarker Study was conducted in 2004–2005, an average of 6.5 years after the Year 1 FFQ data collection in the Dietary Modification trial, and four years after the Year 3 FFQ collection in the Observational Study. The calibrated energy consumption and BMI data analyzed were highly correlated and pertained to a single point in time. More comprehensive analysis of the interplay between energy consumption, body fat accumulation, and CVD risk may be possible from longitudinal data on these factors in a cohort study context. The biomarkers of energy and protein reflect expenditure, and an assumption of energy balance is needed for these assessments to reflect (short-term) consumption. Also, while we controlled for a measure of recreational physical activity, this measure may also be subject to random and systematic measurement error, and it targets only a small fraction of total activity-related energy expenditure. Analyses that incorporate objective measures of both nutrition and physical activity may be needed to more fully elucidate their joint contributions to disease risk. The study is limited to energy and protein associations because these are among the few nutrients with an established recovery biomarker. There is a strong need for the development of biomarkers that adhere to a classical measurement model for other nutrients and dietary components. Such data in conjunction with objective measures of activity-related energy expenditure and its components in appropriate subsets of well-characterized study cohorts have much potential for progress in understanding the roles of nutrition and physical activity in health maintenance.

In summary, energy consumption is positively associated with coronary heart disease risk. This association appears to be explained by body fat accumulation. Energy and protein consumption are inversely associated with ischemic stroke risk. Protein density is inversely associated with the risk of coronary heart disease but not with the risk of stroke. Measurement error correction via nutrient biomarkers has the potential to enhance the reliability of results from association studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources: This work was supported by the National Heart, Lung, and Blood Institute at the National Institutes of Health, U. S. Department of Health and Human Services [contracts N01WH22110, 24152, 32100-2, 32105-6, 32108-9, 32111-13, 32115, 32118-19, 32122, 42107-26, 42129-32, and 44221]. Dr. Prentice's work was partially supported by grant P01 CA53996 from the National Cancer Institute at the National Institutes of Health.

We thank the WHI investigators and staff for their outstanding dedication and commitment. A list of key investigators involved in this research follows. A full listing of WHI investigators can be found at the following website: http://www.whi.org. Clinical Trials Registration: ClinicalTrials.gov identifier: NCT00000611.

Program Office: (National Heart, Lung, and Blood Institute, Bethesda, Maryland) Elizabeth Nabel, Jacques Rossouw, Shari Ludlam, Linda Pottern, Joan McGowan, Leslie Ford, and Nancy Geller.

Clinical Coordinating Center: (Fred Hutchinson Cancer Research Center, Seattle, WA) Ross Prentice, Garnet Anderson, Andrea LaCroix, Charles L. Kooperberg, Ruth E. Patterson, Anne McTiernan; (Wake Forest University School of Medicine, Winston-Salem, NC) Sally Shumaker; (Medical Research Labs, Highland Heights, KY) Evan Stein; (University of California at San Francisco, San Francisco, CA) Steven Cummings.

