# STUDY PROTOCOL

**Version 1.9**
July 11, 2017

# Vitamin D and type 2 diabetes

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Principal Investigator: Anastassios G. Pittas, MD MS
NIDDK Project Scientist: Myrlene Staten, MD
NIDDK Program Official: Saul Malozowski, MD

Prepared by the D2d Coordinating Center
Division of Endocrinology, Diabetes, and Metabolism at Tufts Medical Center
800 Washington Street, Box #268 | Boston, MA 02111
www.d2dstudy.org | Facebook/d2dstudy | Twitter/d2dstudy
d2d@tuftsmedicalcenter.org | Tel 617-636-3232

**PROTOCOL SIGNATURE PAGE**

**Version 1.9 – July 11, 2017**

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and all relevant documentation (e.g. Manual of Procedures) to the study personnel that are under my supervision in relation to the D2d study. I will discuss this material with them and ensure that they are fully informed regarding the study procedures and the conduct of the study according to 21 Code of Federal Regulations parts 50, 54, 56 and 312, to Good Clinical Practices as described in International Conference on Harmonization guideline E6, and by Institutional Review Boards.

________________________________________
Investigational Institution(s)

________________________________________ ______________________
Site Principal Investigator Signature Date

________________________________________
Site Principal Investigator Printed Name

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## PROTOCOL CHANGES FROM PRIOR VERSIONS

### Protocol changes to the (DSMB-approved) version 1.8 | date 2015.9.25

8.1, 8.2 Clarifications were made in regards to the End-of-Study (EOS) visit. The last scheduled visit (semi-annual or annual) will serve as the end-of-study visit. At the end-of-study visit, activities will include those that would occur at the scheduled semi-annual (e.g., fasting labs) or annual visit (e.g., OGTT). Thus, the medical examination and physical examination at the EOS visit are replaced with interim medical history.

There is no interim phone call at 6 weeks after the EOS visit.

9.1 Clarifications were made in regards to the assessment for diabetes at the EOS visit. At the EOS visit, the procedures for the scheduled (semi-annual or annual) visit will be completed and the respective algorithm (Figures 9.1.1 and 9.1.2) will be followed to assess for incident diabetes.

11.1 Clarifications were made to Table 1 in regards to the EOS visit.

**Protocol changes to the (DSMB-approved) version 1.7 | date 2015.4.2**

8.1 Text was added to emphasize the importance of site staff educating participants at every visit or follow-up contact, the importance to *contact the site as soon as possible* after a clinician outside of the D2d study makes the diagnosis of diabetes or if the clinician plans to initiate diabetes-specific pharmacotherapy (for any reason).

9.1 Two clarifications were made in the section, *Physician-diagnosis of diabetes or use of diabetes-specific pharmacotherapy between scheduled visits*. Clarifications were made in the adjudication process.

Participants are advised to contact their site, as soon as possible after their health care provider makes the diagnosis of diabetes or if the health care provider plans to initiate diabetes-specific pharmacotherapy, so that participants return to the clinic to test for diabetes before they start any diabetes-specific medication, but only if the participant's provider agrees to withhold use of a diabetes medication and also not initiate pharmacotherapy for pre-diabetes or other diagnoses (e.g., metabolic syndrome), and allow the participant to be followed-up for diabetes progression within D2d, as per protocol. Otherwise, the diagnosis of diabetes or reason for starting a medication will be adjudicated.

If a participant has started diabetes-specific pharmacotherapy but has been exposed to the medication for no more than 31 days (cumulative, since the last D2d scheduled visit e.g., M06, M24) the participant will return for a visit to undergo D2d-specific glycemic testing, following an appropriate washout period (2 or 4 weeks depending on type of medications) provided *the prescribing clinician and participant agree to stop the medication and allow follow-up for diabetes progression within D2d (i.e., by testing analyzed by the Central Lab).* Otherwise, the diagnosis of diabetes or reason for starting a medication will be adjudicated.

The Clinical Outcomes Committee reviewers will review the material to determine whether the outside laboratory results meet glycemic criteria for diabetes. During the adjudication process, the reviewers will follow, as closely as possible, the D2d glycemic algorithms. However, because it is highly unlikely that routine clinical practice will follow the strict D2d algorithms, the Clinical Outcomes Committee should follow the ADA glycemic criteria and use clinical judgment when adjudicating cases of diabetes.

**Protocol changes to the (DSMB-approved) version 1.6 | date 2014.5.1**

6.5 Sites may use any laboratory as their "local" laboratory, with the primary goal being to maximize the correlation between the Hemoglobin A1c and Fasting Plasma Glucose values obtained at screening and those obtained by the D2d Central Laboratory (LCBR at University of Vermont) at the baseline visit. Towards that goal, sites may elect to use the LCBR in lieu of their "local" laboratory at the screening visit.

7.4 A clarification was made to indicate that if a participant is unable to come for a semi-annual visit (e.g., month 18, month 30) and had a normal safety assessment done at the last annual visit, the semi-annual visit may be conducted over the phone and, provided there are no safety concerns, a 6-month supply of study pills may be shipped to the participant. The participant needs to return for the next annual visit, before any more study pills are dispensed.

Other A few minor language improvements throughout the document were made to improve clarity and internal consistency (e.g. the different age range for specific races is also described in page 30).

**Protocol changes to the (DSMB-approved) version 1.5 | date 2014.1.6**

5 Inclusion/exclusion criteria were modified or clarified as follows:

Criterion 2: Reduced lower age criterion to ≥ 25 years for people of the following races: American-Indian, Alaska Native, Native Hawaiian or Other Pacific Islander.

Criterion 5b: added “at the baseline visit.”

Criterion 6: added “symptomatic and asymptomatic (i.e., by radiographic criteria)” nephrolithiasis.

Criterion 9: Modified criterion to allow for a range (8-12 weeks) in the washout time period for people participants who are taking vitamin D supplements at total doses higher than 1000 IU/day.

Other Various language improvements throughout the document were made to improve clarity and maintain internal consistency of the document.

**Protocol changes to the (DSMB-approved) version 1.4 | date 2013.7.15**

5. Inclusion/exclusion criteria were clarified as follows: BMI range increased (24.0 [22.5 for Asians)] to 42.0 inclusive) [criterion 3]; Volunteers with squamous cell cancer of the skin, which was completely excised and with no evidence of metastases are eligible [criterion 19]. Epidural or intra-articular glucocorticoid injections are not exclusions, but study visits need to be conducted at least a week after the injection. Persons with adrenal insufficiency treated with physiologic doses of glucocorticoids who are otherwise stable are *not* excluded [criterion 20].

6.5 Screening glycemic criteria were modified to allow for sites to develop and use site-specific algorithms to maximize the randomized-screened ratio.

A combined screening/baseline visit is allowed if a potential participant has glycemic tests (FPG and HbA1c), measured at the local laboratory outside of D2d, that meet the screening criteria.

10.1.1 Nausea and vomiting have been added to the risks of the oral glucose tolerance test as these are rare, but known, adverse effects of the glucose drink

Other Various language improvements throughout the document were made to improve clarity and maintain internal consistency of the document.

**Protocol changes since (DSMB-approved) version 1.4 | date 2013.7.3**

5. Inclusion/exclusion criteria were clarified as follows: BMI cutoff values are shown to the first decimal point, e.g., 40.0 instead of 40 kg/m$^2$; gastric banding more than 2 years ago with self-reported weight stability (defined as weight change no greater than 3 kg during the prior 6 months) is not an exclusion criterion; menopausal hormone therapy started within 3 months is an exclusion criterion; platelet donation is not an exclusion criterion.

6.5. The physical examination by the study physician was moved to the baseline visit (during the second phase of the screening).

8.1. At the end-of-study visit, the algorithm for the semi-annual visit (Protocol Figure 9.1.2) will be followed to assess for incident diabetes.

8.2.3. The plan to occasionally notify sites and participants that confirmatory testing is needed despite negative result for diabetes at a scheduled visit was removed. In lieu of this approach, to lessen the chance that participants would dramatically alter their lifestyle after a positive glycemic test on initial testing or be unduly alarmed, study personnel will emphasize to participants that an initial positive test for diabetes may often not be verified at confirmatory testing and that, rarely, repeat testing may be required for quality control purposes.

10.1.1. The list of “expected” adverse events was modified to include only what would be reasonably expected to occur as a result of study pills and the study intervention.

Other Various language improvements throughout the document were made to improve clarity, e.g. “pregnancy testing is done during the study if the participant reports missing two consecutive menstrual periods, based on the woman’s typical menstrual cycle,” “tanning booths” changed to “tanning devices.”

**Protocol changes since version 1.3 | date 2013.4.15**

1. The *Synopsis* section and *Schedule of Procedures* table were changed to reflect protocol changes.

7.3.3. If after unmasking occurs to evaluate an adverse event, it is determined that study pills are not related to the adverse event, participant will resume taking study pills consistent with the intention-to-treat principle.

8.1. An end-of-study visit has been added and FPG and HbA1c will be measured. Serum calcium, serum creatinine and urine calcium-creatinine will also be measured at that visit.

8.1. There will be a phone call approximately 6 weeks after the end-of-study visit to assess for adverse events due to potential residual effects from the study pills.

8.2. Results of laboratory outcome tests (i.e., fasting plasma glucose, HbA1c, 2-hour post-load glucose) are not provided to site staff, participants or health care provider(s) with the exception of the results obtained at the screening and baseline visits. During the study, the web-based electronic data capture (EDC) system will notify sites only that results do not meet criteria for diabetes or do meet criteria for diabetes and confirmatory testing is needed. After a participant reaches the primary endpoint of diabetes, glycemic results will be shared with site staff, participants and their health care provider(s).

8.2.3. After participants reach the diagnosis of diabetes, they will continue taking study pills without unmasking and will complete all subsequent planned visits.

8.6. For participants who become pregnant during the study, study pills and study procedures will be held, i.e. participant will become "inactive" (defined as stopping study pills and not attending study visits) but she will not go off study and will be encouraged to remain in the study and return for scheduled assessments after completion of pregnancy. If the woman agrees to remain in the study, laboratory testing will resume at 8 weeks post-partum. Study pills will resume after participant ceases lactation or after 8 weeks post-partum (whichever is later).

11. Study design was changed to an event-driven trial, which means that D2d will continue until the required number of events is reached. The main implication of this change is that follow up periods are estimates only and will depend on rates of enrollment and retention. It is anticipated that participants will be followed for approximately 4 years for the primary outcome, diabetes. We have changed the protocol and main ICF to indicate that study duration will be "approximately 4 years."

Other To minimize stopping study pills in participants with potentially false positive results, monitoring for hypercalcemia was modified to allow for a wider range for re-testing. Specifically, re-testing will be done when serum calcium falls within the upper limit of normal and the upper limit of normal plus 1 mg/dL (see DSMP figure 2).

Other Various language improvements throughout the document were made to improve clarity (e.g. measurement of 25OHD will not be done in real-time [section 4.3.7]; avoid duplication with the DSMP document (e.g. section 10.2.1 and 10.2.3 in protocol were shortened).

## LIST OF ABBREVIATIONS

| | |
|---|---|
| 2hPG | Plasma glucose 2 hours after a 75-g oral glucose tolerance test |
| 25OHD | Blood (plasma or serum) 25-hydroxyvitamin D |
| ADA | American Diabetes Association |
| AE | Adverse Event/Adverse Experience |
| BAS | Baseline |
| BMI | Body Mass Index |
| BP | Blood Pressure |
| CBC | Complete Blood Count |
| CC | Coordinating Center |
| CFR | Code of Federal Regulations |
| CONSORT | Consolidated Standards of Reporting Trials |
| CRP | C Reactive Protein |
| DCC | Data Coordinating Center |
| DDC | Drug Distribution Center |
| DHHS | Department of Health and Human Services |
| DSMB | Data and Safety Monitoring Board |
| DRI | Daily Recommended Intake |
| EDC | Web-based electronic data capture system |
| eCRF | Electronic Case Report Form |
| GFR | Estimated Glomerular Filtration Rate |
| FDA | Food and Drug Administration |
| FPG | Fasting Plasma Glucose |
| FWA | Federal Wide Assurance |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonization |
| ICMJE | International Committee of Medical Journal Editors |
| IFG | Impaired Fasting Glucose |
| HIPAA | Health Insurance Portability and Accountability Act |
| HbA1c | Hemoglobin A1c |
| HOMA-IR | Homeostatic Model Assessment Insulin Resistance |
| HTE | Heterogeneity of Treatment Effect |
| ICD | International Classification of Diseases |
| IGT | Impaired Glucose Tolerance |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| IU | International Units |
| IWRS | Interactive Web Response System |
| M | Month |
| MOP | Manual of Procedures |
| N | Number (typically refers to participants) |
| NIDDK | National Institute of Diabetes and Digestive and Kidney Diseases |
| NIH | National Institutes of Health |
| OGTT | Oral Glucose Tolerance Test |
| PI | Principal Investigator |
| QA | Quality Assurance |
| QC | Quality Control |
| SAE | Serious Adverse Event/Serious Adverse Experience |
| SOP | Standard Operating Procedure |
| UAP | Unanticipated Problem |
| UL | Upper Intake Level |
| US | United States |
| WHO | World Health Organization |

## 1. PROTOCOL SYNOPSIS

| | |
|---|---|
| *Title* | Vitamin D and type 2 diabetes study (**D2d** study). |
| *Funding (primary)* | National Institute of Diabetes and Digestive and Kidney diseases (NIDDK) National Institutes of Health (NIH). |
| *Study Objective* | To assess whether, in participants with pre-diabetes, oral daily vitamin $D_3$ (cholecalciferol) supplementation reduces the rate of progression from pre-diabetes to diabetes. |
| *Study Design* | Multicenter, randomized (1:1), double-masked, placebo-controlled, parallel-group, primary prevention clinical trial with 2 arms (oral daily vitamin D vs. placebo) in participants at high risk for diabetes (with pre-diabetes) who will be followed for approximately 4 years after randomization for incident diabetes. |
| *Intervention* | |
| *Active* | -Cholecalciferol ($D_3$) 4,000 IU, one soft-gel pill daily |
| *Placebo* | -One soft-gel pill daily |
| *Other* | -At baseline, all participants will receive information on the current lifestyle recommendations for prevention of type 2 diabetes. Twice a year during follow-up, participants will be invited to join a support and education program, set up as a group meeting, to discuss issues on nutrition and physical activity relevant to type 2 diabetes. |
| *Study Population* | Persons at increased risk for type 2 diabetes. |

**Inclusion Criteria**

1. Pre-diabetes ("at increased risk for diabetes") defined by meeting 2-out-of-3 of the following glycemic criteria, established by the American Diabetes Association (ADA) in the 2010 clinical practice guidelines, at the baseline visit:
   *a.* Fasting plasma glucose (FPG) 100-125 mg/dL, inclusive
   *b.* 2-hour plasma glucose (2hPG) 140-199 mg/dL, inclusive
   *c.* Hemoglobin A1c (HbA1c) 5.7-6.4%, inclusive
2. Age ≥ 30 years (≥25 years for people of the following races: American-Indian, Alaska Native, Native Hawaiian or Other Pacific Islander).
3. Body Mass Index ≥ 24.0 (22.5 for Asians) and ≤ 42.0 kg/$m^2$
4. Provision of signed and dated written informed consent prior to any study procedures.

**Major Exclusion Criteria**

5. Diabetes based on *either* of the following criteria:
   *a.* History (past 1 year) of hypoglycemic pharmacotherapy (oral or injectable medication approved by the FDA for type 2 diabetes) used for any condition (e.g. pre-diabetes, diabetes, polycystic ovarian syndrome).
   *b.* Meeting a glycemic criterion for diabetes, as defined by the ADA guidelines (FPG ≥ 126 mg/dL, 2hPG ≥ 200 mg/dL or HbA1c ≥ 6.5%) at the baseline visit.
6. History (past 3 years) of hyperparathyroidism, symptomatic or asymptomatic (i.e. radiographic) nephrolithiasis or hypercalcemia.

7. Any medical condition (past 3 years) that in the opinion of the site investigator may increase risk for nephrolithiasis or hypercalcemia during the trial (e.g., sarcoidosis).
8. Use of tanning devices within 12 weeks of the baseline visit and unwilling to stop use of tanning devices for the duration of the study

*Medications and Supplements*

9. Use of supplements containing vitamin D at total doses higher than 1000 IU/day within 8-12 weeks (depending on dose, as described in the Manual of Operations) of the baseline visit and unwillingness to limit vitamin D supplementation dosage to no higher than 1000 IU/day for the duration of the study.
10. Use of supplements containing calcium at total doses higher than 600 mg/day within 1 week of the baseline visit and unwillingness to limit calcium supplementation dosage to no more than 600 mg/day for the duration of the study.
11. Current use of medications or conditions (e.g. untreated celiac disease) that would interfere with absorption or metabolism of vitamin D.
12. Current use of medications approved by the Food and Drug Administration (FDA) for weight management.
13. Use of thiazide diuretics at a total dose greater than 37.5 mg/day.
14. Use of anticonvulsant drug started within 6 months of screening. Stable regimen of anticonvulsants is allowed.
15. History of intolerance to vitamin D supplements.

*Other Medical History*

16. Severe symptomatic cardiovascular disease based on history and physical examination (unstable angina, dyspnea on exertion, paroxysmal nocturnal dyspnea, arrhythmia, congestive heart failure NYHA class II or higher, claudication)
17. History (past 1 year) of myocardial infarction, percutaneous coronary intervention or coronary artery bypass graft.
18. History (past 1 year) of cerebrovascular disease (stroke, transient ischemic attack).
19. Any type of cancer (past 5 years) except for basal cell skin cancer. Prostate cancer (for men over age 55) or well-differentiated thyroid cancer not expected to require treatment (except for suppression with thyroid hormone) over the next 4 years, are not exclusions. Volunteers with history of with squamous cell cancer of the skin, which was completely excised and with no evidence of metastases, are eligible.
20. History (past 6 months) of treatment with oral (for > 7 days) or intravenous glucocorticoids or disease likely to require oral or intravenous glucocorticoid therapy during the study. Inhaled glucocorticoid is use is not an exclusion. Epidural or intra-articular glucocorticoid injections are not exclusions, but study visits need to be conducted at least a week after the injection. Persons with adrenal insufficiency treated with physiologic doses of glucocorticoids who are otherwise stable are *not* excluded.
21. History (past 1 year) of substance abuse or unstable psychiatric disorder that in the opinion of the site investigator would impede competence or adherence with study procedures or hinder completion of the study or increase risk.

22. History of bariatric surgery (e.g., Roux-en-Y gastric bypass, gastric sleeve) or planned bariatric surgery in the next 4 years. Participants with gastric banding more than 2 years ago with self-reported weight stability (defined as weight change no greater than 3 kg during the prior 6 months) are *not excluded.*
23. A life-threatening event within 30 days of screening or currently planned major surgery.
24. Any other unstable active medical condition (including but not limited to liver disease, wasting illness, AIDS, tuberculosis, oxygen-dependent chronic obstructive pulmonary disease, organ transplant, Cushing's syndrome) that in the opinion of the site investigators would impede competence or adherence with study procedures or increase risk.
25. Uncontrolled hypertension (systolic blood pressure > 160 mm Hg or diastolic blood pressure > 100 mm Hg).
26. Poor venous access.

*Laboratory Evaluation*

27. Serum liver transaminase higher than 3 times the normal range for the clinical site's laboratory
28. Anemia (hematocrit < 32 for women, < 36 for men), whole blood transfusion (within 6 months of screening) or chronic requirement, whole blood donation (within 3 months of screening) or other condition (hemolysis, hemoglobinopathy) rendering HbA1c results unreliable as indicator of chronic glycemia. Participants who donate platelets are not excluded. Whole blood transfusion or donation does *not* exclude participant, but screening and study visits need to be timed appropriately.
29. Low platelet count (< 50,000).
30. Chronic kidney disease, defined as estimated glomerular filtration rate [GFR] < 50 mL/min per 1.73 $m^2$ from creatinine level measured at the clinical site's laboratory and GFR calculated centrally.
31. Hypercalcemia, defined as serum calcium concentration greater than or equal to the upper limit of normal, measured at the clinical site's laboratory.
32. Hypercalciuria, defined as spot urine (morning void) calcium-creatinine ratio > 0.275.

*Other*

33. Participation (within 30 days of screening) in another interventional research study.
34. Previous randomization in D2d. Participants who did not qualify after screening may be screened again if the prior reason for exclusion has been addressed (e.g. high blood pressure is treated).
35. Any other reason that in the opinion of the site investigator would impede adherence with study procedures or hinder completion of the study or increase risk.

*Women only*

36. Pregnancy (past 1 year by report or positive pregnancy test at screening), intent to become pregnant in the next 4 years or unprotected intercourse. History of gestational diabetes is *not* an exclusion criterion.

| | |
|---|---|
| | 37. Currently breastfeeding.<br>38. Use of oral contraceptives or menopausal hormone therapy started within 3 months of baseline. Stable regimen of oral contraceptives or any other hormonal method of contraception (e.g. implantable) is allowed. |
| *Environment* | U.S. based research centers representative of the population at risk for both vitamin D insufficiency/deficiency and diabetes. |
| *Recruitment Strategy* | |
| *Prescreening* | -Site-specific based on what has worked well at each participating site. |
| *Screening* | -Medical history, vital signs, fasting labs (fasting plasma glucose, hemoglobin A1c, safety labs). |
| *Baseline* | -75-gram oral glucose tolerance test, urine for calcium-creatinine, physical examination. |
| *Recruitment Period* | -Approximately 2 years |
| *Study Duration* | -Approximately four years; Because D2d is an event-driven trial, study duration is approximate only. |
| *Visits* | -Thirteen scheduled visits: Screening, Baseline (BAS), Randomization (RAD), M03, M06, M12, M18, M24, M30, M36, M42, M48 and end-of-study. Additional visits may be required for confirmatory glycemic testing or safety evaluation, including scheduled visits beyond M48. |
| *Phone calls* | -Eight scheduled telephone calls in between visits: M09, M15, M21, M27, M33, M39, M45. Additional calls may be required beyond M48. |
| *Primary Outcome* | Time to development of diabetes |
| *Secondary Outcomes* | -Variability of response to vitamin D supplementation by several baseline characteristics: (1) race and ethnicity (as proxies for skin pigmentation); (2) BMI; (3) waist circumference; (4) age; (5) geographic location (proxy for sun exposure); (6) calcium intake (based on Food Frequency Questionnaire); (7) 25OHD concentration.<br>-Variability of response to vitamin D supplementation by adherence based on pills counts and by achieved 25OHD concentration.<br>-HbA1c, FPG and 2hPG as continuous variables.<br>-Insulin resistance and beta cell secretion (indices derived from the OGTT).<br>-Plasma 25OHD concentration and identification of phenotypic, including seasonal and geographic, characteristics associated with variability on achieved plasma 25OHD concentration.<br>-Blood pressure.<br>-Safety and tolerability of vitamin D supplementation. |
| *Sample Size (total)* | -2,382 participants randomized to vitamin D or placebo |
| *Participants per site* | -Variable, depending on site's experience (~100 to 150 per site) |
| *Attrition rate* | -15% (maximum) of the original cohort over the study period |
| *Analysis Strategy* | Kaplan-Meier estimates of "time to confirmed diabetes" distributions will be calculated for each treatment group. The log-rank test will then be used to perform an adjusted comparison of the time-to-event distributions in the two treatment groups. Cox proportional hazard models will be used to calculate an estimate of the adjusted hazard ratio. Pre-specified subgroup analyses will be performed in participant subgroups defined by baseline variables, and other variables, to gain information on predictors of response to therapy. |

## Schedule of Procedures

| Point of Contact | Screening[1] | Baseline | Randomization | M03 | M06 | M09 (Phone) | M12 | Interim Phone | Semi-annual | Annual | Confirm | As needed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day -49 to -7 | Day -21 to -5 | Day 0 | Day 77 to 105 | Day 169 to 197 | Day 260 to 288 | Day 351 to 379 | Midpoint between visits (M15, M21, M27, M33, M39, M45, etc. | Midpoint between annual visits (M18, M30, M42 etc.) | (M24, M36, M48 etc.) | | |
| Written informed consent | X | | | | | | | | | | | |
| Medical history | X[2] | [3] | | [3] | [3] | [3] | [3] | [3] | [3] | [3] | | X |
| Physical examination | | X[2] | [2] | [4] | [4] | | [4] | | [4] | [4] | | X |
| Non-study medication review | X | X | | X | X | X | X | X | X | X | X | X |
| Vital signs [5] | X | X | | X | X | | X | | X | X | | X |
| Waist circumference | | X | | | | | | | | | | |
| Questionnaires | | | | | | | | | | | | |
| FFQ | | X | | | | | X[6] | | | X[6] | | |
| Physical Activity | | X | | | X | | X | | | X | | |
| Lifestyle counseling [7] | | X | | | | | | | | | | |
| Randomization | | | X | | | | | | | | | |
| Study pill distribution, teaching | | | X | | X | | X | | X | X | | |
| Study pill adherence | | | | | X | | X | | X | X | | |
| Laboratory specimen collection [8] | | | | | | | | | | | | |
| CBC, LFT, Pregnancy [9] | L | | | | | | | | | | | L |
| Serum calcium, creatinine (GFR) | L | | | L | | | L | | | L | | L |
| HbA1c [10] | L[11] | C | | | C | | C | | C | C | C[13] | C |
| FPG [10] | L[11] | C | | | C | | C | | C | C | C[13] | C |
| 2hPG (OGTT) [10,12] | | C | | | | | C | | | C | C[14] | |
| $Glu_{30}$ (OGTT) [12] | | C | | | | | C | | | C | | |
| 25-hydroxyvitamin D | | C | | | | | C | | | C | | |
| Insulin [12] | | C | | | | | C | | | C | | |
| Urine calcium-creatinine ratio[10] | | C | | C | | | C | | | C | | |
| Plasma and serum for storage | | C | | | C | | C | | | C | | |
| Urine for storage | | C | | | | | C | | | C | | |
| Whole blood for DNA | | C | | | | | | | | | | |
| Adverse event review | | X | X | X | X | X | X | X | X | X | X | X |
| Letter to physician | | | X[15] | | X[16] | | X[16] | | X[16] | X[16] | X[16] | |

FFQ, food frequency questionnaire; M=month since baseline; L= laboratory analysis performed at the clinical site's (local) laboratory and reported to sites, participants and their health care provider(s), C= laboratory analysis performed at the central laboratory; CBC, complete blood count includes white blood cell count without differential, hemoglobin & hematocrit and platelet count; LFT, liver function tests (AST, ALT); GFR, estimated glomerular filtration rate; OGTT, 75-gram oral glucose tolerance test; $Glu_{30}$ plasma glucose 30 minutes after 75 gram glucose load during OGTT; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; 2hPG, plasma glucose at 2 hours after a 75-gram glucose load during OGTT.

