**Supplementary Appendix:**

Randomization and Follow-up:

In the double-blind, randomized, placebo-controlled VITamin D and OmegA-3 TriaL (VITAL), individuals were randomized to interventions between November 2011 and March 2014 using a computer-generated table of random numbers. Randomization was generated within sex, race, and 5-year age groups in blocks of eight individuals, with two individuals in each of the four treatment combinations.

In the bone health subcohort, baseline DXA scans took place between November 2012 and March 2014, and baseline pQCT scans took place between January 2013 and March 2014. Two-year follow-up scans occurred between October 2014 and July 2016.

Eligibility Criteria:

Participants in this sub-study were enrolled from the New England area from the overall VITAL cohort. In the overall cohort from which this subcohort was formed there was an attrition of 65% during the placebo run-in. In order to be eligible for the trial randomization, participants had to: (1) demonstrate good compliance in pill taking, defined as taking ≥2/3 of the study pills during the run-in; (2) report no new history of cancer, MI, stroke, TIA, angina pectoris, CABG, PCI, hypercalcemia, sarcoidosis, or other serious illness during the run-in; and (3) remain willing to comply with limits on non-study use of supplemental vitamin D and calcium and fish oil.

Participants were eligible for this bone health ancillary study if they were not on bone active medications including bisphosphonates within the past 2 years or denosumab, teriparatide, calcitonin, raloxifene, tamoxifen, or systemic estrogens within the past year..

Serum measures:

Total 25(OH)D and plasma phospholipid omega-3 fatty acids were measured by liquid chromatography tandem mass spectrometry (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA). Calcium was measured by spectrophotometry (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA). Albumin was measured using the Beckman Coulter Clinical Chemistry AU analyzer (Quest Diagnostics Nichols Institute, Chantilly, VA). Future Diagnostics developed the FVD ELISA assay in collaboration with DIAsource ImmunoAssays S.A. This is a competitive ELISA two‐step immunoassay based on patented monoclonal antibody and available at DIAsource. To minimize potential batch effects, all baseline and year 2 sample pairs were shipped and measured at the same time in tandem, in a manner blinded to the analyzing labs. Quest Diagnostics performed the 25(OH)D, calcium, and omega-3 measurements at no cost to the trial. Other than the blinded assay analyses, Quest Diagnostics and Future Diagnostics were not involved in the study design, data analysis, or manuscript preparation. VITAL investigators took part in the vitamin D standardization program of the Centers for Disease Control and Prevention.58

DXA:

Least significant change (LSC) is 0.024 g/cm2 at the spine, 0.021 g/cm2 at the femoral neck, 0.017 g/cm2 at the total hip, and 0.008 g/cm2 for males and 0.010 g/cm2 for females at the whole body. At baseline, the whole body bone measurements of 27 participants were excluded due to metal artifacts; an additional 29 were excluded due to new metal at year 2. When the non-dominant hip was unavailable due to metal artifact(s), the contralateral hip was used. In patients with severe osteoarthritis at the hip, femoral neck measures were excluded at baseline (n=5).59 There were no new cases of severe hip osteoarthritis in the non-dominant hip at 2-year follow-up. A total of 51 spine aBMD measures were excluded because of spinal metal, severe scoliosis and/or degenerative disc disease. At year 2, there were an additional 10 spine aBMD measures excluded. There were two participants who had bilateral breast implants who were excluded from whole body bone density and body composition analyses due to uncertain effects on bone density, fat and lean mass. For the DXA measures, default sex and ethnicity-matched Hologic APEX Software 4.2 databases were used to generate T-scores, and age, sex, and ethnicity-matched results from the same databases were used to generate Z-scores.60 Hispanic white participants were compared to the Hispanic database61 and American Indians and Alaskan Natives were compared to the white database.62

pQCT:

Precision at our site is very good with % CV ranging from 0.02% to 2.87% at the radius and tibia.63 Voxel size was 0.40 mm, slice thickness was 2.2 mm, and scan speed was 20 mm/s. Total and trabecular vBMD and bone strength index (BSI) were obtained at the 4% radius and tibia sites using contour mode 3 at 169 mg/cm3. BSI was calculated as the product of total area and the square of total vBMD. Cortical vBMD, cortical thickness, and polar stress strength index (SSI), the product of the torsional axis and the ratio of measured cortical density to physiologic bone density, were obtained at the 33% radius site and the 38% tibia site using cortical contour mode 2. Two thresholds were applied at the cortical sites: cortical density and area related results used 710 mg/cm3, while strength related results used 480 mg/cm3.