Clinical Centers: (Albert Einstein College of Medicine, Bronx, NY) Sylvia Wassertheil-Smoller; (Baylor College of Medicine, Houston, TX) Aleksandar Rajkovic; (Brigham and Women's Hospital, Harvard Medical School, Boston, MA) JoAnn Manson; (Brown University, Providence, RI) Annlouise R. Assaf; (Emory University, Atlanta, GA) Lawrence Phillips; (Fred Hutchinson Cancer Research Center, Seattle, WA) Shirley Beresford; (George Washington University Medical Center, Washington, DC) Judith Hsia; (Los Angeles Biomedical Research Institute at Harbor- UCLA Medical Center, Torrance, CA) Rowan Chlebowski; (Kaiser Permanente Center for Health Research, Portland, OR) Evelyn Whitlock; (Kaiser Permanente Division of Research, Oakland, CA) Bette Caan; (Medical College of Wisconsin, Milwaukee, WI) Jane Morley Kotchen; (MedStar Research Institute/Howard University, Washington, DC) Barbara V. Howard; (Northwestern University, Chicago/Evanston, IL) Linda Van Horn; (Rush Medical Center, Chicago, IL) Henry Black; (Stanford Prevention Research Center, Stanford, CA) Marcia L. Stefanick; (State University of New York at Stony Brook, Stony Brook, NY) Dorothy Lane; (The Ohio State University, Columbus, OH) Rebecca Jackson; (University of Alabama at Birmingham, Birmingham, AL) Cora E. Lewis; (University of Arizona, Tucson/Phoenix, AZ) Tamsen Bassford; (University at Buffalo, Buffalo, NY) Jean Wactawski-Wende; (University of California at Davis, Sacramento, CA) John Robbins; (University of California at Irvine, CA) F. Allan Hubbell; (University of California at Los Angeles, Los Angeles, CA) Lauren Nathan; (University of California at San Diego, LaJolla/Chula Vista, CA) Robert D. Langer; (University of Cincinnati, Cincinnati, OH) Margery Gass; (University of Florida, Gainesville/Jacksonville, FL) Marian Limacher; (University of Hawaii, Honolulu, HI) David Curb; (University of Iowa, Iowa City/Davenport, IA) Robert Wallace; (University of Massachusetts/Fallon Clinic, Worcester, MA) Judith Ockene; (University of Medicine and Dentistry of New Jersey, Newark, NJ) Norman Lasser; (University of Miami, Miami, FL) Mary Jo O'Sullivan; (University of Minnesota, Minneapolis, MN) Karen Margolis; (University of Nevada, Reno, NV) Robert Brunner; (University of North Carolina, Chapel Hill, NC) Gerardo Heiss; (University of Pittsburgh, Pittsburgh, PA) Lewis Kuller; (University of Tennessee, Memphis, TN) Karen C. Johnson; (University of Texas Health Science Center, San Antonio, TX) Robert Brzyski; (University of Wisconsin, Madison, WI) Gloria E. Sarto; (Wake Forest University School of Medicine, Winston-Salem, NC) Mara Vitolins; (Wayne State University School of Medicine/Hutzel Hospital, Detroit, MI) Susan Hendrix.

Women's Health Initiative Memory Study: (Wake Forest University School of Medicine, Winston-Salem, NC) Sally Shumaker.

References

1. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65. [PubMed: 4014201]
2. Kaaks R, Ferrari P, Ciampi A, Plummer M, Ribole E. Uses and limitations of statistical accounting for random error correlations, in the validation of dietary questionnaire assessments. Public Health Nutr 2002;5:969–976. [PubMed: 12638598]
3. Subar AF, Kipnis V, Troiano RP, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. Am J Epidemiol 2003;158:1–13. [PubMed: 12835280]
4. Kipnis V, Subar AF, Midthune D, et al. Structure of dietary measurement error: results of the OPEN biomarker study. Am J Epidemiol 2003;158:14–21. [PubMed: 12835281]
5. Schoeller DA. Recent advances from application of doubly-labeled water to measurement of human energy expenditure. J Nutr 1999;129:1765–1768. [PubMed: 10498745]
6. Bingham SA. Urine nitrogen as a biomarker for the validation of dietary protein intake. J Nutr 2003;133:921S–924S. [PubMed: 12612177]
7. The Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. Control Clin Trials 1998;19:61–109. [PubMed: 9492970]
8. Neuhouser ML, Tinker L, Shaw PA, et al. Use of recovery biomarkers to calibrate nutrient consumption self-reports in the Women's Health Initiative. Am J Epidemiol 2008;167:1247–1259. [PubMed: 18344516]
9. WHO Technical Report. Vol. 916. World Health Organization; 2003. Diet, Nutrition and the Prevention of Chronic Diseases: Report of a joint WHO/FAO expert consultation; p. 88
10. World Cancer Research Fund/American Institute for Cancer Research. Nutrition and the Prevention of Cancer: a Global Perspective. Washington DC: American Institute for Cancer Research; 1997. Food; p. 371
11. Hu FB, Stampfer MJ, Manson JE, et al. Dietary protein and risk of ischemic heart disease in women. Am J Clin Nutr 1999;70:221–227. [PubMed: 10426698]

