

Biomarker-calibrated estimates for dietary intake for Women's Health Initiative (WHI) analyses – When and How to Apply

When to apply

Calibrate dietary intakes of energy (kcal/day), protein (g/day), protein density (% energy from protein), sodium, or potassium assessed by the WHI Food Frequency Questionnaire (FFQ) in WHI analyses when one or more of these nutrients (or nutrient densities) is a primary exposure. This applies to data from Clinical Trial (CT) or Observational Study (OS) participants.

The NBS and/or NPAAS data are needed to compute the calibrated the calibrated estimates of dietary intakes. It is incorrect to use published estimates from other papers. See methods for guidelines.

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How to Apply

Considerations for the WHI Trial or OS components

- Dietary Modification Trial Year 1 or follow-up years. Follow the foundational guidelines from the Nutritional Biomarkers Study (NBS) biomarker analyses ¹ or the Nutrition and Physical Activity Assessment Study (NPAAS) ² depending on dietary assessment instrument of interest (FFQ, 4-day food record [4DFR] or 24hour dietary recall [24HR]) and nutrient of interest (energy, protein, protein density ^{1,2}, or sodium, potassium. ³ Use DM Trial Year 1 as the starting point for analyses. Do not use baseline data due to the truncated mean and resultant upwardly biased mean due to the WHI DM Trial exclusion criterion of FFQ-reporting <32% energy from fat. ⁴ Exclude outcomes of interest prior to year 1. Covariates from year 1 are preferable to baseline if available.
- Observational Study Baseline or year 3. Follow the foundational guidelines from the Nutrition and Physical Activity Assessment Study (NPAAS) biomarker analyses for the Food Frequency Questionnaire (FFQ) for energy, protein, protein density², or sodium, potassium. ³
- Baseline Hormone Trial. Follow the foundational guidelines that best align with the study sample of interest. Either the NBS or NPAAS foundational references may be used. The Hormone Trial participants completed an FFQ at baseline.

NBS and NPAAS Data Dictionaries and Data Access

- The NBS and NPAAS data dictionaries are available from the WHI website study pages without needing a login. ([W8 for NBS](#), [AS 218 for NPAAS](#)).

- The NBS and NPAAS data will be made available for download once the following criteria have been met: (1) being a lead author of an approved paper proposal using biomarker-calibrated data, (2) having signed and returned per directions the Data Use Policy Acknowledgement, (3) having requested WHI investigator data set access and having received a login, and (4) for WHI ancillary studies, having returned study data to the WHI per WHI [Ancillary Studies Policy](#).

Methods

1. Develop the calibration equations. For the FFQ, refer to the details presented in the appendix from Neuhouwer et al. ¹ The paper describes the basic participant characteristics to include in the regression calibration estimator. Each analysis needs to consider participant characteristics as well as parameters specific to the outcome of interest. The following list is suggested:
 - a. Subject characteristics as described in Neuhouwer et al. ¹ for the DM Trial, Prentice et al. ² for the OS, Huang et al. for sodium and potassium. ³
 - b. Covariates and confounders pertinent to the outcome of interest

Biomarker calibration equations are unique to the analyses of interest, which in combination with the necessary bootstrapping to compute estimated standard errors, precludes computation and posting of a “standard” set of calibrated intake or activity estimates.

After developing the calibration equations, regress the covariates or confounders on the intake biomarkers as described in the relevant papers listed above. Determine which covariates and confounders to retain based on *a priori* selection or statistical significance (per investigator decision).

2. Consider how to address possible outliers, e.g., values from the dietary assessment instruments falling outside the interquartile range by more than 3 times its width may be excluded as outliers. ² The exact handling may vary depending on analysis of interest per investigator discretion.
3. Estimate biomarker-calibrated intakes for the analytic cohort by applying the regression calibration equations to the WHI sample pertinent to the analytic aim. If NBS or NPAAS participants are among the sample, then either the direct biomarkers may be used or the calibration equations may be applied.
4. Conduct the outcome analysis, e.g., Cox proportional hazards model.
 - a. Estimate the standard errors and significance levels (i.e. p-values) for all estimated parameters using bootstrap procedures. Sample bootstrapping r code is posted to the study pages ([W8 for NBS](#), [AS 218 for NPAAS](#)).