**Supplemental Table 1. aBMD From Baseline to Year 2**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Vitamin D**3 **Group (n=388)** | | **Placebo Group  (n=383)** | |  |
| **Bone Measures** | **N** | **Mean (SD)** | **N** | **Mean (SD)** | **P-Value** |
| Spine aBMD |  |  |  |  |  |
| Baseline | 368 | 1.022 (0.161) | 352 | 1.022 (0.169) |  |
| Year 2 | 327 | 1.028 (0.164) | 307 | 1.028 (0.171) |  |
| % Change | 326 | 0.33% | 307 | 0.17% | 0.55 |
| Femoral Neck Hip aBMD |  |  |  |  |  |
| Baseline | 380 | 0.767 (0.129) | 381 | 0.765 (0.125) |  |
| Year 2 | 336 | 0.765 (0.131) | 339 | 0.766 (0.124) |  |
| % Change | 336 | -0.27% | 339 | -0.68% | 0.16 |
| Total Hip aBMD |  |  |  |  |  |
| Baseline | 384 | 0.936 (0.145) | 381 | 0.934 (0.149) |  |
| Year 2 | 340 | 0.931 (0.150) | 339 | 0.935 (0.148) |  |
| % Change | 340 | -0.76% | 339 | -0.95% | 0.23 |
| Whole Body aBMD |  |  |  |  |  |
| Baseline | 371 | 1.152 (0.131) | 369 | 1.146 (0.130) |  |
| Year 2 | 310 | 1.154 (0.132) | 318 | 1.153 (0.127) |  |
| % Change | 310 | -0.22% | 318 | -0.15% | 0.60 |