1. Prior to screening, there will be a site-specific pre-screening phase (e.g. contact over the phone or via the web). Pre-screening phase may also include an additional visit.
2. Complete medical history will be done at the screening visit; physical examination will be done at the baseline visit or at the randomization visit (prior to randomization);
3. Interim medical history will be done at the baseline visit, all follow-up visits and scheduled phone calls.
4. Symptom-directed physical examination will be done as needed (e.g. if adverse events are reported at a follow-up visit).
5. Vital signs include height, weight, blood pressure and heart rate.
6. Food frequency questionnaire (FFQ) will be completed at baseline, and at visits M12 and M36 only in participants free of diabetes.
7. Lifestyle counseling includes written educational materials at baseline. During the trial, participants will join the D2d Support and Education Program.
8. Laboratory and urine testing is done after 8 hours of overnight fasting.

9. Pregnancy test is required for women of reproductive potential at screening. Point-of-care urine testing will be done followed by confirmatory blood testing, if point-of-care test is positive. If point-of-care urine testing is not an option, a urine or serum test will be done at the site's laboratory. Pregnancy testing is done during the study if the participant reports missing two consecutive menstrual periods, based on the woman's typical menstrual cycle. During pregnancy, participant becomes "inactive" and resumes participants after delivery.
10. HbA1c, FPG, 2hPG and urine calcium-creatinine ratio are measured by the Central Laboratory in real time (see text for details on reporting of results). Other outcome laboratory tests (e.g. 25OHD, insulin) are done at a later time
11. At screening, HbA1c and FPG may be done by the site's local laboratory or any other laboratory (including the LCBR at U. Vermont, which serves as the study's Central Laboratory).
12. 2hPG, $Glu_{30,}$ and insulin ($Ins_0$, $Ins_{3o,}$ $Ins_{120}$) are performed at baseline, and at annual visits in participants free of diabetes.
13. Semi-annual confirmatory testing: If FPG ≥ 126 <u>or</u> HbA1c ≥ 6.5% at the semi-annual visit, a confirmatory visit is conducted and only the abnormal test is repeated.
14. Annual confirmatory testing: If FPG ≥ 126 <u>or</u> HbA1c ≥ 6.5% or 2hPG ≥ 200 mg/dL at the annual visit, a confirmatory visit is conducted that may include an OGTT (see text for details).
15. Letter informing participant's physician(s) of her patient's participation in D2d.
16. Letter informing participant's physician(s) of her patient's continued participation in D2d, and the outcome of glycemic testing or diabetes diagnosis according to the study's criteria.

The last scheduled visit (semi-annual or annual) serves as the end-of-study visit. Procedures done will depend upon the scheduled visit with which it coincides (see text).

## 2. BACKGROUND, RATIONALE AND SIGNIFICANCE

### 2.1 Background and Rationale

The incidence of diabetes is increasing at an alarming rate both nationally and worldwide with 1.9 million new cases diagnosed in 2010 in the US alone,[1] with nearly 9 out of 10 new cases due to type 2 diabetes. The diabetes population and related costs are expected to more than double in the next quarter century,[2] as more than 79 million Americans are at risk of developing diabetes.[1,3,4] In clinical trials, lifestyle changes aiming at weight loss are successful at reducing risk of diabetes.[5-9] However, long-term weight-maintenance in the clinical setting has proved elusive. Moreover, even after successful weight loss, substantial residual risk (~40-50%) remains and may be attributable to modifiable factors.[10] Several medications that are used to treat established diabetes have also been studied for prevention of diabetes and many have been shown to delay incident diabetes;[9,11-17] however, the role of pharmacologic agents for prevention of diabetes is not clear.[9] Therefore, identification of easily modifiable risk factors that are safe, inexpensive and acceptable is urgently needed to prevent type 2 diabetes and decrease disease burden.

Based on recent evidence,[18-44] which has been synthesized in systematic reviews,[45-48] suboptimal vitamin D status has emerged as a potential contributor to the pathophysiology of type 2 diabetes. However, the evidence to support general supplementation for prevention of type 2 diabetes does not currently exist because the favorable association between vitamin D status and type 2 diabetes risk is based almost exclusively on observational studies, which may be confounded by a variety of factors and there are no published trials specifically designed and powered to test the effects of vitamin D supplementation on the development of type 2 diabetes. The D2d study will test this hypothesis by examining the causal relationship between vitamin D and development of diabetes in persons at risk for diabetes. If the hypothesis of a link between vitamin D and type 2 diabetes is confirmed by the proposed trial, the results will have significant public health implications since vitamin D status has declined in the US over the last decade[49] and vitamin D supplementation can be implemented easily and inexpensively in clinical practice.

### 2.2 Biologic plausibility of an association between vitamin D and type 2 diabetes

When glucose intolerance and type 2 diabetes develop, impaired pancreatic beta-cell function, insulin resistance and systemic inflammation are often present.[50,51] There is evidence that vitamin D influences these mechanisms, as described next.

*Vitamin D and pancreatic beta-cell function / insulin secretion*

In *in vitro* and *in vivo* studies, vitamin D deficiency impairs glucose-mediated insulin secretion from beta-cells,[52-55] while vitamin D supplementation restores insulin secretion.[52,54-57] Vitamin D may have a direct effect on beta-cell function mediated by binding of the circulating active form, 1,25$(OH)_2$D, to the vitamin D receptor, which is expressed in pancreatic beta-cells.[58,59] Furthermore, mice lacking a functional vitamin D receptor show impaired insulin secretory response following a glucose load, attributed to a decrease in insulin synthesis resulting in a reduction in the amount of insulin stored in the beta cell.[58] The presence of the vitamin D response element in the human insulin gene promoter[60] and transcriptional activation of the human insulin gene by 1,25$(OH)_2$D[61] further support a direct effect of vitamin D on insulin synthesis and secretion. Alternatively, activation of vitamin D also occurs within the pancreatic beta cell by the 25-hydroxyvitamin D-1α-hydroxylase enzyme (CYP27B1), which is expressed in pancreatic beta cells.[62] Such a mechanism allows for a paracrine effect of circulating 25OHD. An indirect effect of vitamin D on the beta cell may be mediated via its regulation of extracellular calcium concentration and calcium flux through the beta cell.[63] Insulin secretion is a calcium dependent process,[64] therefore, alterations in calcium flux can have an effect on insulin secretion.[65-67] Vitamin D also regulates calbindin, a cytosolic calcium-binding protein found in many tissues including beta cells.[59,68] Calbindin is a modulator of depolarization-stimulated insulin release via regulation of intracellular calcium.[69] Finally, vitamin D may promote beta-cell survival by modulating the generation (e.g. through inactivation of nuclear factor-kB [NF-kB]) and effects of cytokines.[70,71] In some but not all cross-sectional human studies, an association between the blood 25OHD concentration and insulin secretion has been reported.[72,73,74]

*Vitamin D and insulin sensitivity*

In peripheral insulin-target cells, vitamin D may enhance insulin sensitivity in several ways. Vitamin D may directly augment insulin sensitivity by stimulating the expression of insulin receptors;[60,61,75,76] The active form, 1,25$(OH)_2$D, enters insulin-responsive cells and interacts with the vitamin D receptor, activates the vitamin D receptor-retinoic acid X-receptor (RXR) complex which, in turn, binds to a vitamin D response element found in the human insulin receptor gene promoter. The result is enhanced transcriptional activation of the insulin receptor gene, which increases the total insulin receptor number without altering receptor affinity. Vitamin D may also enhance insulin sensitivity by activating peroxisome proliferator-activated receptor delta (PPAR-δ), a transcription factor implicated in the regulation of fatty acid metabolism in skeletal muscle and adipose tissue.[77] An indirect effect of 1,25$(OH)_2$D on insulin sensitivity might also be exerted via its important and well-recognized role in regulating extracellular calcium concentration and flux through cell membranes. Calcium is known to be essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue,[78,79] with a very narrow range of intracellular calcium needed for optimal insulin-mediated functions.[80] Changes in intracellular calcium in insulin target tissues may contribute to peripheral insulin resistance [80-87] via impaired insulin signal transduction[87,88] leading to decreased glucose transporter activity.[87-89] Hypovitaminosis D also leads to increased parathyroid hormone concentration, which has been associated with increased insulin resistance.[90,91] Vitamin D may also affect insulin resistance indirectly through the renin-angiotensin-aldosterone system.[92-95] Finally, vitamin D insufficiency is associated with increased fat infiltration in skeletal muscle, independent of body mass, which is thought to contribute to decreased insulin action.[96] In observational human studies, low vitamin D status (assessed by self-reported vitamin D intake or blood 25OHD concentration) has been associated with simple indices of insulin resistance, including measurements of fasting insulin and homeostasis model assessment (HOMA-IR),[20,29,35,72,73,97-101] but the association is not consistent.[74,99,102]

*Vitamin D and systemic inflammation*

Systemic inflammation, via an increase in pro-inflammatory cytokines, plays an important role in the pathogenesis of type 2 diabetes, mostly by promoting insulin resistance; however, pancreatic beta cell function may also be affected via cytokine-induced apoptosis.[50,103-105] Vitamin D can lessen the effects of systemic inflammation on type 2 diabetes risk in several ways. For example, 1,25$(OH)_2$D may improve insulin sensitivity and protect against beta cell cytokine-induced apoptosis by directly modulating the expression and activity of cytokines.[71,106-108] One such pathway may be through down-regulation of NF-kB, which is a major transcription factor for TNF-a and other inflammatory mediators.[109] Another pathway that may, at least in part, mediate the anti-apoptotic effect of 1,25$(OH)_2$D on beta cell is through counteracting cytokine-induced Fas expression.[110] Several other immune-modulating effects of 1,25$(OH)_2$D (e.g. blockade of dendritic cell differentiation, inhibition of lymphocyte proliferation, inhibition of foam cell formation and cholesterol uptake in macrophages, enhanced regulatory T-lymphocyte development)[107,111] may provide additional pathways of protection against inflammation-induced type 2 diabetes risk. In observational human studies, low vitamin D status (assessed by self-reported vitamin D intake or blood 25OHD concentration) has been associated with elevated concentration of markers of systemic inflammation in some [101,112,113] [44] but not all studies.[20,114-116]

## 2.3 Evidence from human studies for a link between vitamin D and type 2 diabetes

Observational Studies

The strong data in humans that link vitamin D to type 2 diabetes are primarily from observational studies. Cross-sectional studies have generally reported inverse associations between vitamin D status and prevalent hyperglycemia.[35,38,40,41,45,73,97,99,117-131] In a large cross-sectional study with data from the U.S. based National Health Nutrition Examination Survey (NHANES), serum 25OHD concentration was inversely associated with prevalence of diabetes in a dose-dependent pattern in non-Hispanic whites and Mexican-Americans, after multivariate adjustment, including BMI.[97] In this study, there was no association in non-Hispanic blacks despite lower 25OHD concentration found in this racial group, which may be explained by the observation that non-whites exhibit a different vitamin D, calcium and PTH homeostasis compared to whites.[132] However, a subsequent analysis from NHANES did not find an interaction between blood 25OHD concentration and race or ethnicity on glycemic outcomes.[35] More recent studies using NHANES data have repeatedly confirmed the inverse association between 25OHD and glycemia,[35,121,133-135] which has also been reported in other large cohorts from the U.S.[91], Europe[136] and China.[100] Cross-sectional studies, however, are difficult to interpret, as the directionality of the association (cause and effect) cannot be determined.

To overcome the inability of cross-sectional studies to establish the direction of the causality between vitamin D status and type 2 diabetes related parameters, longitudinal observational studies have been conducted where vitamin D status is assessed prior to the development of the outcome of interest, incident type 2 diabetes. There are 14 studies (from 15 cohorts) that have reported on the longitudinal association between vitamin D status (intake *or* 25OHD) and risk of type 2 diabetes.[18,21,22,25,26,28,36,37,39,42-44,137] Nearly all of them have reported an inverse association between vitamin D status (intake or 25OHD concentration) and incident type 2 diabetes. A recent meta-analysis, examining only 25OHD concentration as the predictor,[138] identified 9 publications that provided data from 13 cohorts involving 65,721 participants and 3,567 incident cases of diabetes.[22,25,28,37,39,42-44,137] Comparing the highest to the lowest category of 25OHD, the pooled relative risk for incident diabetes was 0.65 (95% CI, 0.55-0.77). A linear trend meta-regression analysis showed that each 4 ng/mL increment in 25OHD was related to a 4% lower risk.

Intervention Studies

Several trials have reported the effect of vitamin D supplementation on glycemia[19,34,139-147] or incident diabetes by self-report. In nine trials that included participants with *normal glucose tolerance*, supplementation with vitamin D had overall a neutral effect on glycemic measures or incident diabetes.[19,34,139,141,142,144,145,148,149] However, several of these trials were designed for non-glycemic outcomes and the analyses on diabetes were *post-hoc* and *all trials* (except for the Women's Health Initiative trial, WHI) *were underpowered for glycemic outcomes.* In several trials, adherence with supplementation was suboptimal. For example, in a post-hoc analysis of the RECORD trial (a community-based trial designed for bone outcomes),[148] 800 IU/day of vitamin $D_3$ did not change risk of *self-reported type 2 diabetes*; however, among study participants who were highly compliant with supplementation, there was a notable trend towards reduction in type 2 diabetes risk with vitamin $D_3$ (odds ratio 0.68; 95%CI 0.40, 1.16), which *highlights the importance of efficacy vs. effectiveness trials.* Importantly, several trials supplemented with infrequent (weekly or monthly) large doses of vitamin D, a commonly used clinical approach, which may not be a desirable physiologic method for supplementation and may be counterproductive.[150]

The potential effect of vitamin D appears to be more prominent among persons with pre-diabetes. In a post-hoc subgroup analysis conducted using data from a completed trial designed for fractures, combined vitamin $D_3$ (700 IU/day) and calcium carbonate (500 mg/day) supplementation improved fasting plasma glucose (FPG) and insulin resistance (HOMA-IR) among adults with glucose intolerance at baseline,[19] suggesting that *vitamin D may benefit only individuals at high risk (e.g. pre-diabetes).* In this study, *the reduction in FPG over 3-years was similar to the reduction in FPG achieved with metformin or lifestyle, in the Diabetes Prevention Program, which was associated with a 31-58% decrease in incident diabetes.*[5] In the Calcium and Vitamin D for type 2 Diabetes Mellitus (CaDDM) study, a 2x2 factorial design trial, vitamin D supplementation improved - disposition index, a composite measure of beta cell function that accounts for the prevailing insulin sensitivity, in participants with pre-diabetes but without regard to baseline vitamin D status or calcium supplementation.[24] In another intervention study, very similar to the CaDDM trial, where vitamin D was given without a placebo, insulin sensitivity improved after 4 weeks of vitamin D administration in persons with pre-diabetes.[151] *On the basis of these observations, in the proposed D2D trial, a pre-diabetes population is targeted.*

## 2.3 Summary of human studies

Although the data from published studies suggest a strong link between vitamin D and diabetes risk, the evidence to support general supplementation with vitamin D for diabetes prevention does not currently exist. The evidence is based almost exclusively on observational studies, which may be confounded by many factors and there are no published trials designed and powered to test the effects of vitamin D supplementation on reducing diabetes risk; therefore, definitive conclusions cannot be drawn on the role of vitamin D for prevention of type 2 diabetes. There have been numerous previous occasions where highly encouraging data from observational studies led to irrational exuberance and widespread adoption of the intervention which proved premature, as subsequent trials did not confirm benefit (e.g. hormone therapy, vitamin E).[152-158] The D2d study will address the issue of causality and quantify the protective benefit of vitamin D in type 2 diabetes risk, if present, in a target population most likely to benefit.

## 2.4 Potential impact on human health

The "excitement" surrounding the role of vitamin D for diabetes, and other chronic diseases, has led to dramatic increases in 25OHD assays done as part of routine medical care and in supplementation with very high doses of vitamin D to "improve" vitamin D status. Medicare payments for vitamin D testing nearly quadrupled between a 2-year span, 2006 and 2008, to $129 million. A decade ago, these payments were only about $1 million. Concurrently, spending on vitamin D supplements has increased tenfold in the last 8 years to $425 million in 2009, which represents a growth of 81% from 2008.[159] Manufacturers of vitamin D assays and supplements have rushed to claim a piece of the "vitamin pie;" however, such enthusiasm is premature as the evidence is extrapolated from observational studies, which are severely limited, as outlined above. Furthermore, simply raising 25OHD level does not always translate to favorable outcomes.[150,160]

The need for such a trial has been recognized in the recent literature in editorials, reviews and book chapters.[32,46,47,148,161-184] Of importance, the 2011 Institute of Medicine report on dietary reference intakes (DRI) for calcium and vitamin D recognized as a major limitation in setting the DRI the lack of long-term trials with vitamin D supplementation, and *identified diabetes as one of the most promising non-skeletal areas that require further research with rigorously done trials to confirm the promising results seen in observational and mechanistic studies.*[185] The Endocrine Society guidelines also note that "trials that evaluate the effects of vitamin D doses in the range of 2,000 to 5,000 IU/day on non-calcemic health outcomes are desperately needed." [176]

The proposed trial, therefore, addresses an important and timely question and has the potential for significant impact in the clinically important areas of vitamin D and type 2 diabetes prevention with extensive public health implications especially given that the cost of supplementation with vitamin D is inexpensive compared to treating the chronic disease and its complications. We expect the D2d study results to define the role of vitamin D supplementation in modifying type 2 diabetes risk. If the trial confirms a favorable benefit/harm ratio of raising 25OHD in pre-diabetes, then vitamin D supplementation will be integrated into conventional medical approaches to prevent type 2 diabetes and ameliorate personal and societal disease burden in this high-risk population. Moreover, the study will define subgroups that may benefit preferentially from optimizing vitamin D status (e.g. obese vs. non-obese, white vs. non-white). The study can also serve as the backbone for ancillary analyses to meet mechanistic and related research needs identified by the NIH and others.

# 3. HYPOTHESES AND SPECIFIC AIMS

## 3.1 Study Objectives

The *objectives* of the D2d study are to evaluate the safety of oral daily vitamin D supplementation and its effect on the time to onset of clinical diabetes in participants with pre-diabetes (at risk for type 2 diabetes).

## 3.2 Specific Aims

### 3.2.1 Primary Specific Aim

The *primary aim* of the study is to assess whether, in participants with pre-diabetes, oral daily vitamin $D_3$ supplementation reduces the rate of progression from pre-diabetes to diabetes. The underlying hypothesis is that, compared to placebo, vitamin $D_3$ supplementation will reduce the rate of incident diabetes.

### 3.2.2 Secondary Specific Aims

Secondary specific aims will assess:

- Variability of response to vitamin D supplementation in subgroups defined by baseline characteristics: (1) race and ethnicity (as proxies for skin pigmentation);[186] (2) BMI; (3) waist circumference;[187,188] (4) age; (5) geographic location (proxy for sun exposure); (6) calcium intake (based on Food Frequency Questionnaire); (7) 25OHD concentration.
- Variability of response to vitamin D supplementation by adherence based on pills counts and by achieved 25OH concentration.
- Effect of vitamin D supplementation on HbA1c, FPG and 2hPG as continuous variables.
- Effect of vitamin D supplementation on insulin resistance and beta cell secretion (indices derived from the OGTT).
- Effect of vitamin D supplementation on plasma 25OHD concentration and identification of phenotypic, including seasonal and geographic, characteristics associated with variability on achieved plasma 25OHD concentration.
- Effect of vitamin D supplementation on blood pressure.
- Safety and tolerability of vitamin D supplementation.

### 3.2.3 Ancillary Studies

Other outcomes (e.g. effect of vitamin D supplementation on cardiovascular risk factors [cholesterol profile, C-reactive protein, urine albumin excretion]), as part of distinct ancillary studies requesting additional funds, may be developed in parallel. Additional blood (serum and plasma) and urine samples will be collected at the baseline, 6-month and annual visits for banking. These samples will become available to ancillary studies. Long-term storage of blood and urine samples will be transitioned to the NIDDK Central Repositories. In addition, at baseline, the study will collect whole blood for future DNA extraction, which may be done as part of a genetic ancillary study.

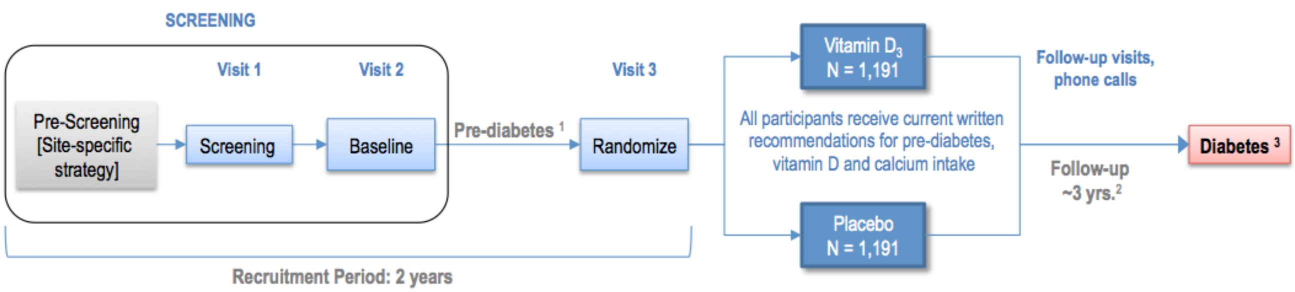
# 4. STUDY DESIGN, INTERVENTION AND PROCEDURES

## 4.1 Overview of Study Design

The D2d study is a multicenter, randomized (1:1), double-masked, placebo-controlled, parallel-group primary prevention clinical trial with 2 arms (oral daily vitamin $D_3$ vs. placebo) in participants at high risk for diabetes (with pre-diabetes) who will be followed for approximately 4 years after randomization for incident diabetes (Figure 4.1).

