Clinical Intervention Protocol

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Précis

Specific Aims. Diabetes affects 1 in 10 persons in the United States and is projected to increase to 1 in 4 persons by 2050 (1), resulting in a 72% increase in health care costs (2, 3). Women with type 2 diabetes have worse glycemic control and diabetes self-care behaviors than men with type 2 diabetes (4-6). One reason for these poor outcomes is the influence of depression which affects over 25% of women with diabetes (7).

Depression interferes with self-care behaviors and significantly impacts glycemic control in women with type 2 diabetes (8-12). Although there is significant research to indicate that depression can negatively affect diabetes self-management (8-10), there is limited evidence on whether treating depression can improve self-management (13-15). Antidepressants can effectively relieve depression and its related symptoms in persons with type 2 diabetes (16, 17). However, these medications can also disrupt glycemic control (18-20) and cause weight gain (21, 22) making diabetes self-management more challenging.

Vitamin D is being studied for its effects on depression (23-26). Cross sectional studies report that low vitamin D levels are associated with depressive symptoms (depression) (27-30). Several intervention studies have reported that vitamin D supplementation can reduce depression (31-33); however consistent limitations were the lack of significant depressive symptomology or depression as inclusion criteria. One such study, a large, randomized clinical trial (RCT) trial of healthy, overweight and obese adults, found that weekly vitamin D supplementation (40,000 IUs) improved depressive symptoms; suggesting its use for amelioration of these symptoms (23). Vitamin D supplementation is associated with few side effects and is inexpensive (34-36). To our knowledge, there are no published studies on vitamin D supplementation for persons with diabetes who have depression.

We recently completed a six month proof of concept study using a one group, pre-post test design to examine the effects of weekly vitamin D supplementation (50,000 IUs weekly) in women (n=50) with type 2 diabetes who had significant depressive symptoms (a score $\geq$ 16 on the Center for Epidemiologic Studies Depression Tool (CES-D) (NIH 5P60DK020595, pilot project). We found a significant decrease in depression and improvements in diabetes self-management. We also found a decrease in systolic blood pressure. Depression is associated with hypertension and an increased risk of stroke (37-39). Evidence suggests that vitamin D supplementation may lower blood pressure (BP) by direct and indirect vascular activity (40-44).

We now propose a randomized trial to determine the efficacy of vitamin D_3 supplementation on depressive symptoms, self-management, and blood pressure in women (n=180) with type 2 diabetes who have significant depressive symptoms (CES-D $\geq$ 16). Using a stratified block randomization (strata based on depression

symptom severity) women will be assigned to either weekly vitamin D_3 supplementation of 50,000 IUs or 5000 IUs for a period of six months. Since guidelines recommend assessing vitamin D levels at three months upon initiating this dose, study measurements will collected at baseline, three, and six months. The following outcomes will be measured: depressive symptoms, diabetes self-management (self-efficacy, diabetes distress, diabetes self-care behaviors), and systolic blood pressure. We will also explore a possible mechanistic effect of vitamin D_3 supplementation on depression. Evidence indicates that in depression, pro-inflammatory biomarkers, notably cytokines and C-reactive protein (CRP), are elevated (45-48). Several studies (not depression studies) have reported that Vitamin D can decrease levels of inflammatory biomarkers (49-51). Thus we will explore whether vitamin D_3 supplementation decreases inflammatory biomarkers (CRP, interleukin-6, and tumor necrosis factor- α) providing evidence for a possible mechanism of antidepressant activity.

Aim 1: To determine the effect of 50,000 IUs of vitamin D_3 supplementation on depressive symptoms (primary outcome), self-management (secondary outcome), and systolic BP (exploratory outcome) compared to 5000 IUs.

Hypothesis: Women receiving 50,000 IUs of vitamin D_3 supplementation will report fewer depressive symptoms, increased diabetes self- management mediated by depression improvement, and have a lower systolic BP compared to those taking 5000 IUs at three and six months follow-up.

Aim 2: To explore the mechanistic effect of vitamin D supplementation on inflammatory biomarkers and their association with depression.

Hypothesis: Women receiving 50,000 IUs of vitamin D_3 supplementation will have a decrease in inflammatory biomarkers which will be associated with fewer depressive symptoms compared to those taking 5000 IUs at three and six months follow-up.

UPDATED AIMS

A sub-study was added to investigate family functioning in women with Diabetes and Depression titled *Family Matters in Diabetes and Depression (FAMDD)*. This study is also funded under LU# 208808
This sub-study of the Sunshine 2 Study has the following specific aims and hypotheses:

Aim 1: Describe the impact of family functioning on self-management, barriers to self-management, and FQOL in women with depression and T2DM. (qualitative)

Aim 2: Determine the relationship of depressive symptoms, family functioning and FQOL.

Hypothesis 1a: Depressive symptoms will be associated with poor family functioning.
Hypothesis 1b: Higher depressive symptoms will be associated with poor FQOL.
Hypothesis 3c: Depressive symptoms mediate the relationship of family functioning and FQOL.

Aim 3: Determine the relationship of family functioning, self-management and FQOL.

Hypothesis 2a: Better family functioning will be associated with more self-management behaviors.
Hypothesis 2b: Better family functioning will be associated with better FQOL.

A sub-study was added to investigator the urinary microbiome and lower urinary tract symptoms in women with type 2 diabetes titled "The Urinary Microbiome and Vitamin D in Women with Type 2 Diabetes" study has recently been submitted for review for potential funding to Novo Nordisk under LU# 208880.

This substudy of the Sunshine 2 Study has the following specific aims:

Primary Objectives: (1) To determine the FUM composition in women with T2DM, using high throughput 16S rRNA gene sequencing & enhanced quantitative urine culture (EQUC), and determine the FUM's association with LUTS in women with T2DM. **(2)** To determine the impact of glycemic control on LUTS and the FUM composition in women with T2DM.

Secondary Objective: To determine whether there is an association between vitamin D status (sufficient, insufficient, and deficient), the FUM and LUTS in women with T2DM.

Exploratory Aim: To examine the relationship of the phage activity with glycemic control, and Urine PH in biobanked urine samples in the laboratory of Catherine Putonti. Proctocal additions from Putonti lab included in Modifications included in section

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I. Significance.

Diabetes and Depression. Depression occurs more often in women with diabetes than men with diabetes (7, 52) and over 25% of women with diabetes have depression (7). For women having both depression and diabetes, the risk of mortality is significantly higher (RR=3.11) than for having either diabetes (RR=1.71) or depression (RR=1.76) (53). Of significance is that the cost of treating diabetes and co-morbid depression is almost four times higher ($247 million) than treating diabetes alone ($55 million) (54, 55).

Depression Treatment and Diabetes. Drug therapy for treatment of depression can disrupt glycemic control (18-20) and cause weight gain (21, 22). In the Diabetes Prevention Program for persons who were at risk for diabetes, continuous antidepressant use was associated with an increased risk of diabetes in both the placebo (RR=2.60) and the intensive lifestyle (RR=3.39) study arms (56). Although cognitive behavioral therapy is an effective treatment for depression and depressive symptoms in persons with diabetes (57-59); lack of available trained personnel, poor access to mental health services for many individuals, and cost can be challenges for its greater use (60, 61). Thus, exploration of other strategies to treat depression are needed.

Vitamin D and Depression. Evidence from observational studies suggests a relationship between low levels of vitamin D and depression (25-30), but no randomized clinical trials in persons with depression are available to confirm this evidence. However, reports indicate that Vitamin D receptors in the brain may have an important role in neuroendocrine functioning (62-64). For example, low levels of serotonin are present in depression, and vitamin D has been linked with the production of serotonin (65). Research also indicates that lack of vitamin D negatively affects growth, cellular signaling, and neural activity in the brain (62, 66). Thus, other mechanisms may be involved in the role of vitamin D and depression.

There have been several studies examining the impact of vitamin D supplementation on mood. In earlier studies, improvements in mood were not observed (67-69); however, variation in the amount of vitamin D given, frequency of dosing (daily, weekly, monthly), duration of dose and lack of achieving therapeutic vitamin D levels could have been reasons (25). More recent studies report improved feelings of depression and well being for persons who are not depressed (31-33). One large trial of healthy, overweight and obese individuals (n=400) randomized to vitamin D_3 supplementation (40,000 D_3 IU weekly) or placebo for a year reported that symptoms of depression significantly decreased (23). However, a study limitation was that significant depression symptoms (depression) were not criteria for enrollment. Recently, a substudy of the VITAL trial (randomized trial of vitamin D and Omega A-3 examining cardiovascular health outcomes in healthy adults aged 55+) began assessing for depressive symptoms, but the dose of vitamin D (2000 IUs daily) may be insufficient for obese individuals (70, 71).

Vitamin D supplementation for the treatment of persons who have significant depressive symptoms has not been studied. Our pilot study included women with type 2 diabetes who had significant depressive symptoms (depression), but was a one-arm study (treatment only) Following supplementation, we found a significant improvement in depressive symptoms and as well as other outcomes (See pilot). We now are proposing a randomized trial targeting treatment of depressive symptoms with vitamin D_3 supplementation (Figure).