All analyses adjusted for age, sex, and race

**Supplemental Table 2. Absolute 2-year Change in aBMD according to Subgroup, Comparing the Vitamin D3 Group with the Placebo group**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Subgroup** | **Femoral neck aBMD** | | | | | |
|  | **Vitamin D3 Group** | | **Placebo Group** | | **P-value** | **P-value for Interaction** |
|  | **N** | **Absolute Change (SD) g/cm2** | **N** | **Absolute Change (SD) g/cm2** |  |  |
| Sex |  |  |  |  |  | 0.21 |
| Female | 150 | -0.004 (0.027) | 150 | -0.011 (0.026) | P= 0.06 |  |
| Male | 186 | -0.000 (0.028) | 189 | -0.001 (0.029) | P= 0.82 |  |
| Low Bone Density |  |  |  |  |  | 0.81 |
| Normal | 112 | -0.002 (0.032) | 118 | -0.006 (0.030) | P= 0.29 |  |
| Osteopenia/ Osteoporosis | 214 | -0.002 (0.025) | 206 | -0.005 (0.027) | P= 0.310 |  |
| Race |  |  |  |  |  | 0.54 |
| Non-Hispanic White | 278 | -0.003 (0.028) | 277 | -0.006 (0.026) | P= 0.18 |  |
| Black | 27 | 0.003 (0.026) | 28 | -0.005 (0.035) | P= 0.32 |  |
| BMI (median) |  |  |  |  |  | 0.65 |
| < Median (26.45 kg/m2) | 171 | -0.002 (0.024) | 166 | -0.004 (0.026) | P= 0.39 |  |
| ≥ Median (26.45 kg/m2) | 165 | -0.002 (0.031) | 173 | -0.006 (0.029) | P= 0.26 |  |
| FMI |  |  |  |  |  | 0.51 |
| < Median (9.42 kg/m2) | 181 | -0.001 (0.023) | 161 | -0.002 (0.027) | P= 0.49 |  |
| ≥ Median (9.42 kg/m2) | 152 | -0.003 (0.031) | 178 | -0.008 (0.029) | P= 0.21 |  |
| Vitamin D supplement use at baseline ≤800 IU/day |  |  |  |  |  | 0.30 |
| Yes | 141 | -0.000 (0.025) | 148 | -0.006 (0.028) | P= 0.098 |  |
| No | 195 | -0.003 (0.029) | 191 | -0.004 (0.028) | P= 0.62 |  |
| Calcium supplement use at baseline ≤1200 mg/day |  |  |  |  |  | 0.10 |
| Yes | 59 | 0.002 (0.023) | 54 | -0.009 (0.028) | **P= 0.029** |  |
| No | 277 | -0.003 (0.028) | 285 | -0.005 (0.028) | P= 0.53 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.889 |
| <75 nmol/L | 213 | -0.002 (0.028) | 183 | -0.004 (0.028) | P=0.35 |  |
| ≥75 nmol/L | 122 | -0.003 (0.026) | 156 | -0.006 (0.028) | P=0.36 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.36 |
| < Median (70 nmol/L) | 182 | -0.002 (0.028) | 148 | -0.002 (0.027) | P= 0.63 |  |
| ≥ Median (70 nmol/L) | 153 | -0.003 (0.026) | 191 | -0.007 (0.029) | P= 0.19 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.55 |
| <50 nmol/L | 61 | -0.001 (0.035) | 60 | -0.001 (0.029) | P= 0.78 |  |
| ≥50 nmol/L | 274 | -0.002 (0.025) | 279 | -0.006 (0.028) | P= 0.15 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.52 |
| <37 nmol/L | 30 | -0.001 (0.030) | 20 | 0.002 (0.034) | P= 0.82 |  |
| ≥37 nmol/L | 305 | -0.002 (0.027) | 319 | -0.006 (0.028) | P= 0.17 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.99 |
| <30 nmol/L | 13 | 0.003 (0.031) | 10 | 0.000 (0.036) | P=0.81 |  |
| ≥30 nmol/L | 322 | -0.002 (0.027) | 329 | -0.005 (0.028) | P=0.18 |  |
| Baseline FVD |  |  |  |  |  | 0.35 |
| < Median 14.2 pmol/L | 172 | -0.002 (0.029) | 159 | -0.003 (0.028) | P= 0.59 |  |
| ≥ Median 14.2 pmol/L | 163 | -0.002 (0.025) | 180 | -0.007 (0.028) | P= 0.15 |  |
| **Subgroup** | **Whole Body aBMD** | | | | | |
|  | **Vitamin D3 Group** | | **Placebo Group** | | **P-value** | **P-value for Interaction** |
|  | **N** | **Absolute Change (SD) g/cm2** | **N** | **Absolute Change (SD) g/cm2** |  |  |
| Sex |  |  |  |  |  | 0.67 |
| Female | 135 | -0.007 (0.025) | 136 | -0.008 (0.024) | P= 0.97 |  |
| Male | 175 | 0.001 (0.022) | 182 | 0.003 (0.022) | P= 0.45 |  |
| Low Bone Density |  |  |  |  |  | 0.46 |
| Normal | 104 | 0.000 (0.027) | 111 | -0.001 (0.023) | P= 0.63 |  |
| Osteopenia/ Osteoporosis | 196 | -0.004 (0.022) | 193 | -0.002 (0.023) | P= 0.45 |  |
| Race |  |  |  |  |  | 0.15 |
| Non-Hispanic White | 259 | -0.003 (0.024) | 262 | -0.003 (0.024) | P= 0.86 |  |
| Black | 23 | 0.000 (0.027) | 25 | 0.010 (0.020) | P= 0.13 |  |
| BMI (median) |  |  |  |  |  | 0.99 |
| < Median (26.45 kg/m2) | 164 | -0.004 (0.022) | 159 | -0.003 (0.023) | P= 0.72 |  |
| ≥ Median (26.45 kg/m2) | 146 | -0.001 (0.025) | 159 | -0.000 (0.023) | P= 0.75 |  |
| FMI |  |  |  |  |  | 0.94 |
| < Median (9.42 kg/m2) | 174 | -0.003 (0.021) | 158 | -0.002 (0.022) | P= 0.64 |  |
| ≥ Median (9.42 kg/m2) | 136 | -0.002 (0.027) | 160 | -0.002 (0.025) | P= 0.81 |  |
| Vitamin D supplement use at baseline ≤800 IU/day |  |  |  |  |  | 0.79 |
| Yes | 132 | -0.002 (0.022) | 139 | -0.000 (0.025) | P= 0.56 |  |
| No | 178 | -0.003 (0.025) | 179 | -0.003 (0.022) | P= 0.89 |  |
| Calcium supplement use at baseline ≤1200 mg/day |  |  |  |  |  | 0.24 |
| Yes | 56 | -0.007 (0.024) | 49 | -0.002 (0.023) | P= 0.24 |  |
| No | 254 | -0.002 (0.023) | 269 | -0.002 (0.023) | P= 0.97 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.81 |
| <75 nmol/L | 196 | -0.002 (0.024) | 173 | -0.001 (0.021) | P=0.79 |  |
| ≥75 nmol/L | 113 | -0.004 (0.023) | 145 | -0.003 (0.026) | P=0.54 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.75 |
| < Median (70 nmol/L) | 166 | -0.002 (0.025) | 140 | 0.000 (0.020) | P= 0.51 |  |
| ≥ Median (70 nmol/L) | 143 | -0.004 (0.022) | 178 | -0.003 (0.026) | P= 0.76 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.12 |
| <50 nmol/L | 54 | -0.006 (0.027) | 55 | 0.001 (0.018) | P= 0.12 |  |
| ≥50 nmol/L | 255 | -0.002 (0.023) | 263 | -0.002 (0.024) | P= 0.90 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.91 |
| <37 nmol/L | 28 | 0.002 (0.025) | 19 | 0.002 (0.019) | P= 0.97 |  |
| ≥37 nmol/L | 281 | -0.003 (0.023) | 299 | -0.002 (0.024) | P= 0.53 |  |
| Baseline Total 25(OH)D Level |  |  |  |  |  | 0.43 |
| <30 nmol/L | 12 | 0.001 (0.031) | 10 | 0.011 (0.020) | P=0.40 |  |
| ≥30 nmol/L | 297 | -0.003 (0.023) | 308 | -0.002 (0.023) | P=0.70 |  |
| Baseline FVD |  |  |  |  |  | 0.32 |
| < Median 14.2 pmol/L | 158 | -0.003 (0.024) | 146 | -0.000 (0.022) | P= 0.28 |  |
| ≥ Median 14.2 pmol/L | 151 | -0.002 (0.024) | 172 | -0.003 (0.025) | P= 0.80 |  |