Adults at increased risk for type 2 diabetes (*pre-diabetes*), defined as meeting two-out-of-three ADA glycemic criteria established in 2010 (FPG 100-125 mg/dL or 2hPG 140-199 mg/dL or HbA1c 5.7-6.4%), will be randomized to receive, once daily, either a single soft-gel of vitamin $D_3$ (cholecalciferol, 4,000 IU) or matching placebo. Study participants will be seen every 3 months during the first 6 months and every 6 months for the remainder of the study. At the midpoint of each 6-month interval after the month 6 visit, participants will receive a telephone call to encourage compliance, assess for adverse events and receive a reminder of their upcoming visit. The primary outcome will be *time to diabetes*, defined by laboratory criteria derived from a 75-gram OGTT [to obtain 2hPG] done annually, FPG and HbA1c done every 6 months or when symptoms consistent with hyperglycemia are reported (Figure 9.1).

Figure 4.1. Schematic of Study Design




[1] Two out of 3 glycemic criteria are met: FPG 100-125 mg/dL (5.5-6.9 mmol/L); HbA1c 5.7-6.4% (39-46 mmol/mol); 2hrPG 140-199 mg/dL (7.7-11.0 mmol/L)
[2] Mean follow-up is estimated to be approximately 3 years. Because D2d is an event-driven trial, follow-up period is an estimate
[3] Based on laboratory criteria: 1 out of 3 glycemic criteria are met: FPG ≥126 mg/dL (7 mmol/L); HbA1c ≥6.5% (47.5 mmol/mol); 2hrPG ≥200mg/dL (11.1 mmol/L)

## 4.2 Performance Locations

The D2d study will be conducted at multiple collaborating clinical sites in the United States. To ensure study representation of the US population, several sites are located at high latitude (to capture low UVB exposure) while other sites have high proportion of minority populations.

## 4.3 Principles Guiding Selection of Specific Intervention and Study Duration

### 4.3.1 Choice of Specific Vitamin D Supplementation as the Active Intervention

#### 4.3.1.1 Choice of Vitamin D Formulation

Cholecalciferol (vitamin $D_3$, inactive form) was chosen instead of $1,25(OH)_2D$ (active form) because administration of the latter would increase risk of hypercalcemia. Moreover, certain critical tissues, such as the beta cell, express 1-alpha-hydroxylase (CYP27B1) and can convert inactive vitamin D to its active metabolite.[62] Cholecalciferol ($D_3$) was chosen over ergocalciferol ($D_2$) because supplementation with $D_3$ may result in a greater and sustained increase in 25OHD level and because at high doses, $D_2$ may be less effective than $D_3$.[185,189-193] Lastly, vitamin $D_3$ is the most commonly consumed vitamin D form; therefore, use of vitamin $D_3$ will increase the study's translational potential.

#### 4.3.1.2 Choice of Vitamin D Dose (amount and frequency)

*Rationale for amount of vitamin D.* Based on the available literature, the planning committee has determined that the selected dose of 4,000 IU/day for vitamin D provides the best possible balance of safety and efficacy in terms of obtaining a large-enough difference in 25OHD concentration between active and placebo groups and reaching a high enough 25OHD that will have an effect on the outcome of interest, as described below.

Based on published observational and short-term mechanistic intervention studies,[18-22,27,28,34,45,47,194] it appears that a plasma 25OHD concentration of approximately 30-50 ng/mL is required to detect a protective effect of vitamin D in relation to type 2 diabetes, if present. In an observational study in the Nurses' Health Study, the median 25OHD in the highest quartile, which was associated with an approximate 50% risk reduction in incident type 2 diabetes, was 33 ng/mL.[22] This level was consistent with another observational study by Knekt et al (mean 25OHD in the highest quartile, 30 ng/mL).[27,28] Of particular relevance to the D2d trial, in a longitudinal observational study in the Diabetes Prevention Program (DPP) cohort, participants with pre-diabetes with baseline plasma 25OHD≥30 ng/mL had a 28% risk reduction in incident diabetes over a 3-year period, after adjustment for the DPP lifestyle intervention, while participants with 25OHD concentration ≥50 ng/mL had a 56% risk reduction.[25] In the Stockholm Diabetes Prevention Program, progression from pre-diabetes to type 2 diabetes was reduced by 62% among those with 25OHD>28 ng/mL compared to those with 25OHD<18 ng/mL.[195] In a recent meta-analysis, the pooled relative risk of type 2 diabetes comparing the highest with the lowest quartile of 25OHD was 0.59 (0.52, 0.67), with little heterogeneity between the 11 studies included (3,612 cases and 55,713 non-cases).[196] In another meta-analysis,[138] 9 publications were identified that provided data from 13 independent cohorts involving 65,721 participants and 3,567 incident cases of diabetes.[22,25,28,37,39,42-44,137] Comparing the highest to the lowest category of 25OHD levels, the pooled relative risk for incident diabetes was 0.65 (95% CI, 0.55-0.77). A linear trend meta-regression analysis showed that each 4 ng/mL increment in 25OHD levels was related to a 4% lower risk. In the CaDDM trial, among participants with pre-diabetes (which is the target population in the D2d study), daily supplementation with 2,000 IU of vitamin $D_3$ achieved a mean 25OHD concentration of 31 ng/mL in the active arm, which was associated with improvements in beta cell function (disposition index) by approximately 40% and glycemia (HbA1c) by approximately 50% after 4 months.[24] In a six-month trial by von Hurst et al, insulin resistance with vitamin D supplementation improved the most when end-of-study 25OHD concentration was higher than 32 ng/mL.[34] Although one can extrapolate these data to recommend a much higher target 25OHD concentration, there is very limited longitudinal observational data[25] and no intervention studies to support targeting much higher levels.

Study participants, who will be overweight/obese and in many cases non-Caucasian, are expected to have a mean 25OHD concentration of approximately 20 ng/mL at study entry.[19,22,24,49,197-200] Approximately 4,000 IU/day of vitamin D will be needed to increase participants' mean 25OHD to 35-40 ng/mL, especially during the winter.[24,197,200-205] Of high relevance and importance to the D2d trial, in the CaDDM trial a dose of 2,000 IU/day of vitamin $D_3$ over 4 months achieved a mean 25OHD concentration of 31 ng/mL in a cohort of participants with pre-diabetes, which is identical to the target population in the D2d trial. The results from the CaDDM trial are in line with studies by Vieth et al,[200] Aloia et al[203] and Talwar et al where a daily vitamin $D_3$ dose of between 3,800 and 5,000 IU raised 25OHD above 30 ng/mL in all participants studied, including African American women. A recent dose response vitamin D supplementation study confirmed that 4,000 IU/day is an appropriate dose to raise 25OHD to ~40 ng/mL in white post-menopausal women including those with obesity.[205] Of note, the 2011 Institute of Medicine set the Tolerable Upper Intake Level (UL) of Vitamin D for adults at 4,000 IU/day;[185] The UL is defined as the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population; however, the report also emphasized that the "UL is not specified as an 'upper limit' for clinical research and it may be appropriate to conduct clinical research with doses exceeding the UL, as long as there is monitoring and the protocol is carefully considered." Vitamin D-related acute toxicity is not expected; however, the study will carefully assess and adjudicate all serious adverse effects reported by participants to gain insight into any potential risk of higher-dose vitamin D supplementation than that recommended by the Institute of Medicine for the majority of the healthy population, as described below.

Based on these arguments, the chosen dose balances efficacy (i.e. very likely to reach optimal 25OHD concentration that differentiates intervention from placebo) with safety.

*Rationale for daily dose.* Raising 25OHD can be achieved equally well with daily, weekly, or monthly vitamin D supplementation.[202,206] Although non-daily dosing (e.g. weekly, monthly) is convenient and would reduce costs related of manufacturing, shipping, storage and distribution of study pills, the planning committee decided to test daily dosing because: (1) it is physiological in terms of how vitamin D exposure occurs and may be more likely to have an effect compared to infrequent and/or lower doses;[150,160,185,207,208] (2) there is evidence that high infrequent (i.e. non-daily) doses of vitamin D may be metabolized differently as compared to daily doses and may provide either no benefit or result in an unfavorable benefit/risk ratio;[150,160,185,207-211] (3) it is straightforward to apply among all sites; (4) it will maximize the study's external validity.

#### 4.3.1.3 Rationale for Lack of a Target 25OHD Concentration

The D2d study is designed so that most participants reach adequate plasma 25OHD concentration with adequate differentiation between the 2 study arms. A supplementation strategy that aims for a specific 25OHD threshold will not be used for the following reasons: (1) although 25OHD is a well-established biomarker of total vitamin D exposure (from intake and biosynthesis), it is not a validated health outcome surrogate i.e., simply aiming for a specific 25OHD threshold may not translate to favorable outcomes.[150,185] The study will test for such thresholds in the planned mediation analyses. (2) Aiming for and achieving a specific 25OHD level is complicated, as it depends on a variety of contributing factors (e.g. baseline 25OHD, age, BMI, genetic predictors [gender, skin color, genetic polymorphisms], dietary and environmental factors). (3) The approach to not target a specific 25OHD concentration is practical and will increase generalizability of results. (4) Aiming for a specific 25OHD target would add significant expense and burden without proportional benefit.

### 4.3.2 Choice of Comparison Group

Selecting a comparison group is challenging for trials with vitamin D given the evolving nature of the evidence. The following ethical and scientific principles were considered to select an appropriate control: (1) From a "subject protection" point of view, control participants should be allowed to receive the "standard-of-care" vitamin D intake. For bone-related health, there are recent age-specific recommendations for vitamin D intake set by the Institute of Medicine and these will be included in the overall recommendations provided to all participants (see below).[185] *Notably, in relation to the outcome of interest (diabetes), there are no recommendations for vitamin D intake;*[185,212] (2) Exclusively on the basis of observational studies, several investigators advise routine intake of doses higher than the Institute of Medicine recommended doses. However, such recommendations are not based on results from randomized controlled trials, which is the premise behind the D2d trial. Indeed, the current knowledge with vitamin D in relation to type 2 diabetes, but also for many other outcomes, is similar to other interventions (e.g. hormone replacement therapy, vitamin E)[152-154,158,213-217] that were strongly supported by observational studies. Several investigators had even questioned the need for trials to test these interventions; however, when prospective controlled trials were completed, these interventions showed harm or no benefit, a result with a direct impact on clinical care. After taking these issues into consideration, the planning committee concluded that *all participants will be advised* *to follow* the Recommended Dietary Allowance (RDA) for vitamin D, which is 600 (up to age 71) or 800 IU/day (age 71 and older).[185] The RDA is the intake that meets or exceeds the requirements for 97.5 percent of the population. The control participants will be given a true placebo, instead of supplying them with their IOM age-recommended level of vitamin D intake, as the latter would complicate the study design and increase cost. The study will not ask participants to specifically avoid sun, but will provide recommendations for sensible sun exposure.

The decision to use a true placebo: (1) balances all competing factors; (2) is consistent with current practice; (3) does not compromise participants' health; (4) favors adequate differentiation in 25OHD concentration between active and placebo arms; and (5) is consistent with the principle of 'equipoise' in relation to the underlying hypothesis.

### 4.3.3 Rationale for no randomization to calcium

A potential mechanism for the effect of vitamin D on t2DM risk may be indirect, via optimizing calcium status; however, the D2d study will not randomize to calcium supplementation because: (1) results from the CaDDM trial (Section 1.c.1) showed no effect of calcium supplementation alone and there was no interaction between vitamin D and calcium on outcomes;[24] (2) there is no evidence that high calcium alone improves t2DM related outcomes;[36,39,218-220] (3) there is concern that calcium supplementation may increase risk of cardiovascular events;[221-223] (4) unlike vitamin D, calcium intake in the U.S. (~700-1000 mg/day) is closer to the current recommended levels[18,185,224-226] and (5) adding a calcium pill may decrease adherence and increase gastrointestinal side effects, hypercalciuria and nephrolithiasis.[227] All participants will receive written information describing the latest recommendations by the Institute of Medicine for calcium intake, as described below.

### 4.3.4 Case for Permitting Supplements Outside of the Study

Based on NHANES data, the average intake of vitamin D in the D2d target age group was approximately 400 IU/day, including from diet and supplements. The study will encourage all participants to optimize their dietary vitamin D intake and supplement their intake of vitamin D from supplements up to 600 or 800 IU per day (depending on their age); however, participants will be discouraged from taking vitamin D-containing supplements on their own throughout the trial, *beyond what is recommended by the Institute of Medicine for their age group (600 or 800 IU/day),* unless specifically prescribed by a physician. The planning committee recognized that for practical reasons, participants may take up to 1000 IU/day of vitamin D on their own from all supplemental sources combined (stand-alone vitamin D supplements, multivitamins, medications containing vitamin D [e.g. Fosamax Plus D]), if they wish. The maximum allowable dose of 1000 IU/day was chosen because it is the dosage contained in many commercially available supplements and also commonly recommended by health care providers. Participants who are unwilling to limit outside-of-study vitamin D intake from supplements to 1000 IU/day for the duration of the study will be excluded from participating. During screening, potential participants who are taking more than the allowed vitamin D dose from supplements will be excluded from the study, unless they agree to lower their supplemental intake to no more than 1000 IU/day for 8-12 weeks (depending on dose) prior to initiating the protocol and also agree not to exceed this supplemental threshold during the entire study.

Depending on the level of 25OHD threshold required to affect type 2 diabetes risk, it is plausible that allowing all participants to receive the age-appropriate Institute of Medicine recommended vitamin D intake may potentially reduce the study's ability to detect the effect of the intervention on diabetes; however, based on available data, the threshold for type 2 diabetes benefit, if present, appears to be no lower than 30-35 ng/mL (without an apparent plateau as 25OHD level increases), which we expect participants in the active group to achieve, while very few in the placebo group will do so, ensuring differentiation between the two arms in achieved 25OHD concentration. Finally, a potential challenge may be that participants will keep changing their vitamin D intake on their own during the study as more information about benefits/harms becomes available; however, this is currently much less likely to happen as the findings of the 2011 Institute of Medicine report, which have been disseminated widely,[185,207,228] call for only a moderate increase in intake of vitamin D compared to the previous guidelines.

*Calcium Supplements:* The Institute of Medicine recommendation for total (dietary and supplemental) calcium intake for adults is 1000-1200 mg/day from either food or supplements; however, there is concern that high calcium intakes from supplements may be associated with adverse cardiovascular effects and development of nephrolithiasis. The current recommendation is to optimize calcium intake through diet with supplementation only as needed to reach the recommended total intake. In the CaDDM trial, among persons with pre-diabetes – a population identical to D2d – total calcium intake was 976 mg per day, nearly all of it coming from dietary sources (859 mg per day), which is consistent with calcium intake in the general population.[18,185,224-226] Therefore, participants will not be allowed to take more than 600 mg/day of calcium on their own from all supplemental sources combined (e.g. stand-alone calcium supplements, multivitamins, medications containing calcium [e.g. Actonel with Calcium]), unless specifically prescribed by a physician. Participants who are unwilling to limit calcium supplementation to 600 mg/day for the duration of the study will be excluded from participating. During the pre-screening, potential participants who are reporting more than the allowed calcium intake from supplements will be excluded from the study, unless they agree to lower their supplemental intake to no more than 600 mg/day or less for 1 week prior to the screening visit and also agree not to exceed this supplemental threshold during the study.

*Other supplements:* Participants are free to take additional vitamins or supplements that do not contain vitamin D or calcium on their own. All supplement use (with or without vitamin D) will be recorded, based on self-report, at each visit.

### 4.3.5 Duration of Intervention and Follow-up Period

It is anticipated that participants who are randomized early will be followed for approximately 4 years for development of the primary outcome, diabetes. Because D2d is an event-driven trial, follow up periods are estimates and will depend on rates of enrollment and retention. There are no definitive data to determine the optimum duration of exposure to vitamin D required to affect diabetes risk. In an observational study in the Nurses' Health Study, women with the highest 25OHD concentration at baseline had a reduction in diabetes by 40% over an approximate 10-year follow-up period.[22] In the Framingham Offspring Study, participants in the highest tertile of predicted 25OHD score had a 40% lower incidence of diabetes during a 6-year follow-up period.[21] Of particular relevance to the D2d trial, in the Diabetes Prevention Program (DPP) cohort, participants with pre-diabetes who had a plasma 25OHD concentration ≥30 ng/mL at baseline had a 28% risk reduction in incident diabetes over a 3-year period, after adjustment for the DPP lifestyle intervention.[25] In the Stockholm Diabetes Prevention Program, among participants with pre-diabetes, the risk of progression to type 2 diabetes was reduced by 62% among those with blood 25OHD above 28 ng/mL compared to those with 25OHD lower than 18 ng/mL over a 8-year follow-up, which corresponds to a 23% reduction for each 4 ng/mL increase in blood 25OHD concentration.[195] These results are consistent with other observational cohorts.[26-28] A post-hoc analysis of a completed trial showed a reduction of approximately 90% in FPG and insulin resistance (HOMA-IR) in participants with pre-diabetes after 3 years of supplementation with vitamin D.[19] In the RECORD trial, a trend towards reduced diabetes risk was seen with vitamin D supplementation after 2-4 years of follow up.[148] In the CaDDM trial, among participants with pre-diabetes, 2,000 IU/day of vitamin $D_3$ improved measures of beta cell function (disposition index) by approximately 40% and glycemia (HbA1c) by approximately 50% after 4 months.[24] In addition to vitamin D-specific mechanisms, the planning committee also took into consideration additional issues, including practical ones, when deciding on the follow-up period, as follows: (1) a shorter follow-up period will minimize "study fatigue" by study personnel and participants, and will maximize retention; (2) a shorter follow-up period will allow the study to be completed faster and results disseminated to the scientific community and public earlier, although with a shorter follow-up period, more participants need to be recruited, to ensure sufficient statistical power; (3) adequate follow-up is required to allow progression to diabetes to occur; (4) supplementation is expected to achieve steady state level of plasma 25OHD concentration within 4 months in the majority of participants;[24,229-231] (4) the rate of progression from pre-diabetes to diabetes is expected to be about 10% per year in the control arm, which is a rate high enough to allow for a statistical comparison with the active arm. After considering all these issues, an average follow up period of approximately 3 years is expected, with early enrollees being followed for approximately 4 years and the sample size was calculated accordingly.

*Rationale for lack of run-in period.* A run-in period before randomization could identify participants whose 25OHD concentration may not respond to vitamin D or develop side effects and also could identify participants that are likely to become low adherers, thereby increasing adherence to study medications and retention. However, a run-in period was not included in the study design because it would add considerable expense without proportional benefit as the proposed intervention and assessment of outcomes are relatively low-burden and vitamin D supplementation raises 25OHD concentration in all participants (although the degree of rise may vary). Furthermore, external validity may be lessened in studies that include a run-in period.[232]

### 4.3.6 Advice on Lifestyle

Eligible participants are at increased risk for diabetes. In accordance with the latest ADA Standards of Care,[233] at baseline, the study will: (1) provide enrolled participants with written information on the current recommendations for prevention of type 2 diabetes, which emphasizes lifestyle intervention aiming to achieve and maintain at least a 5% weight loss and increased physical activity to at least 150 min/week of moderate activity;[233] (2) send a letter to participants' primary care providers indicating that participants are at risk for diabetes and to advocate efforts at weight loss and increasing physical activity. This approach is consistent with the lifestyle advice that was provided to participants in other type 2 diabetes prevention trials that have compared medications with placebo.[11-13,16] Importantly, during the study, all participants will be invited to join the D2d Support and Education Program (SEP). As part of this program, participants will attend group meetings, held twice yearly at each site, to discuss specific topics in nutrition, exercise and diabetes (e.g. healthy eating strategies for the holidays). These meetings will allow the opportunity to meet other participants and will also serve as a way to enhance retention. The Recruitment and Retention subcommittee will suggest topics that the sites can utilize for these meetings. Finally, participants will receive a D2d study newsletter at regular intervals, which will include a section on healthy nutrition and exercise. Participants may lose some weight during the study, but this is not expected to confound the results as it is anticipated that weight change will be similar between arms.

### 4.3.7 Measurements of Other Exposures

*Vitamin D status. Plasma 25OHD concentration* will be measured as a proxy for vitamin D status at baseline and yearly during follow-up to assess the efficacy of the intervention in the active arm at improving vitamin D status and to evaluate for heterogeneity of treatment effect by achieved 25OHD concentration. Measurement of 25OHD will not be done in real-time (see 7.6). *Self-reported vitamin D intake* will be estimated from a validated self-administered semi-quantitative food frequency questionnaire administered at baseline, month 12 and month 36 in participants free of diabetes.[18,234-237]

*Calcium status.* There are no widely accepted measures of adequacy of calcium status. Calcium intake will be used as proxy of calcium adequacy at baseline and during the intervention. *Self-reported calcium intake* will be estimated from a validated food frequency questionnaire administered at baseline, month 12 and month 36 in participants free of diabetes.[18]

### 4.3.8 Principles Guiding Selection of Patient Population

***Rationale for selection of a population with pre-diabetes.*** The study will recruit and follow participants with pre-diabetes. The target population was selected based on published data from post-hoc analyses of a completed trial designed for skeletal outcomes,[19] which showed that vitamin D supplementation had a favorable effect on FPG and insulin resistance (HOMA-IR) only among participants with pre-diabetes at baseline. These findings were confirmed by results from the CaDDM trial among participants with pre-diabetes, which showed that oral vitamin $D_3$ supplementation (2,000 IU/day) improved the disposition index (a measure of beta cell function that accounts for the prevailing insulin sensitivity) and glycemia.[24] In contrast, other studies (most were underpowered) did not report an effect of vitamin D supplementation on glycemia among those with normal glucose tolerance or established diabetes.[32,33,45,47,166] Therefore, D2d is focused on a population with pre-diabetes, which would be the most likely group to benefit from the proposed intervention.

The ADA guidelines define that a pre-diabetes state ("at increased risk for diabetes") exists when a patient does not meet criteria for diabetes and meets one of the following criteria: FPG 100-125 mg/dL **or** 2hPG 140-199 mg/dL **or** HbA1c 5.7-6.4%.[233] The addition of HbA1c as a criterion for the diagnosis of pre-diabetes is expected to increase the number of patients diagnosed with pre-diabetes but may also identify patients at different risk compared to the definition using only the FPG or 2hPG criteria. *For the D2d trial, pre-diabetes is defined if 2-out-of-3 ADA glycemic criteria for pre-diabetes are met.* This definition was chosen to identify a population that is at somewhat higher risk for developing diabetes than when diabetes is defined by only 1-out-of-3 criteria. As a result, the D2d target population will: (1) potentially benefit the most from the proposed intervention and (2) have a higher conversion rate to clinical diabetes, thereby lowering the required sample size.

***Rationale for chosen age range.*** The study will include participants equal to or older than 30 years (≥25 years for people of the following high-risk races: American-Indian, Alaska Native, Native Hawaiian or Other Pacific Islander). Risk for pre-diabetes increases with age but we chose a lower age than what is typically considered higher risk (>45 years), because lower age at diagnosis of pre-diabetes is associated with higher progression to diabetes.[5] Also, this age cutoff would reduce "contamination" with type 1 diabetes or Latent Autoimmune Diabetes of Adults and minimize loss to follow-up due to social mobility. The planning committee selected no upper age limit to increase the generalizability of findings and to allow for heterogeneity of treatment effect (subgroup) analyses by age.

