Biomarker-calibrated protein intake and bone health in the Women's Health Initiative clinical trials and observational study[1–3]

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ABSTRACT

Background: The effects of dietary protein on bone health are controversial.

Objective: We examined the relation between protein intake with fracture and bone mineral density (BMD) within the Women's Health Initiative (WHI).

Design: This prospective analysis included 144,580 women aged 50–79 y at baseline in the WHI clinical trials (CTs) and observational study (OS) that recruited participants in 1993–1998 with follow-up through 2011. Self-reported clinical fractures were collected semiannually through the original end of the trials (WHI CTs) and annually (WHI OS) by questionnaires. Hip fracture was adjudicated by a central review of radiology reports. BMDs for total body, hip, and spine were measured at baseline and 3 and 6 y in 9062 women at 3 WHI clinics by using dual-energy X-ray absorptiometry. Protein intake was assessed via food-frequency questionnaire and calibrated by using biomarkers of energy and protein intakes. Associations between protein intake and fracture were estimated by using Cox proportional hazards regression, and the relation between protein intake and BMD was estimated by using linear regression.

Results: Median biomarker-calibrated protein intake was 15% of energy intake. Per 20% increase in calibrated protein intake (percentage of energy), there was no significant association with total fracture (HR: 0.99; 95% CI: 0.97, 1.02) or hip fracture (HR: 0.91; 95% CI: 0.84, 1.00), but there was an inverse association with forearm fracture (HR: 0.93; 95% CI: 0.88, 0.98). Each 20% increase in calibrated protein intake was associated with a significantly higher BMD for total body (mean 3-y change: 0.003 g/cm^2; 95% CI: 0.001, 0.005 g/cm^2) and hip (mean 3-y change: 0.002 g/cm^2; 95% CI: 0.001, 0.004 g/cm^2).

Conclusions: Higher biomarker-calibrated protein intake within the range of usual intake was inversely associated with forearm fracture and was associated with better maintenance of total and hip BMDs. These data suggest higher protein intake is not detrimental to bone health in postmenopausal women. The WHI program was registered at clinicaltrials.gov as NCT00000611. *Am J Clin Nutr* 2014;99:934–40.

INTRODUCTION

The effects of dietary protein intake on bone health are controversial. A supply of protein is required for bone maintenance, and low protein intake has adverse effects on bone health (1). However, high protein intake increases urinary calcium to counteract the acidifying amino acids released after protein digestion, and there has been debate over whether the source of the calcium is bone or increased intestinal absorption (2, 3). If increased intestinal absorption of calcium is the source, higher protein intake may be detrimental to bone if calcium intake is low (4). A systematic review of dietary protein, bone mineral density (BMD)[4], and fracture risk studies reported a positive association between protein intake and BMD and an inverse association with bone resorption markers, but there was no significant association between protein and fracture risk (5). Another systematic review of health effects of protein intake in healthy adults suggested that previous studies that evaluated the association between protein and bone health were often weakened by limited information about the quality of the dietary assessment methods, use of measures that did not include total energy intake, or lack of distinction between animal and vegetable protein sources (6). Studies that have a large number of events with sufficient follow-up are needed to discern whether the positive association observed with BMD translates into a long-term benefit as measured by lower rates of fracture.

An approach for statistically correcting for the measurement error by using biomarkers for total energy and protein has been

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[4] Abbreviations used: BMD, bone mineral density; CT, clinical trial; DM, dietary modification; FFQ, food-frequency questionnaire; OS, observational study; WHI, Women's Health Initiative.

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developed by investigators from the Women's Health Initiative (WHI) (7). This approach provides an opportunity to examine associations between diet and health outcomes while taking into account the measurement error that attends self-reported protein intake. This prospective analysis examines the role of biomarker-calibrated protein intake in bone health measured by the change in BMD (total, hip, and spine) and incidence of fracture (any, hip, spine, and forearm) in postmenopausal women in the WHI.

SUBJECTS AND METHODS

Study population

The WHI includes an observational study (OS; n = 93,676) and clinical trials (CTs; n = 68,132) of postmenopausal hormone therapy, dietary modification (DM), and calcium and vitamin D supplementation. As previously described, women aged 50–79 y were recruited between 1 October 1993 and 31 December 1998 at 40 clinical centers in the United States (8). This analysis included 144,580 women with follow-up through 2011 in women enrolled in the OS and CT components who reported plausible energy intakes on a food-frequency questionnaire (FFQ) (600–5000 cal/d) and had complete data for model covariates. All protocols were approved by institutional review boards at participating institutions, and all women documented a willingness to participate via signed informed consent forms.