All analyses adjusted for age, sex, and race

**Supplemental Table 3. Two Year Achieved Total 25(OH)D Levels in the Vitamin D3 group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **aBMD stratified by Achieved Total 25(OH)D** | | | | | |
|  | **< median (97.3 nmol/L)** | | **≥ median (97.3 nmol/L)** | |  |
|  | **N** | **Mean (SD)** | **N** | **Mean (SD)** | **P-value** |
| **Spine aBMD** | | | | | |
| Baseline | 167 | 1.031 (0.161) | 174 | 1.008 (0.161) |  |
| Year 2 | 162 | 1.039 (0.165) | 165 | 1.018 (0.163) |  |
| % Change | 162 | 0.60% | 164 | 0.05% | 0.12 |
| **Femoral Neck aBMD** | | | | | |
| Baseline | 171 | 0.773 (0.129) | 181 | 0.757 (0.128) |  |
| Year 2 | 165 | 0.773 (0.131) | 171 | 0.757 (0.131) |  |
| % Change | 165 | 0.00% | 171 | -0.54% | 0.20 |
| **Total Hip aBMD** | | | | | |
| Baseline | 171 | 0.949 (0.140) | 185 | 0.923 (0.149) |  |
| Year 2 | 165 | 0.947 (0.145) | 175 | 0.917 (0.154) |  |
| % Change | 165 | -0.39% | 175 | -1.11% | 0.02 |
| **Whole Body aBMD** | | | | | |
| Baseline | 167 | 1.158 (0.131) | 176 | 1.148 (0.131) |  |
| Year 2 | 151 | 1.159 (0.131) | 159 | 1.150 (0.133) |  |
| % Change | 151 | -0.18% | 159 | -0.26% | 0.84 |