Depression and Self-Management. We also observed an improvement in diabetes self-management (specifically, self-care behaviors and diabetes distress) in our pilot study. We believe that this effect was due to an improvement in depression. Evidence indicates that depression negatively impacts diabetes self-management (72-76), especially for women (6). Self-management includes beliefs (i.e., self-efficacy), emotional responses (i.e., diabetes distress), as well as the ability to engage in self-care behaviors (diet, activity, medication adherence) (77). For women, depression is associated with lower self-efficacy (78, 79) and increased diabetes distress (80, 81), both of which can contribute to poor self-care behaviors (82). Although there is significant research to indicate that depression impacts diabetes self-management, there is limited evidence as to whether treatment of depression improves diabetes self-management (13-15). This study will add to that body of knowledge. For this study, we propose that if vitamin D supplementation reduces depression, self-management will improve as individuals begin to feel better and more actively participate in their care. If depression subsides, we believe that self-efficacy, diabetes distress, and self-care behaviors will improve (Figure).

Vitamin D and High Blood Pressure. We also observed an improvement in blood pressure in our pilot study. Definitive mechanistic studies of vitamin D and blood pressure are currently in progress. Both in vitro and in

vivo studies of vitamin D receptor knockout mice have supported the hypothesis that Vitamin D may affect blood pressure by regulating activity of the renin-angiotensin system through suppression of renin biosynthesis (40, 44). Vitamin D may also be effective in reducing deleterious effects of glycation end products on the endothelium (41), thus allowing for endothelium-dependent vascular relaxation (42, 43).

The Nurses' Health Study reported that women were twice as likely to develop hypertension if vitamin D levels were less than 15 ng/ml (83). Women have vitamin D levels as low as 15-20 ng/ml (84, 85), especially those with type 2 diabetes (86). There have been few vitamin D trials in women to examine blood pressure effects, and results have been inconsistent (87-89). The Women's Health Initiative did not find a change in blood pressure with vitamin D supplementation; however, the dose (400 IU) daily may not have been sufficient (90). Yet Pfeifer and colleagues (91) did report a 7% decrease in systolic blood pressure in vitamin D deficient women following 800 IUs of vitamin D_3 after 6 weeks.

High blood pressure is a major risk factor for persons with diabetes which contributes significantly to their renal complications. Epidemiologic evidence indicates that low levels of vitamin D are associated with a higher systolic blood pressure (92). Because persons with diabetes are at high risk for hypertension, this outcome is important in assessing as an additional benefit of vitamin D supplementation. In addition, because depression increases the risk for hypertension and stroke, this is another important reason for its study (37-39). Thus, we propose that vitamin D supplementation will decrease systolic blood pressure (Figure).

Vitamin D and Diabetes**.** Our pilot study did not demonstrate improvements in glycemic control following vitamin D supplementation; although women had type 2 diabetes for an average of 8 years and were under good metabolic control (see pilot data). Some epidemiologic evidence suggests that vitamin D may prevent diabetes (93, 94), however, few RCTs are published (95, 96). The Women's Health Initiative reported that that vitamin D did not prevent the development of type 2 diabetes (97). Other studies have been inconclusive regarding the effects vitamin D supplementation on glycemia, with most showing no significant benefit (98, 99).

Summary. Depression is higher in women with type 2 diabetes and is costly to treat (7, 54-55). Evidence from observational studies suggests a relationship between low levels of vitamin D and depression (27-30), but no randomized clinical trials in persons with depression are available to confirm this evidence. Studying vitamin D supplementation as a treatment option can have significant implications. The cost for vitamin D supplementation is low (as low as cost $3 per month) and it has few side effects (34, 36). Given that 1 in 10 Americans now take antidepressants (100) which can have negative effects on weight and metabolic control (18-22), this study could provide evidence as a treatment option for depression. Since women with diabetes have low vitamin D and high levels of depression, this group is being studied as efficacy is more likely to be demonstrated.

II. Innovation.

Vitamin D for Depression. The proposed study is innovative in that a randomized trial to examine the impact of vitamin D supplementation for treatment of depressive symptoms has not been conducted. To target women with type 2 diabetes that are at highest risk for depression is innovative. If vitamin D supplementation improves depressive symptoms, it may also improve diabetes self-management which is another novel aspect of this study. Although there is evidence to suggest that depression impacts self-management, there are few studies to examine whether this is actually true. Finally, to study whether vitamin D supplementation can improve systolic blood pressure is also innovative.

Dosing of Vitamin D. Although the Institute of Medicine recommends 600 IUs day for women up to age 70 (101), information regarding doses for persons who have chronic diseases like depression or diabetes are almost nonexistent. It has been reported that higher doses are needed to reach sufficient levels in persons who are overweight and obese (23, 35). Evidence recommends that in obese patients, doses of at least 6000 to 10,000 IUs day of vitamin D for at least eight weeks followed by similar dosing to maintain the level above 30 ng/ml (34, 35). A recent study reported that taking high doses (10,000 IUs daily) for almost one year did not have adverse effects (49, 96)**.** Most persons with type 2 diabetes are overweight or obese. The proposed study will be administering a dose of vitamin D that is adequate for replacement for overweight and obese persons (23, 35, 102) and is another novel aspect of this study.

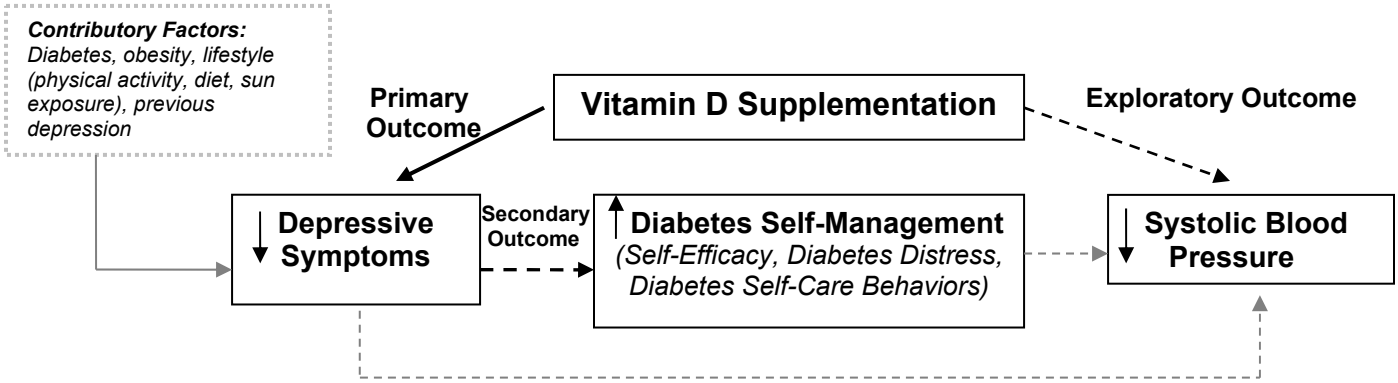
Depression and Vitamin D Mechanism of Action. The Cytokine Theory of Depression evolved when studies identified that inflammation played a significant role in the pathophysiology of depression (103-105). Research indicates that pro-inflammatory cytokines alter the metabolic pathway of tryptophan by shunting it away from serotonin synthesis via stimulation of the enzyme indoleamine 2,3-dioxygenase (IDO) (106). This cytokine-serotonin interaction also explains the neurodegeneration observed in depression (106). Several studies (not depression studies) have reported that Vitamin D can decrease levels of pro-inflammatory biomarkers (49-50). Since individuals with depression have elevated inflammatory markers (CRP, IL-6, and TNF-α) (45-48), to explore whether vitamin D supplementation will decrease inflammation and subsequently ameliorate depressive symptoms is innovative and may provide information regarding a potential mechanism of action.

Urinary microbiome and vitamin D supplementation. Because Vitamin D_3 supplementation has been associated with decreased inflammation (Burton et al., 2010; Hopkins et al., 2011; Wobke et al., 2014), it *is plausible that this decreased inflammatory response reduces ower urinary tract symptoms (LUTS)*. Indeed, two studies (one animal, one human) showed that Elocalcitol, a biologically active vitamin D_3 analogue given for treatment of overactive bladder, reduces nonvoiding urinary contractions (Shapiro et al, 2013) and improved perceptions of bladder condition and reduced episodes of urinary incontence (Digesu et al., 2012). Since detrusor overactivity is associated with overactive bladder, vitamin D_3 supplementation may alleviate symptoms by reducing overactive detrusor muscle activity, which is a significant problem for women with diabetes (James et al., 2014).

Another potential mechanism for improved urinary symptoms following vitamin D_3 supplementation in postmenopausal women is the increased synthesis of the human antimicrobial peptide cathelicidin, which has been shown to be up-regulated during *E. coli* infection, potentially to protect the urinary tract (Hertting et al., 2010*).* Nseir et al. (2013) reported *a*n association between serum levels of vitamin D and recurrent UTIs in premenopausal women; women with <15 ng/ml vitamin D, as measured by serum 25 (OH) D, were 4X more likely to have recurrent UTIs. *The data collected in this study will provide insight regarding the relationship of vitamin D supplementation to UTI occurrence*.

III. Conceptualization.

For this study, we hypothesize that the vitamin D supplementation will reduce depressive symptoms. Diabetes self-management will improve as a result of the decrease in depression. We also hypothesize that vitamin D supplementation will improve blood pressure.



Contributory factors such as diabetes, obesity, and lifestyle may impact depressive symptoms and will be assessed and included in the data analysis. Diabetes is associated with more depression, especially for women (7). Most women with type 2 diabetes are obese (107). Evidence indicates that obesity may increase the risk of depression, particularly for women (108-110). Obesity is also associated with low vitamin D levels for women (111) and inflammation (94), which may contribute to depression. Physical activity can influence depression (112) and is also important to diabetes self-management. Other lifestyle factors such as dietary

intake and sun exposure can affect vitamin D levels, which impact depression. Finally, because previous depression may increase the potential for future depression episodes, this will be evaluated as well.