Outcome ascertainment

Fracture

Total fractures were defined as all reported clinical fractures other than those of the ribs, sternum, skull or face, fingers, toes, and cervical vertebrae. Self-reported clinical fractures were collected by questionnaires semiannually through the original end of the CTs and annually thereafter; for the OS, fractures were reported annually. Participants were asked the following question: "Since (last reporting date), has a doctor told you that you had a broken, fractured, or crushed bone?" If the answer was "yes," women were asked to answer the question, "Which bone did you break, fracture, or crush?" by designating *1*) hip, *2*) upper leg (not hip), *3*) pelvis, *4*) knee (patella), *5*) lower leg or ankle, *6*) foot (not toe), *7*) tailbone (coccyx), *8*) spine or back (vertebra), *9*) lower arm or wrist (forearm), *10*) hand (not finger), *11*) elbow, *12*) upper arm or shoulder, or *13*) other (specify). Additional questions were asked regarding whether fractures were diagnosed or treated during an overnight hospital stay and whether an X-ray or imaging scan (MRI) was taken at the same medical facility where fractures were treated. Hip fracture was adjudicated by a central review of radiology reports. Other fracture outcomes (spine, forearm, and any fractures) were centrally adjudicated during the CTs and self-reported otherwise. On average, the agreement between self-reported fracture and medical records was >70% for single-site fractures, with a higher agreement for hip and forearm fractures compared with fractures at other sites (9).

Measurement of BMD

BMDs at the hip, posterior-anterior spine, and total body were measured at baseline and 3 and 6 y at 3 clinical centers (Pittsburgh, PA; Birmingham, AL; and Phoenix and Tucson, AZ) in 9062 women by using dual X-ray absorptiometry with a Hologic QDR densitometer (Hologic Inc). Standard protocols for positioning and analysis were used by trained technicians, and an ongoing quality assurance program was conducted.

Protein exposure

WHI FFQ

All WHI women completed the FFQ at baseline. The self-administered FFQ included 122 items for individual foods and food groups, 19 adjustment items, and summary questions (10). Protein intake was characterized as total intake (g), as a percentage of total kilocalorie intake (percentage of kcal), and relative to body weight (g/kg).

Calibrated protein estimation

As previously described (7), the WHI Nutritional Biomarkers Study was conducted in 2004–2005 to further assess the measurement properties of the FFQ by using objective biomarkers of total energy expenditure (equivalent to energy intake in weight-stable persons) and protein intake. A total of 544 women from the Dietary Modification trial participated in a doubly labeled water protocol to estimate total energy expenditure over a 2-wk period and a urinary nitrogen protocol to estimate protein consumption over a 24-h period to be compared with concurrent self-reported dietary intake data. These results showed that FFQ total energy was considerably underestimated, and protein was modestly underestimated, whereas the percentage of energy from protein was overestimated. Calibration equations were developed separately for energy, protein (g), and the percentage of energy from protein by using a linear regression of log-biomarker estimates on corresponding log-FFQ estimates, BMI (in kg/m^2), age, and other participant characteristics.

Potential confounders

Information on all covariates was obtained by self-report at baseline. Baseline questionnaires ascertained information on race-ethnicity, history of fracture, and current and past smoking. Information was collected by self-report of several physician-diagnosed conditions. BMI was calculated from measured weight divided by height squared. Self-reported leisure physical activity was summarized as metabolic equivalent tasks (11). Dietary intake of calcium was measured by using a semiquantitative FFQ, and total calcium intake was defined as the sum of calcium from diet and supplements.

Energy intake was estimated from the FFQ and calibrated by using regression equations (7). Dietary supplement use was assessed by using an inventory-type questionnaire in which study staff recorded nutrients from participants' bottles brought to a clinic visit. Smoking status was classified as current, past, or never. Postmenopausal hormone therapy was categorized as current, past, or never use of any estrogen with or without progestin.