***Rationale for chosen weight/body mass index range.*** The study will include participants with a BMI equal to or higher than 24 (22.5 $kg/m^2$ for Asians) but not higher than 42 $kg/m^2$ because overweight/obesity is a significant risk factor for type 2 diabetes and because those with severe obesity require much higher doses of vitamin D to achieve the desired 25OHD concentration.[238]

***Rationale for lack of "low vitamin D status" as an inclusion criterion:*** Vitamin D status (assessed by blood 25OHD concentration) at baseline is not an inclusion criterion for the following reasons: (1) in small trials, vitamin D benefited those with pre-diabetes irrespective of baseline 25OHD concentration;[19,24] (2) the definition of 'optimal' vitamin D status is controversial and no consensus exists on optimal blood 25OHD level;[172,176,185,239-243] (3) suboptimal vitamin D status is widespread in the U.S. adult population, especially among overweight and obese persons;[49,198,244-250] (4) to ensure that the study is as "pragmatic" as possible and results are generalizable to clinical practice;[251] (5) baseline 25OHD is a treatment selection marker to enter the heterogeneity of treatment effect (subgroup) analyses and to assess its performance, participants with a wide range of 25OHD must be enrolled;[252] (6) 25OHD concentration varies by season;[185,253] and may decrease during acute phase response;[254,255] (7) screening with 25OHD would be cumbersome and expensive. All participants will be encouraged to take the IOM recommended dose of vitamin D for their age.

***Other clinical characteristics****:* Study participants with pre-diabetes will have baseline clinical characteristics (age, gender, race, ethnicity, BMI) representative of the U.S. adult population with pre-diabetes. The target cohort will aim to include a large number of minorities (30-40%, including Black or African American, American Indian/Alaska Native, Asian, Native Hawaiian/Pacific Islander, Hispanic) and women (50%). Participating clinical sites, where recruitment will take place, are selected to ensure adequate distribution of these characteristics in the cohort.

**Potential Differences by Race and Ethnic Composition –** In the preliminary studies, there was no difference among whites vs. non-whites in the association between 25OHD and incident diabetes in the DPP[25] or with vitamin D supplementation (CaDDM trial).[24] Nevertheless, given that non-whites have higher risk of t2DM and different vitamin D-calcium homeostasis, D2d aims to recruit a large proportion, of *non-whites and analyses will test for heterogeneity of treatment effect by race and ethnicity*. R*ace or ethnicity appear to be better determinants of 25OHD variability than objective measures of skin color*[186] suggesting that behavioral variables or physiological variables, other than biosynthesis, may be important in determining 25OHD concentration. Therefore, D2d has not incorporated objective measures of skin color in the study design, because these methods are labor-intensive and difficult to standardize among sites. Data on race and ethnicity data are collected by participant self-report.

## 5. STUDY POPULATION

Specific participation (inclusion/exclusion) criteria are described below. A brief justification for each inclusion/exclusion criterion, if necessary, is shown in brackets [...]

### 5.1 Inclusion Criteria

1. Pre-diabetes ("at increased risk for diabetes") defined by meeting 2-out-of-3 of the following glycemic criteria, established by the ADA in the 2010 clinical practice guidelines, at the baseline visit:[233]
    a. FPG 100-125 mg/dL, inclusive
    b. 2hPG 140-199 mg/dL, inclusive
    c. HbA1c 5.7-6.4%, inclusive
2. Age ≥ 30 years, (≥25 years for people of the following races: American-Indian, Alaska Native, Native Hawaiian or Other Pacific Islander). [Age is a major risk factor for type 2 diabetes; avoid "contamination" with type 1 Diabetes or Latent Autoimmune Diabetes of Adults, conditions that have a different pathophysiology; minimize loss to follow-up due to social mobility; facilitate recruitment and increase applicability of findings; onset of type 2 diabetes is earlier in the certain high-risk ethnic groups]
3. BMI ≥ 24.0 (22.5 for Asians) and ≤ 42.0 $kg/m.^2$ [Overweight/obesity is a major risk factor for type 2 diabetes; those with severe obesity require higher doses of vitamin D[238]]
4. Provision of signed and dated written informed consent prior to any study procedures.

### 5.2 Exclusion Criteria

Exclusion Criteria were selected to: (1) ensure participants' safety; (2) avoid conditions that would affect the outcomes (i.e. minimize competing risk); (3) make recruitment targets realistic; (4) amplify generalizability of study results; (5) maximize participants' adherence with study procedures.

5. Diabetes based on *either* of the following criteria:
    a. History (past 1 year) of hypoglycemic pharmacotherapy (oral or injectable medication approved by the FDA for type 2 diabetes) used for any condition (e.g. pre-diabetes, diabetes, polycystic ovarian syndrome).
    b. Meeting a glycemic criterion for diabetes, as defined by the ADA guidelines (FPG ≥ 126 mg/dL, 2hPG ≥ 200 mg/dL or HbA1c ≥ 6.5%) at the baseline visit.
6. History (past 3 years) of hyperparathyroidism, symptomatic or asymptomatic (i.e., radiographic) nephrolithiasis or hypercalcemia. [Safety]
7. Any medical condition (past 3 years) that in the opinion of the site investigator may increase risk for nephrolithiasis or hypercalcemia during the trial (e.g. sarcoidosis). [Safety]
8. Use of tanning devices within 12 weeks of the baseline visit and unwilling to stop using tanning devices for the duration of the study [interference with intervention]

    *Medications and Supplements*

9. Use of supplements containing vitamin D at total doses higher than 1000 IU/day within 8-12 weeks (depending on dose, as described in Manual of Operations) of the baseline visit initiating the protocol and unwillingness to limit vitamin D supplementation dosage to no higher than 1000 IU/day for the duration of the study. [Safety]
10. Use of supplements containing calcium at total doses higher than 600 mg/day within 1 week of the baseline visit initiating the protocol and unwillingness to limit calcium supplementation dosage to no more than 600 mg/day for the duration of the study. [Safety]
11. Current use of medications or conditions (e.g. untreated celiac disease) that would interfere with absorption or metabolism of vitamin D.

12. Current use of medications approved by the FDA for weight management.
13. Use of thiazide diuretics at a total dose greater than 37.5 mg/day.
14. Use of anticonvulsant drug started within 6 months of screening. Stable regimen of anticonvulsants is allowed.
15. History of intolerance to vitamin D supplements. [Safety]

*Other Medical History*

16. Severe symptomatic cardiovascular disease based on history and physical examination (unstable angina, dyspnea on exertion, paroxysmal nocturnal dyspnea, arrhythmia, congestive heart failure NYHA class II or higher, claudication).
17. History (past 1 year) of myocardial infarction, percutaneous coronary intervention or coronary artery bypass graft. [Safety]
18. History (past 1 year) of cerebrovascular disease (stroke, transient ischemic attack). [Safety]
19. Any type of cancer (past 5 years) except for basal cell skin cancer. [Safety] Participants with prostate cancer (for men over age 55) or well-differentiated thyroid cancer that are not expected to require treatment (except for suppression with thyroid hormone) over the next 4 years are *not excluded.* Volunteers with history of squamous cell cancer of the skin, which was completely excised and with no evidence of metastases, are eligible.
20. History (past 6 months) of treatment with oral (for > 7 days) or intravenous glucocorticoids or disease likely to require oral or intravenous glucocorticoid therapy during the study). [Interference with outcome assessment] Inhaled glucocorticoid use in not an exclusion. Epidural or intra-articular glucocorticoid injections are not exclusions but study visits need to be conducted at least a week after the injection. Persons with adrenal insufficiency treated with physiologic doses of glucocorticoids who are otherwise stable are *not* excluded.
21. History (past 1 year) of substance abuse or unstable psychiatric disorder that in the opinion of the site investigator would impede competence or adherence with study procedures or hinder completion of the study or increase risk. [Safety, adherence] Use of marijuana with a medical prescription is permitted.
22. History of bariatric surgery (e.g., Roux-en-Y gastric bypass, gastric sleeve) or planned bariatric surgery in the next 4 years. Participants with gastric banding more than 2 years ago with self-reported weight stability (defined as weight change no greater than 3 kg during the prior 6 months) are *not excluded.* [Interfere with vitamin D absorption]
23. A life-threatening event within 30 days of screening or currently planned major surgery.
24. Any other unstable active medical condition (including but not limited to liver disease, wasting illness, AIDS, tuberculosis, oxygen-dependent chronic obstructive pulmonary disease, organ transplant, Cushing's syndrome) that in the opinion of the site investigators would impede competence or adherence with study procedures or increase risk. [Safety, adherence, plasma 25OHD may decrease as an acute-phase response[254,255]] Such conditions will be assessed based on self-report and/or review of medical records (if available).
25. Uncontrolled hypertension (systolic blood pressure > 160 mm Hg or diastolic blood pressure > 100 mm Hg). [Safety]
26. Poor venous access. [Safety]

*Laboratory Evaluation*

27. Serum liver transaminase (ALT or AST) higher than 3 times the normal range for the clinical site's laboratory [Safety]
28. Anemia (hematocrit < 32 for women, < 36 for men), whole blood transfusion (within 6 months of screening) or chronic requirement, whole blood donation (within 3 months of screening) or other condition (hemolysis, hemoglobinopathy) rendering HbA1c results unreliable as indicator of chronic glycemia. [Interference with outcome assessment] Participants who donate platelets are

not excluded. Whole blood transfusion or donation does *not* exclude participant, but screening and study visits need to be timed appropriately.

29. Low platelet count (< 50,000). [Safety for blood draws]
30. Chronic kidney disease, defined as estimated glomerular filtration rate [GFR] < 50 mL/min, from creatinine measured at the clinical site's laboratory and GFR calculated centrally. [Vitamin D homeostasis changes as GFR declines. These changes start when GFR falls around 40-60 mL/min per 1.73 $m^2$.[256,257] The planning committee selected 50 mL/min as the exclusion cutoff to ensure that participants maintain GFR > 40 mL/min during the study] Please note: to prevent potential confusion, GFR units will be denoted as mL/min throughout the protocol and associated documents.
31. Hypercalcemia, defined as serum calcium concentration greater than or equal to the upper limit of normal, measured at the clinical site's laboratory. [Safety]
32. Hypercalciuria, defined as spot urine (morning void) calcium-creatinine ratio > 0.275.[258] [Safety]

*Other*

33. Participation (within 30 days of screening) in another interventional research study. [Conflict, "contamination"]
34. Previous randomization in the D2d study. Participants who did not qualify after screening may be screened again if the prior reason for exclusion has been addressed (e.g. high blood pressure is treated).
35. Any other reason that in the opinion of the site investigator would impede adherence with study procedures or hinder completion of the study or increase risk (e.g. use of non-approved or experimental drugs, inability to follow instructions or understand the informed consent, dementia, unable to remain in the program for the duration of the study, inability to comply with the study protocol for any reason). [Safety, adherence]

*Women only*

36. Pregnancy (past 1 year by report or positive pregnancy test at screening), intent to become pregnant in the next 4 years or unprotected intercourse. [Safety] History of gestational diabetes is *not* an exclusion criterion.
37. Currently breastfeeding. [Safety]
38. Use of oral contraceptives or menopausal hormone therapy started within 3 months of baseline. Stable regimen of oral contraceptives or any other hormonal method of contraception (e.g. implantable) is allowed. [Safety, interference with intervention]

## 6. RECRUITMENT AND ENROLLMENT OF PARTICIPANTS

### 6.1 Recruitment overview

Potential participants will be recruited after IRB approval of the protocol. The screening and informed consent process is staged in 2 parts, *Pre-screening* phase followed by a formal *Screening* visit, (Figure 4.1) to: (1) maximize the prospective participant's understanding of the study purpose and procedures required for an informed decision regarding participation; (2) promote efficiency.

The pre-screening phase will be site-specific (i.e. over the phone and/or web pre-screening) followed by a screening visit. Each site, based on their prior experience, will provide a detailed site-specific recruitment plan that includes a pre-screening recruitment strategy to identify individuals with high likelihood of pre-diabetes that will be invited in for the formal screening visit, which will be identical for all sites. The intent of the staged pre-screening phase is to allow collaborating sites flexibility in their approach of recruiting participants, while maintaining a standard study-specific set of inclusion/exclusion criteria. Publicity and recruitment efforts are the responsibility of the sites; however, the CC will assist with centrally developed publicity and recruitment procedures and tools (e.g. brochures, flyers, and posters) that the sites may adapt. Active recruitment will take place year round at regular rates of enrollment to ensure equal exposure of all participants to UV-B.

### 6.2 Recruitment Progress

Recruitment of about 100-150 participants per site is expected to take place over a 2-year period. At each site, the research coordinator will work with the site PI and D2d Project Manager to develop a written site-specific recruitment plan that will be submitted to and reviewed by the Recruitment and Retention subcommittee prior to study initiation. Recruitment will be closely monitored and short-term recruitment goals will be established (based on total recruitment goals for each site) and reviewed monthly at each site and by the CC, quarterly by the Recruitment and Retention subcommittee and yearly by NIDDK, DSMB and local IRB. The overall recruitment goal is 4-8 enrolled participants per site per month. Based on review of recruitment progress, changes in the pre-screening strategy and site-specific recruitment plan may be required.

### 6.3 Informed Consent Process

At first contact with participants, prior to any study specific procedures, the informed consent process will be started. If the first contact is over the phone, a sample site-specific script will be read to the potential participant providing a brief overview of the study, informing him/her that they will be asked questions to determine initial eligibility, and if potentially eligible, callers will be invited to the research site for a screening visit. If the first contact is over the web, a web-based version of the script will be provided and the potential participant will be informed that they can discontinue the web based questionnaire at any time.

At the initial visit, written informed consent will be obtained prior to any study procedures. A qualified member of the site research team (e.g. site PI, co-investigator, research coordinator, research assistant or clinical research nurse) will discuss with the potential participant the study's purpose, procedures, risks, potential benefits, and rights as a participant. Once all questions have been answered and concerns addressed, the potential participant will be asked to sign the written informed consent form. The informed consent process is ongoing and interactive. Participants will be given the opportunity to ask questions throughout their participation in the study. Participants will be told that they can cease participating in the study at any time for any reason. A verbal or written "withdrawal of consent" will be requested for participants who elect to discontinue active participation from the study (i.e. go "off study").

## 6.4 Pre-Screening

Clinical sites will employ a variety of sources including electronic databases (e.g. electronic medical records and research volunteer databases); community-based advertising (e.g. hospital newsletters, and specific local newspapers); targeted outpatient hospital clinics (e.g. primary care, cardiology); mailings to primary care physicians in the each metropolitan area; social media (e.g. craigslist), and study press releases to local news media to recruit potential participants. A public web page has been created for D2d that each site can refer to in their local advertisements. The web site will provide national exposure and will provide interested participants with the contact information to the local sites. Because pre-diabetes is not a diagnosis with a clear ICD code, the study will emphasize pre-screening based on presence of self-reported major risk factors (age, weight, family history etc.) and objective data (e.g. HbA1c) that are available through electronic medical records. The goals of the pre-screening phase are to: (1) identify potentially eligible participants; (2) initiate the informed consent process; (3) conduct a preliminary verification of eligibility criteria; (4) promote efficiency by pre-selecting candidates with high likelihood of eligibility after formal screening. The pre-screening strategy is site-specific and is based on what has worked well previously at the participating site. Each site will describe in detail their procedures for identifying participants likely to have pre-diabetes, based on meeting the major inclusion/exclusion criteria that will be invited for a formal screening visit. For example, a site may employ a diabetes-risk engine (e.g. ADA Diabetes Risk Score) to pre-screen volunteers over the phone, via the web or in-person.

## 6.5 Screening & Assessment of Eligibility

The goals of the formal screening visit are to: (1) complete the informed consent process and obtain written informed consent; (2) assess eligibility. Volunteers will be seen at each site in the morning after an overnight fast for a screening visit lasting approximately 1.5 hours. At this visit, after written informed consent is obtained (unless it has been obtained already during a pre-screening optional visit), the following will be performed: medical history, vital signs (height, weight, blood pressure and heart rate), and laboratory measurements to assess eligibility for the study (complete blood count, liver function tests [AST, ALT], serum calcium, serum creatinine and estimation of creatinine clearance, pregnancy test for women with reproductive potential, FPG and HbA1c). Laboratory measurements will be performed locally, (i.e., by the site's clinical laboratory) or by any other laboratory (e.g., LCBR at U. Vermont, which serves as the study's Central Laboratory), depending on site's preference. All screening laboratory results will be reported to volunteers and their health care provider(s).

Volunteers will be invited to the baseline visit if they meet the following criteria at the screening visit: (see Figure 6.1):

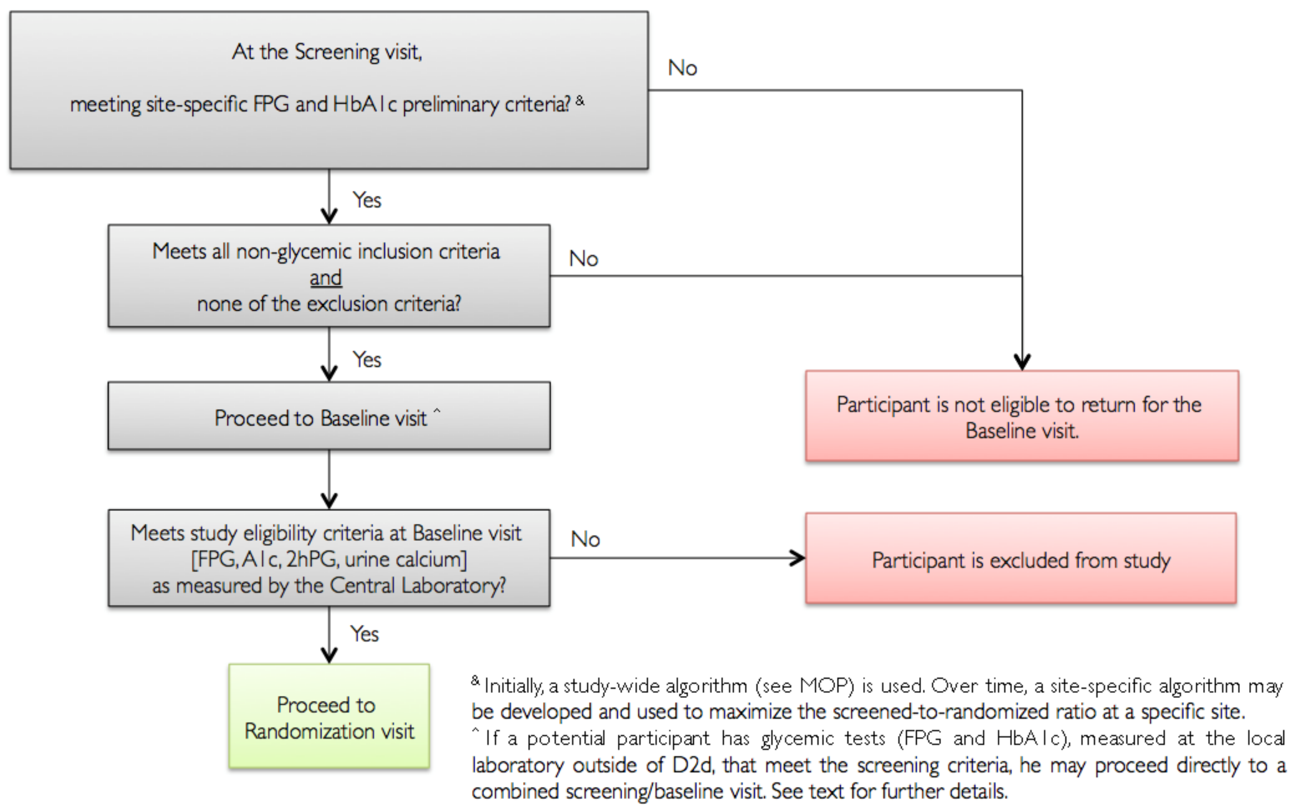
a) FPG and HbA1c results are within range, as defined by site-specific criteria. At the screening visit, sites use a specific algorithm to determine whether the FPG and HbA1c values fall within range to proceed to the baseline visit. A default algorithm has been developed for all sites to use initially (shown at the bottom of Figure 6.1). As needed, the success of the default algorithm for that site is reviewed and, if appropriate, a site-specific algorithm can be developed to maximize the screened-to-randomized ratio.
b) All other non-glycemic inclusion criteria and meet none of the exclusion criteria

At the baseline visit, a 75-gram OGTT will be performed to obtain blood for FPG, 2hPG and HbA1c to confirm the glycemic eligibility criteria, urine will be collected to assess the calcium-creatinine ratio eligibility criterion and physical examination will be done. The Central Laboratory will conduct the analyses of tests obtained at the baseline visit. Volunteers who meet the eligibility criteria at the screening visit *and* the physical examination, and glycemic and urine criteria at the baseline visit (measured by the Central Laboratory) will be invited to the randomization visit.

Multiple screenings are allowed for participants who missed the inclusion/exclusion criteria by a small margin. For example, if a participant does not qualify due to high blood pressure, she may return for screening after her hypertension is treated. It will be up to the site PI to decide when a participant may return for re-screening.

If a potential participant has recent glycemic results (FPG and HbA1c) that meet the screening criteria, he may proceed directly to a combined screening/baseline visit, as long as the visit occurs within 6 months of these tests. During this combined visit, all screening procedures will take place, including blood tests that have not been done by the local laboratory within the last 6 weeks. At this combined visit, blood and urine will be collected for baseline measurements and processed but sent to the central laboratory *only if the participant meets all other screening criteria.*

Figure 6.1: Flow diagram of assessment of eligibility at screening and baseline visits




## 6.6 Retention of Participants / Avoidance of Missing Data

Each site will build on its experience to maximize participant retention. In general, the following will be implemented: (1) Participants will be followed until study end, even if they discontinue randomized treatment, have initiated other interventions or unmasking has occurred. Participants will be educated on the importance of completing all assessments. (2) At every visit, participants will receive information and tips to promote retention and adherence to study procedures.[259,260] (3) Participants who are lost to follow-up but have not gone formally "off study" will be termed "inactive" to reflect the possibility that they will resume adherence with study medication and will return for outcome measurements. (4) Sites will employ a variety of methods to promote retention by maximizing rapport with participants and their families as described in section 7.6.

In addition, as part of the Support and Education Program (described earlier), participants will be invited to attend group meetings, held twice yearly at each site, to discuss specific topics in nutrition, exercise and diabetes (e.g. healthy eating strategies for the holidays). These meetings will allow the opportunity to meet other participants and will serve as a way to enhance retention. Finally, at regular intervals, the CC will develop a D2d study newsletter and provide to the sites, which they can further customize by adding their logo and any other relevant site-specific information and send to participants by mail or e-mail. The newsletter will have a section on healthy nutrition and exercise and will also include specific tips to promote adherence.

### 6.7 Participant Study Stipend

Participants will be reimbursed for their time and effort during the study visits and for the cost of transportation and/or parking. The rates, timing and form of reimbursement will be determined by the expected time requirement and complexity of the study visit and may differ by site. A site-specific stipend schedule will be described in the informed consent form for each site.

# 7. STUDY INTERVENTION

## 7.1 Summary

Participants will be randomized to two equal groups and assigned to vitamin $D_3$ or matching placebo. Treatment assignment will be double masked.

## 7.2 Study Pills

### 7.2.1 Description of Vitamin D and Placebo Pills

Vitamin D or placebo will be dispensed as a single pill taken daily. The vitamin D pill will be a white soft-gel containing 4,000 IU of cholecalciferol [$D_3$] and additional (inert) ingredients. The placebo pill will be a soft-gel identical looking in size, shape, texture, color, odor and taste with the same inert ingredients as the active pill, but without vitamin D. The bottles that will hold the active or placebo pills for distribution to the participants will also be identical. Based on stability studies, the manufacturer has established the expiration period for both vitamin D and placebo pills to be at least 24 months at the required storage conditions. The actual contents of both active and placebo pills will be confirmed at the beginning of each manufacturing lot, as described below.

### 7.2.2 Manufacturing, Formulation, Packaging and Labeling

Tishcon Corp. will prepare the vitamin $D_3$ and matching placebo soft-gel pills intended for oral administration according to the United States Pharmacopeia standards and Good Manufacturing Practices. The manufacturer will ship study pills to the Drug Distribution Center in bulk where they will be packaged into bottles with enough pills (vitamin $D_3$ or placebo) for a 6-month period plus enough additional pills in case participants need to postpone their follow-up visit for up to 8 weeks. The Drug Distribution Center will code each bottle with a unique serial number that links to its contents (vitamin D or placebo). This number will be linked to the randomization scheme and drug dispensation at baseline and during each 6-month visit. The expiration date and any other information required by local and federal regulations will be printed on the bottle. The bottles and labels for the vitamin D and placebo pills will look identical except for the unique serial number.