It is known that depressive symptoms may impact the management of your diabetes. Family resources and family quality of life may impact the ability to manage one's health (i.e., diabetes and/or mood). Family functioning is defined as the attributes of a family system that characterize how they operate or behave (McCubbin & McCubbin, 1987). Family functioning is an important variable in the study of adaptation to chronic conditions. Thus, these measures will also be studied.

IV. Preliminary Studies.

The PI and members of the team have been developing and testing treatments for women with type 2 diabetes who have significant depressive symptoms. We have demonstrated our ability to recruit, retain and effectively treat women with type 2 diabetes for these symptoms in our previous work.

Previous work in depression treatment for women. Initially, to gain an understanding of the psychological impact of living with diabetes, we conducted focus groups. This study was funded by the American Nurses Foundation (Gloria Smith Health Disparities Award) and findings were published (4). Results indicated that women with type 2 diabetes experience depression and other emotions which significantly impact their well being, relationships with others, and diabetes self-management. Based on these findings, a nurse-delivered group cognitive behavioral intervention (CBT) intervention for the education and treatment of dysphoric symptoms (SWEEP) in depressed women with type 2 diabetes was developed and tested. This was funded by NIH-NINR (K23 009240) with presentations and publications (113, 114). The final study results were just reported in the Annals of Behavioral Medicine, a multidisciplinary journal (Impact factor 4.3, E-Pub Head, July 2012) (59). Findings indicated that a nurse delivered CBT intervention was more effective than usual care for the treatment of depressed women with type 2 diabetes as there were significant improvements in depression, anxiety, and anger and improvements in metabolic outcomes.

Preliminary work for pilot study. During the SWEEP study, there was evidence to suggest that insufficient vitamin D may impact depression and other health outcomes. Our literature reviews on this topic were published in the Issues in Mental Health Nursing, Circulation, and Diabetes Educator (25, 44, 94). With funds from our School of Nursing and IRB approval, we were able to assess the vitamin D levels in our SWEEP participants. Findings indicated that 72% of our women from SWEEP had vitamin D levels less than 30 ng/ml ($\geq$ 30 is normal), and there was a significant relationship between vitamin D deficiency and depression (CES-D) ($r=-.39$, $p<.05$). Relationships also existed for HBA1c ($r=-.20$) and fasting glucose ($r=-.28$), but were not statistically significant.

Pilot study description and findings. Using this preliminary data from SWEEP, we applied and were funded for a "proof of concept study" to examine whether vitamin D supplementation in women with 2 diabetes with low Vitamin D levels could improve depression and metabolic outcomes (NIH 5P60DK020595 pilot study, University of Chicago Diabetes Research and Training Center). It was branded as the "Sunshine Study". A one-group pre-post test design study was used to test for feasibility, compliance, and the impact of vitamin D_2 supplementation. Weekly ergocalciferol (D_2) of 50,000 IUs was administered for six months. This formulation of vitamin D is approved for the treatment of low vitamin D, and was being used at our clinical practice site at the time of the study. Women were enrolled if they had: type 2 diabetes greater than 6 months, significant depressive symptoms (CESD $\geq$ 16), were not taking vitamin D supplements or had stopped them for at least 1 month prior to enrollment, had a vitamin D level < 30 ng/ml, and were not hypercalcemic. These same inclusion and exclusion criteria are being proposed in this study. Over 300 women were phone screened for participation. Fifty women were enrolled at baseline. They were a mean age of 55 years, had diabetes an average of 8 years with a mean HBA1c of 6.8%, and 60% were white. At three months, 49 or 98% continued and at six months, 46 or 92% were retained. Procedures for dispensing the vitamin D and follow-ups are the same being proposed for this study (see procedures). Women came within 10 days following baseline screening to obtain their prescription of vitamin D, were then sent a weekly message (their preferred method as phone, text, or e-mail) to take their vitamin D, and called at 4 to 6 weeks. This was done to determine if any

concerns with the supplementation and to ensure that their depression did not worsen (and it did not). Women were then seen at three months for follow-up measures and for prescription refill. For all 3 time points, patients completed questionnaires that included: depression (CES-D) and diabetes self-management which included diabetes self-care inventory (SCI) and diabetes distress using the Problem Areas in Diabetes Scale (PAIDS). For the pilot study, labs and physical measurements were also assessed and included vitamin D, calcium, PTH, HbA1c, blood pressure, and weight. Depression was verified at baseline using the Diagnostic Interview Schedule (DIS). Following completion of the study, all patients were given their labs and referred to their healthcare provider for future follow-up.

Repeated measures ANOVA results indicated that depression symptoms decreased significantly over time. In addition, there was a significant improvement in self-care behaviors and less diabetes distress. Vitamin D levels increased to a therapeutic level, and there were no significant changes in labs (calcium and parathyroid hormone). Although there was no improvement in HBA1c, the average baseline value indicated very good control (<7%). Other investigators have reported no change in HBA1c with vitamin D supplementation (97-98). A clinically and statistically significant decrease in systolic blood pressure was observed. There was also a small decrease in weight.

Variables	Baseline Mean (SD)	Three Months Mean (SD)	Six Months Mean (SD)	P value
Depression-CES-D	26.83 (7.6)	15.13 (8.9)	12.15 (8.3)	<.001
Self Care (SCI)	2.76 (.58)	2.87 (.57)	2.88 (.64)	.041
Distress (PAIDS)	29.00 (14.2)	21.29 (14.4)	19.36 (14.9)	.001
Vitamin D (ng/ml)	18.78 (7.0)	34.39 (9.4)	37.5 (9.5)	<.001
Systolic BP (mmHG)	140.43 (17.1)	135.04 (17.1)	132.46 (14.3)	.007
Weight (pounds)	226.10(59.0)	224.64 (59.9)	223.58 (59.4)	.026

Given our preliminary data, we hypothesize a significant decrease in depression symptoms, improved diabetes self-management, and a lowering of systolic blood pressure in the treatment group (vitamin D) compared to the control group using a RCT. In our proposed study, we will measure weight since it can impact on both blood pressure and vitamin D levels (102). Since vitamin D levels could be affected by external factors such as sun exposure and dietary intake (27, 115, 116), we measured these as well. Women reported an average of 10% body exposure to the sun (largely their head and upper forearm) with no change over time in our pilot study. Dietary intake of vitamin D was low (153 IUs/ day), but given the limited natural food sources for vitamin D, this finding was not surprising. Since other research has addressed these factors, we will continue to measure these factors and address them in the data analysis.

Pilot work on depression and inflammation. Dr. Halaris, co-investigator and psychiatrist, has studied depression and inflammation but not vitamin D. His study of 48 patients with major depressive disorder (MDD assessed by DSM IV criteria) and 23 healthy controls (HC) were compared for pro-inflammatory cytokines (IL-6, TNFα) and hs CRP using a compact biochip array system (Randox Technologies; Evidence Investigator TM). Depressed patients demonstrated significantly higher levels of inflammation than healthy controls as reflected in plasma levels of TNF-α (MDD 2.15+/- 0.04 pg/ml and HC 1.59+/-1.59 pg/ml, p=0.05) as well as plasma IL-6 (MDD 0.810 +/- 0.012 and HC 0.665+/- 0.023 pg/ml, p=0.05). Similarly, hs-CRP in depressed individuals was significantly higher than in healthy control subjects (MDD 3.53 +/-0.4 mg/l; HC 1.79 +/- 0.7 mg/l, p<0.05). Given Dr. Halaris' success in measuring inflammatory biomarkers in depressed patients, we have proposed exploration of these measures following treatment with vitamin D supplementation where we hypothesize women will have a significant improvement in depressive symptoms.

Team Composition. In summary, we have a strong interdisciplinary team of nursing (Dr. Penckofer), mental health (Drs. Halaris and Mumby), endocrinology (Drs. Emanuele and Camacho), nutrition (Drs. Kouba and Sheean) and preventive medicine (Dr. Durazo) that has conducted the pilot studies necessary for this application. We have demonstrated success on the recruitment, retention, implementation, and measurement of the outcomes proposed for this RCT. We also have outstanding consultants who have had success with vitamin D implementation in research studies (Dr. Pittas) as well as clinical practice (Dr. Wallis). We believe that the uniqueness and strength of this team makes this application strong for its success if awarded.

V. Approach.

1. Design. This is a randomized, double-blind, controlled trial of six months duration. Women will be randomly assigned to either 50,000 IU of vitamin D_3 (n=75) or 5000 IUs of vitamin D_3 (n=75) (both dispensed by the Loyola University Medical Center pharmacy).

a. Treatment Schedule and Dose. The supplements will be administered once a week for six months. The dose of vitamin D_3 (50,000 IUs) is recommended for overweight and obese individuals to achieve adequate blood levels (34, 35, 102). In addition, doses such as these have been safely used (23, 49, 96).

The weekly dosing is being used to increase compliance to therapy (i.e., not having to remember to take it daily). A once a week administration schedule has been very successful in treating women for other disorders (117). To further increase compliance, women will receive a weekly reminder (preferred method of telephone, e-mail or text) to take their supplement. This was very successful in our pilot study.