12. Kelemen LE, Kushi LH, Jacobs DR Jr, Cerhan JR. Associations of dietary protein with disease and mortality in a prospective study of postmenopausal women. Am J Epidemiol 2005;161:239–249. [PubMed: 15671256]

13. Rosner Preis S, Stampfer MJ, Spiegelman D, Willett WC, Rimm EB. Lack of association between dietary protein intake and risk of stroke among middle-aged men. Am J Clin Nutr 2010;91:39–45. [PubMed: 19889826]

14. Prentice RL, Shaw PA, Bingham SA, et al. Biomarker-calibrated energy and protein consumption and increased cancer risk among postmenopausal women. Am J Epidemiol 2009;169:977–989. [PubMed: 19258487]

15. Kristal, AK.; Shattuck, AL.; Williams, ME. Food frequency questionnaires for diet intervention research. 17th National Nutrient Databank conference proceedings; Baltimore, MD: International Life Sciences Institute; 1992. p. 110-125.

16. Schakel SF, Buzzard IM, Gebhardt SE. Procedures for estimating nutrient values for food composition databases. J Food Compost Anal 1997;10:102–114.

17. Curb JD, McTiernan A, Heckbert SR, et al. for the WHI Morbidity and Mortality Committee. Outcomes ascertainment and adjudication methods in the Women's Health Initiative. Ann Epidemiol 2003;13:S122–S128. [PubMed: 14575944]

18. Cox DR. Regression analysis and life tables (with discussion). J R Statist Soc B 1972;34:187–220.

19. Prentice RL, Sugar E, Wang CY, Neuhouser M, Patternson RE. Research strategies and the use of biomarkers in studies of diet and chronic disease. Public Health Nutr 2002;5:977–984. [PubMed: 12633522]

20. Sugar E, Wang CY, Prentice RL. Logistic regression with exposure biomarkers and flexible measurement error. Biometrics 2007;63:143–151. [PubMed: 17447939]

21. Hu FB, Stampfer MJ, Manson JE, et al. Dietary fat intake and the risk of coronary heart disease in women. N Engl J Med 1997;337:1491–1499. [PubMed: 9366580]

22. Gillman MW, Cupples LA, Millen BE, Ellison RC, Wolf PA. Inverse association of dietary fat with development of ischemic stroke in men. JAMA 1997;278:2145–2150. [PubMed: 9417007]

23. Howarth NC, Murphy SP, Wilkens LR, Hankin JH, Kolonel LN. Dietary energy density is associated with overweight status among 5 ethnic groups in the multiethnic cohort study. J Nutr 2006;136:2243–2248. [PubMed: 16857848]

24. Savage JS, Marini M, Birch LL. Dietary energy density predicts women's weight change over 6 y. Am J Clin Nutr 2008;88:677–684. [PubMed: 18779283]

25. Halbesma N, Bakker SJL, Jansen DF, et al. for the PREVEND Study Group. High protein intake associates with cardiovascular events but not with loss of renal function. J Am Soc Nephrol 2009;290:1797–1804. [PubMed: 19443643]

Table 1

Baseline characteristics for women in the Women's Health Initiative Dietary Modification Trial Comparison Group and Observational Study