**Supplementary Table 4. Two Year Achieved Free Vitamin D Levels in the Vitamin D3 group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **aBMD stratified by Achieved Free Vitamin D** | | | | | |
|  | **< median (21.3 pmol/L)** | | **≥ median (21.3 pmol/L)** | |  |
|  | **N** | **Mean (SD)** | **N** | **Mean (SD)** | **P-value** |
| **Spine aBMD** | | | | | |
| Baseline | 175 | 1.043 (0.167) | 166 | 0.994 (0.151) |  |
| Year 2 | 169 | 1.050 (0.172) | 158 | 1.006 (0.153) |  |
| % Change | 169 | 0.53% | 157 | 0.10% | 0.22 |
| **Femoral neck aBMD** | | | | | |
| Baseline | 175 | 0.776 (0.136) | 177 | 0.754 (0.120) |  |
| Year 2 | 169 | 0.773 (0.139) | 167 | 0.757 (0.123) |  |
| % Change | 169 | -0.44% | 167 | -0.09% | 0.33 |
| **Total hip aBMD** | | | | | |
| Baseline | 175 | 0.954 (0.152) | 181 | 0.918 (0.136) |  |
| Year 2 | 169 | 0.949 (0.157) | 171 | 0.914 (0.141) |  |
| % Change | 169 | -0.65% | 171 | -0.87% | 0.49 |
| **Whole body aBMD** | | | | | |
| Baseline | 169 | 1.161 (0.137) | 174 | 1.144 (0.125) |  |
| Year 2 | 155 | 1.165 (0.137) | 155 | 1.144 (0.126) |  |
| % Change | 155 | -0.12% | 155 | -0.32% | 0.39 |