Patients will be seen at baseline, 3 and 6 months with physical assessment and lab data as with our pilot study. Published guidelines recommend monitoring calcium, parathyroid hormone (PTH), and vitamin D at baseline and 3 months when initiating vitamin D at this dose (50,000 IU) to assess for a therapeutic level (35, 44). In addition, since treatment of depression is usually a minimum of 12 weeks, we will capture the time when typically evaluated if receiving treatment for depression (57, 59). Women will also be contacted by telephone at months 2, 4, for assessment of depressive symptoms as well as any potential side effects of the supplement.

Vitamin D_3 also known as cholecalciferol, has been selected as the treatment since it is the natural form of vitamin D, the more widely used supplement, and now recommended for clinical practice (34). The Institute of Medicine recommends 600 IUs of Vitamin D per day for individuals up to age 70 (101). Thus, the 5000 IUs dose will approximate follow that recommendation (600 IUs x 7 days= 4200 IUs) and be given once per week as well. The use of a dose with the minimum recommended amount for comparison is consistent with other studies examining the benefit of vitamin D supplementation on health outcomes (35, 95). Bio Tech Pharmacal, Inc. will prepare the vitamin D_3 supplements. This company complies with all applicable Food and Drug Administration regulations and follows Good Manufacturing Practices that are designed to ensure that the products meet the highest quality standards.

b. Treatment Randomization. A stratified block randomization will be used with blocks of random size 2, 4 and 6. There will be two strata based upon depression symptom severity using the CES-D guidelines (118). These strata will be (1) moderate severity for CES-D scores of 16 to 26 and (2) high severity for CES-D scores greater than 26. In our previous work, about half of the women were in each stratum at baseline. With stratified randomization, factors associated with depression should be more evenly distributed as randomization with stratification induces more balanced groups (119). A randomization list will be developed by Dr. Durazo, statistician and co-investigator. The list will be retained by the research pharmacist at Loyola, who is independent from those involved with the conduct of the study. The research pharmacist will use the list to assign subjects to the treatments..

c. Blinding. The study supplements will be administered by the pharmacist in a blinded fashion during the study so that the patient and clinical site personnel (except for the pharmacist) will not be aware of the patient's assigned treatment.

d. Treatment side effects. Published reports suggest that 50,000 IU per day of vitamin D (note that the proposed study dose is 50,000 IU per week) can increase vitamin D levels to more than 150 ng/dl and cause hypercalcemia (35). The current dose for this study is one capsule of 50,000 IU per week which equates to about 7000 IU per day. The dose at which the Vitamin D is being administered has minimal side effects, which may include gastrointestinal symptoms of constipation, stomach upset, and loss of appetite. Symptoms associated with vitamin D toxicity include bone pain, nausea, and vomiting (35, 120). We will be monitoring patient labs and for these symptoms at all study visits (baseline, 3, 6 months). Telephone interview (months 2, 4) will be used when patients are not seen for these assessments as with our pilot study (see procedures).

e. Safety. The vitamin D levels will be sent to the endocrinologist who will monitor the labs for the study, and inform the PI if the supplement needs to be stopped. This will be done when a study subject has either: (1) hypercalcemia or (2) vitamin D level of greater than 100 ng/ml with normal serum calcium or symptoms of vitamin D previously listed that may be indicative of possible toxicity (35). Patients will be requested to return for a serum level of vitamin D within one month (since the half life is approximately two weeks) of stopping therapy (120) and then again at the end of the study. The depression assessment will be reviewed with the psychiatrist prior to enrollment and subjects will be monitored for depression over the course of the study when they are seen (baseline, 3, 6 months) as well as when they are not seen (months 2, 4) by telephone. Subjects will be withdrawn if there is significant worsening of depression (see Human Subjects). These protocols were followed for the pilot study and no adverse effects occurred.

2. Sample.

a. Criteria for Participation. Inclusion criteria are: 1) women aged 21 and older, 2) having significantly elevated depressive symptoms as measured by a score $\geq$ to 16 using the Center for Epidemiologic Studies Depression Tool (CES-D) (118,121) and/or taking antidepressant medication and having a score $\geq$12 using the CES-D and/or CES-D score average of $\geq$16 of phone screen and face to face screen, 3) type 2 diabetes and under the care of a health care provider, (4) level of vitamin D also known as 25 hydroxyvitamin D, 25 (OH) D less than 32 ng/dl. **Exclusion criteria are:** 1) current alcohol or substance abuse disorders, 2) a history of bipolar depression or any other psychotic disorder, 3) debilitating chronic illness (e.g., cancer, multiple sclerosis), 4) severe complications of diabetes (e.g., blindness or amputation) since these will impact depression, 5) malabsorption disorders (e.g., crohn's disease, celiac sprue, gastric bypass) since this impacts vitamin D absorption (122), 6) elevated serum calcium since vitamin D may increase serum calcium (123). 7) taking St. John's Wort unless they stop for 3 weeks prior to enrollment since it can impact mood (124). 8) use of vitamin D supplements (1000 IUS or greater) in past 2 months and unwillingness to discontinue for at least 1 month prior to study 9) Pregnant or planning a pregnancy. 10) Baseline systolic blood pressure greater than 160 mmHG or diastolic greater than 100 mmHG. Having active treatment for depression (e.g., antidepressant therapy) will not be exclusion criteria if the patient has been under treatment for six weeks or more. Similar criteria were used for our pilot.

b. Recruitment. We contacted diabetes centers within a 20 mile radius of LUMC (most are within a 5 to 10 mile radius) with the closest facilities. The diabetes educators provided estimates of potential patients with type 2 diabetes per month. Support letters have been provided by the recruitment sites. To facilitate recruitment, a meeting will be held at each site (with educators and endocrinologists) to discuss the study, review study brochure, procedures for participating, and follow-up information. We used these sites and this recruitment strategy for our pilot and SWEEP study. We will also work with our marketing department for recruitment of study participants.

Diabetes Center Sites	# Patients/Mo	Diabetes Center Sites	# Patients/Mo
Loyola University Health Systems, Maywood IL (includes 10 satellites)	800	Elmhurst Hospital, Elmhust IL	240
MacNeal Hospital, Berwyn IL	85	Gottlieb Hospital, Melrose Park IL	15
Rush Oak Park, Oak Park IL	280	Edward Hospital, Naperville IL	350
LaGrange Medical, LaGrange IL	100	Lutheran General Hospital, Park Ridge, IL	85
DuPage Medical, Dupage County	800		
Total Patients per Month 2775			

In order to allow patients from Loyola to know about the study, we will have the endocrinologists (Camacho, Emanuele, Charnogusky, Mazhari, Michelfelder, Bading) send letters about the study to their patients. A waiver of HIPPA authorization will be used to identify patients from these physicians who meet the study criteria using CPT codes. We have contacted the diabetes nurses at these various locations and have provided them a copy of the approved patient letter that is being used for our mailing. However, we would also like to provide flyers at the Diabetes Centers and Primary Care Centers for patients to contact us. This is the

same flyer that was used in Sunshine 1 study. Additional recruitment is delineated in Appendix 1. (See Appendix 1 at end of protocol after references)

3. Procedures

a. Phone Screening of Participants. We will use the protocol established in our previous studies. Interested participants will be given a phone interview to describe the study. If interested, age, health information, and questions regarding diabetes and mental health will be asked to screen for potential eligibility. The CES-D is administered on all participants. Candidates who are not eligible to participate in the trial because of enrollment criteria but have elevated CESD scores will be asked to speak with their primary healthcare provider about their symptoms. We may also send these excluded candidates our resource list of local mental healthcare providers developed by Dr. Mumby. If participants meet eligibility criteria, they will be scheduled for a baseline visit.

b. Baseline Screening and Enrollment. As with our previous work, the baseline screening will occur within a week to reduce the potential for change in depression over time. Subjects will be able to come to the Loyola School of Nursing (SON) Research Room in Maywood, IL on weekday or Saturdays. Women will fast for 10 hours prior to their appointment (they will be allowed to drink water) and to bring their supplements with them. They will be informed that first baseline testing will last between 2 and 3 hours and subsequent visits will last between 1 to 2 hours. Upon arrival at the site, informed consent will be obtained and their blood will be drawn by a study nurse. The physical measures (i.e. weight and height) will then be done. Study subjects will then be provided breakfast, reminded to take their prescribed supplements, and instructed to complete the questionnaires. The researcher will review the questionnaires for missing data and assess if the items were skipped or not answered due to personal choice.

The diagnostic interview schedule (based on the DSM-IV) to assess current and past mental health will be administered by a trained study nurse (25 to 30 minutes). Exclusionary criteria such as bipolar and psychotic disorders and alcohol/substance abuse will be verified. Active suicidal ideation will also be assessed using a form developed by Dr. Mumby (attached). If a participant has active suicidal ideation, Dr. Halaris, psychiatrist and co-investigator and/or Dr. Patricia Mumby, psychologist and co-investigator, will be contacted and the safety protocol followed (See Human Subjects). These subjects will not be enrolled. The same procedures will be implemented at all follow-up visits**.** In addition, if the patient answers question #9 on the PhQ-9 depression tool with a response other than “Not at all”, the suicide assessment form will also be completed on these patients as well and similar procedures followed if active suicidal ideation.

At the baseline visit, both groups will receive a list of resources for mental health services developed by the psychiatrist (as with our previous study) as they will not be prevented from getting help if needed as a safety measure. In our previous depression study, this occurred infrequently (<3%), as 1 woman started an antidepressant and 1 woman restarted therapy.