### 7.2.3 Quality Control

The manufacturer (Tishcon Corporation) performs quality control analyses on each lot that is shipped from the manufacturing plant. A Certificate of Analysis is generated by Tishcon Corp. and supplied to the Drug Distribution Center and the CC documenting quality control, including assaying for the amount of the active ingredient (vitamin $D_3$) to ensure that variation from the labeled claim is within United States Pharmacopeia specifications. In addition, when the Drug Distribution Center receives the study pills, it conducts an independent quality control that includes potency analysis of the contents prior to release of the product to the sites. If the results of the initial testing by the independent source (Drug Distribution Center) are outside of the desired range, Tishcon Corp. will manufacture a new lot and the above process will be repeated. In addition, the Drug Distribution Center will perform long-term stability testing to confirm that the potency of vitamin D has remained stable during the 24-month shelf life.

### 7.2.4 Accountability Procedures for the Investigational Product(s)

Bottles with study pills will be stored at the Drug Distribution Center until shipment to sites. Study pills will be shipped from the Drug Distribution Center to each site's research pharmacy where pills will be stored under Good Clinical Practices. If a site does not have a research pharmacy available, an alternative storage and distribution plan must be provided to and approved by the CC and the Drug Distribution Center. The Drug Distribution Center will work closely with the CC, the study pill manufacturer and all sites, using a web-based real-time inventory and randomization system (Interactive Web Response System), to monitor supply chain and manage inventory and to ensure that an adequate supply of bottles with vitamin D and placebo soft-gels are present at each site at all times. Upon receipt of the study pills, the site pharmacist or designee will review the shipping document and confirm receipt of the study pills on the Interactive Web Response System. After the initial shipment, subsequent shipments to sites will be determined based on recruitment rate and site inventory that will be actively monitored in real time via the Interactive Web Response System.

At randomization and every six months, the site research pharmacy or designee, using the Interactive Web Response System, will distribute study pills to the research staff for distribution to participants according to the randomization code.

Study staff will review study pills returned by participants at each visit. Pill counts will be completed by a member of the research staff and documented. The returned unused pills will then be destroyed following local guidelines and disposal will be documented.

### 7.3 Randomization and Masking

#### 7.3.1 Randomization Process and Sequence Generation

Randomization will be in a stratified and blocked fashion in a 1:1 ratio. Stratification factors will be by site, BMI ($<30$ or $\geq 30$ kg/m$^2$) and race (White or non-White [e.g. American Indian, Asian, Pacific Islander, Black]). Using stratified randomization helps balance the number of participants in each group for BMI and race, which are major determinants of vitamin D status and diabetes risk. The use of permuted blocks within each stratum will guarantee the balance between the two treatment arms during the course of randomization. The randomization will be administered by the Drug Distribution Center via the Interactive Web Response System.

Upon the participant's arrival at the randomization visit, the site research coordinator (or his/her approved designee) will log into the Interactive Web Response System and will enter the required stratification information (site, BMI and race). The system will use the stratification information to randomize the participant and assign a pill bottle (vitamin D or placebo). The research coordinator will then notify the site research pharmacist of the pill bottle number. The site pharmacist or designee will dispense a six-month supply of masked study pills to the research coordinator or nursing staff for distribution to participants.

#### 7.3.2 Allocation Concealment (Masking)

Assignment will be double-masked. Participants will be masked as to the composition of their pills. All study personnel, including site investigators, research coordinators, nurses, pharmacists and laboratory staff, will also be masked as to composition of study pills.

Results of laboratory outcome (glycemic) tests will *not* be provided to sites, participants or health care provider (s) with the exception of the results obtained at the screening and baseline visits. After a participant reaches the primary endpoint of diabetes, glycemic results will be shared with the site, participant and her health care provider(s). Results of safety tests and certain other variables (medical history and physical examination, vital signs, waist circumference) will be reported to participants.

The randomization code will be masked until data files have been cleaned and locked and data analyses are completed.

### 7.3.3 Breaking of Randomization Code (unmasking)

At no time will the code of the treatment assignment be broken without the expressed knowledge and consent of the site PI and the CC. Unmasking will only occur if there is a serious adverse event (SAE) or any other adverse event (AE) that is “severe” and “probably/definitely related” to the study pill, *and* the site PI (and site study physician, if site PI and physician are not the same person) determines it is necessary for the care of the participant to be unmasked. It is expected that almost all AE and SAEs will be handled without unmasking. If it is determined that the masking needs to be broken, then study pills will be temporarily held and the assignment will be disclosed only to research personnel that need to know (e.g. site PI, site study physician and/or research coordinator). Participants who discontinue study pills will not go “off study” and will remain in the study and return for all remaining scheduled follow-up visits and procedures, consistent with the intention-to-treat principle. It is expected that unmasking will be exceedingly rare as it will be restricted to situations in which knowing the assignment will change the course of care of the participant. When unmasking occurs, the site PI will review and report to the CC and IRB the circumstances that led to it. If it is determined that the study pills are not related to the adverse event, participant will resume taking the study pills, consistent with the intention-to-treat principle.

## 7.4 Administration of Study Pills

Participants will be asked to take the study pills daily in the morning, preferably with breakfast, until the end of the trial and to bring the pill bottles with them to all scheduled visits for adherence assessment. During the scheduled 6-month visits, participants will return the study pill bottles and receive new bottles with a supply of pills for the next 6 months (plus enough additional pills in case participants need to postpone their follow-up visit for up to 8 weeks).

If a participant is unable to come for a semi-annual visit (e.g. month 18, month 30) and had a normal safety assessment done at the last annual visit, the semi-annual visit may be conducted over the phone and, provided there are no safety concerns, a 6-month supply of study pills may be shipped to the participant. The participant needs to return for the next annual visit, before any more study pills are dispensed.

Every time participants receive a new bottle of pills, they will receive written instructions on proper administration of the study pills and the need to report any changes in their health or medications to the research staff. The research staff will also review instructions verbally. Participants will also receive information and tips to promote adherence with study pills.

## 7.5 Modification of Study Intervention

If a participant is unable to tolerate the study pills due to safety concerns (i.e. symptom, sign or laboratory abnormality) or for any other reason, study pills will be temporarily held of permanently discontinued (depending on the reason). Specifically, study pills will be permanently discontinued for: hypercalcemia, nephrolithiasis, hypercalciuria and kidney dysfunction, per criteria defined in the Data and Safety Monitoring Plan (DSMP). Study pills will also be held or discontinued at participant’s request. Study pills may be temporarily held for an adverse event (that is unrelated to study pills) or for other reasons. Study pills will be held during pregnancy and lactation. Participants who permanently discontinue study pills will remain in the study and return for all remaining scheduled follow-up visits until study end, consistent with the intention-to-treat principle. The importance of remaining in the study, even if they stop taking the pills, will be emphasized to participants during the informed consent process and at every visit.

### 7.6 Adherence with Study Procedures and Study Pills

Participants' adherence to study procedures is critical to the successful conduct of D2d, especially because of its impact on power; therefore, the study will aim to maximize adherence, by taking the following steps: (1) Prevent/minimize potential adherence problems before participant enrollment, as follows: the study design has kept the intervention (i.e. single daily dose study pill regimen) and follow-up procedures uncomplicated; the informed consent process will emphasize the importance of adherence; exclusion criteria include those with higher probability of non-adherence. (2) Maintain/enhance adherence during the trial, as follows: participants will be seen three times during the first 6 months (at baseline, 3-month and 6-months) and every 6 months for the remainder of the study; at the midpoint of each 6-month interval, participants will receive a telephone call to encourage adherence, assess for adverse events or changes to medical history and concomitant medication use and be reminded to report the diagnosis of diabetes outside of D2d or planned (or actual) use of any new medications (especially diabetes medications) and be reminded of their upcoming visit (alternatively, participants may also be contacted via email, or text based on their preference); study staff will provide participants with a personalized study calendar with visit dates; at every visit, participants will also receive information and tips to promote retention and adherence to study procedures; each site PI, co-investigators, research coordinator and staff will be available to answer participants' questions and will maintain a flexible schedule to meet the participants' needs; sites will also employ a variety of site-specific methods to promote retention by maximizing rapport with participants and their families (e.g. holiday and/or birthday cards). (3) Monitor adherence during the study based on pill counts (at every 6-month visit). Individual adherence to study medication will be defined as a pill intake over 80% based on pill counts. If a participant does not meet the adherence minimum target of 80% at any visit, s/he will be reminded of the importance of adherence to the study's objectives, the administration instructions will be reinforced and the site research staff will work with the participant to identify causes that contribute to suboptimal adherence and develop approaches to overcome barriers to adherence. Adherence during the study will also be monitored by measurement of the physiological response biomarker to vitamin D supplementation (plasma 25OHD concentration) at yearly intervals. A pre-specified absolute level of increase in 25OHD concentration cannot be used for adherence for each participant given the individual variation in response to supplementation, although it is expected that adherent participants will have a rise in 25OHD concentration from baseline. *The 25OHD concentration will not be available in real-time as measurements will be conducted at the end of the study*. Planned analyses will test for effect modification by adherence based on (1) adherence based on pills counts and (2) achieved plasma 25OHD concentration (see Section 11).

## 8. PROCEDURES AND OUTCOME MEASURES

### 8.1 Study Visits and Phone Calls

The baseline visit (BAS) should occur after the results of screening lab analyses have been reviewed and preliminary eligibility determined, within 6 weeks of the screening visit. The site investigator will review the results of the baseline visit laboratory measures and determine if the participant meets the study eligibility criteria. If eligibility is confirmed, the participant will return for the randomization visit, within 3 weeks of the baseline visit.

Follow-up visits will occur at 3 months (M03), 6 months (M06), and every 6 months thereafter (e.g. M12, M18) until study end. Follow-up visits should occur within ±2 weeks of the scheduled date. However, follow-up visits may be postponed for up to 8 weeks if a temporary concomitant condition exists that would affect glucose tolerance (e.g. infection) or its assessment (e.g. blood transfusion or blood donation) or for any other administrative or social reason. The reason for the postponement of the visit will be documented in the electronic data capture system.

The last scheduled visit (semi-annual or annual) will serve as the end-of-study visit. When D2d is approximately within 2 months of reaching its goal of the number of diabetes events needed, a study-wide announcement will notify sites that all subsequent scheduled visits will be considered the end-of-study visit. At the end-of-study visit, activities will include those that would occur at the scheduled semi-annual (e.g., fasting labs) or annual visit (e.g., OGTT).

During each follow-up visit, the research coordinator will assess study pill adherence, and question the participant regarding the occurrence of adverse events or changes to medical history and concomitant medication use.

Phone contact is scheduled at the midpoint of each 6-month follow-up visit after the 6-month visit (e.g. M09, M15). Participants will be contacted by the research coordinator to encourage adherence, assess for adverse events or changes to medical history and concomitant medication use, and be reminded of their upcoming visit. Participants may be contacted at other times between visits for invitations to Support and Education Program events, to respond to questions, or other study-related issues. These contacts may be conducted via email, text, or other means of communication, based on participants' preference.

Unscheduled visits may occur between scheduled visits for the following reasons:

- Evaluation of adverse events.
- Confirmatory testing for assessment of the primary outcome, as described in section 9.
- Primary outcome met outside of D2d, as described in section 9.

In addition, participants will receive reminder communication (phone call, letter, email, or text) one week prior to each scheduled visit.

At every contact (visit or phone call), site staff will educate participants to *contact the Research Coordinator as soon as possible* after a clinician outside of D2d makes the diagnosis of diabetes or if the clinician plans to initiate diabetes-specific pharmacotherapy (for any reason).

### 8.2 Laboratory Procedures and Outcomes

Laboratory samples will be collected during scheduled visits and also in-between visits, as needed, to evaluate symptoms consistent with diabetes or adverse events. All laboratory tests and procedures will be done in the morning and participants will be instructed to fast overnight prior to the visit for at least 8 hours. Safety assessments (with the exception of urine calcium-creatinine ratio) will be analyzed at each site's chosen clinical laboratory. Laboratory samples for assessment of outcomes (at baseline and follow-up visits) will be collected and processed at each site and then sent to the Central Laboratory for storage and analyses.

### 8.2.1 Screening Laboratory Tests

The following laboratory tests will be drawn and analyzed *at each site's chosen clinical laboratory*, *during the screening visit and* results will be used to determine participant's preliminary eligibility for the study. Results will be provided to participants and their health care provider(s).

- Complete blood count (CBC: white blood cell count without differential, hemoglobin/hematocrit, platelet count)
- Liver transaminases (AST, ALT)
- Serum calcium
- Serum creatinine and estimated creatinine clearance (GFR, calculated centrally)
- Pregnancy test for women of reproductive potential. Point-of-care urine testing will be done followed by confirmatory blood testing, if point-of-care urine test is positive. If point-of-care urine testing is not an option, a urine or serum test will be done at the site's laboratory.
- FPG
- HbA1c

The following laboratory tests will be drawn and analyzed *at the Central Laboratory during the baseline visit.* Results will be provided to participants and their health care provider(s).

- Urine calcium-creatinine ratio (early morning spot urine specimen)
- FPG
- HbA1c
- 2hPG (OGTT)

### 8.2.2 Safety Laboratory Tests during the Study

To monitor safety, the following laboratory tests will be drawn and analyzed at each *site's clinical laboratory* at M03, M12, at yearly visits thereafter, and as needed to evaluate symptoms and/or physical signs. Results will be provided to participants and their health care provider(s).

- Serum calcium
- Serum creatinine and estimated creatinine clearance (GFR, calculated centrally)

To monitor safety, the following laboratory test will be analyzed at the Central Laboratory at BAS, M03, M12, at yearly visits thereafter, and as needed. The Central Laboratory will analyze the urine calcium-to-creatinine (instead of the site's clinical laboratory) because: (1) this variable also serves as a safety outcome of interest and would be important to standardize its measurements; (2) the result does not need to be communicated back to the participant urgently. Results will be reported to the clinical site and also provided to participants and their health care provider(s).

- Urine calcium-creatinine ratio

To monitor safety, the following laboratory test will be drawn and analyzed at each *site's clinical laboratory*.

- Pregnancy test for women of reproductive potential who report missing two consecutive menstrual periods *based on the participant's typical menstrual cycle.*

### 8.2.3 Outcome Laboratory Tests

At scheduled visits, as shown below, blood and urine samples will be collected and processed (prepared for freezing and shipping) at each site and then sent to the Central Laboratory for immediate analyses of the outcome measures and for storage for subsequent planned analyses and future ancillary analyses.

**At baseline (BAS) and annually**, the following will be collected and analyzed upon receipt of the specimens by the Central Laboratory. Results will be reported to the clinical site, as described below.

- FPG, HbA1c, 2hPG (from the OGTT)

*At baseline*, the numerical values of these tests will be reported to the sites to confirm participant's eligibility for the study. If a participant meets the study criteria for diabetes at baseline, he will not be randomized and his participation will end. The participant will be referred to his primary care provider. Results will also be provided to participants and their health care provider(s).

*At annual visits* after the baseline visit, numerical values will *not* be reported to the sites. The web-based electronic data capture (EDC) system will notify the sites only that results do not meet criteria for diabetes or meet criteria for diabetes and confirmatory testing is needed.

Results of the following tests are not required in real-time and will not be reported to the sites.

- Glucose (from the OGTT at 30 minutes)
- Insulin (fasting, 30 minutes and 120 minutes from the OGTT)
- 25OHD

The following will be stored for future studies:

- Blood (plasma and serum) and urine
- Whole blood for DNA (collected at BAS visit only)

**At each of the in-between 6-month visits (e.g. M06, M18…),** the following will be collected and analyzed upon receipt of the specimens by the Central Laboratory. Numerical values will *not* be reported to the sites. The EDC system will notify the sites only that results do not meet criteria for diabetes or meet criteria for diabetes and confirmatory testing is needed.

- FPG, HbA1c

The following will be collected at the M06 visit only and stored for future studies:

- Blood (plasma and serum)

**At the M03 visit,** the following will be collected and analyzed upon receipt of the specimens by the Central Laboratory:

- Urine calcium-creatinine ratio (see section 8.2.2)

**Confirmatory testing**. If any one of the glycemic measures (FPG, HbA1c, 2hPG) meets criteria for diabetes during a scheduled visit, including the end-of-study visit, a confirmatory visit will be completed to repeat the measures as described in section 9. The Central Laboratory, via the EDC, will notify the clinical site of the need for confirmatory testing. A diagnosis of diabetes will be made when any two sequential glycemic measures are positive for diabetes as described in section 9.1. Numerical values will *not* be reported to the sites, participants and health care providers unless criteria for diabetes are met at the confirmatory visit. Otherwise, the EDC system will notify the sites only that results do not meet criteria for diabetes.

To lessen the chance that participants would dramatically alter their lifestyle after a positive glycemic test on initial testing or be unduly alarmed, study personnel will emphasize to participants that an initial positive test for diabetes may often not be verified at confirmatory testing and that, rarely, repeat testing may be required for quality control purposes.

**After participants reach the diagnosis of diabetes,** they will continue in the study, including taking the study pills without unmasking and will complete all subsequent planned visits. OGTT and serum for insulin will not be drawn after the diagnosis of diabetes.

The following blood and urine samples will continue to be collected after diabetes diagnosis, per study schedule, and results will provided to the site, participant and her healthcare provider(s):

- FPG
- HbA1c
- Serum calcium
- Serum creatinine
- Urine calcium-creatinine ratio

The following blood and urine samples will also continue to be collected, per study schedule, but results will not be reported to the site.

- 25OHD
- Blood and urine for storage

**End-of-study visit**. The end-of-study visit will coincide with a scheduled visit (semi-annual or annual), and the activities will include those that would occur at the scheduled semi-annual (i.e., FPG, HbA1c) or annual visit (e.g., FPG, HbA1c, 2hPG, serum calcium and creatinine, urine calcium-creatinine ratio, blood and urine for storage, FFQ).

### 8.3 Vital Signs

Vital signs composed of height, weight, blood pressure and heart rate will be assessed at screening, baseline and all follow-up and unscheduled visits. Waist circumference will be measured at baseline only. Results of these measurements will be reported to participants, as it is not feasible to keep these data masked from participants.

### 8.4 Other Measures / Assessments

- Food Frequency Questionnaire

A validated self-administered semi-quantitative food frequency questionnaire will be used to estimate self-reported vitamin D and calcium intake and other nutrients of interest at baseline, month 12 and month 36 in participants free of diabetes.

- Physical Activity Questionnaire

*Physical Activity* (kcal/d) will be measured by the 7-day International Physical Activity Questionnaire at baseline, 6-month visit and annually.[261]

- NDEP Questionnaire (optional)

At baseline, all participants will be provided with the written educational material “Small Steps. Big Rewards. GAME PLAN” developed by the National Diabetes Educational Program (NDEP) per section 4.3.6. As part of an approved ancillary study, approximately 500 participants will be given the option to complete a pre- [at BAS visit] and post- [at M03 visit] survey to assess the usability of the

"Small Steps, Big Rewards, GAME PLAN" material. Once 300 matched BAS-M03 questionnaire pairs have been completed, administration of the NDEP questionnaire will be discontinued.

### 8.5 Withdrawal from the study (going "off study")

The D2d study distinguishes between "non-retention" (being permanently off study) and "non-adherence" (being off study medication). Participants can go "off study" only for withdrawal of consent, which is defined as no longer wishing to participate in all aspects of the trial. Proper use of the term "withdrawal of consent" will be monitored during the study. The site investigator may also withdraw participants for a safety reason, but that will be a rare occurrence.

Unless participants go "off study", they will be asked to return for all scheduled follow-up evaluations to collect outcome and safety data, even after they have reached the primary endpoint. Participants will be educated during the consent process and will be reminded at every visit of the importance of completing planned assessments.

### 8.6 Inactive status

Participants, for safety reasons, personal choice or any other reason, may need to go "off study medication." However, they will continue with outcomes assessment as planned. Participants who are lost to follow-up but have not gone formally "off study" (i.e. have not provided a verbal or written *withdrawal of consent*) will be termed "inactive" to reflect the possibility that they will resume adherence with study medication and will return for outcomes measurements. Both "inactive" and "off study" participants are included in the estimated attrition rate that went into the sample size calculations.

#### 8.6.1 Inactive status due to pregnancy

For participants who become pregnant during the study, study pills and study procedures will be held, i.e. participant will become "inactive," but she will not go off study and will be encouraged to remain in the study and return for scheduled assessments after completion of pregnancy. If the woman agrees to remain in the study, laboratory testing will resume at 8 weeks post-partum. Study pills will resume after participant ceases lactation and after 8 weeks post-partum (whichever is later).

## 9. ASCERTAINMENT OF OUTCOMES & POTENTIAL CONFOUNDERS

All interactions with study participants and all outcome measurements will be performed by study staff that is masked to study group assignment.

### 9.1 Primary Endpoint (Outcome)

The primary outcome will be *time to progression from pre-diabetes to incident (new-onset) diabetes, defined by laboratory criteria*, as follows:

*Laboratory diagnosis of diabetes,* based on the following ADA glycemic criteria[262] measured at study visits: FPG ≥ 126 mg/dL, 2hPG ≥ 200 mg/dL **and/or** HbA1c ≥ 6.5% (Figure 9.1.1 and 9.1.2).

All three glycemic measures will be assessed at scheduled yearly visits by conducting a 75-gram OGTT (Figure 9.1.1). If all three glycemic measures are negative for diabetes, then the participant does not have diabetes and will continue on the assigned treatment. If two or all three of the glycemic measures are positive for diabetes, the participant will be considered to have reached the diabetes outcome and no confirmatory testing is required. If only FPG or HbA1c is positive for diabetes, then a confirmatory visit to repeat the *same glycemic test that was positive* will be completed within 8 weeks. If the repeat measure is also positive for diabetes, then the participant will be considered to have reached the diabetes outcome. For example, HbA1c = 6.5% and FPG = 119 mg/dL at the scheduled annual follow-up visit and HbA1c = 6.6% at the confirmatory visit. If only the 2hPG is positive for diabetes, then a confirmatory visit (with an OGTT) to repeat all three glycemic measures will be completed within 8 weeks. If the repeat 2hPG is positive for diabetes, then the participant will be considered to have reached the diabetes outcome. If the repeat 2hPG is negative for diabetes but *both* repeat HbA1c and FPG are positive for diabetes, then the participant will be considered to have reached the diabetes outcome. Otherwise, the participant does not have diabetes and will continue on the assigned treatment.

Fasting plasma glucose and HbA1c will be assessed at scheduled semi-annual visits (Figure 9.1.2). If both glycemic measures are negative for diabetes, then the participant does not have diabetes and will continue on the assigned treatment. If both glycemic measures are positive for diabetes, the participant will be considered to have reached the diabetes outcome and no confirmatory testing is required. If only one of two glycemic measures (FPG or HbA1c) is positive for diabetes, then a confirmatory visit to repeat the *same glycemic test that was positive* will be completed within 8 weeks. If the repeat measure is also positive for diabetes, then the participant will be considered to have reached the diabetes outcome. For example, HbA1c = 6.3% and FPG = 127 mg/dL at the scheduled semi-annual follow-up visit and FPG = 129 mg/dL at the confirmatory visit.

