

## **Biomarker-calibrated intake estimates of energy, protein and protein density from the WHI food frequency questionnaire (FFQ) – When and How to Apply**

### When to apply:

Apply when intake of energy (kcal/day), protein (g/day), protein density (% energy from protein), sodium, or potassium is the primary exposure.

### How to Apply:

#### Foundational References

- 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 24-hour dietary recall [24HR]) and nutrient of interest (energy, protein, protein density (1, Prentice, 2011 #4130), 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).
- 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 density (2), or sodium, potassium (3)
- Baseline Hormone Trial Baseline. 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.

#### Foundational Data

- Biomarker-estimated intake data for total energy expenditure (TEE), protein (g/day), protein density (% energy from protein), sodium and/or potassium from:
  - NBS when analyzing DM Trial data
  - NPAAS when analyzing OS data.
  - Although using NBS for the DM Trial data and NPAAS for the OS data may be preferable, either NBS or NPAAS may be used.
  - Request these data from the CCC.
- FFQ-assessed energy, protein, protein density data collected during NBS or NPAAS, respectively.

#### Methods

1. Develop the calibration equations. Details of the method are provided in the appendix from Neuhaus et al. (1). The paper describes the basic subject characteristics to include in the regression calibration estimator. Each analysis needs to consider those parameters as well as parameters specific to the outcome of interest. The following list is suggested:
  - a. Subject characteristics as described in Neuhaus et al. (1) for the DM Trial or in Prentice et al. (2) for the OS.
  - b. Covariates and confounders pertinent to the outcome of interest

Then, regress the covariates or confounders on the intake biomarkers as described in (1, 2) 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 TEE (for energy intake) or protein biomarker data (for protein or protein density) may be used, or the calibration equations may be applied.
4. Conduct the outcome analysis, e.g., Cox proportional hazards model. Estimate the standard errors and significance levels (i.e. p-values) for all estimated parameters using bootstrap procedures.
5. Examples of WHI papers. Several WHI published papers describe diet-disease association studies using biomarker-calibrated estimates of intake (5-9). Search the WHI Bibliography ([www.whi.org](http://www.whi.org)) as papers are added upon publication.
6. Evolving methodology: The methodology of the biomarker calibration equations is dynamic and evolving. These guidelines reflect current thinking. The foundational references provide details relative to their time of publication, and thus analyses conducted today may not exactly replicate earlier work.

#### 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 thus 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 Huang (10) and Zheng and colleagues (11). The latter manuscript is available upon request by lead authors of approved paper proposals where biomarker-calibrated estimates of energy, protein or protein density are the primary exposures.
2. Combining OS and DM Trial. Depending on the objectives of the analysis, select the biomarker foundational references and data from the NBS or NPAAS, or combine NBS and NPAAS foundational data to calculate the biomarker calibration equations.

#### References

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