Prior to leaving the data collection site, the participants will be asked to partake in a brief history and physical that will be conducted by a trained health care professional. This assessment will take 20 minutes.

c. Study Visits. Following the first visit, the lab tests (vitamin D, calcium, PTH, Cardiometabolic profile as recommended by the FDA) will be reviewed by Dr. Emanuele,endocrinologist and co-investigator to ensure that blood enrollment criteria are met. If so, the patient will be scheduled for a second visit within 10 days of their initial visit At that visit, the patient will have a body composition scan to assess for body composition (which includes bone, lean and fat mass) which should take 30 minutes. Prior to the scan, a pregnancy test will be administered to ensure the subject is not pregnant (see human subjects). Upon completion, a small conference room at that site will be used for the research nurse to provide the study supplement, review the weekly schedule, and discuss all subsequent contacts (phone and scheduled visits). The study nurse will also review potential side effects and reiterate the reproductive risks associated with the treatment at baseline visit 2 and 3 month visit when the supplement is provided. The patient will be told that an automated message (preferred as phone, e-mail or text) will remind them to take their weekly supplement. They will be informed that at three and six months, they will return for data collection of physical measurements and questionnaires. They will be told that in the months (2, 4,) when they don’t have a scheduled visit, the study nurse will conduct a formal phone call follow-up where they will be asked about their compliance, side effects, and be screened for their depressive symptoms (CES-D). These procedures will be followed for all phone call visits (Appendix).

Upon completion of these verbal and written instructions, the supplement will be dispensed. There will be a total of 12 pills for the period of 12 weeks. Following the dispensing directions, the nurse will ask the subject if they have any questions to ensure there is an understanding and to call the study nurse if questions arise.

The third visit (three months following initiation of treatment) will occur at the SON. The protocol established at baseline will be followed by the nurse (blood will be drawn, physical measures, questionnaires, and interview). Questionnaires that do not address mental health will be sent to participants prior to their visit with a reminder appointment letter. This is to limit the time spent onsite for data collection. It will not be mandatory that they bring them completed as they can complete them onsite if they so choose. The nurse will also request the study bottle and count the remaining pills (if any). If supplement is remaining, the nurse will record the reasons and the bottle will not be returned to the patient. All study questionnaires will be reviewed. Upon completion of these procedures, study supplement will be dispensed and the protocol for taking the supplement will be reviewed. To protect subjects, their depression level will be assessed and safety protocol implemented (if needed) using the same procedures described at baseline. The labs drawn at this time will be reviewed by the endocrinologist to inform the PI if patients need to stop their prescribed therapy (Study Design: Safety-1e). The fourth and final visit (six months following initiation of treatment) will occur at the SON. The patient will have the following assessed at end of study: vital signs, weight, walk test, neurosensory exam of feet, and assessment for any complaints. Questionnaires will be administered and reviewed as delineated in the protocol as well. The participant will be informed of optional interview at the six month visit. An optional informed consent will be signed at the time of the interview and participants will also complete the family questionnaires. The procedures previously described will be followed, although no supplement will be dispensed and patients will be informed to follow-up with the primary care provider regarding future supplementation. The patient will be sent their study lab tests (performed by Quest) and DEXA scan in the mail, informed that the dose of the medication will not be known until the end of the study, thus following up with their healthcare provider for future dosing of vitamin D is recommended. Letter to study participant is attached.

Patients will be called 3 months after completion of the Sunshine Study treatment intervention (which is 9 months from baseline) to see if they have followed up with their healthcare provider about the final test results mailed to them. They will be asked general questions about their physical well-being and the CES-D for depression symptom assessment will be conducted. (See attached, 9 month revised phone interview). Protocol for this phone screen will be congruent with procedures for baseline phone screen (see Protocol 3a)

Patients may be asked to provide clean urine sample using a clean catch midstream voided urine samples with 60 ml urine cups at study visits as well as complete several questionnaires on bladder and pelvic symptoms (see measurements).

d. Optional Biobanking. Participants may *optionally* agree to reposit up to six tablespoons of blood (i.e., approximately two tablespoons) and 8 tablespoons of urine at each at Baseline, Month 3, and Month 6) into CRO-BIOREP (LU# 204853) for future research purposes. Participants will sign a second informed consent document should they agree to reposit their blood and/or urine. The research team members and Biorepository staff will each retain a copy of the signed repository informed consent document. It is important to point out that participants do not need to agree to reposit blood and/or urine in order to participate in the trial.

All patients who agree to reposit blood specimens into CRO-BIOREP (LU# 204853) will be assigned a unique code using our SMART-ID system. This number will be used to connect coded specimens and coded data to other studies conducted by the Clinical Research Office in which they may participate. To receive this number, we will ask participants for sex at birth, month of birth, day of birth, year of birth, and social security number. This information is entered into a secure website where it is hashed into a unique ID using a java script. The participant and research team will receive the unique ID number. Once the number is created, the information that was entered is immediately deleted. This means the website will not retain any of the information that is entered to create the SMART-ID. However, should anyone need to retrieve a SMART-ID number, you will be able to get it again at a later date by going to the website and entering the same information (i.e., sex, date of birth, and social security number) that was entered before.

Freezerworks, the biospecimen tracking software used in the repository, will also automatically assign specimen numbers to incoming specimens. These numbers will identify all donated specimens. The list that links these codes with patients' identifying information will be kept separate from all research specimens and clinical data.

Information that may readily identify patients will never be shared with anyone.

d. Retention, Adherence, and Drop Outs. To enhance retention, participants will be scheduled for visits on a day that is convenient for them (oftentimes Saturdays). Free parking will be provided for visits. Honorarium for data collection points will be given in a stepped manner ($25-baseline 1, $25 baseline 2, $30-three months, $35-six months, $40 optional qualitative interview after 6 month visit) with a personalized thank you note. We have published on these strategies (114). To enhance adherence to treatment we will have automated weekly reminders to take supplements (preferred method: phone, e-mail, or text). Retention in our pilot study was 92% using these techniques. To assess for treatment adherence, we are obtaining blood vitamin D levels and requesting that patients bring their bottle (to count for the number of unused pills, if any) at both 3 and 6 months. For persons who drop out, we will assess the reason for drop out and the extent to which they require further treatment with referral to their provider as stated in informed consent document (Human Subjects).

4. Measurements (Appendix). Variables, measures, and times are delineated below. In our pilot study, respondent burden was not an issue. Measurements are in the Appendix.

	Variables	**Measures**	**Timing**
Aim 1	**Depression (Primary)**	Center for Epidemiologic Studies (CES-D), Patient Health Questionnaire (PhQ-9) Diagnostic Interview Schedule (DIS) Suicidal Assessment Form (if applicable)	0, 3, 6 months (Phone CES-D @ months 2, 4,and 9mos) Baseline 0, 3, 6 months
	Self- Management (Secondary) Self-Efficacy Diabetes Distress Self-Care Behaviors	 Diabetes Self-Efficacy Scale Problem Areas in Diabetes Diabetes Self-Care Inventory , Summary of Diabetes Self-Care Activities Appraisal of Diabetes Scale	 0, 3, 6 months 0, 3, 6 0, 3, 6
	Blood Pressure (Exploratory)	Blood Pressure (mm HG)	0, 3 , 6 months
Aim 2 Exploratory	**Inflammatory Biomarkers** Vascular Markers	e.g., CRP, IL-6, TNF alpha e.g., VEG-F and clotting factors	0,3, 6 months
Treatment	**Vitamin D, CA, PTH Levels** **Cardiometabolic Profile (CMP)**	Serum Vitamin D, CA, PTH Serum labs Pill counts	0, 3, 6 months Baseline 3, 6 months
Contributory **Obesity** **Diabetes** **Lifestyle Factors**	 **Bone, Lean and Fat Mass** **Body Mass Index** **Glycemic Control** **Diabetes History and Care** **Physical Activity** **Dietary Intake** **Sun Exposure** **Depression History**	 Body Composition Scan Height, Weight HBA1c, Fasting glucose Diabetes Care Profile Godin Leisure-Time Exercise NHS 4 –item Questionnaire Block Diet Inventory Sun Exposure Tool s Diagnostic Interview Schedule	 0 months 0, 3, 6 months 0, 3, 6 months 0, 3, 6 months 0, 3, 6 months 0, 3, 6 months 0, 3, 6 months
Other Factors	Sleep Quality of Life Diabetes Symptoms Anxiety Stress	Pittsburg Sleep Quality Index Ferrans Quality of Life Index Diabetes Symptom Scale State Trait Anxiety Cohen Stressor Scale	0, 3, 6 months