Characteristic		Dietary Modification Trial (n = 21,573)[a] % (No.)	Observational Study (n = 59,157)[a] % (No.)
Age (years)	50–59	30 (6402)	19 (11,241)
	60–69	48 (10,427)	43 (25,372)
	70–79	21 (4620)	34 (20,430)
	80–85	1 (124)	4 (2114)
BMI (kg/m^2)	Normal (<25.0)	26 (5687)	42 (25,089)
	Overweight (25.0–29.9)	36 (7718)	34 (20,318)
	Obese (≥ 30)	38 (8168)	23 (13,750)
Race/Ethnicity	White	83 (17,789)	86 (51,114)
	African-American	10 (2124)	6 (3634)
	Hispanic	3 (716)	3 (1736)
	Mixed or Other	4 (944)	5 (2673)
Income (total yearly)	< $20,000	15 (3194)	14 (8036)
	$20,000–$34,999	25 (5312)	23 (13,541)
	$35,000–$49,999	21 (4548)	21 (12,249)
	$50,000–$74,999	21 (4521)	21 (12,497)
	$75,000+	18 (3988)	22 (12,834)
Education	< High school diploma	4 (888)	4 (2134)
	High school diploma/GED	17 (3786)	16 (9301)
	School after high school	40 (8530)	36 (21,433)
	College degree or higher	39 (8369)	44 (26,289)
Smoking	Current	6 (1374)	6 (3280)
	Past	52 (11,294)	51 (30,224)
	Never	41 (8905)	43 (25,653)
Recreational physical activity (METs/week)	< 1.5	24 (5295)	16 (9417)
	1.5–6.2	25 (5349)	20 (11,726)
	6.3–14.7	26 (5507)	27 (15,727)
	> 14.8	25 (5422)	38 (22,287)
History of cardiovascular disease	Yes	3 (702)	4 (2114)
Family history of premature cardiovascular disease	Yes	19 (4005)	18 (10,382)
Hypertension	Yes	41 (8801)	37 (21,764)
Treated diabetes	Yes	4 (955)	3 (1818)
Statin use	Yes	6 (1381)	8 (4609)
Aspirin use	Yes	19 (4053)	21 (12,604)
Postmenopausal hormone usage	Current user	45 (9801)	50 (29,371)
	Never user	41 (8751)	37 (21,829)
	Past user	14 (3021)	13 (7957)

Characteristic		Dietary Modification Trial (n = 21,573)[a] % (No.)	Observational Study (n = 59,157)[a] % (No.)
Postmenopausal hormone treatment in the WHI Hormone trial	Estrogen placebo	3 (751)	NA
	Active estrogen	3 (741)	NA
	Estrogen plus progestin placebo	5 (984)	NA
	Active estrogen plus progestin	5 (1032)	NA
	Not randomized	84 (18,065)	NA

[a]Number of subjects for whom there were no missing values for the nutrients regression calibration or for cardiovascular-event hazard-ratio analysis.

NA indicates not applicable

Table 2

Incidence of cardiovascular events per 1000 person-years in the Women's Health Initiative Dietary Modification Trial Comparison Group and Observational Study following food-frequency data collection in Year 1 and Year 3, respectively.

Cardiovascular Disease	Dietary Modification Trial (n = 21,573)[a]		Observational Study (n = 59,157)[a]		Total (n = 80,730)[a]	
	No. cases	Incidence	No. cases	Incidence	No. cases	Incidence
Total CHD	557	3.7	959	3.2	1516	3.4
Nonfatal MI	438	2.9	702	2.4	1140	2.6
Coronary death	142	1.0	290	1.0	432	0.5
CABG and PCI	750	5.1	1305	4.4	2055	4.6
Total stroke	399	2.7	825	2.8	1224	2.7
Ischemic stroke	245	1.6	496	1.7	741	1.7
Hemorrhagic stroke	75	0.5	142	0.5	217	0.5
Total CVD: CHD and stroke	914	6.2	1713	5.8	2627	5.9
Total CVD including CABG and PCI	1356	9.3	2561	8.7	3917	8.9

[a]The number of subjects in the cohort for whom there were no missing values for the nutrients calibration or for cardiovascular hazard ratio analysis.

CHD indicates Coronary Heart Disease; CVD, Cardiovascular Disease; CABG, Coronary Bypass Graft; MI, myocardial infarction; PCI, Percutaneous Coronary Intervention

Table 3

Geometric mean consumption for uncalibrated dietary consumption as estimated by the Women's Health Initiative food frequency questionnaire, and for calibrated consumption using nutritional biomarker data, in the WHI Dietary Modification Trial Comparison Group and Observational Study.