5. For nutrient biomarker analyses, use models of an increased percentage of nutrient of interest, e.g., 20% increased energy. Several examples have been published. A list of publications from 2008-2026 is presented in the Appendix. You may prefer to search the WHI [Papers and Proposals](#).
6. Evolving methodology: The methodology of the biomarker calibration equations is dynamic and evolving. These guidelines reflect thinking as of 2015. The foundational references provide details relative to their time of publication, and thus analyses conducted today may not exactly replicate earlier work. **Contact the [WHI Help Desk](#) if you have any questions.**

Special Considerations

1. Body mass index (BMI). When examining the associations of biomarker-calibrated estimates of energy intake on disease risk, including BMI in the outcomes model may over-adjust for BMI. The reason, and assumption, being that BMI is the result of an energy over-balance, and thus the biomarker-calibrated energy intake estimate is the sufficient parameter to include in the analytic model. Further including BMI may over-adjust the model. However, additional or alternate effects of BMI on the disease of interest– in addition to long-term energy intake surplus– may exist and thus not including BMI may under-adjust the model. Investigators are encouraged to analyze the models both with and without BMI. Further discussion of this important concept may be found in the work of Prentice and Huang¹⁸ and Zheng and colleagues.¹⁹
2. Combining OS and DM Trial data for FFQ analyses. The NBS and NPAAS data may be combined when computing dietary intake biomarker calibration equations.
3. Cubic spline analyses. Calibrated intake estimates themselves incorporate quite a lot of 'noise', precluding them to be used directly to study dose response shape using complex models, like cubic splines. This is why we have confined our analyses to linear HR models for log-transformed intake under which ordinary regression estimates based on calibrated intake is appropriate (under normality of log transformed intakes). Statistical methods are not available for handling a (large) noise component of the calibrated intakes under more complex HR models.
4. Calibrating covariate exposures. In general, calibrating covariates is not recommended. An exception is if the covariate is a major risk factor for the disease outcome, e.g., sodium as a covariate with hypertension or CVD as the outcome. In this example, calibrating sodium is recommended.
5. Dietary Quality Indices. Analyses and papers are have been published on dietary quality indices, such as the Healthy Eating Index (HEI), Alternative Healthy Eating Index (AHEI), Dietary Approaches to Stop Hypertension (DASH), Alternative Mediterranean Index (aMed) to investigate calibrating dietary quality indices. See the Appendix at the end of this document, or search WHI [Papers and Proposals](#).

6. Protein sub-groups. Multiplying self-reported protein subgroup comparisons by biomarker-based total protein intake is not a procedure to be recommended, though may be something to try as an exploratory analysis. As a check on the reliability of the approach one could multiply DLW energy by the self-reported proportion of energy from protein, and compare the result with UN protein. These points should be included in the paper's discussion.

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Appendix
PUBLICATIONS LIST 2008-2026 (as of June 2026)
National Cancer Institute grants R01 CA119171 and P30 CA015704.
Search [WHI Papers and Proposals](#) for newer publications

NBS and NPAAS PUBLICATIONS

2008

1. Neuhouser ML, Tinker L, Shaw PA, Schoeller D, Bingham SA, Van Horn L, Beresford SAA, Caan B, Thompson C, Satterfield S, Kuller L, Heiss G, Smit E, Sarto G, Ockene J, Stefanick ML, Assaf A, Runswick S, Prentice RL. Use of recovery biomarkers to calibrate nutrient consumption self-reports in the Women's Health Initiative. *Am J Epidemiol* 2008;167(10):1247-1259.

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1. Ms5045p – Prentice RL, Aragaki AK, Lampe JW, Neuhaus ML, Manson JE, Raftery D, Gowda GAN, Huang Y, Tinker LF, Navarro SL, Lane DS, Zheng C. Serum-based

biomarker development for dietary macronutrient densities and their association with breast and colorectal cancer risk in a cohort of postmenopausal females. Under review for AJCN, 2026.