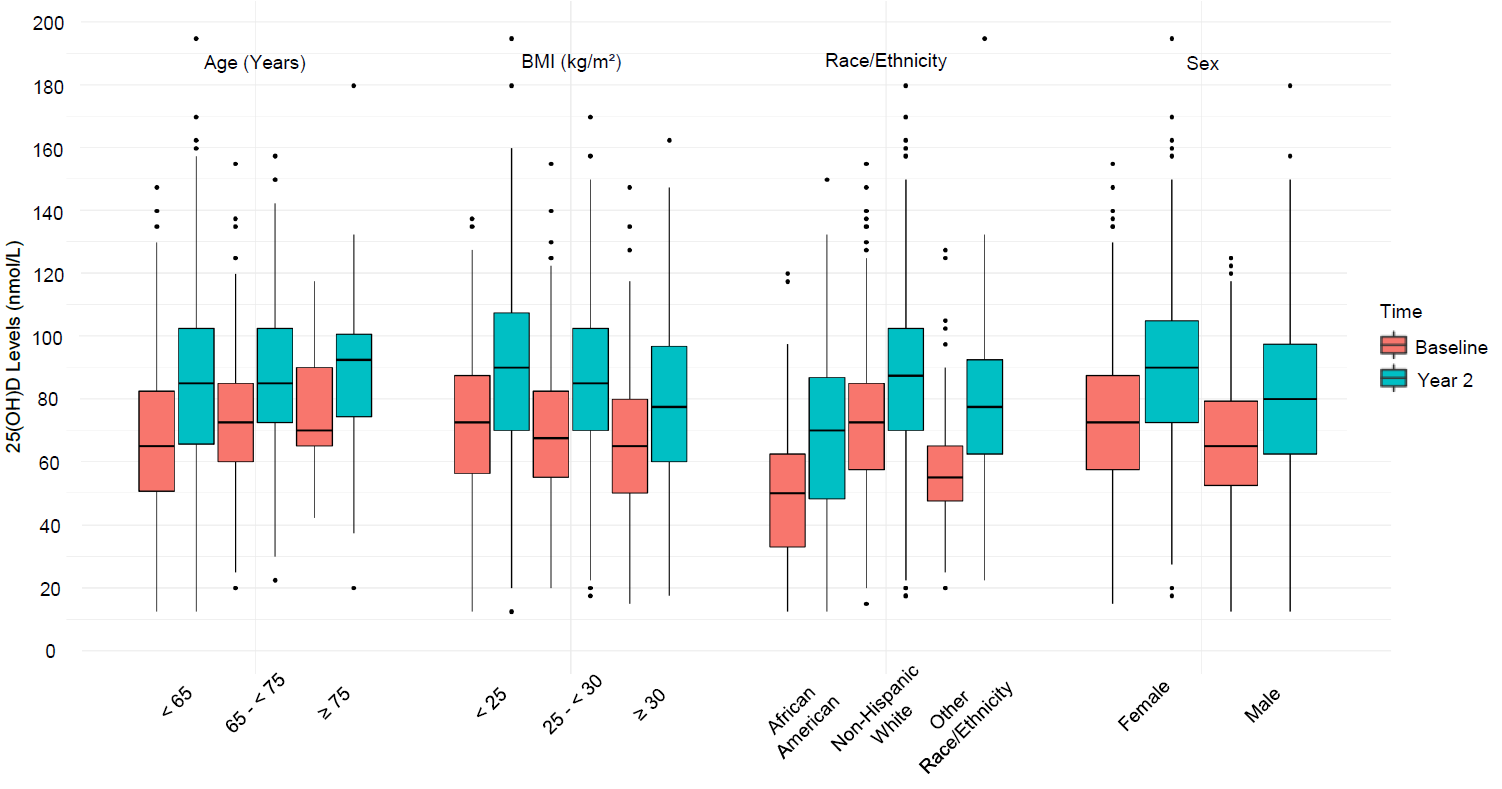
**Supplementary Table 5. Participant-reported adherence with the vitamin D and placebo study pills (percent of pills taken) at time points over 2 years**

1. **Among those answering the compliance question by questionnaire**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Vitamin D Group** | **Placebo Group** |
| **Time** | **N** | **Mean (SD)** | **Mean (SD)** |
| 6 months | 752 | 95.2 (10.5) | 95.2 (9.8) |
| 1 year | 755 | 94.3 (11.7) | 93.2 (14.9) |
| 2 years | 746 | 93.0 (16.2) | 92.1 (17.5) |

1. **Including nonrespondents to questionnaires and assuming noncompliance among all nonrespondents**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Vitamin D Group** | **Placebo Group** |
| **Time** | **N** | **Mean (SD)** | **Mean (SD)** |
| 6 months | 768 | 92.3 (19.5) | 94.2 (13.8) |
| 1 year | 763 | 93.3 (15.1) | 92.3 (17.6) |
| 2 years | 761 | 91.8 (19.2) | 89.7 (22.7) |



n=127

n=181

n=317

n=35

n=31

n=236

n=136

n=16

n=209

n=179

**Supplementary Figure 1. Mean Baseline and Year 2 Serum 25(OH)D Levels (nmol/L) by Demographic Variables (sex, age, race/ethnicity) and BMI in the bone health subcohort (placebo group is not graphed: <2.5 nmol/L change at year 2)**

Members of the VITAL Research Group

*VITAL Steering Committee:*

JoAnn E. Manson (Chair), Julie E. Buring (Chair), Nancy R. Cook, I-Min Lee, William Christen, Shari S. Bassuk, Samia Mora, Heike Gibson, David Gordon, Trisha Copeland, Denise D’Agostino, Georgina Friedenberg, Claire Ridge, Vadim Bubes, Edward L. Giovannucci, Walter C. Willett (all at Brigham and Women’s Hospital, Harvard Medical School, Boston; Drs. Manson, Buring, Cook, Lee, Giovannucci and Willett are also at the Harvard T.H. Chan School of Public Health).