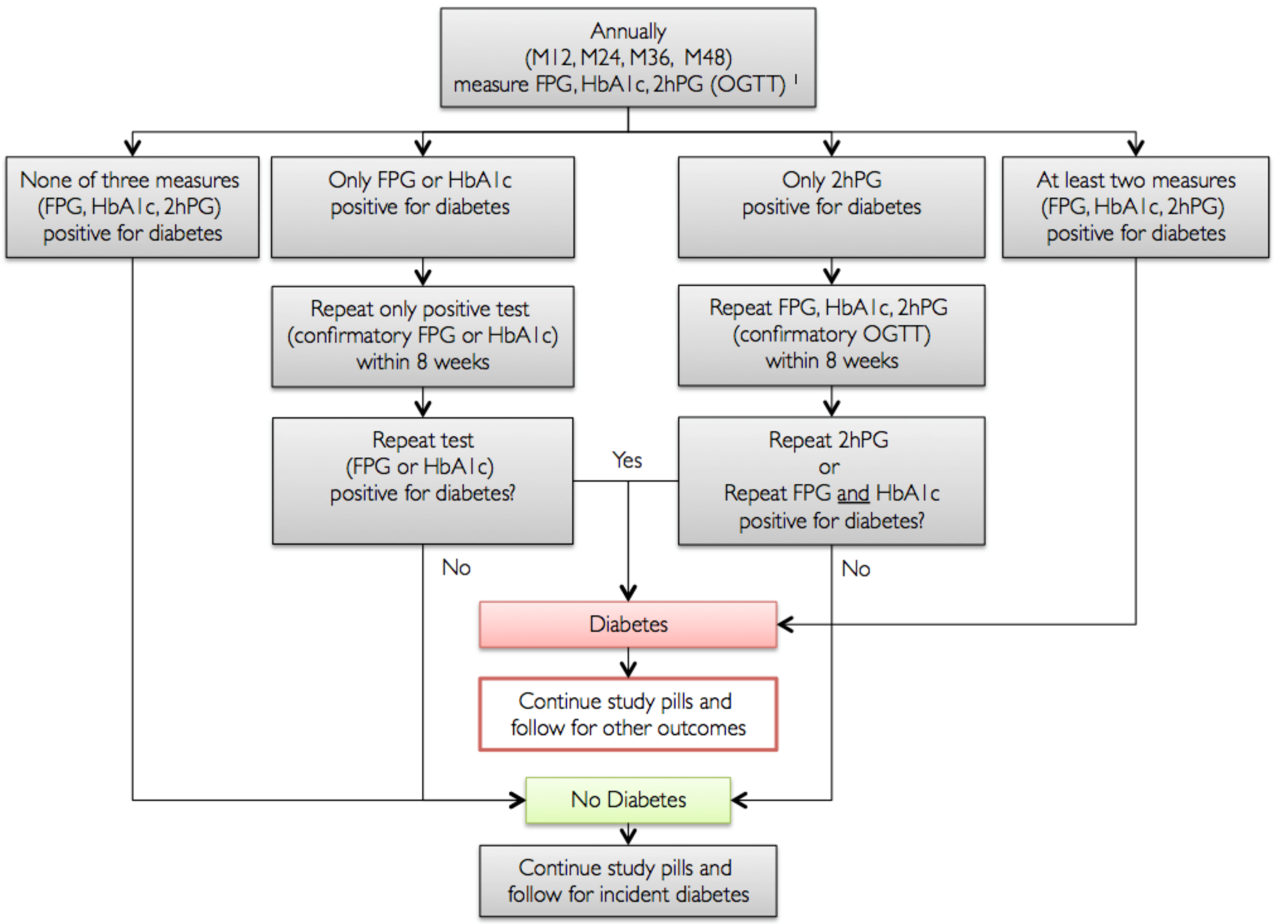
At any time when symptoms consistent with hyperglycemia are reported, FPG and HbA1c will be measured, outside of a scheduled study visit. The algorithm for the semi-annual visit, described above, will be followed.

At the end-of-study visit, the procedures for the scheduled (semi-annual or annual) visit will be completed and the respective algorithm (Figures 9.1.1 and 9.1.2) will be followed to assess for incident diabetes.

Blood for plasma glucose (FPG, 2hPG) and HbA1c will be drawn locally and shipped to the Central Laboratory for measurements. Results will be available to the site typically within 5 business days from day of shipment. The date of onset of diabetes is defined as the date of the first diagnostic glycemic value. When diabetes is diagnosed, study pills will be continued without unmasking and participants will continue in the study and complete all subsequent planned visits.

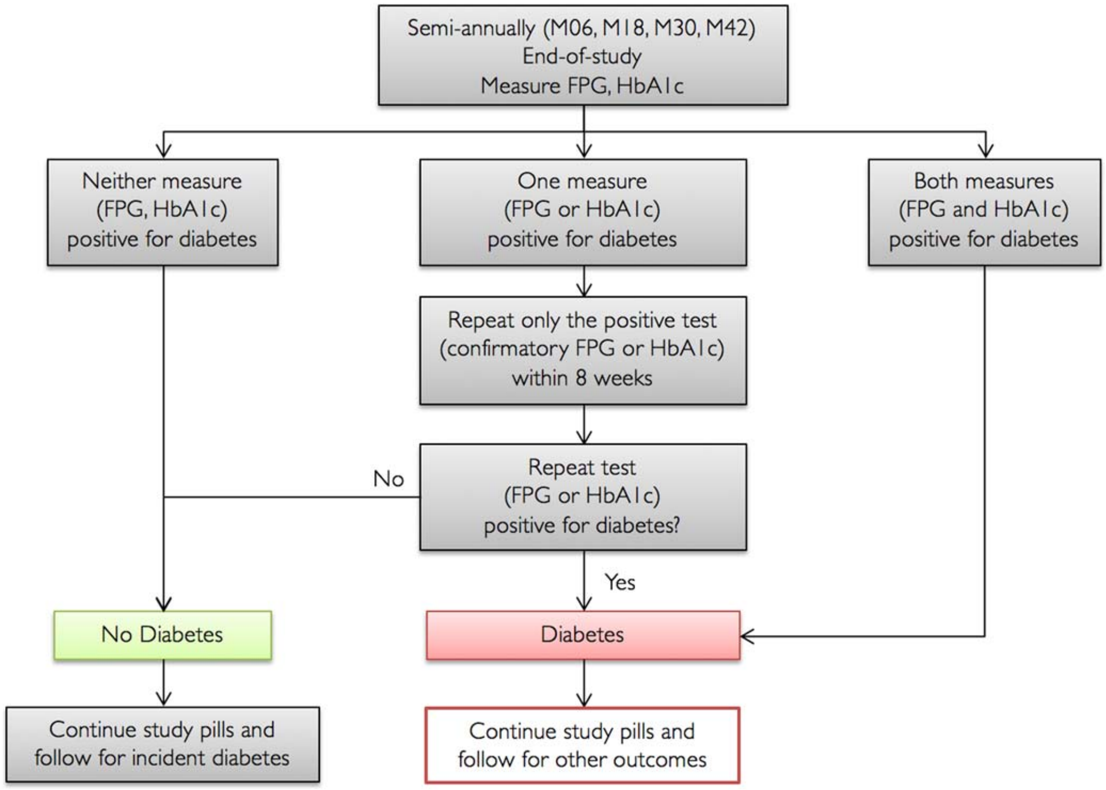
Tests for glycemia will be performed without interrupting the assigned treatment. Testing at one of the scheduled follow-up visits or confirmatory visits will be postponed for up to 8 weeks if a temporary concomitant condition exists that would affect glucose tolerance (e.g. active infection, hospitalization which may also require temporary use of a diabetes medication), its assessment (e.g. blood transfusion or blood donation) or for any other administrative or social reason.

Figure 9.1.1. Flow diagram of laboratory evaluation for diabetes at the annual visit




[1] During the annual OGTT, the following will also be drawn: insulin at 0, 30 and 120 minutes and glucose at 30 minutes

Figure 9.1.2. Flow diagram of laboratory evaluation for diabetes at the semi-annual visits




*Physician-diagnosis of diabetes or use of diabetes-specific pharmacotherapy between scheduled visits* self-reported by participants will be validated by laboratory testing as part of the study or by review of medical records by the Clinical Outcomes Committee, which is independent of the D2d investigators (Figure 9.2). Participants will be advised to contact their site, as soon as possible after their health care provider makes the diagnosis of diabetes or if the health care provider plans to initiate diabetes-specific pharmacotherapy, so that participants may return to the clinic to be tested for diabetes *before* they start any diabetes-specific medication (defined as any FDA-approved medication for diabetes, <u>and</u> any other medication that contains an active substance approved for diabetes, e.g. Saxenda [liraglutide]) *even if the indication for usage was not diabetes*). Testing for diabetes will be done after the site investigator (or study physician) promptly discuss the case with the participant's health care provider <u>and</u> the provider agrees to withhold use of a diabetes medication <u>and</u> also not initiate pharmacotherapy for pre-diabetes or other diagnoses (e.g., metabolic syndrome), *and allow follow-up for diabetes progression within D2d (i.e.,* by glycemic testing analyzed by the Central Lab*).* If the provider (or participant) is determined to start diabetes-specific pharmacotherapy regardless of the results of D2d-specific glycemic testing, then the diagnosis of diabetes or indication for starting a medication will be adjudicated. The participant will continue on the assigned D2d treatment and be followed for incident diabetes, in accordance with the intention-to-treat (ITT) principle.

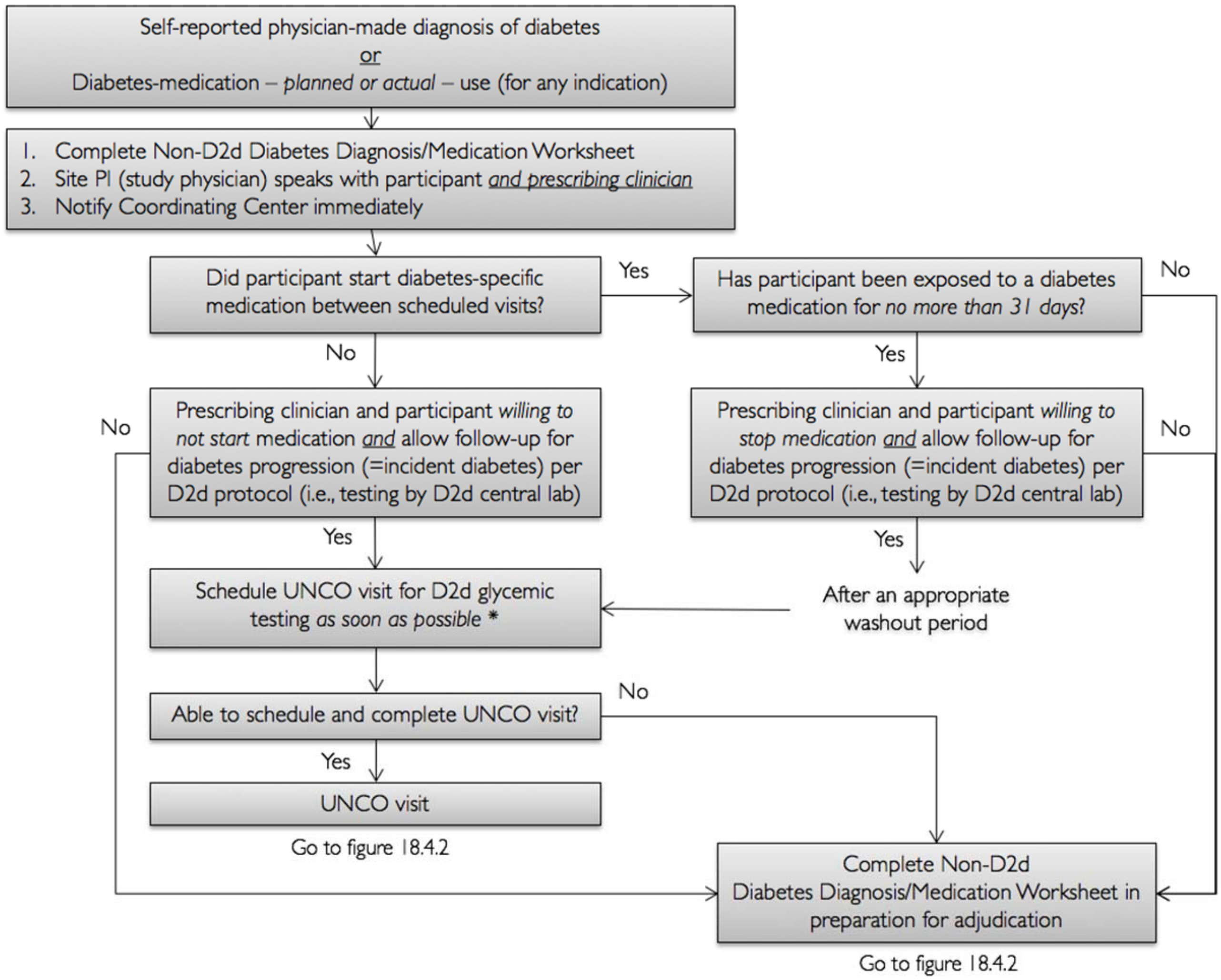
At the study visit, participants will be asked questions related to their non-study visit with their health care provider and laboratory testing for diabetes will be conducted outside of the study schedule (unscheduled visit [UNCO]) or at the next scheduled D2d visit (e.g. M06, M12), if the next visit scheduled visit is approaching (Figures 9.2.1 and 9.2.2). If testing does not confirm the diagnosis, the participant will not be counted as an incident case of diabetes. The site investigator will communicate the outcome of the glycemic results (FPG/HbA1c) to the participant's health care provider and will reinforce the principles of the study. If diabetes-specific pharmacotherapy is not initiated based on D2d-specific testing, participant will continue on the assigned D2d treatment and be followed for incident diabetes. If diabetes-specific pharmacotherapy is started despite D2d-specific testing not confirming diabetes, *and despite the pre-testing discussion between the site principal investigator and participant's provider,* then the participant will still continue on the assigned D2d treatment and be followed for incident diabetes, in accordance with the ITT principle.

Participants who have started diabetes-specific pharmacotherapy but have been exposed to the medication for no more than 31 days (cumulative, since the last D2d scheduled visit e.g., M06, M24) will return for a visit to undergo D2d-specific glycemic testing, following an appropriate washout period (2 or 4 weeks depending on type of medication), *provided the prescribing clinician and participant agree to stop the medication and allow follow-up for diabetes progression within D2d (i.e., by glycemic testing analyzed by the Central Lab) (Figure 9.2.1 and 9.2.2).* If a participant has taken diabetes-specific medication between study contacts for more than 31 days or the prescribing clinician and participant do not agree to stop the diabetes medication, then the diagnosis of diabetes or indication for the medication is adjudicated. The participant will continue on the assigned D2d treatment and be followed for incident diabetes, if adjudication does not confirm diabetes.

Adjudication process: Study staff will request medical records from the health care provider, with special emphasis on obtaining the most recent laboratory values for FPG, HbA1c or 2hPG and any glucose results from fingersticks, before any diabetes-specific medication was started. Medical records will be centrally reviewed and the diagnosis of diabetes adjudicated by the independent Clinical Outcomes Committee to determine whether the outside laboratory results meet glycemic criteria for diabetes. Because HbA1c and glucose (FPG, 2hPG) are standardized tests across laboratories, this method is sufficiently robust to make the diagnosis of diabetes. The Clinical Outcomes Committee will review the material to determine whether the outside laboratory results and relevant medical history are sufficient to make the diagnosis of diabetes. During the adjudication process, the reviewers are encouraged to follow, as closely as possible, the glycemic algorithms shown above (Figure 9.1.1 and 9.1.2). However, because it is highly unlikely that routine clinical practice will follow the strict D2d algorithms *(e.g., repeating testing is not commonly done in clinical practice and may be limited by insurance reimbursement rules)*, the Clinical Outcomes Committee should follow the ADA threshold criteria and use clinical judgment when adjudicating cases of diabetes. For example, a single HbA1c measured in clinical practice that meets ADA criteria (e.g., 6.5%) may suffice for the committee to make the diagnosis of diabetes. If laboratory data from the health care provider cannot be obtained to adjudicate the diagnosis of diabetes, then the participant will be considered as not having reached the diabetes outcome, and will be coded as "insufficient information for adjudication." Sensitivity analysis will be conducted to include as diabetes cases the occurrences when a diabetes medication was started but the events could not be adjudicated as confirmed diabetes. Based on other diabetes prevention trials, it is expected that less than 5% of diabetes outcomes will be made exclusively based on adjudication.[12]

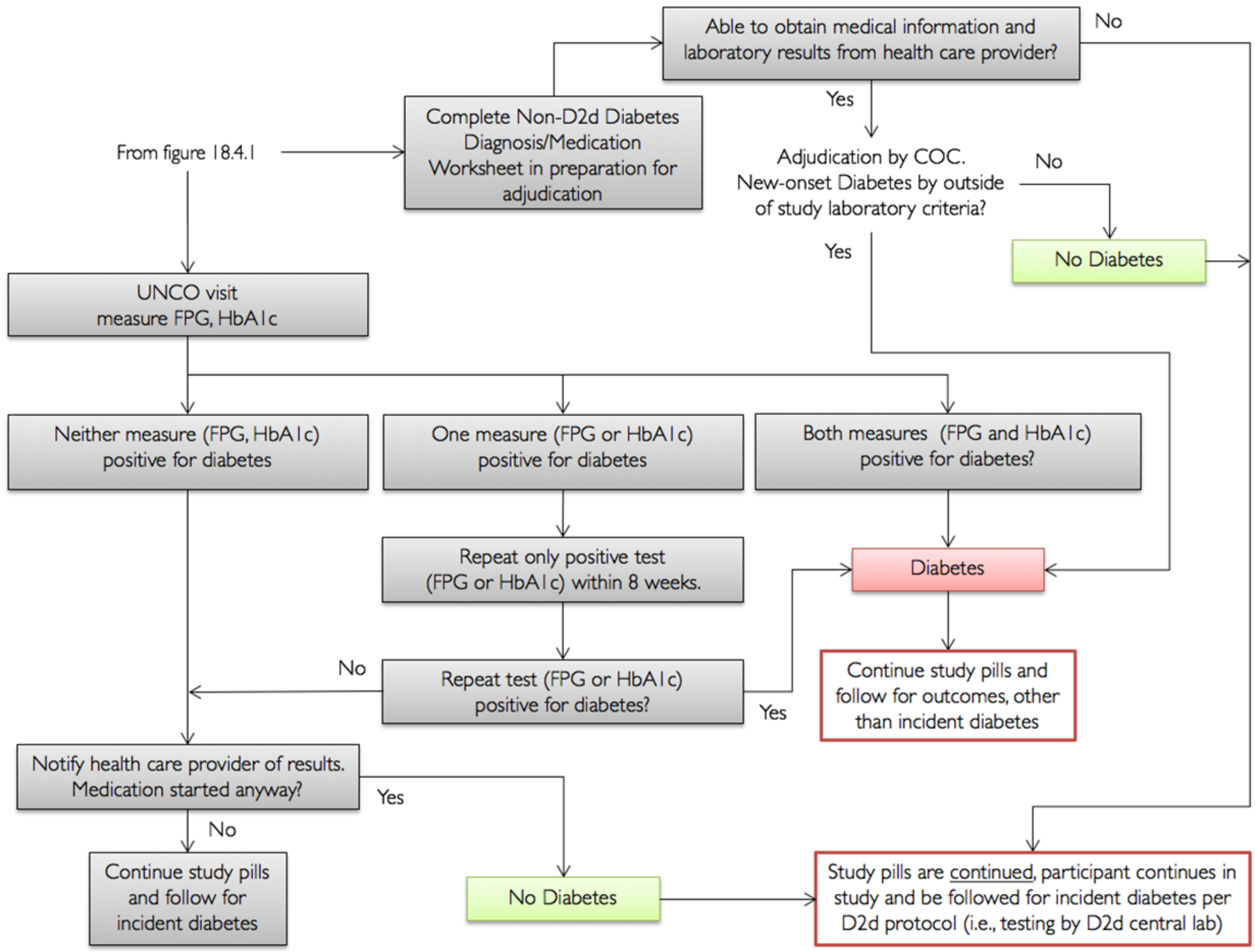
When the participant meets a laboratory criterion for diabetes, the Central Laboratory will notify the collaborating site of the results and the need for repeat confirmatory testing via the electronic web-based data capture system. After the primary outcome has been reached (by D2d criteria or adjudication), participants will continue in the study, including taking the study pills without unmasking and will be referred to their physician for further care in relation to diabetes. Participants will continue to return for scheduled visits until the end of the study for assessment of other outcomes. At the annual visits, the HbA1c and FPG will be collected but the 75-gram OGTT for 2hPG will not be collected.

Figure 9.2.1. Flow diagram of evaluating self-reported physician-diagnosis of diabetes or initiation of diabetes-specific pharmacotherapy between scheduled visits.




- In lieu of an Unscheduled Confirmatory (UNCO) visit, a regular D2d visit may be done provided the visit window is approaching

Figure 9.2.2. Flow diagram for (planned or actual) use of diabetes-specific pharmacotherapy between scheduled visits.



## 9.2 Secondary Endpoints (Outcomes) & Subgroup Analyses

The secondary outcomes were selected based on their importance to the underlying hypothesis of a potential link between vitamin D and type 2 diabetes (e.g. identify mechanisms that explain the effect of vitamin D supplementation on the primary outcome). Additional secondary outcomes that are not directly relevant to type 2 diabetes may be included, depending on budget and burden consideration, to increase our understanding of vitamin D physiology and its relevance to other outcomes, thereby maximizing the yield of the study. Secondary outcomes will be assessed in the entire cohort or in subsets of the study population, depending on statistical power needs, feasibility and availability of funding.

- Variability of response to vitamin D supplementation in subgroups defined by baseline characteristics: (1) race and ethnicity (as proxies for skin pigmentation);[186] (2) BMI; (3) waist circumference;[187,188] (4) age; (5) geographic location (proxy for sun exposure); (6) 25OHD concentration.
- Variability of response to vitamin D supplementation by adherence based on pills counts and by achieved 25OH concentration.
- Effect of vitamin D supplementation on HbA1c, FPG and 2hPG as continuous variables.
- Effect of vitamin D supplementation on insulin resistance and beta cell secretion (indices derived from the OGTT).
- Effect of vitamin D supplementation on plasma 25OHD concentration and identification of phenotypic, including seasonal and geographic, characteristics associated with variability on achieved plasma 25OHD concentration.
- Effect of vitamin D supplementation on blood pressure.
- Safety and tolerability of vitamin D supplementation.

## 9.3 Potential Confounders or Effect Modifiers

Measurements to ascertain other exposures or variables that serve as potential confounders or effect modifiers will be performed by staff masked to study group assignment.

*Vitamin D status. Plasma 25OHD concentration* will be measured *as a proxy* for vitamin D status at baseline and during the intervention to assess the efficacy of supplementation in the active arm and to compare between the two arms. Measurement of 25OHD will not be done in real-time and will not be reported to sites, participants or health care provider(s). *Self-reported vitamin D intake* will be estimated from a validated self-administered semi-quantitative food frequency questionnaire.[18,234-237]

*Calcium status.* There are no widely accepted measures of adequate calcium status. Self-reported calcium intake will be used *as a proxy* of calcium adequacy at baseline and during the intervention. *Self-reported Calcium Intake* will be estimated from a validated self-administered semi-quantitative food frequency questionnaire.[18,234-237]

*Other Potential Confounders or Effect Modifiers.* In addition to vital signs (height, weight [BMI calculation], blood pressure and heart rate), waist circumference will be measured at baseline. *Physical Activity* (kcal/d) will be measured by the 7-day Modifiable Activity Questionnaire at baseline, M06 and annual visits.[263]

## 9.4 Concomitant Medications / Treatments

Participants will be instructed to follow the IOM recommendations for vitamin D and calcium intake. During the study, exogenous vitamin D supplementation cannot exceed 1000 IU/day, and calcium supplementation cannot exceed 600 mg/day, unless specifically prescribed by a physician.

Detailed information, *using source documents,* will be collected on all concomitant medications participants take during the study, including prescription medications, vitamins and dietary supplements (prescribed or over-the-counter); however, detailed information on only certain classes of medications (i.e., diabetes, obesity, hypertension, hypercholesterolemia) and critical supplements (i.e., containing vitamin D or calcium) will be entered in the electronic data capture (EDC) system.

### 9.5 Stored Specimens

In participants who provide informed consent for specimen repository, samples of plasma, serum and urine will be stored in small aliquots for future ancillary studies. At baseline, the study will collect whole blood for future DNA extraction, which will be done as part of a genetic ancillary study.

## 10. DATA AND SAFETY MONITORING

### 10.1 Risk-benefit analysis

The potential risks (probability and magnitude) to participants from the study intervention or procedures described in the research proposal are small and reasonable in relation to the anticipated benefits that may be reasonably expected to result from this study.

Please refer to the D2d Data Safety Monitoring Plan for additional details.