	Social support Sociodemographic Information Family Demographics Family Resources Family Quality of Life Qualitative Interview Urinary Microbiome Overactive Bladder Tool Pelvic Floor Distress Inventory Putonti Lab Protoco (Appendix 2) Physical Exam	MOS Social Support Sociodemographic Form Questionnaire FIRM Global Family Quality of Life Scale Interview Guide (Optional Consent) Clean Catch Urine Specimen Microbiome and Phage activity OAB-q PFDI ICIQ History and Physical Exam (UTI history)	 0 (baseline and visit 2), 3, 6 months, and time of the qualitative interview. After 6 month visit 0 (baseline and visit 2), 3 and 6 months 0, 6 months

a. Depression Outcomes. The CES-D and the Diagnostic Interview Schedule (DIS) will be used.
(1) The CES-D (20 items) is well accepted for depression screening. The Center for Epidemiologic Studies Depression Tool is 20 items and measures symptoms of depression and their severity (0=none to 3=most of the time). A cutoff score of $\geq$ 16 has been used to indicate depression. The tool has excellent internal reliability (Cronbach alpha from .85 to .90). It has also been reported that the CES-D and the Beck Depression Inventory performed comparably as depression screening tools (118, 121). In our SWEEP trial, the CES-D was reliable (Cronbach alpha from.87 to .92) and was validated by the DIS. We were able to detect changes in level of depression symptoms and whether patients changed from the depressed to the non-depressed category using the CES-D guidelines (59). The Patient Health Questionnaire 9 (PHQ-9) is used in clinical practice to assess for depression and will be used to compare to CES-D and DIS (Kroenke et al., 2001).
(2) The DIS will be used to screen and verify mental information. It is a structured mental health interview that uses the criteria specified in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) to generate diagnoses that can be used for research purposes (125, 126). It has been reported that results of the DIS correlate significantly with clinical diagnoses (127-128). It will be used to obtain a history of mental illness, depression characteristics (e.g., suicidality, # of previous depressive episodes, age of onset, and duration of episodes) and to verify the CES-D measure. (3) Suicidal Assessment form was developed by Dr. Mumby and will be used to assess for those patients who have positive ideation on the DIS and/or positive score on the PhQ-9 with item #9.

b. Self-Management Outcomes include Self-Efficacy, Diabetes Distress, and Self Care Behaviors.
(1) Diabetes Self-Efficacy Scale was developed by Lorig and based on previous chronic-disease self-efficacy scales (130). It consists of 8 items that assess an individual's confidence in caring for their diabetes on a likert scale from "1=not confident" to "10=totally confident". It has established reliability of .83 (131) and has been reported to be effective in capturing self-efficacy for persons in diabetes clinical trials (132, 133).
(2) Diabetes-Related Distress (PAIDS) is a 20-item tool that measures the current burden of diabetes and its treatment. A sample question includes the following: Feeling discouraged with your diabetes treatment plan? which is scored on a 5-point scale (0=not a problem to 4=serious problem). Higher scores indicate greater emotional distress. The PAIDS has high internal reliability (alpha= .95). Predictive as well as concurrent validity have been established by correlations with other measures (e.g., psychosocial factors, regimen adherence, and glycemic control) (134, 135).The responsiveness of the tool to detect changes in distress has also been established using data from seven prospective diabetes intervention studies (136).
(3) Self-Care Behaviors: (a) Self-Care Inventory was developed to assess patient's perceptions of the degree to which they adhere to treatment recommendations for diabetes self-care (1= never do it to 5= always do it). The tool has 16 items that addresses the areas of medication adherence, dietary regulation, exercise, and management of blood sugar (137, 138). The Self-Care Inventory unlike measures that only assess the frequency of self-care behaviors doesn't presume that all individuals have the same treatment prescription and thus is able to assess adherence to different treatment regimens (e.g., insulin or not taking insulin). Studies have used the tool which has demonstrated psychometrics of reliability (Cronbach alpha .87) and validity (correlations with diabetes distress, depression, and HBA1c) (137-139). (b) Summary of Diabetes Self-Care

Activities has 11 questions about self-care activities and 14 questions about recommended activities (Toobert et al. 2000) (c) Appraisal of Diabetes Scale (ADS) which is items to assess patient perceptions of diabetes (Carey et al., 1991). **c. Blood Pressure Outcome.** Blood pressure will be measured in a standardized fashion using calibrated equipment in accordance with JNC guidelines (140). The protocol and training developed for the ongoing international studies assessing blood pressure by Dr. Durazo, co-investigator will be used. Blood pressure will be measured using the Omron Automatic Digital Blood Pressure Monitor (model HEM-7471c, Omron Healthcare, Bannockburn, IL, USA). After at least 5 minutes of resting, with the antecubital fossa at heart level, 3 blood pressure readings will be taken 5 minutes apart and the mean of the last 2 used for analysis (141).

d. Inflammatory and Vascular Biomarkers. (1) C-Reactive Protein (high sensitivity) will be measured by either Dr. Halaris team or Quest Lab depending upon price agreement. **(2) Cytokines (e.g.,** TNF-α, IL-6 IL-1) will be measured by Dr. Halaris and his team in their lab (see Resources) using the **radioimmunoassay or with the** technique of Randox Technologies, "Evidence InvestigatorTM". This compact Biochip array system is comprised of a super-cooled Charge Coupled Device (CCD) camera and unique image processing software. We have already validated this Biochip technology for blood samples in preliminary studies. The results of the Biochips are comparable to results obtained by individual ELISAs for each of the individual parameters. Several cytokines, chemokines and growth factors can be analyzed simultaneously. (3) Vascular Factors (e.g., VEG-F) will be measured by Dr. Halaris using similar methodologies described above.

e. Treatment (1) Labs: Serum vitamin D, calcium and PTH will be measured for each time by **Quest Laboratories** which is accredited by the College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendments (CLIA) and has performed these assays for our pilot study at minimal cost. Quest will send the results either electronically or by fax with the patient ID number, initials, and date of birth. There will be no other identifiers on the labs. **Serum vitamin D** will be measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS) which is the standard for the measurement of vitamin D and its components. This method provides a total 25 hydroxyvitamin D (25 OH- D) which includes 25 (OH) D_2 and the 25 (OH) D_3. Thus, we will be able to determine the effectiveness of vitamin D_3 supplementation. **PTH Intact and calcium** will be measured by Quest. The PTH is measured via an immunoassay and Calcium is measured by spectrophotometry

Cardiometabolic Profile will be measured by Quest Laboratories to assess normal lab function prior to enrollment as recommended by the FDA. This test includes the assessment of: Albumin, Albumin/Globulin Ratio (calculated), Alkaline Phosphatase, ALT, AST, BUN/Creatinine Ratio (calculated), Calcium, Carbon Dioxide, Chloride, Creatinine with GFR Estimated, Globulin (calculated), Glucose, Potassium, Sodium, Total Bilirubin, Total Protein, Urea Nitrogen. **(2) Vitamin D Pill counts**: At 3 and 6 month visits, supplement bottle will be checked for number of unused pills and reason for not taking.

f. Contributory Factors include obesity, diabetes, and lifestyle factors.
(1) Obesity (a) Body composition (which includes bone, lean and fat mass) will be measured using dual-emission x-ray absorptiometry (DXA) with a whole body scanner (Discovery W by Hologic) provided by Dr. Durazo (co-investigator) who has an international study on vitamin D. The protocol for that study will be used: (a) an intrinsic CV will be determined by the DXA machine by the scanning of a phantom and (b) all DXA operators will be required to complete an intra-observer CV. The method for assessing body composition using DXA has been reported to be precise, accessible, and requires short scanning (142-144). The percent body fat measured by DXA has been used in examining vitamin D and its relationship to weight in postmenopausal women (145-146). **(b) Body Mass Index** will be calculated from weight using an electronic calibrated scale and height using a stadiometer.
(2) Diabetes: (a) Glycemic Control will be measured by hemoglobin A1C using the DCA 2000 (Miles Diagnostic Division). The within CV is 2.1 to 4.5% and the between CV is 0.8 to 4.4%. Fasting blood sugar will also be measured by Quest Laboratory from the Cardiometabolic Profile previously listed. **(b) Diabetes History and Care** will be measured using the Diabetes Care Profile from the Michigan Diabetes Research and Training Center. This has information on demographics and medical information such as current treatment, comorbidities, all medications (e.g., diabetes, depression), and previous diabetes education. Development, validity, and reliability of the Diabetes Care Profile have been reported (147). Demographics related to family composition (e.g., children) will be measured as well.

(3) Lifestyle: (a) Physical Activity: Short physical activity scale called the Godin Leisure-Time exercise Questionnaire which is 2 questions and assesses level of activity and if it is regular enough to work up a sweat (http://www.godin.fsi.ulaval.ca/Fichiers/Quest/Godin%20leisure-time.pdf). In addition, 4 items from the Nurses Health Study that asks the number of minutes per week for recreational outdoor activities, indoor activities (e.g., standing, sitting), usual walking pace, and number of flights of stairs per day.

(b) Food Dietary Intake (which includes vitamin D and calcium will be assessed by the Block Questionnaire(s). These have been developed using a data-based approach from the National Health and Nutrition Examination Survey (N-HANES) to create the selection of the foods, appropriate portion sizes and nutrient composition selected. The questionnaire for this study has about 110 food items to estimate usual and customary intake of a wide array of nutrients and food groups. The food list and nutrient database for its analysis were developed from the Block 1998 Food Frequency Questionnaire. The food list was developed from (NHANES) 1998 to 2002 dietary recall data and the nutrient database was developed from the USDA Food and Nutrient Database for Dietary Studies. Food is quantified in terms of portion size and pictures are provided to enhance accuracy (151, 152). The 110 item will be administered at second baseline visit and 6 months and the shorter version which assesses vitamin D and calcium only will be measured at baseline to capture this information for all enrolled subjects.