*Scientific consultants:*

John Baron (University of North Carolina, Chapel Hill)  
Michael Holick (Boston Medical Center)  
Bruce Hollis (University of South Carolina).

*Bone Health Scientific consultants:*

**Consultants on VITAL; Effects on Bone Structure and Architecture (NCT01747447)**

Joel S. Finkelstein (Massachusetts General Hospital, Boston)   
Mary L. Bouxsein (Beth Israel Deaconess Medical Center and Massachusetts General Hospital, Boston)

**Consultants on VITAL: Fracture, Vitamin D, and Genetic Markers (NCT01704859)**

Christopher Gallagher (Creighton University, Omaha)  
Peggy Cawthon (California Pacific Medical Center, San Francisco)

Doug Bauer, Dennis Black (University of California San Francisco, San Francisco)

*Other Members of the VITAL Research Group:*

(Brigham and Women’s Hospital): Christine M. Albert, Diane Gold, Meryl LeBoff, Olivia Okereke, Aruna Pradhan, Howard Sesso, Wendy Chen, Paulette Chandler, J. Michael Gaziano, Olga Demler, Kathryn Rexrode, Karen Costenbader, John Forman, Erik Alexander, Sonia Friedman, Jeffrey Katz, Shumin Zhang, Jennifer Lin, Joseph Walter, Julie Duszlak, Kate Kalan, Jean MacFadyen, Natalya Gomelskaya, David Bates, Ara Sarkissian, Mary Breen, Yeulolani Andrade, Manickavasagar Vinayagamoorthy, Chunying Li,Eunjung Kim, Franco Giulianini, Gregory Kotler, Marty Van Denburgh, Rimma Dushkes, Yanyan Liu, Eduardo Pereira, Lisa Fields-Johnson, George Menjin, Lucy Liu, Lauren Girard, Scott Zeller, Naomi Riches, Katelyn Hasson, Ellen Bhang, Maria Revilla, Elena McCarthy, Alex Moran, Kristen Haise, Leah Arsenault, Philomena Quinn, Sancia Grimes, Ivan Fitchorov, Kurt Schwerin, Shamikhah Curry, Annie Murray, Angela Zhang, Diana Walrond-Williams, Alison Weinberg, Chris Pfeffer, Margarette Haubourg, Viviane Nguyen, Henry Ouellette, Rolando Rodriguez, Tony Montgomery, Keith Morse, Vincent Guzman, Megan Perry, Sandra Weekes, Doug Smith, Allison Clar, Sara Curran, Yaneve Fonge, David Hibbert, Louisa Paine, Kelly Royce, Courtney Splaine, Jennifer McMahon, David Eldridge, Laura Hand, Kay Inandan, Meghan Rieu Werden, Harriet Samuelson, Andrea Hrbek, Megan Mele, Eileen Bowes, Mary Anne Ryan

(Massachusetts General Hospital, Boston): Carlos Camargo, Jacqueline Danik, Ravi Thadhani

(Vanderbilt University, Nashville): Thomas Wang

(Rush University Medical Center, Chicago): Raj C. Shah

(University of California, San Francisco): Michelle A. Albert

(Emory University): Carlos Kase

(Centers for Disease Control and Prevention, Vitamin D Standardization Program): Hubert Vesper and Julianne Botelho.

*Data and Safety Monitoring Board:*

(Voting Members): Nanette Wenger, MD (Chair); Lawrence S. Cohen, MD; Theodore Colton, ScD; Mark A. Espeland, PhD; Craig Henderson, MD; Alice H. Lichtenstein, ScD; and Rebecca A. Silliman, MD, PhD. Ex-officio members include Josephine Boyington, PhD, MPH; Rebecca Costello, PhD; Cindy Davis, PhD; Peter Greenwald, MD; Gabriela Riscuta, MD; and Harold Seifried, PhD.