#### 10.1.1 Potential risks related to this study

The potential risks that may be seen in this study are shown below.

"Expected" AE related to the *intervention, vitamin D supplementation* (uncommon).

- Hypercalcemia
- Hyperphosphatemia
- Nephrolithiasis
- Hypercalciuria
- Nephrotoxicity
- Anemia
- Polyuria
- Nausea
- Vomiting
- Poor appetite
- Weakness
- Fatigue
- Insomnia
- Headache
- Metallic taste

"Expected" AE related to *study procedures for outcome assessment (testing)*

Related to blood draws

- Minor discomfort from introducing the needle/catheter under the skin or skin bruise (common)
- A skin infection from the needle/catheter (rare)
- Mild anemia from repeated blood draws (rare)
- Nausea, vomiting and syncope (uncommon)

Related to the OGTT

- Risks associated with insertion of needle/catheter and mild anemia as described above
- Phlebitis (rare)
- Symptoms or signs of hypoglycemia following the oral glucose load (uncommon)
- Nausea, vomiting

Other risks related to overall trial participation

- Social-psychological risk due to inadvertent disclosure of confidential medical information (rare)

### 10.1.2 Protection Against Risk

Site staff working on D2d will be trained and certified by IRB in the ethical conduct of human research.

**Before the start of the study**, to minimize the occurrence of "expected" AE and overall risk, the following measures will be taken:

- Participants who may be predisposed to AE as a result of the study intervention or procedures will be excluded.
- Renal stones are an unusual but not a rare complication of vitamin D supplementation, especially when combined with calcium. In this proposal, total calcium intake from supplements will be limited to 600 mg per day and participants will be randomized to 4000 IU of vitamin D daily or placebo. Although the 4000 IU per day dose is the tolerable upper limit (UL) for safety as determined by the Institute of Medicine (IOM), there is some risk that that kidney stones could occur in the active treatment group at a higher rate than the control group. Hence, to minimize risk of nephrolithiasis, volunteers with high serum calcium at baseline, hyperparathyroidism, history of nephrolithiasis or other medical condition (e.g. sarcoidosis) that may increase risk for nephrolithiasis or hypercalcemia, will be excluded, as described in exclusion criteria. The study will monitor urine calcium excretion as a safety measure.
- Women of reproductive potential will be instructed to use an effective method of birth control of their choice during the trial. Pregnancy testing will be conducted at the screening visit and as needed during the study.
- As part of the informed consent process, "expected" AE will be described to the participants and they will be instructed to report any changes in their health, complaints or problems during and between regular visits, whether they think the problem may be related to the study or not.
- As part of their informed consent process, participants will be instructed to contact the site PI (or his designee) or site research coordinator at any time for any questions related to the study.
- Rules for discontinuation of study medications due to safety concerns are defined (please see section 7.5).
- The site PI will review the protocol and Manual of Procedures with study personnel (co-investigators, site research coordinator, nurses, etc.) and ensure that all personnel understand their responsibilities. All study personnel will be instructed to monitor for AE and be familiar with the reporting requirements for the different types of AE.

**During the study**, to minimize the occurrence of "expected" AE and overall risk, the following measures will be taken:

<u>Intervention</u>

- Serum calcium and creatinine (GFR), and urine calcium excretion will be monitored at regular intervals.
- At each contact (phone or visit), participants will be questioned in regards to changes in their health or medications (prescription or non-prescription, including supplements).

<u>Outcome Measures</u>

- For all blood draws/catheter insertion, research staff will follow the usual sterile techniques and only experienced personnel will perform the procedure to minimize discomfort, bruising and risk of infection or bruising.
- To minimize the risk of anemia from repeated blood draws, the smallest amount of blood required for each testing procedure will be drawn.

Trial Participation

- A site study physician (or his designee) will be available at all times to evaluate and provide the necessary medical intervention in the event of an adverse event, or make a referral for the appropriate care.
- Risk to patient confidentiality is minimal because all study records are confidential within the study staff environment. Although the consent form will specify what protected health information will be collected and with whom it will be shared, an authorization form in addition to the consent form will be signed by participants from institutions that require a separate document in accordance with their interpretation of the Health Insurance Portability and Accountability Act (HIPAA) guidelines. Safety precautions and encryption of data will ensure that electronic systems do not pose a risk to participant confidentiality. Study results will be published, but participant identity will not be revealed in any articles or scientific presentations and records will be kept as required by federal and state laws and regulations.

#### 10.1.3 Potential Benefits of the Proposed Research to the Subjects and Others

The main tangible benefits of this study to participants include:

- During Screening phase (screening and baseline visits), access to the results of their medical examination and laboratory tests. Participants will be advised to contact their personal physician if any unexpected medical condition or problem is identified.
- During the trial, participants will receive testing for the diagnosis of diabetes.
- During the trial, participants will participate in the Support and Education Program, which will provide advice on making important lifestyle changes to delay the onset of diabetes.
- Many participants obtain personal satisfaction from participating in nutritional studies.

Despite these potential direct benefits to study participants, the main benefits of this study will be to others in the future based on the knowledge gained upon completion of the study.

#### 10.1.4 Importance of the Knowledge to be Gained

The knowledge to be gained as a result of the proposed research is important because it may lead to implementation of a successful nutritional intervention for prevention of type 2 diabetes.

### 10.2 Adverse Events

Adverse events will be collected and documented in accordance with Good Clinical Practice guidelines, local, state and federal regulations.

#### 10.2.1 Definitions and classification of Adverse Event & Serious Adverse Event

**Adverse event** (AE) is defined as any untoward or unfavorable and unintended medical occurrence (including symptom, physical sign, laboratory finding or disease) observed in or experienced by a participant that is not a benefit to the participant whether or not it is considered study-related by the research staff.

**Adverse Event Classification** All AE will be documented in the electronic data capture system characterized by the following criteria: (1) seriousness, (2) expectedness, (3) relatedness, (4) severity, (5) frequency, (6) outcome and (7) action taken.

**(1) Seriousness** is classified as:

- **Serious AE (SAE)** is any event that results in any of the following outcomes:
  - Death.
  - Life-threatening condition (e.g. event that places the participant at immediate risk of death).
  - New inpatient hospitalization or prolongation of existing hospitalization.
  - Persistent or significant disability or incapacity.
  - Congenital anomaly or birth defect.
  - Any other significant hazard that, based upon appropriate medical judgment by the investigators, may jeopardize the participant's health and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

- **Non-Serious** is any event that does not meet the above criteria for *Serious*

**(2) Expectedness** is classified as:

- **Expected** event is known to be associated with the intervention or condition under study, in terms of nature, severity or frequency, and has been described as a potential AE in the IRB-approved research protocol, supporting documents and informed consent forms.
- **Unexpected** event has not been previously described as a potential AE in the IRB-approved research protocol, supporting documents or the informed consent forms, in terms of nature, severity or frequency. Unexpected also refers to an adverse event that has not been observed before (i.e. has not been published in medical literature).

**(3) Relatedness** is classified as:

- **Unrelated.** The adverse event is clearly not related to the study and it is due to extraneous causes (e.g., underlying disease, environment)
- **Unlikely.** The adverse event is doubtfully related to the study.
- **Possible.** The adverse event is possibly related to the study.
- **Probable.** The adverse event is likely related to the study.
- **Definite.** The adverse event is clearly related to the study.

**(4) Severity** is classified based on intensity of symptoms, degree of limitation of usual daily activities, or level of abnormality of clinical signs or laboratory parameters, as:

- **Mild.** Awareness of symptoms or signs, but AE is easily tolerated and is of minor irritant type and does not interfere with the participant's usual activity or cause loss of significant time from normal activities. AE may not require therapy or a medical evaluation and is transient and resolves without sequelae.
- **Moderate.** Adverse event introduces a low level of inconvenience or concern to the participant and may interfere with daily activities but participant is able to function with minimal interference. A moderate AE may improve without any therapeutic measure or with simple therapeutic measures.
- **Severe.** Adverse event interrupts the participant's normal daily activities and generally requires systemic drug therapy, major surgery or other treatment; adverse event may be incapacitating.

**(5) Frequency** is classified as:

- **Single Event**
- **Re-occurring Event**

**(6) Outcome**. The clinical outcome is classified as:

- **Resolved.** The participant returned to baseline status.
- **Condition still present and under treatment**. Participant has not recovered and symptoms or signs continue.
- **Death**.

**(7) Action taken (participant related)** for a specific adverse event in relation to study intervention and procedures is classified as:

- **No action.**
- **Study pills temporarily held,** participant continues in the study.
- **Study pills permanently discontinued,** participant continues in the study.
- **Study pills and participation temporarily held,** participant is “inactive” and will return to the study (e.g., pregnancy).
- **Participation in study permanently discontinued and participant has gone “off study”.** This is expected to be a rare event and written (preferably) or verbal “withdrawal of consent” will be requested from the participant.
- **Intervention, new medication**
- **Intervention, other (**e.g. surgery, acupuncture, physical therapy).

### 10.2.2 Definition of Unanticipated Problem

An unanticipated problem (UAP) is defined as any adverse event, incident, experience, or outcome that meets *all of the following three criteria:*

1. **Unexpected**, in terms of nature, severity, or frequency, given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent documents; and (b) the characteristics of the study population.
2. **Possibly, probably or definitely related** to participation in the research (i.e. study procedures or intervention).
3. Suggests that the research places participants or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized. *This criterion (“greater risk of harm”) is always met if the event is a SAE.*

In general, an unanticipated problem may require specific action, such as: modification of the research protocol (e.g. changes in inclusion/exclusion criteria; implementation of additional procedures for monitoring participants), suspension of enrollment of new participants, suspension of research procedures in enrolled participants, changes to the informed consent forms or a combination of the above.

### 10.2.3 Safety Surveillance and Reporting of Adverse Events

The D2d study Safety and Outcomes subcommittee (SOS) has been established to review all SAEs and UAPs as they are reported, review periodic safety reports of all AE and oversee study safety. The subcommittee chair or designee will assess each SAE and UAP, as it occurs, to determine if immediate action is required in response to the event. In general, the subcommittee will meet three times a year (or more frequently as needed) by conference call. During these meetings, the subcommittee discusses all SAE, UAP and summary reports of non-serious AE. The subcommittee also evaluates whether there is any clustering of AE by clinical site. The subcommittee remains masked to patient treatment group during these evaluations. The subcommittee considers whether changes in the protocol (monitoring, consent process, etc.) are indicated based on the occurrence, frequency, or severity of AE, SAE or UAP and provides its recommendations to the Steering

Committee and Executive Committee for further discussion and subsequently to the DSMB for approval and implementation.

Below is an overview of reporting requirements for each type of AE. Details on review and management of AE are found in the D2d DSMP.

**Non-serious Adverse Events (AE)** must be reported to the CC by completing the AE-specific eCRF in the EDC system and providing relevant information, within fifteen (15) business days of the site becoming aware of the event.

**Serious Adverse Events (SAE)** must be reported to the CC by completing the AE-specific eCRF in the EDC system as soon as possible, and providing all relevant information always within five (5) business days of the site becoming aware of the event.

**Unanticipated Problems (UAP):** By definition, UAP are events that may require corrective action by the D2d Study Group; therefore, timely report and evaluation of UAP is of utmost importance. An UAP must be reported to the CC by completing the AE-specific eCRF in the EDC system as soon as possible, and providing relevant information always within two (2) business days of the site becoming aware of the event.

### 10.3 Data Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) has been established by the primary sponsor, NIDDK, to oversee the safety and other aspects of the study. The role of the D2d DSMB is to provide independent oversight and ensure that the study is conducted according to currently established safety and ethical standards. The CC will oversee the provision of data to the DSMB. In general, it is anticipated that the DSMB meets twice a year. The DSMB reviews and evaluates all SAEs, UAPs and outcome measures and also receives a summary report for all non-serious AE. The DSMB charter outlines the method and schedule for AE reporting. Unless specific action is required, the results of the analyses reviewed by the DSMB will not be shared with site investigators. The DSMB will also develop rules for stopping the study. The CC will distribute DSMB meeting summary reports to collaborating clinical sites for submission to the sites' IRB.

### 10.4 Ethical and Regulatory Responsibilities & Statement of Compliance

This study will be carried out in compliance with the IRB-approved protocol and related documents and in accordance with Good Clinical Practice guidelines, the applicable regulatory requirements of the Department of Health and Human Services, the International Conference on Harmonization (ICH) Guidelines and state and local legal and ethical requirements. The following documents contain the policies and procedures designed to ensure adherence to Good Clinical Practices:

1. United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
2. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Patients, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).
3. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996

All investigators agree to adhere to the instructions and procedures described in the protocol, thereby adhering to the principles of Good Clinical Practice. All key persons and research staff will have completed educational modules on human subjects protection and are certified by their local IRB. If new information emerges that may affect participants' willingness to continue to participate in the study, the information will be conveyed to participants via a revised informed consent form.

## 10.5 Confidentiality

All information and data generated as part of the study concerning participants are considered confidential. Access to these files will be restricted to authorized staff of the local investigational team, the CC and Central Laboratory. Authorized regulatory personnel have the right to inspect and copy all records pertinent to this study.

All data will be entered and managed with the use of a 21 CFR Part 11 compliant web-based EDC system, which is platform independent. In compliance with regulations, the EDC features multiple security levels including data element (e.g. restricted access to fields), user (e.g. password authentication access with frequent password changes and lockout after a period of inactivity) and application (e.g. role based access to features, access audit trails).

Each site will maintain a list of participant names with the associated study ID and no other site or entity will have access to this code. All information used in the data analyses and reporting of study results will have no identifiable reference to participants.

## 10.6 Protocol Deviations

A protocol deviation is any departure from the study protocol and Manual of Procedures requirements or Good Clinical Practice guidelines. Protocol deviation may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

## 10.7 Site Monitoring

A representative from the CC (Project Manager and/or assigned Clinical Research Associate) will visit the collaborating clinical sites at periodic intervals to monitor the collection of data, adherence to the study protocol and regulatory requirements. The case files of a minimum of 10 percent of participants will be reviewed. More frequent monitoring and or additional participant source files may be monitored if warranted based upon requests by the Executive Committee, DSMB, NIDDK or the CC.

During monitoring visits, for each participant selected for review the source documentation verification will be completed for all eCRF data collected up until the date of the visit. Emphasis will be placed on the informed consent process and documentation, study pill storage and accountability and primary outcome assessment.

## 10.8 Conflict of Interest

The Executive Committee in conjunction with NIDDK has established a policy for all members of the investigative team to disclose all potential (real or perceived) conflicts of interest.

## 10.9 ClinicalTrials.gov Requirements

The proposed study is a clinical trial and will be registered in ClinicalTrials.gov.

## 10.10 Other regulatory requirements and Requirements for Investigational New Drug (IND)

The FDA has determined that an IND is not required for the D2d study.

Participant medical insurance companies will not be billed for any study visits or study procedures. Payment for research-related injury will be determined by each site. Study records will be maintained for a minimum of 7 years. If D2d related records will be moved to a different location, the site is required to inform the CC of the new location.

## 11. SAMPLE SIZE CALCULATIONS AND DATA ANALYSIS

### 11.1 Sample Size Calculation (Power Analysis)

The primary aim of D2d is to assess whether, in participants at risk for diabetes, oral daily vitamin $D_3$ supplementation will reduce the rate of progression to incident diabetes when compared to placebo.

The D2d study is designed as an event-driven trial to assure that the intended power to detect the hypothesized treatment effect (hazard ratio = 0.75, see below) is achieved irrespective of the event rate in the placebo arm.

The following considerations were used to determine the required number of events:

1) The primary study outcome is “time to confirmed diabetes”.
2) The primary hypothesis will be tested at a two-sided type 1 error rate (alpha) of 0.05.
3) If vitamin D therapy is superior to placebo, the hazard ratio will be 0.75 in the vitamin D arm as compared to the placebo arm.
4) The study is designed to have power of 90% to detect a hazard ratio of 0.75.

Based on these assumptions, the required number of events is 508[264]. To achieve the intended power, D2d will continue until 508 primary events have occurred.

Based on the following assumptions, it is estimated that the required (i.e. randomized) sample size will be 2,382 participants.

5) Participants will be recruited over a 2-year period (“accrual period”)
6) The expected study duration will be approximately 4 years plus an end-of-study visit over a period not to exceed three months.
7) The incidence rate of confirmed diabetes will be 10% per year (hazard rate = 0.1054) in the placebo group.
8) The loss-to-follow-up (i.e. going “off study”) rate will be up to 5% per year of follow-up (hazard rate = .0513).
9) If vitamin D therapy is superior to placebo, the hazard ratio will be 0.75 in the vitamin D arm as compared to the placebo group.

The table immediately below displays the expected numbers of incident diabetes cases in the placebo group at each semi-annual visit based on transition probabilities derived from the assumed incidence rates of progression and loss-to-follow-up, and the assumption of constant hazard rates for events and loss-to-follow-up. This calculation also assumes that, when study closeout begins, all participants will return for an end-of-study visit, which will include testing for incident diabetes. The table also displays the expected number of events in the vitamin D supplementation arm if treatment reduces the hazard rate by 25%. Given the assumed uniform rate of recruitment over a period of 2 years, the number of events expected to occur within 2 years after the date of last enrollment or at the close-out visit is 586 (293*2) under the null hypothesis and 521 (293 + 228) under the alternative hypothesis. Thus, the study will be very likely to reach the required number of events within four years if vitamin D supplementation is not effective and all other assumptions are satisfied, and will reach that target of events within the same time period with probability greater than 0.50 if treatment reduces diabetes incidence by 25%.

**Table 1.** Expected number of events at each six-month visit in the placebo and vitamin D arm (under the alternative hypothesis).

| | Visit | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | M06 | M12 | M18 | M24 | M30 | M36 | M42 | End-of-study | Total |
| **Placebo** | 60.4 | 55.9 | 51.7 | 47.8 | 33.1 | 20.4 | 9.4 | 14.3 | 293 |
| **Treatment** | 45.6 | 42.7 | 40.0 | 37.5 | 26.3 | 16.5 | 7.7 | 11.6 | 228 |

Number of events is approximate. End-of-study visit will coincide with a scheduled visit (semi-annual or annual).

### 11.1.1 Rationale for the Assumed Conversion Rate of 10% in the Control Arm

The primary outcome of the D2d trial is "time to confirmed diabetes". The diagnosis of diabetes is based on ADA-based cutoffs for FPG, 2hPG and HbA1c, as defined in section 9. The incidence rate of diabetes can vary considerably based upon a number of factors. In prior type 2 diabetes prevention trials, conducted in a variety of populations worldwide,[5,6,11-14,17,265-269] the diabetes incidence rates (based only on FPG/2hPG criteria) varied from 2.4% to 23% per year. The most relevant data for the proposed D2d trial come from the DPP (U.S.)[5] and DREAM (21 countries)[12] studies. The DPP trial recruited participants with FPG 95-125 mg/dL, 2hPG 140-199 mg/dL, and BMI $\geq$ 24 kg/m$^2$. The DPP placebo group had a cumulative incidence of diabetes of ~29% during the 2.8-year mean follow-up (approximately 10% per year). The DREAM trial started recruitment with impaired fasting glucose and impaired glucose tolerance (defined as FPG 110-125 mg/dL and 2hPG<200 mg/dL) or impaired glucose tolerance (defined as FPG≤125 mg/mL and 2hPG 140-199 mg/dL) but later expanded the criteria to include isolated impaired fasting glucose (defined as FPG 110-125 mg/dL and 2hPG<140 mg/dL). The DREAM study had a cumulative incidence of diabetes in the placebo arm of 25% over a 3-year mean follow-up (estimated ~8-9% annually). In two other large type 2 diabetes prevention trials with alpha-glucosidase inhibitors, the cumulative incidence rates in the placebo arm were 42% over 3.3 years (reported 12.1% annually; STOP-IT/Europe)[11] and 36% over 2.8 years (estimated ~12-13% annually; Voglibose trial/Japan).[13] In the CANOE trial (Canada), the cumulative incidence rates in the placebo arm were 42% over 3.9 years (estimated ~10% annually).[269] Importantly, in nearly all these studies, the observed annual incidence rate was higher than that assumed in the pre-trial sample size calculations (6.5% [predicted] vs. 11% [actual] in DPP; 4.5% vs. ~8-9% in DREAM; 7% vs. 12 in STOP-IT; 7.7% vs. 12-13% in the Voglibose trial).

We expect the diabetes incidence rate in the D2d study to be at the high end of the rate that would be predicted from the mean annual incidence rate (~9-11%) reported in previous diabetes prevention studies because: (1) the study is targeting a population similar to the DPP (U.S. adults at high risk for type 2 diabetes with similar BMI and age at entry); (2) the definition of 'at risk for diabetes' requires 2-out-of-3 glycemic criteria, which will identify participants at higher risk for progression to diabetes; (3) the study will use the current ADA glycemia criteria for diagnosis of diabetes (addition of HbA1c), which will likely raise the incidence rate of diabetes compared with the above studies.

In relation to point (3) above, only one of the published studies on prevention of type 2 diabetes included HbA1c as a criterion for both inclusion and outcome assessment.[13] The study reported a conversion rate to diabetes in the placebo arm of 36% over 2.8 years. However, this study was conducted in Japan and its definitions of pre-diabetes or diabetes were not consistent with the current ADA criteria; therefore, its relevance to the D2d study is not clear. Adding HbA1c alters the epidemiology of pre-diabetes and diabetes and makes it difficult to predict incidence rates. We reviewed relevant data from recent cross-sectional observational studies that have evaluated the impact of adding HbA1c as a diagnostic criterion. As expected, there is considerable overlap between the HbA1c and FPG/2hPG criteria. Overall, the addition of HbA1c appears to increase prevalence of both pre-diabetes and diabetes.[270-275] For example, Lu et al identified 37% more individuals as having pre-diabetes by HbA1c 5.6-6.5% and identified 9% more individuals as having diabetes by HbA1c >7%.[275] Data from NHANES show that an additional ~6% of participants are identified as having diabetes when HbA1c >6.5% criterion is used in addition to FPG/2hrPG.[271,273] In a recent systematic review that examined ranges of HbA1c and progression to diabetes, the A1c range of 6.0 to 6.5% was associated with a 25 to 50% incidence of diabetes over 5 years.[276] In the CaDDM trial,[24] among 202 fully screened participants with available data on FPG/2hPG/HbA1c, the addition of HbA1c≥6.5% increased the prevalence of newly diagnosed diabetes from 2% to 7% (unpublished data provided by Dr. Pittas). Finally, in the Pima Indian Longitudinal Study (unpublished data provided by Dr. Knowler), after excluding participants with diabetes at baseline (based on clinical and the 2010 ADA glycemic criteria), there were 392 participants with pre-diabetes (by 2010 ADA criteria) with mean age of 42 years, BMI 39 kg/m$^2$ and HbA1c 5.7%. The cumulative incidence of diabetes during a 5-year follow-up period increased from 36% using the old criteria, to 39% using the new expanded criteria, which represents an increase of 12% in the annual incidence rate if HbA1c is added to the FPG/2hPG criteria.