(c) Sun Exposure: Participants will be enrolled in the fall or early spring as these are the times that we have identified depresses subjects in our previous studies. To assess for sun exposure two methods will be used: amount of sun exposure and seasonality. (1) Amount of sun exposure has been developed using a method develop by Barger-Lux and Heaney (153) where sun exposure is assessed by the amount of time in the sun and usual attire worn. A quantifiable sun exposure is generated. (2) Season of study enrollment will also be recorded as a measure of sun exposure as done by other studies (116). (3) Cancer Sun Exposure Tool which is 14 items to screen for sun exposure (Glanz et al., 2008).

(d) Other Factors: Factors that contribute to the overall health of persons with diabetes will also be assessed and include sleep using the validated Pittsburg Sleep Index (Buyssee et al., 1998), perceived quality of life using Ferrans Quality of Life Index (Ferrans, 2013), and diabetes symptoms developed by Grootenhuis (1994), and state-trait anxiety developed by Speilberger (1983), the Perceived Stressor Scale developed by Cohen (1983) and the MOS Social Support tool developed by Sherbourne and Stewart (1991). Addition of assessment for sarcopenia, muscle function, and falls has been added as Appendix 2 with scientific justification.

(e). Family Quality of Life Outcomes: (1) Family Inventory of Resources for Management (FIRM). Used to assess internal family and social support services. Reliability (α= 0.87-0.92) has been strong in families with chronic illness (Ridosh, Sawin & Brei, 2013; Sawin, Brei, Buran, & Fastenau 2003). (2) FQOL is the "overall appraisal of the domains of life important to the family" measured with a 3-item scale, The G-FQOL Scale (parent perception of their off-spring QOL, their own QOL and their FQOL) reported on a scale from 0 (poor) to 100 (excellent). The G-FQOL Scale has strong preliminary reliability (α = 0.86 – 0.90) in a study of women of children with chronic condition (Ridosh, Sawin, Brei & Schiffman, 2015). (3) Qualitative Interview Questions developed by Ridosh to assess how patients manage their health after the study and how the family may impact their ability to manage their health (i.e., diabetes and/or mood) will be conducted after the 6 month study visit. An optional consent to participate will be obtained. The consent indicates that the audiotape will be transcribed word for word into a written document (transcript). The audiotape and transcription will be destroyed when the study is complete.

(f) **Urinary Microbiome and Self-Report Measures**. (1) Assessment of the urinary microbiome will occur in the laboratory of Dr. Alan Wolfe at LUMC. Three tests will be conducted on the urine: the *(i)* general screen of bacteria, *(ii)* 16S rDNA sequencing, and *(iii)* both EQUC and standard urine cultures. Each sample will be labeled with the participant's identification number from the parent grant and refrigerated. Samples not used will be biobanked (repository consent approved). (2) Assessment of urinary symptoms will include the use of the use of the (a) Overactive Bladder Questionnaire (QAB-q) which has been used in women with diabetes

(Palleschi et al., 2014) and .(b) Pelvic Floor Distress Inventory (Barber et al., 2001; 2011) which asks about urinary symptoms and is able to classify individuals into categories of urinary continence. (c) Urinary Incontinence Questionaire to determine impact of this variable on health and quality of life (International Consultation on Incontinence Modular Questionnaire; ICIQ- http://www.iciq.net/structure.html) . (d) Urinary tract history completed by patient or administered during nurse administered physical exam. (2) Assessment of the urine for inflammatory markers in the laboratory of Dr. Iwashima Makio at LUMC. This will be done by biochip for cytokine analysis.
Putonti Lab protocol (e-mail 4-12-19_Specimens transported using FEDEx through Wolfe Lab protocal. Urines stored -80.C and padlocked. Working protocal to isoloate and congreate phages from urines in Appendix 2 of protocol.

5. Data Management and Analysis
a. Data Management: The goals of data management are to: (a) ensure that data collected during the study are properly and accurately entered and documented; (b) ensure that data will be stored in an electronic format that will allow the primary investigators of the project to retrieve data easily and to export data to statistical packages; and (c) ensure the confidentiality of subjects. After the data are entered and checked, several data quality checks will also be conducted, including descriptive statistics and graphic plots to detect outliers and influential observations. All computer data will be stored in a password-protected network server with back-up. The original raw data will be stored in a locked cabinet accessed only by key project personnel. We will run univariate and bivariate analyses for all variables prior to our main analyses. We will evaluate continuous variables using t-tests or F-tests and categorical variables using χ^2. We will examine data distributions and test all variables for linear relationships or non-linear relationships. Descriptive statistics (means, standard deviation, frequency) and correlations (both Pearson and Spearman) between variables will be conducted to preliminarily assess the data. Necessary transformations and imputations will be conducted on based the raw data.

b. Data Analysis: The conceptual relations in the model for the study (Figure) are being used to guide the testing of the primary, secondary, and exploratory outcomes.

SUNSHINE PRIMARY STUDY:
Hypothesis 1: Women receiving 50,000 IUs vitamin D supplementation will report fewer depressive symptoms (primary outcome), increased diabetes self-management mediated by depression improvement (secondary outcome), and have a lower systolic BP (exploratory) compared to those 5000 IUs at three and six months follow-up.

For analysis of the primary outcome (depressive symptoms) generalized, linear, mixed models (GLMM) for repeated measures will be used (154). The proposed model will have one between-subjects variable GROUP (vitamin D supplementation; control) with two levels and one within-subjects variable TIME with three levels (baseline, 3 and 6 months**).** The test of the hypotheses will hinge on the GROUP by TIME interaction as an indication of group differences in the variable of interest over time. A given hypothesis will be supported with the finding of a statistically significant GROUP by TIME interaction, and the finding that the means are in the hypothesized direction. GLMM will be implemented using SPSS which will provide estimates of the means for each outcome variable by time and treatment groups. The results will be summarized with adjusted means and 95% confidence intervals. A compound symmetric covariance structure will be assumed. If necessary, other covariance structures, such as Huynh-Feldt, will be investigated. An intention-to-treat analysis (ITT) which includes an analysis of completers and noncompleters will also be done using GLMM. Stratification by depression severity should provide comparable groups at baseline; however, differences will be assessed. All analyses will control for baseline values of body composition (body fat), race (white compared with nonwhite), and time at entry into the study (as season of the year, winter, summer, spring, fall) as previously done by our consultant, Dr. Pittas, in his vitamin D trials. Other covariates, related to diabetes (length of time with diabetes, HBA1c), lifestyle factors, (physical activity, diet), and previous depression will be added to generate a multivariate model which is parsimonious and based on the clinical relevance and statistical significance of these factors.

For analysis of the secondary outcome (self-management), regression analysis will be used to test whether an improvement in self-management is mediated by an improvement in depressive symptoms (Figure). The Preacher and Hayes macro add-on for SPSS will be used. This technique estimates the path coefficients and

generates bootstrap confidence intervals for total and specific indirect effects of the independent variable (vitamin D supplementation) on the dependent variable (diabetes self-management) through one or more mediator variables (depressive symptoms). This method allows for testing indirect effects through a mediating variable without the estimation of a direct link between and independent variable on an outcome, and also adjusts for the potential influence of covariates (155, 156).

For the exploratory outcome (blood pressure), GLMM will be used to examine for the direct effect of vitamin D on blood pressure since there is physiologic evidence to support this. The GLMM statistical procedures described above will be used.

Hypothesis 2: Women receiving 50,000 IUs vitamin D supplementation will have a decrease in inflammatory biomarkers which will be associated with fewer depressive symptoms compared to 5000 IUs at three and six months follow-up.

For this exploratory hypothesis, to determine if there is a decrease in the inflammatory markers, the same procedures (GLLM) will be used. If significant, then correlational methods (parametric and non-parametric) will be used to explore the association between vitamin D supplementation, depression, and the inflammatory bio markers.

1.For the sub-study FAMDD, the qualitative component **(Aim 1)** interview data is being transcribed. Following transcription, there will be verification of the transcription by Dr. Ridosh. Once these data have been verified, a narrative analysis will be conducted. Narrative inquiry was chosen to focus on the relationship between individuals' life stories and it guides synthesis of data to represent the participants' stories as a whole. Particular modifiable factors that mitigate health risks for family members and factors that facilitate self-management will be identified. Study team involved in this component of qualitative analysis includes Dr. Penckofer, Dr. Ridosh, Dr. Roux and Ms. Meghan Meehan.
For the analysis of the quantitative measures, descriptive statistics will be used to generate information that will allow for exploring the relationship of these measures to the other study variables being collected (e.g., severity of depression and diabetes) **(Aim 2)**. The descriptive data will also allow comparison of these data to other studies that have used these tools. Reliability will be established for each tool at each time point using Cronbach alpha. The current analysis will test *The Global Family Quality of Life Scale-Adult Women Version* and detail its psychometric properties, specifically construct validity and reliability. Descriptive statistics will include mean age, gender, level of education, race and ethnicity. Data from total sample will be analyzed. Regression analysis will be used to address **Aims 2 and 3**. Mediational analyses will be used as appropriate

2. For the sub-study on the Urinary Microbiome, the following analysis was proposed.

Primary Objectives

(1) To determine the FUM composition in women with T2DM using high throughput 16S rRNA gene sequencing & enhanced quantitative urine culture (EQUC), and to determine the FUM's association with LUTS in women with T2DM.

Hypothesis: Among women with T2DM, their FUM composition is a salient predictor of LUTS.

Planned Analyses: To test this hypothesis, LUTS is the response variable and FUM is the primary explanatory variable. FUM will be measured in at least two ways: First as a microbial diversity index (e.g., Shannon, Chao 1) and as an individual microbial taxa count. When diversity indices are used, a log transformation may be applied to adjust for heterogeneity or lack of linearity.