2. Ms5318 – Prentice RL, Vasan S, Zheng C, Tinker LF, Manson JE, Navarro SL, Raftery D, Gowda GAN, Song X, Huang Y, Orchard T, Brasky TM, Lampe JW, Neuhouser ML. Biomarker-calibrated dietary macronutrient densities and their association with chronic disease risk in a cohort of postmenopausal females (spinoff of Ms4952). Revised for Am J Clin Nutr, 2026.

Approved Manuscripts In Development

Ms2600 - Physical activity assessments: comparing biomarker and accelerometer measured energy expenditure among older women (Chongzhi Di)

Ms4566 - Feasibility of estimating empirical dietary inflammatory pattern (EDIP) and empirical dietary index for hyperinsulinemia (EDIH) scores from feeding study data (Fred Tabung)

MS4815 – Biomarker-calibrated estimates of animal protein ratio and associations with cardiovascular disease outcomes (Diane O'Brien, Johanna Lampe, Marian Neuhouser, Lesley Tinker, Ross Prentice). Approved by P&P.

MS 4828 -- Generalizability and external validation of WHI NPAAS derived macronutrient biomarkers in controlled feeding studies in other populations (Navarro, Lampe, Neuhouser, Tinker, Prentice, Zheng). Approved by P&P.

Ms4837 -- Nutrient and food group intake in the WHI dietary modification trial and chronic disease risk (Prentice, Tinker, Lampe, Zheng, Huang, Neuhouser) – Approved by P&P, in analysis.

Ms5042p -- Biomarker-calibrated estimates of added sugar intake and associations with chronic disease outcomes in postmenopausal women (J Johnson et al). To be submitted to P&P.

Ms5109 – Calibrated sodium and potassium intakes and cancer risk among postmenopausal women (Cheng E, Albert Einstein). Approved by P&P.

Ms5247 -- Navarro SL, Zheng C, Raftery D, Lampe JW, Neuhouser ML, Prentice RL. Comparison of biomarker macronutrient densities using different metabolomics platforms in a controlled feeding study. Approved by P&P.

Ms5417 – Ward L et al. Development of new predictive fat-free mass algorithm for use in predicting energy expenditure in adults aged 65-79 years and ≥80 years

ABSTRACTS

Understanding the role of nutrition in health promotion and disease prevention in the Women's Health Initiative (WHI) using biomarkers; Co-Presenters: Marian L Neuhouser and Lesley F Tinker for WHI Meeting, May 2020.

The carbon isotope ratio of serum alanine predicts added sugar intake in a controlled feeding study of US postmenopausal women. Hee Young Yun, Lesley F. Tinker, Marian L. Neuhouser, Dale A. Schoeller, Yasmin Mossavar-Rahmani, Linda Snetselaar, Linda V. Van Horn, Charles B. Eaton, Ross L. Prentice, Johanna W. Lampe, Diane M. O'Brien. Accepted for podium presentation at the 2020 International Conference on Diet and Physical Activity Assessment Methods, The Netherlands. Conference cancelled due to COVID-19

Total energy expenditure measured using doubly labelled water compared with estimated energy requirements in older adults. Judi Porter, Kay Nguo; Helen Truby; Catherine Huggins; Jorja Collins; Nicole Kellow; Simone Gibson; Zoe Davidson; Dale Schoeller; Linda G. Snetselaar; Marian Neuhouser; Ross Prentice. Accepted for oral presentation at ISBNPA (International Society for Behavioral Nutrition and Physical Activity) 2020, Auckland, NZ. Conference cancelled due to COVID-19.

Health and lifestyle factors associated with metabolism. Navarro S. Presented at the University of Washington Northwest Metabolomics Research Center/Nutrition and Obesity Research Center Symposium. October 7, 2022.

Demographic, Health, and Lifestyle Factors Associated with the Metabolome (Abstract 1521). Navarro S. Presented at the 19th Annual Conference of the Metabolomics Society, Niagara Falls, Canada, June 18-22, 2023.

Beyond Traditional Nutrition Markers for Assessing Dietary Quality and Chronic Disease Risk. Lampe JW. Presented at the US NASEM Food Forum Dietary Patterns and Diet-Related Chronic Diseases Across the Lifespan, Washington, DC, August 15-16, 2023.

Developing Metabolomic Biomarkers of Dietary Intake for Personalized Health, 7th Munich Metabolomics Meeting, Nov 13-14, 2020, (online).

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