Statistical analysis

Characteristics of women by quintile of calibrated protein intake (ie, calibrated percentage of calories from protein) at baseline were compared by using chi-square tests (for categorical

TABLE 1
Baseline characteristics by calibrated protein intake (calibrated percentage of calories from protein) in the WHI (n = 144,580)[1]

Characteristic	Quintile 1 (<13.3%)	Quintile 3 (14.2–14.8%)	Quintile 5 (≥15.6%)
Age (y)	66.0 ± 7.2[2]	63.7 ± 6.9	59.6 ± 6.4
BMI			
Underweight (<18.5 kg/m^2)	262 (0.9)	200 (0.7)	332 (1.1)
Normal (18.5–24.9 kg/m^2)	8250 (28.7)	9656 (33.7)	12,357 (42.5)
Overweight (25.0–29.9 kg/m^2)	9481 (33.0)	10,057 (35.0)	10,242 (35.3)
Obese (≥30 kg/m^2)	10,780 (37.5)	8781 (30.6)	6121 (21.1)
Ethnicity			
White	22,283 (77.2)	24,308 (84.7)	24,725 (85.1)
Black	4149 (14.4)	2128 (7.4)	1731 (6.0)
Hispanic	1110 (3.9)	981 (3.4)	1189 (4.1)
American Indian	157 (0.5)	99 (0.3)	116 (0.4)
Asian/Pacific Islander	676 (2.3)	781 (2.7)	902 (3.1)
Unknown	458 (1.6)	397 (1.4)	389 (1.3)
Family history of fracture	9834 (34.2)	10,657 (37.1)	11,162 (38.4)
History of fracture (at age ≥55 y)	4473 (15.5)	3778 (13.2)	2498 (8.6)
Calibrated energy intake (kcal)[3]	2122 ± 233	2143 ± 214	2143 ± 171
Physical activity (METs/wk)	9.9 ± 12.6	12.6 ± 13.6	15.0 ± 14.9
Smoking			
Never	12,116 (42.1)	15,368 (53.6)	15,538 (53.5)
Past	10,050 (34.9)	12,544 (43.7)	13,364 (46.0)
Current	6607 (23.0)	782 (2.7)	150 (0.5)
Hormone use			
Never	14,687 (51.0)	12,244 (42.7)	10,903 (37.5)
Past	5083 (17.7)	4519 (15.7)	4092 (14.1)
Current	8981 (31.2)	11,902 (41.5)	14,033 (48.3)
Corticosteroid use	299 (1.0)	227 (0.8)	200 (0.7)
Glucocorticoid use	294 (1.0)	215 (0.8)	199 (0.7)
General health status			
Excellent/very good	14,309 (49.7)	16,891 (58.9)	19,226 (66.2)
Good	10,868 (37.8)	9466 (33.0)	7942 (27.3)
Fair/poor	3596 (12.5)	2337 (8.1)	1884 (6.5)
Medical history			
Arthritis	15,199 (52.8)	13,823 (48.2)	11,718 (40.3)
Rheumatoid arthritis	1791 (6.2)	1412 (4.9)	1155 (4.0)
Diabetes (treated with pills or shots)	1176 (4.1)	1287 (4.5)	1299 (4.5)

[1] $P < 0.0001$ for all baseline characteristics across quintiles of calibrated protein intake. P-value testing did not include participants who were not randomly assigned to the trial. MET, metabolic task hours; WHI, Women's Health Initiative.

[2] Mean ± SD (all such values).

[3] All values are geometric means ± SDs, because calibrated energy was back-transformed from the log scale.

variables) or ANOVA (for continuous variables). HRs for fracture per 20% difference in calibrated protein intake were computed from Cox proportional hazards survival models for each fracture outcome. SEs were estimated from a bootstrap procedure (1000 replicates), whereby the nutrient intake–calibration equations were refitted for each bootstrap sample.

Women contributed follow-up time until the occurrence of fracture, death, or end of follow-up, whichever came first. Models were stratified on the WHI component participation (ie, CT treatment arm; OS) and were adjusted for age, race-ethnicity, BMI, general health, physical activity, history of fracture at age ≥55 y, history of parental fracture, current smoking, hormone therapy use, corticosteroid use, glucocorticoid use, treated diabetes, and rheumatoid arthritis. For the survival modeling, the proportional hazards assumption was evaluated by examining plots of the baseline hazard as a function of the exposure variables of interest as well as by testing an interaction term of protein intake by the log follow-up time.