	Energy (kcal/day)		Protein (g/day)		% of Energy from Protein	
	Uncalibrated Geometric Mean (95% CI)	Calibrated[a] Geometric Mean (95% CI)	Uncalibrated Geometric Mean (95% CI)	Calibrated Geometric Mean (95% CI)	Uncalibrated Geometric Mean (95% CI)	Calibrated Geometric Mean (95% CI)
Dietary Modification Trial Comparison Group (n = 21,573)	1485 (686–3219)	2141 (1788–2565)	61.5 (26.7–141.6)	78.1 (58.5–104.4)	16.6 (11.5–23.9)	14.4 (12.0–17.3)
Observational Study (n = 59,157)	1390 (648–2983)	2056 (1723–2453)	58.9 (25.2–137.5)	74.3 (55.0–100.5)	16.9 (11.5–24.9)	14.4 (11.9–17.6)

[a]Calibrated using biomarker only for women in the Nutrition Biomarker Study, and otherwise using equations developed on the basis of FFQ nutrients measure and other factors.[8] Specifically, calibrated log-energy (kcal) was given by 7.61 + 0.062(log FFQ energy – 7.27) + 0.013(BMI – 28.2) − 0.005(age – 70.9) − 0.016[black ethnicity] − 0.004[hispanic ethnicity] − 0.093[other minority ethnicity] − 0.019[(annual household) income <20,000] + 0.037[income 20,000–34,999] + 0.013[income 50,000–74,999] + 0.019[income ≥75,000]; calibrated log-protein (gms) was given by 4.28 + 0.211(log FFQ protein – 4.14) + 0.012(BMI – 28.2) − 0.008(age – 70.9) − 0.130[black ethnicity] − 0.021[hispanic ethnicity] − 0.100[other minority ethnicity] + 0.065[high school, GED or less education] + 0.033[college degree or more] − 0.053[income <20,000] − 0.009[income 20,000–34,999] + 0.042[income 50,000–74,999] + 0.067[income ≥75,000] − 0.009(log FFQ protein – 4.14)(BMI); log-calibrated % of energy from protein was given by 2.66 + 0.439(log FFQ % of energy from protein – 2.85) − 0.004(BMI – 28.2) − 0.005(age – 70.9), where BMI is defined by weight in kg/height $(m)^2$ and square brackets denote indicator variables.

Table 4

Hazard Ratio estimates for a 20% increment in uncalibrated FFQ energy (kcal/day) consumption and in calibrated energy consumption, based on data from 80,730 women enrolled in the WHI Dietary Modification Trial Comparison Group or Observational Study.

Cardiovascular Disease	No. Incident cases	Uncalibrated Energy	Calibrated Energy	Calibrated Energy, Adjusted for BMI
		HR[a] (95%CI)	HR[a] (95%CI)[b]	HR[a] (95%CI)[b]
Total CHD	1516	1.00 (0.98–1.03)	1.18 (1.04–1.33)	0.89 (0.62–1.27)
Nonfatal MI	1140	1.00 (0.98–1.03)	1.13 (0.98–1.30)	0.89 (0.60–1.32)
Coronary death	432	1.01 (0.97–1.05)	1.29 (1.07–1.56)	0.92 (0.53–1.60)
CABG and PCI	2055	0.99 (0.97–1.01)	1.18 (1.08–1.29)	0.82 (0.63–1.06)
Total stroke	1224	0.99 (0.97–1.02)	0.86 (0.75–1.00)	0.61 (0.41–0.91)
Ischemic stroke	741	0.98 (0.95–1.02)	0.88 (0.74–1.05)	0.54 (0.34–0.87)
Hemorrhagic stroke	217	1.02 (0.96–1.08)	0.75 (0.53–1.07)	0.93 (0.46–1.85)
Total CVD: CHD plus stroke	2627	1.00 (0.99–1.02)	1.05 (0.95–1.15)	0.77 (0.57–1.05)
Total CVD with CABG and PCI	3917	1.00 (0.98–1.01)	1.11 (1.03–1.19)	0.80 (0.62–1.04)

[a] Hazard ratios were adjusted for ethnicity, education, history of cardiovascular disease, family history of premature cardiovascular disease, smoking status, hypertension, treated diabetes, statin use, aspirin use, prior hormone use, and recreational physical activity.

[b] 95% confidence intervals for analyses that include calibrated energy are based on log-estimated HR ±1.96 × bootstrap standard error.