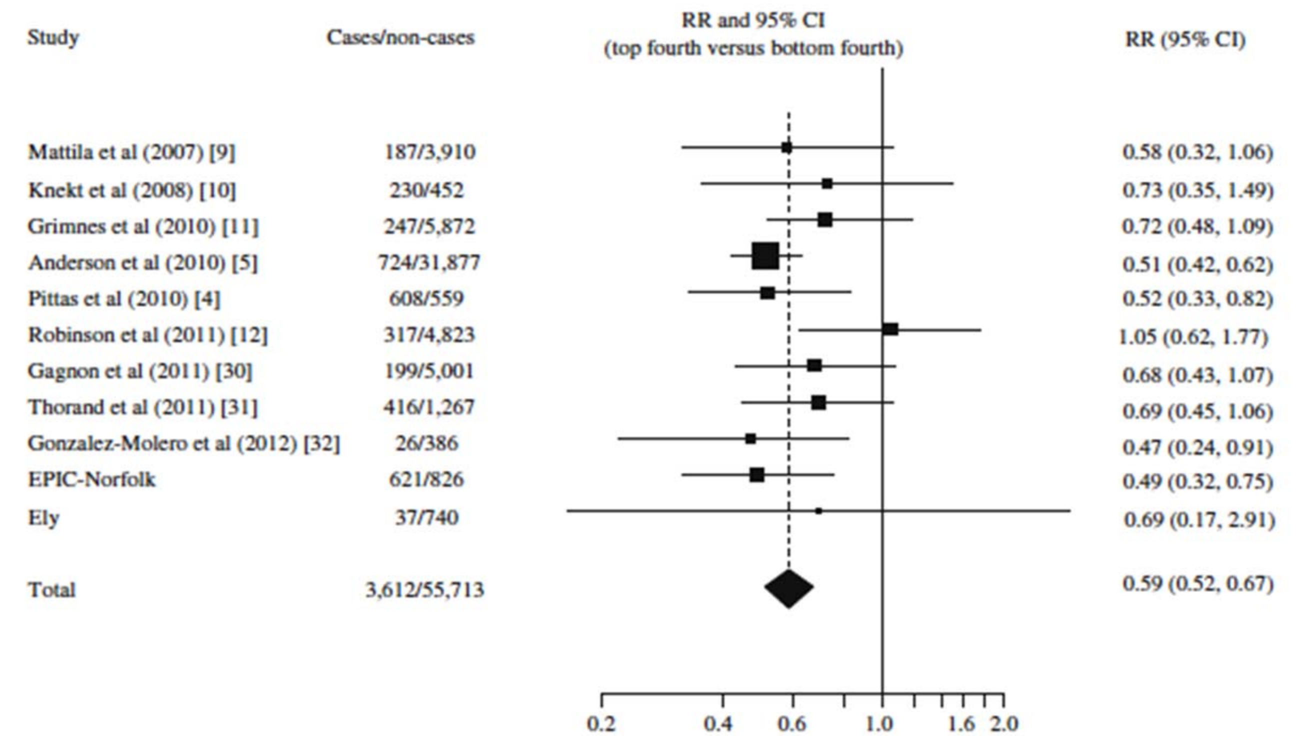
In summary, based on available data in the literature and results of unpublished analyses shown above, it is expected that the addition of HbA1c to the FPG/2hPG diagnostic criteria will increase the number of cases of diabetes diagnosed during follow-up compared to the FPG/2HPG criteria alone. Furthermore, the definition of the main inclusion criterion, pre-diabetes, requiring 2 of 3 criteria (identifying participants at relatively high risk) will increase the incidence rate compared to previous studies. However, the planning committee elected to be conservative in its estimate to ensure adequate power for the primary outcome and has assumed an incidence rate of 10% per year (hazard rate = 0.1054) in sample size calculations.

#### 11.1.2 Rationale for the Assumed Hazard Ratio of 0.75

The planning committee relied on published observational *longitudinal* data and short-term intervention studies to estimate a plausible hazard ratio.

*Observational studies:* In the Nurses' Health Study, the highest 25OHD concentration (>33 ng/mL) was associated with a reduction in incident diabetes by *~50%* over ~10-year follow-up period.[18] In the Framingham Offspring Study, participants in the highest tertile of predicted 25OHD score (>22 ng/mL) had a *40%* lower incidence of diabetes during a 7-year follow-up period.[21] These results are consistent with other recently published observational data.[26-28,195,196] In a recent meta-analysis that combined data from all available longitudinal observational studies, the pooled relative risk of t2DM comparing the highest with the lowest quartile of 25OHD was 0.59 (0.52, 0.67), with little heterogeneity between the 11 studies included (3,612 cases and 55,713 non-cases) (Figure 11.1).[196]

**Figure 11.1**. Meta-analysis (Forouhi et al) of the association between blood 25OHD concentration and incident type 2 diabetes.[196]




In another meta-analysis by Song et al, the pooled relative risk for incident type 2 diabetes was 0.62 (95% confidence interval [CI]:0.54-0.70) for the highest vs. lowest 25OHD concentration (Figure 11.2).[138] A linear trend meta-regression analysis showed that each 4 ng/mL increment in 25OHD level was related to a 4% lower risk of diabetes (RR, 0.96; 95% CI, 0.94-0.98; *p* for linear trend<0.0001; Figure 11.3).[138]

Based on the observational data, if the proposed D2d intervention (4,000 IU/day of vitamin D) increases participants' mean 25OHD to approximately 40-45 ng/mL from ~20 ng/mL,[197,200-202] then risk reduction will be approximately 20-25%.

**Figure 11.2**. Meta-analysis (Song et al) of the association between blood 25OHD concentration and incident type 2 diabetes.[138]

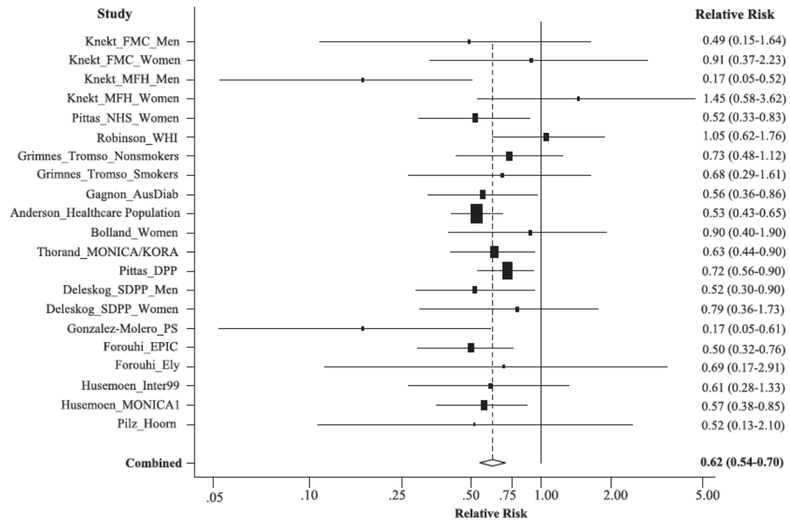
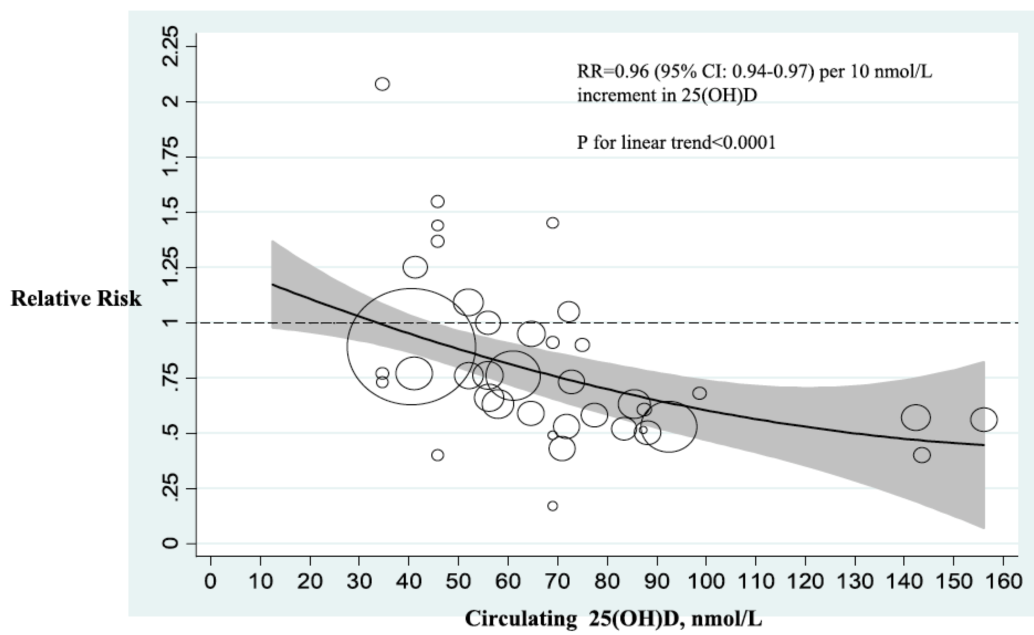



**Figure 11.3**. Relation between blood 25OHD concentration and risk of incident type 2 diabetes, modeled by quadratic spline regression.[138]



There are two observational studies of particular importance and relevance to the D2d study that were not included in the above meta-analyses: In the DPP study, among participants with pre-diabetes, there was an inverse linear association between 25OHD and incident diabetes, after multivariate adjustment including change in BMI and physical activity, with the hazard ratio for incident diabetes being lowest (0.46; 95%CI, 0.23 to 0.90) in the highest category (25OHD≥50 ng/mL) compared to the lowest category (25OHD<12 ng/mL) with no evidence of a threshold.[25] In another observational study, progression from pre-diabetes to type 2 diabetes was reduced by 73% among those with 25OHD>28 ng/mL compared to those with 25OHD<18 ng/mL.[195]

*Intervention studies:* In the CaDDM trial, 2,000 IU/day of vitamin $D_3$ supplementation, which raised 25OHD to 31 ng/mL, improved measures of beta cell function (disposition index) by ~40% and glycemia (HbA1c) by ~50% after 4 months.[24]

After taking into consideration all published data and short-term mechanistic studies on vitamin D and diabetes, a hazard ratio of 0.75 (i.e. 25% reduction in risk in the intervention arm) was used as the assumed treatment effect in sample size calculations. A reduction of this magnitude would clearly be clinically significant to public health.

### 11.2 Data Analysis Plan

*Defining the Study Population and Treatment Groups* - The primary analysis is by intention-to-treat, including all randomized participants in their assigned treatment arm and including all events observed during the study irrespective of adherence to assigned treatment. When participants withdraw or go "off study," follow-up will be censored at the date of the last visit. Exploratory "per-protocol" analyses and analyses in subgroups defined by level of adherence to study treatment will be undertaken as well, but are not considered part of the confirmatory analysis plan.

Every effort will be made to establish eligibility prior to enrollment and randomization; however, a few enrolled participants may subsequently be found not to meet all enrollment criteria. Most such individuals will be at increased risk for incident diabetes even though some eligibility criteria were not assessed or met. Thus, the primary analyses will include all randomized participants. If more than 1% of those randomized do not meet all eligibility criteria, which is an unlikely scenario, we will perform sensitivity analyses to assess the impact of excluding those individuals.

*Baseline Characteristics* – Although the randomization procedure should produce balanced treatment groups, relevant demographic (age, gender, race, ethnicity) and baseline clinical characteristics (e.g., BMI, physical activity, family history of diabetes, and 25OHD concentration) of participants will be compared across groups to characterize study participants and to document the success of the randomization procedure. Standard parametric and nonparametric statistical techniques will be used to test for differences in the distributions of baseline characteristics between treatment groups. Statistical significance will be determined from two-sided tests with an alpha level of 0.05. The large size of the trial and the stratification by BMI and race make it likely that differences in the distributions of these potential confounders between treatment groups, if statistically significant, will not be clinically significant.

*Primary Outcome* – The primary endpoint (outcome variable) is *"time to progression to incident diabetes."* For most participants, incident diabetes will be diagnosed at one of the regularly scheduled study visits, which will occur every 6 months. In some cases, however, participants will notify study personnel that they have received a diagnosis of diabetes, or been placed on diabetes medication, by their personal physician. In such cases, every effort will be made to assess whether the participant has met the study criteria for incident diabetes. When participants have been placed on a diabetes medication and the diagnosis cannot be confirmed by D2d laboratory criteria or adjudicated by the clinical outcomes committee, the participant will be considered to have not reached the primary study outcome and follow-up for incident diabetes will continue per the-intent-to treat principle. Sensitivity analyses will be performed that include these occurrences (i.e., a diabetes-specific medication is prescribed but a D2d-diagnosis of diabetes is not made) as cases of incident diabetes.

*Statistical Methods* - Since the exact time of progression to incident diabetes, defined as the date on which the criteria for incident diabetes would have been met if laboratory tests had been performed, will be unknown for participants who receive a diagnosis of incident diabetes at a regularly scheduled study visit, one could consider statistical methods for interval-censored data for the analysis of the time-to-event variables.[277] However, subjects whose diagnosis is initiated by their personal physician will not have a diagnosis date corresponding to a regular visit, and participants will have variable visit schedules. Thus, we have chosen instead to use standard methods for analysis of right-censored time-to-event data, using the incidence date defined in Section 9.1 for the primary outcome. The interval-censored approach and the right-censored time-to-event approach would give similar estimates and standard errors for treatment effects, because the change in approach would have similar effects on the estimated time-to-event distributions in the two treatment groups. Moreover, the notion that each individual participant has a true exact date of onset of diabetes is a somewhat artificial concept, in that an individual will not consistently meet the diagnostic criteria on successive days as they transition from pre-diabetes to incident diabetes.

Kaplan-Meier estimates of “time to confirmed diabetes” distributions will be calculated for each treatment group. The log-rank test will then be used to perform an unadjusted comparison of the time-to-event distributions in the two treatment groups. Follow-up of participants who withdraw or are lost to follow up will be "censored" on the date of their last follow-up visit. All p values examined for statistical significance will be two tailed, and p-values below 0.05 will be considered statistically significant. Cox proportional hazard models[278] will be used to calculate an estimate of the adjusted hazard ratio. To construct the model for the adjusted analysis, we will first construct a regression model that does not include the indicator for treatment group. Age, race, ethnicity, BMI, and other variables (fasting glucose, 2-hr glucose, HbA1c and 25OHD) to be specified a priori will be forced into the model. Variables will then be added in a step-up fashion form a list of candidates selected a priori. Covariates making a statistically significant contribution to the proportional hazards regression model will be included in the multivariate model. When the step-up procedure has been completed, the covariate for treatment group will be added to the model. The regression coefficient for treatment group in this multiple regression model will be the adjusted estimate of the log hazard ratio.

*Subgroup Analyses* - Variability of response to vitamin D supplementation will be assessed by pre-specified analyses in participant subgroups defined by *baseline* variables, e.g., race (self-reported definitions of White, vs. non-White e.g. Black/African American, American Indian, Alaska Native); ethnicity (Hispanic vs. Non-Hispanic); BMI (two groups based on median value); waist circumference (two groups based on median value);[187] age (two groups based on median value); geographic location (two groups, above or below 42$^{o}$ latitude), calcium intake and 25OHD concentration (two groups based on median value). Subgroup analyses by clinically applicable cutoffs will also be done (e.g. BMI as normal weight, overweight or obese; 25OHD concentration by IOM cutoffs). Each analysis of participant subgroups will include a test for interaction. Effect modification will be claimed only if the test for interaction reaches statistical significance. These analyses, although pre-specified, are considered exploratory as the study is not powered for such analyses and multiple comparisons must be considered when interpreting findings that are nominally statistically significant. We will also assess variability of response to vitamin D supplementation by “per-protocol” and “on-treatment” analyses, where treatment is defined by adherence based on pill count or achieved 25OHD concentration. These analyses will also be considered to be exploratory.

*Missing Data* - Missing data are inevitable in clinical research. Every effort will be made to minimize missing data in D2d. The primary methods of data analysis assume that censoring for time-to-event variables is non-informative. However, as recommended in the recent report of the Institute of

Medicine, we will perform sensitivity analyses to assess the degree to which the results are sensitive to the validity of the assumptions of non-informative censoring or non-informative missingness.

*Data Monitoring Plan* – Methods for interim analysis of accumulating data will be reviewed and approved by the D2d DSMB, which will regularly review accumulating safety and efficacy data to assure that continuation of the study remains scientifically and ethically appropriate. As a specific part of this monitoring activity, a single formal interim analysis of the accumulating primary endpoint data will take place when 70% of the expected events have accrued. The stopping boundary for the interim analysis will be based on the Peto-Haybittle approach.[279,280] With this stopping boundary, the nominal P value representing statistical significance will be 0.001, corresponding to a Z score of 3.09.

The D2d coordinating center will prepare the reports on behalf of the DSMB, whose members will review the data and advise the sponsor (NIDDK) whether (1) there is evidence that the intervention is clearly better or worse than placebo in relation to the primary outcome, and (2) the DSMB recommends a change in the study plan based on safety considerations.

*Seasonal and Geographic Variability* – Given that the cohort will be recruited at a constant rate throughout the calendar year and outcomes will be determined every 6 months, the potential for seasonal variability confounding the association between vitamin D supplementation and outcomes is low. We will test this assumption in the analyses, and if needed, we will adjust for the month of the year at entry into the study. All analyses will adjust for site, as routinely done in multicenter studies, which will also approximate latitude at each of the participating sites. Given the age group, we anticipate few participants moving residence during the study.

## 12. FUNDING, STUDY ORGANIZATION, ADMINISTRATION AND GOVERNANCE

### 12.1 Sponsors

The planning phase of D2d was funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) through the Multi-Center Clinical Study Implementation Planning Grant (U34) to Tufts Medical Center in Boston, Massachusetts (U34DK3491958). The conduct of D2d is supported primarily by NIDDK and the Office of Dietary Supplements through the U01 Multi-Center Clinical Study Cooperative Agreement (U01) to Tufts Medical Center (U01DK098245), which has established sub-award agreements with each collaborating clinical site and core units (e.g. Central Laboratory, drug distribution center) to reimburse each site and core unit for their activities related to D2d. Funding is also provided by the American Diabetes Association (1-14-D2d-01). Educational materials are provided by the National Diabetes Education Program. The primary funding agency (NIDDK) and the study investigators work as partners; however, the primary responsibility for the D2d study planning and conduct is with the study's Principal Investigator and the CC. Representatives from NIDDK participate in all phases of planning, development of the protocol, development of policies and procedures, implementation and monitoring of study performance and safety, data analyses and interpretation and dissemination of study findings.

### 12.2 Organizational Overview

Overall study leadership is provided by the Steering Committee while the Executive Committee acts as the operational arm of the Steering Committee. The D2d Coordinating Center (CC) is established in the Division of Endocrinology at Tufts Medical Center. The CC provides overall study organization and administration, and manages day-to-day operations by working directly with the collaborating sites, other study core units and study committees and subcommittees. The CC implements the study protocol; oversees the development of the Manual of Procedures; monitors participant recruitment and treatment delivery; evaluates data collection and management; oversees quality assurance procedures; monitors participant safety; and implements changes and enhancements to the study as recommended by the Steering Committee. The CC also develops the data collection processes, performs data monitoring, data cleaning and analyses and contributes to manuscript writing. The *Drug Distribution Center*, located at the VA Cooperative Studies Program, Clinical Research Pharmacy Coordinating Center, conducts the randomization and distribution of study drug to the sites. The *Central Laboratory*, located at the University of Vermont in Burlington, Vermont, coordinates collection of study specimens from collaborating sites, conducts laboratory measurements and serves as a central repository for short and intermediate-term storage of human blood and urine samples. Long-term storage of human blood and urine samples will be transitioned to the NIDDK Central Repositories.

### 12.3 Study Leadership and Governance

#### 12.3.1 Executive Committee (EC)

The EC acts as the operational arm of the Steering Committee (SC) and makes scientific, administrative and fiscal decisions, on behalf of the SC, for day-to-day operational issues requiring prompt action. The committee develops timelines for the accomplishment of tasks, appoints (and disbands) subcommittees as the need arises and selects subcommittee members and chairs, presents information to the sponsor, and develops Steering Committee meeting agendas.

### 12.3.2 Steering Committee (SC)

The SC provides scientific leadership for the study. The committee works closely with its operational arm, the EC, and the SC subcommittees. The committee reports to the primary study sponsor (NIDDK), via the EC. The committee is comprised of all standing members of the EC plus the Principal Investigator from each collaborating site and the Central Laboratory

#### 12.3.2.1 Steering Committee Subcommittees

There are 7 standing subcommittees, which are established to monitor specific components of the study conduct and to provide periodic status reports to the Steering and Executive Committees.

The following subcommittees have been established:

**Conflict of Interest Subcommittee (CIS):** The subcommittee reviews all conflict of interest forms in which there has been a disclosure. The committee will determine if a potential conflict of interest exists, and will develop and implement a management plan that will specify the actions that have been and/or will be taken to eliminate or limit the potential impact of such conflict(s) on the study's credibility. The subcommittee works closely with the sponsor to evaluate and mitigate conflicts of interest

**Recruitment and Retention Subcommittee (RRS)**: The subcommittee reviews and approves the collaborating sites' recruitment plans prior to the start of the study and monitors recruitment progress and retention and adherence to study procedures. Rates of participant retention and prompt and complete data capture will serve as quality measures of performance by the sites. Pre-specified site-specific targets for optimal performance are defined and should be met to achieve high quality of trial conduct. The subcommittee reviews these measures regularly and makes recommendations (general or site-specific) to improve these rates and ensure that sites meet their target recruitment/retention goals. The subcommittee makes recommendations to the Executive Committee regarding the need for additional sites, if recruitment is slower than anticipated.

**Support and Education Subcommittee (SES):** The subcommittee develops the Support and Education Program that will be implemented during the study. The subcommittee reviews and approves the education materials on the current lifestyle recommendations for prevention of type 2 diabetes that will be provided to all participants at baseline. The subcommittee also develops program themes and content for use during the SEP group meetings that will be held at each site twice a year. The goals of the SEP meetings will be: (1) to provide participants with up-to-date information on the lifestyle recommendations for the prevention of type 2 diabetes and (2) to promote participant retention. Towards these goals, the SES works closely with the RRS.

**Research Coordinators Subcommittee (RCS)**: The subcommittee is comprised of the research coordinators from each site and representatives from the CC. The major objective of the subcommittee is to assure communication among the sites with respect to overall study coordination and share best ideas and problem solve. The coordinators are closest to the day-to-day issues at the sites; therefore, they are expected to be an invaluable resource to the study and are encouraged to make recommendations regarding the study conduct to the Steering and Executive Committees for review and consideration. The Chairperson of the RCS is a member of the EC.

**Safety & Outcomes Subcommittee (SOS):** The subcommittee implements the Data Safety Monitoring Plan. The subcommittee reviews all serious adverse events (SAE) and unanticipated problems (UAP) as they are reported, reviews periodic safety reports of all adverse events (AE) and oversees study safety. The subcommittee chair or designee assesses each SAE and UAP to determine if immediate action is required in response to the event. The subcommittee meets three times a year (or more frequently if needed) by conference call. During these meetings, the subcommittee discusses SAE, UAP and summary reports of non-serious AE. The subcommittee also evaluates whether there is any clustering of AEs, SAEs or UAPs by clinical site. The subcommittee remains masked to patient treatment group during these evaluations. The subcommittee considers whether changes in the protocol (monitoring, consent process, etc.) are indicated based on the occurrence, frequency, or severity of AE, SAE or UAP and provides its recommendations to the Steering and Executive Committees for further discussion and subsequently to the DSMB for approval and implementation. The subcommittee also provides adjudication for secondary outcomes.

**Ancillary Study Evaluation Subcommittee (ASES)**: The subcommittee is responsible for establishing and overseeing the Ancillary Studies Policies and Procedures and ensuring that the policy is followed. The subcommittee is responsible for evaluating ancillary study applications and making recommendations to the Steering Committee regarding the proposals and monitors progress of approved ancillary studies.

**Publications & Presentations Subcommittee (PPS):** The subcommittee develops and oversees the policies and procedures by which D2d investigators will interpret data analyses and will coordinate publications and presentations of study results. The subcommittee is responsible for establishing and overseeing the Publications and Presentations Policy and ensuring that the policy is followed. The subcommittee reviews and approves all publications and presentations related to the D2d study, including those from ancillary studies, prior to submission. The subcommittee monitors the progress of all proposed manuscripts to ensure prompt completion and publication.

### 12.3.3 Clinical Outcomes Committee (COC)

The committee, which is independent of the D2d Study Group, is formed to review and adjudicate the diagnosis of diabetes that is made outside of the D2d study or initiation of diabetes-specific medication (for any reason) when study-specific glycemic data are not available. The COC is composed of clinical diabetes experts who have no real or perceived conflict of interest related to the D2d study, D2d Study Group, sponsor or study core units.

## 12.4 Role of Industry

Industry may contribute resources to the study and will be acknowledged appropriately; however, industry will play no role in the design and conduct of the study, data analysis, interpretation or publication of study results.

**SUPPLEMENTS AND APPENDICES**

Appendix A Sample D2d Informed Consent Form
Appendix B Sample D2d Research Specimen Repository Informed Consent Form

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