Linear regressions were used to assess the association of baseline BMD with protein intake as well as baseline, follow-up, and annualized changes in BMD according to protein intake. Mean BMDs by protein intake are presented with SEs estimated from a bootstrap procedure, as previously described.

The analysis was conducted in the combined CT and OS cohorts. As published by Howard et al (12), the Dietary Modification intervention significantly increased self-reported total dietary protein in the active intervention group. There was no effect of the DM intervention on fracture incidence, a small decrease in bone density, and an interaction between DM and hormone therapy intervention, whereby women assigned to the active intervention in both trials had a greater reduction in the occurrence of fracture (13). Thus, we first examined associations

TABLE 2
Risk of fracture per 20% increase in daily biomarker-calibrated protein in the WHI (n = 144,580)[1]

Fracture site	No. of events	HR (95% CI)
Any fracture	36,166	0.99 (0.97, 1.02)
Hip	3286	0.91 (0.84, 1.00)
Spine	4836	1.05 (0.98, 1.13)
Forearm	7800	0.93 (0.88, 0.98)

[1] HRs were derived from Cox proportional hazard regression models adjusted for age, BMI, race-ethnicity, calibrated energy intake, general health, physical activity, history of fracture at age ≥55 y, history of parental fracture, current smoking, corticosteroid use, glucocorticoid use, treated diabetes, rheumatoid arthritis, and hormone use. WHI, Women's Health Initiative.

between calibrated protein intake and fracture and bone density within the CTs and OS separately and tested for an interaction (all $P > 0.05$) before combining cohorts. All analyses were conducted with SAS statistical software (version 9.3; SAS Institute Inc).

Analyses to examine the effect modification by key variables (age, BMI, race-ethnicity, and calcium intake) were conducted to determine whether associations between protein use and fracture or the change in BMD were apparent in key subgroups of women. Statistical tests for interactions were conducted for each of these variables to determine whether any stratum-specific differences were strong enough to interpret as potentially important.

RESULTS

Median calibrated protein intake was 15% of energy intake. Women who consumed a lower proportion of their calories from protein were more likely to be older, obese, nonwhite, have a personal, but not family, history of fracture, engage in less physical activity, be current smokers, report a lower health status, and have a history of arthritis (all $P < 0.0001$) (**Table 1**). The annualized incidence of clinical fracture was 2.6% for any fracture, 0.21% for hip fracture, 0.30% for spinal fracture, and 0.50% for forearm fracture.

Women who consumed 20% higher calibrated protein intake (percentage of energy) were 7% less likely to have a forearm fracture (95% CI: 2%, 12%), but there were no significant associations with any, hip, or spinal fractures (**Table 2**). When associations by quintiles and in other units (g/d and g · kg body weight^{-1} · d^{-1}) were examined, associations differed in magnitude but remained consistent in the overall directionality (data not shown).

The directionality of associations by site were similar for BMD compared with fracture (**Table 3**). An increase in calibrated protein intake was associated with a significantly higher BMD (Table 3). Women who consumed 20% higher protein showed more positive changes in total BMD (0.004 g/cm^2; 95% CI: 0.001, 0.007 g/cm^2) after 6 y follow-up (Table 3). There were no longitudinal associations between protein intake and spine BMD. There were no significant interactions by race-ethnicity or calcium intake (data not shown). There was a significant interaction in the association between calibrated protein intake and risk of any fracture by BMI (**Table 4**). The strongest inverse associations between calibrated protein intake and any fracture risk was in women who had lower BMI [HR for women with BMI of 18.5 was 0.95 (95% CI: 0.90, 1.00) compared with 1.02 (95% CI: 0.98, 1.07) in women with BMI of 35); Table 4]. For BMD, there were no significant tests for interaction, and none of the subgroup analyses were significant (**Table 5**).

DISCUSSION

Data from this large, long-term study of postmenopausal women suggested that women who consumed more protein did not have a higher risk of fracture or lower BMD than do women who consumed less protein, irrespective of the bone site measured. Rather, a 20% higher protein intake was associated with 7% lower risk of forearm fracture (95% CI: 2%, 12%). Higher protein intake was also significantly associated with higher baseline BMD overall and at the hip and spine sites. Women who consumed greater protein intake were more likely to preserve BMD over time as well.