Table 5

Hazard Ratio estimates for a 20% increment in uncalibrated FFQ protein (g/day) consumption and in calibrated protein consumption, based on data from 80,730 women enrolled in the WHI Dietary Modification Trial Comparison Group or Observational Study.

Cardiovascular Disease	No. Incident cases	Uncalibrated Protein	Calibrated Protein	Calibrated Protein, Adjusted for BMI
		HR[a] (95%CI)	HR[a] (95%CI)[b]	HR[a] (95%CI)[b]
Total CHD	1516	0.99 (0.97–1.01)	1.01 (0.92–1.10)	0.89 (0.80–0.99)
Nonfatal MI	1140	1.00 (0.97–1.02)	1.00 (0.90–1.10)	0.90 (0.80–1.02)
Coronary death	432	0.99 (0.95–1.03)	1.03 (0.89–1.19)	0.86 (0.72–1.04)
CABG and PCI	2055	1.00 (0.98–1.01)	1.03 (0.96–1.10)	0.90 (0.82–0.98)
Total stroke	1224	0.99 (0.97–1.01)	0.89 (0.82–0.98)	0.87 (0.78–0.98)
Ischemic stroke	741	0.99 (0.96–1.01)	0.91 (0.81–1.02)	0.87 (0.75–1.00)
Hemorrhagic stroke	217	1.02 (0.97–1.08)	0.91 (0.75–1.10)	1.01 (0.81–1.27)
Total CVD: CHD plus stroke	2627	0.99 (0.98–1.01)	0.96 (0.90–1.03)	0.89 (0.82–0.97)
Total CVD with CABG and PCI	3917	1.00 (0.98–1.01)	1.00 (0.94–1.06)	0.91 (0.84–0.98)

[a]Hazard ratios were adjusted for ethnicity, education, history of cardiovascular disease, family history of premature cardiovascular disease, smoking status, hypertension, treated diabetes, statin use, aspirin use, prior hormone use, and recreational physical activity.

[b]95% confidence intervals for analyses that include calibrated energy are based on log-estimated HR ±1.96 × bootstrap standard error.

Table 6

Hazard Ratio estimates for a 20% increment in uncalibrated FFQ protein density consumption and in calibrated protein density, based on data from 80,730 women enrolled in the WHI Dietary Modification Trial Comparison Group or Observational Study.

Cardiovascular Disease	No. Incident cases	Uncalibrated Protein Density	Calibrated Protein Density	Calibrated Protein Density, Adjusted for BMI
		HR[a] (95%CI)	HR[a] (95%CI)[b]	HR[a] (95%CI)[b]
Total CHD	1516	0.95 (0.91–1.00)	0.85 (0.75–0.97)	0.87 (0.78–0.97)
Nonfatal MI	1140	0.97 (0.92–1.02)	0.89 (0.78–1.02)	0.91 (0.80–1.03)
Coronary death	432	0.91 (0.84–1.00)	0.74 (0.59–0.93)	0.77 (0.62–0.95)
CABG and PCI	2055	1.00 (0.96–1.04)	0.94 (0.84–1.05)	0.96 (0.88–1.06)
Total stroke	1224	0.98 (0.93–1.03)	0.94 (0.84–1.06)	0.94 (0.83–1.06)
Ischemic stroke	741	0.99 (0.93–1.06)	0.97 (0.83–1.12)	0.97 (0.83–1.13)
Hemorrhagic stroke	217	1.05 (0.93–1.19)	1.17 (0.90–1.53)	1.13 (0.87–1.48)
Total CVD: CHD plus stroke	2627	0.97 (0.93–1.00)	0.89 (0.81–0.98)	0.90 (0.83–0.98)
Total CVD with CABG and PCI	3917	0.99 (0.97–1.02)	0.94 (0.87–1.02)	0.96 (0.90–1.03)

[a] Hazard ratios were adjusted for ethnicity, education, history of cardiovascular disease, family history of premature cardiovascular disease, smoking status, hypertension, treated diabetes, statin use, aspirin use, prior hormone use, and recreational physical activity.

[b] 95% confidence intervals for analyses that include calibrated energy are based on log-estimated HR ±1.96 × bootstrap standard error.