The inclusion of 36,166 fractures (including 3 286 hip fractures) over more than a decade of study provided us with the unique opportunity to substantially augment data on the relation between protein intake and fracture. Previous studies with fracture as the outcome in women aged >50 y reported inconsistent results, with some studies of higher protein intake reporting an increased risk of fracture (4, 14), whereas others studies showed a decreased risk (15, 16). The meta-analysis including 4 studies reported no significant effect for protein and fracture risk (RR :0.75; 95% CI: 0.47, 1.21) (5). However, because of the magnitude and duration of this study, the preponderance of evidence suggested that, if higher protein has any impact on fracture risk, it results in slightly reduced risk.

Studies of the association between protein intake and BMD also reported inconsistent results, with some studies that showed beneficial associations (17, 18), other studies that reported inconsistent associations (19), and other studies that found adverse associations (20). The systematic review including 61 studies reported a small beneficial association between total protein intake and BMD, estimating that the proportion of BMD attributable to

TABLE 3
Change in mean BMD per 20% increase in the calibrated percentage of calories from protein in the WHI BMD Cohort[1]

BMD site	n	BMD
		g/cm^2
Total body		
Baseline	9062	0.009 (0.004, 0.016)[2]
3-y – baseline Δ	7440	0.003 (0.001, 0.005)
6-y – baseline Δ	6522	0.004 (0.001, 0.007)
Hip		
Baseline	9062	0.010 (0.005, 0.017)
3-y – baseline Δ	7489	0.002 (0.001, 0.004)
6-y – baseline Δ	6553	0.003 (0.000, 0.005)
Spine		
Baseline	9062	0.014 (0.006, 0.023)
3-y – baseline Δ	7499	0.003 (0.000, 0.006)
6-y – baseline Δ	6457	0.003 (0.000, 0.008)

[1] Means were estimated from linear regression models adjusted for age, BMI, race-ethnicity, calibrated energy intake, general health, physical activity, history of fracture at age ≥55 y, history of parental fracture, current smoking, corticosteroid use, glucocorticoid use, treated diabetes, rheumatoid arthritis, and hormone use. BMD, bone mineral density; WHI, Women's Health Initiative.

[2] Mean; 95% CI in parentheses (all such values).

TABLE 4
Risk of any and hip fracture per 20% increase in calibrated protein intake (percentage of kcal) in the WHI at subgroup levels[1]

Outcome	Any fracture	*P*-interaction	Hip fracture	*P*-interaction
Overall	0.99 (0.97, 1.02)	—	0.91 (0.84, 1.00)	—
Age at baseline		0.106		0.429
55 y	1.02 (0.96, 1.07)		0.90 (0.75, 1.05)	
65 y	0.99 (0.96, 1.01)		0.91 (0.82, 0.99)	
75 y	0.96 (0.91, 1.02)		0.92 (0.83, 1.02)	
BMI		0.035		0.191
18.5 kg/m^2	0.95 (0.90, 1.00)		0.87 (0.74, 1.00)	
25.0 kg/m^2	0.98 (0.95, 1.00)		0.91 (0.83, 0.99)	
30.0 kg/m^2	1.00 (0.97, 1.03)		0.94 (0.85, 1.05)	
35.0 kg/m^2	1.02 (0.98, 1.07)		0.97 (0.83, 1.15)	

[1] All values are HRs; 95% CIs in parentheses. HRs were derived from Cox proportional hazard regression models adjusted for age, BMI, race-ethnicity, calibrated energy intake, income, general health, physical activity, history of fracture at age ≥55 y, history of parental fracture, current smoking, corticosteroid use, glucocorticoid use, treated diabetes, rheumatoid arthritis, and hormone use and calculated at the subgroup point of interest. WHI, Women's Health Initiative.

protein was 1–2% (5). A weight-loss feeding study in middle-aged adults showed that a high-protein diet (1.4 g · kg^{-1} · d^{-1}) with 3 dairy servings/d attenuated bone loss relative to a diet consistent with the current Recommended Dietary Allowance for protein (0.8 g · kg^{-1} · d^{-1}) during both weight loss (4 mo) and the maintenance of weight loss (8 mo) (21).

The protein source (ie, animal or vegetable) may influence protein's effect on bone health. Studies that have investigated the role of protein source on bone health have been conducted primarily in postmenopausal women and reported disparate findings. In a cohort of adults aged ≥55 y, higher animal protein intake was associated with higher BMD, whereas vegetable protein intake was inversely correlated with BMD (22). Another study showed no overall association between protein intake and fracture risk but did see a trend toward increased fracture risk with increased intake of animal protein (23). A 2008 study in older women showed increased odds of osteoporosis for total protein but a decrease in odds with increased vegetable protein intake (24). An investigation of postmenopausal women in a large cohort study (the European Prospective Investigation into Cancer and Nutrition, Potsdam) showed an inverse association between increased animal protein and bone structure assessed by ultrasound but a positive association with higher vegetable-protein intake (25).

Because we lacked a biomarker for the protein source (animal compared with vegetable), we were unable to correct for the measurement error in self-reported intake by source. Because significant associations were only observed after we corrected for the measurement error in total protein intake, our analyses focused on total, rather than the type, of protein intake.

Limitations should be considered in interpreting our findings. The FFQ had considerable measurement error and, thus, may have substantially attenuated diet-disease associations (26). However, by using a biomarker of total protein intake, we were able to include a correction for the measurement error in self-reported diet. Also notable is that protein intake did not vary across the entire recommended range of 10–35% of energy intake. Thus, although these inferences applied to typical protein

TABLE 5
Three-year change in total body and hip BMD per 20% increase in calibrated protein intake (percentage of kcal) in the WHI BMD Cohort at subgroup levels[1]

Outcome	Total body	*P*-interaction	Hip	*P*-interaction
	g/cm^2		*g/cm^2*	
Overall	0.003 (0.001, 0.005)	—	0.002 (0.001, 0.005)	—
Age at baseline		0.334		0.154
55 y	0.002 (0.000, 0.005)		0.002 (−0.001, 0.004)	
65 y	0.003 (0.001, 0.005)		0.003 (0.001, 0.005)	
75 y	0.003 (0.000, 0.007)		0.004 (0.001, 0.007)	
BMI		0.496		0.118
18.5 kg/m^2	0.002 (0.000, 0.007)		0.000 (−0.002, 0.004)	
25.0 kg/m^2	0.003 (0.001, 0.005)		0.002 (0.000, 0.004)	
30.0 kg/m^2	0.003 (0.001, 0.005)		0.003 (0.001, 0.005)	
35.0 kg/m^2	0.003 (0.000, 0.006)		0.004 (0.001, 0.007)	

[1] All values are means; 95% CIs in parentheses. Estimates were derived from linear regression models adjusted for age, BMI, race-ethnicity, calibrated energy intake, income, general health, physical activity, history of fracture at age ≥55 y, history of parental fracture, current smoking, corticosteroid use, glucocorticoid use, treated diabetes, rheumatoid arthritis, and hormone use and calculated at the subgroup point of interest. BMD, bone mineral density; WHI, Women's Health Initiative.

intake in the population, data were not available to evaluate lower and upper bounds of recommended ranges of intake. The study population was predominantly non-Hispanic white, and thus, our findings may not be generalizable to other racial-ethnic groups with differences in bone metabolism.

Strengths of the current study included the large sample size of postmenopausal women, which allowed us to examine associations between dietary intake and bone health over more than a decade of follow-up. The excellent follow-up of fracture incidence and longitudinal measures of BMD as measured by dual X-ray absorptiometry provided us with the opportunity to accurately and precisely detect changes in bone health over time. Data were collected on multiple exposures related to bone health in addition to biomarker-calibrated energy and protein intake, such as physical activity and smoking, and these factors were accounted for in the analysis.

In conclusion, data from this large cohort study of postmenopausal women provide evidence that protein intake in the upper range of typical consumption in the United States does not negatively affect bone mass in postmenopausal women. Additional studies in populations consuming protein in the upper end of the recommended range (25–35% of energy from protein) could inform whether higher protein intake contributes to better health outcomes in older women.

A short list of WHI investigators can be found in **Appendix A**. For a list of all of the investigators who have contributed to WHI science, please visit https://cleo.whi.org/researchers/SitePages/Write%20a%20Paper.aspx.

The authors' responsibilities were as follows—JMB and AZL: designed the research; MLN, LFT, RJ, KCJ, AZL, and RLP: conducted the research; JCL and YH: analyzed data and provided statistical expertise; JMB, LS, and CBE: wrote the manuscript; JMB: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. None of the authors declared a conflict of interest.

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APPENDIX A

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