In this repeated measures study (baseline, 3, and 6 months), a linear mixed effects model will be used to estimate the effect of FUM on patients' LUTS severity score while allowing random intercepts for each participant to account for the within subject correlation engendered by the repeated measurements. Since we are documenting UTIs over the trial, we will also explore the odds of experiencing at least one UTI, to be estimated as a function of the FUM using a generalized linear mixed effects model that also allows random intercepts for each participant; a binomial distribution with logit link will be specified to determine the odds ratio for the FUM diversity index and microbial taxa count. In these models, extreme error variances will be monitored using the ratio of the Pearson chi-square fit statistic over its degrees of freedom. When necessary, UTI may be treated as a count variable

(particularly if there are enough participants experiencing multiple UTI events) and a Poisson distribution with negative binomial link may be used to adjust for observed overdispersion.

(**2)** To determine the impact of glycemic control on the LUTS and FUM composition in women with T2DM.

Hypothesis: In women with T2DM, the effect of FUM on LUTS *depends* on glycemic control (HBA1C), which may be measured as a continuous or binary (i.e., >7% versus <= 7%) variable.

Planned Analyses: Testing this hypothesis is largely a continuation of the investigation specified in prior objective, except that each model will evaluate if any effect of the FUM on LUTS severity *depends* on women's level of glycemic control. Consequently, for this aim, an interaction term between FUM and glycemic control will be estimated for each model specified above. If the interaction is not significant, adjusted estimates will be provided for the effect of FUM on LUTS after controlling for the effect of glycemic control, as well as other salient covariates (e.g., race, age, depression level, body mass index, and concomitant medications).

For both hypotheses, all comparisons, including all possible pairwise interaction terms, will be evaluated using an alpha level of 0.05 and the tests will be two tailed, meaning that an effect in either direction will be interpreted.

Secondary Objective:

To determine whether there is an association between vitamin D status (sufficient, insufficient, and deficient), LUTS, and the FUM.

Hypothesis (a): Women with T2DM who have less vitamin D will have will have a different FUM composition than those with sufficient vitamin D.

Hypothesis (b): Women with T2DM who have less vitamin D will have will have more LUTS than those with sufficient vitamin D.

Planned Analyses: For this secondary objective, a generalized linear mixed effects model will be used to determine the odds of at least one UTI as a function of ordinal vitamin D status (deficient, insufficient, and sufficient), again allowing random intercepts for each participant to account for within subject correlation due to repeated measurements; these models will specify a binomial distribution with logit link to determine the odds ratio for each pairwise comparison of vitamin D status. As before, extreme error variances will be monitored using the ratio of each model's Pearson chi-square fit statistic over its degrees of freedom. When necessary, a negative binomial link may be used to account for observed over-dispersion. Finally, a linear mixed effects model will be used to estimate the effect of ordinal vitamin D status on patients' FUM indices and microbial taxa count as well as their LUTS symptom severity score, again allowing random intercepts for each participant. For this objective, all model terms will be evaluated using an alpha level of 0.05 and the tests will be two tailed, meaning that an effect in either direction will be interpreted.

To compare vitamin D, LUTS and the FUM, each individual will function as her own control, with the first time point establishing the baseline FUM profile with which to compare the effect of treatment dose at 6 months (endpoint of trial). We will model the outcome using a GLMM, which takes into account the correlation between 2 observations on the same woman. We will determine if there is a change in a women's FUM baseline to end of study by treatment. We will use similar procedures to determine treatment group differences. Similar data analyses will be conducted on the urinary symptoms reported by each participant across time points and between groups.

Exploratory Aim: To examine the relationship of the phage activity with glycemic control, other labs including Urine PH in biobanked urine samples in the laboratory of Catherine Putonti.

Other Exploratory Analysis: We will be able to make comparisons of the FUM with persons who do not have diabetes. Drs. Wolfe and Brubaker have sequence and culture data from healthy and incontinent (UUI) women from their prior cohort studies (see **Fig. 2**) (Hilt et al., 2014; Pearce et al., 2014). In addition, 180 well-characterized UITN subjects are adult women with stress urinary incontinence (SUI) that have had clean catch midstream voided urine sequenced (see **Fig. 1**) (Thomas-White et al., 2016b). Thus, comparisons with these

data offer a unique opportunity to determine the impact of having the comorbid condition of diabetes on urinary outcomes to those who do not have diabetes.

c. Missing Data. Item missing data (e.g., a subject fails to answer a particular questionnaire item), will be imputed using procedures such as EM algorithm. For unit missing data (where a subject is missing at a data collection time point), we will determine whether missing data are MCAR (data are missing completely at random), MAR (data are missing at random), or NMAR (not missing at random). If missing data are MCAR or MAR, it is likely that the standard multivariate computations will not result in biased standard error estimates. However, if missing data are NMAR, we will use the "pattern mixture" approach to compute a "weighted average" of the parameters that are associated with the missing data to estimate what the data would have been (157).

d. Sample Size Estimation. Based on our power estimates, a sample of 75 women per group will be needed for a final sample of 150 women. We have planned for a conservative attrition (15%) which is higher than our Sunshine pilot (8%) but consistent with our SWEEP trial (59). **Therefore, 180 (90 per group) will be enrolled into the treatment arms.** Thus far, out of 169 patients enrolled, only 83 (49%) have been randomized to treatment arms. Thus, we will enroll 360 as we have had a screen failure of almost 50% to allow for a sufficient number of persons to be randomized. For the power analysis, we used a repeated measures approach formulae developed by Hedeker et al. (158) which allows comparisons of the group means across time while adjusting for baseline values and accounting for the correlation between baseline and follow-up observations. We have assumed a 2 tailed test with a type I error rate of 0.05 for each outcome. **Depression:** There was a 14 point decrease in depression from baseline to 6 months following vitamin D supplementation using the CES-D (see pilot data). Using the control group in the SWEEP study (which had the same enrollment criteria for depression and did not receive treatment), there was a 7 point decrease in depression (28.8 to 21.4) as depression symptoms can improve without treatment. Given these group difference, the effect size is .46. A sample size of 30 per group would provide 95% power**.** We also powered for depression remission (CED-D < 16) as other depression trials (159). In our vitamin D study, 55% of women had remission of their depression at 3 months and 63% at 6 months**.** Using the control group data from the SWEEP study, 30% had remission of depression at 3 months and 20% at 6 months. Having this information, a sample size of 75 per group (n=150) would be needed to detect differences in depression remission at 3 months and a sample of 25 per group (n=50) to see the difference at 6 months. **Diabetes Self-Management:** There were significant differences from baseline to six months (see pilot data) to calculate the effect sizes. We calculated an effect size of .70 for diabetes distress and .33 for self-care. For diabetes self-efficacy, an effect size of .30 based on our previous research (SWEEP) will be used. A sample of 75 per group (n=150) will achieve 85% power on these study outcomes. **Blood Pressure:** We observed a decrease of 8 mmHg in the vitamin D study but no change in a control group from the SWEEP study (136.2 mmHg to 135.9 mmHg). Pfeifer et al. (96) reported a 7% decrease in blood pressure in women after 800 IUs of D_3 for 6 weeks. To be conservative, we will assume a difference of about 5 mmHg to account for the placebo effect and regression to the mean phenomena commonly observed in clinical trials. This difference yields an effect size of about 0.3. A final sample of 150 (75 per group) would provide 80% power to assess this outcome. **Inflammation (Exploratory):** Using pilot data of Dr. Halaris (see pilot data) a sample size of 75 per group provides 85% power to detect differences between groups on depression and inflammation. **For the meditational analyses**, power was estimated using effect sizes from our previous research and current pilot study. Although the depression effect sizes discussed above are large (160), a more conservative approach anticipating medium effect sizes was employed for the mediation analysis. With medium effects for self-care outcomes, a total sample of 135 would achieve 95% power.

Sample size estimation:
For the sub-study (FAMDD), the qualitative component of the study, the sample size proposed was 50 women. For the quantitative component, it is estimated that since these tools have been used previously, that the estimate needed for adequate power will be determined by power analysis. An a-priori sample size by these investigators for multiple regression indicates with a medium effect size, p = .05, power of .80 and up to 6 independent variables a minimum sample of 97 will be needed for quantitative analysis.

6. Potential Problems and Alternative Solutions. Although subject accrual is a concern for all research, we have been successful in our previous studies. We have established relationships with key clinical sites (see diabetes educators, physicians support letters) which have reported a high volume of accessible patients (see

2b, recruitment table). Although attrition is another potential issue, it has been low in our previous studies (6% pilot vitamin D, 15% SWEEP). And, we have provided steps for addressing adherence, retention, and drop-outs (see 3d). To prevent measurement issues, protocols for training of staff to ensure consistency with the collection of data have been established in our prior work. It is possible that factors like diet and sunshine may impact on vitamin D levels. Although the impact of these variables in our pilot work was not substantial, they will be measured. Because body fat may affect vitamin D levels, body composition has been added as measurement. Treatments will be assessed at all time points and evaluated for their contribution to the study outcomes. In our prior work, we did not seen significant changes in medications as women were on established medications prior to enrollment. The placebo effect may have been a factor for the positive findings in our one-arm pilot study; hence a randomized trial is the next logical step. Study staff will be blinded to the treatment and we have protocols if the blind needs to be broken (see 1c-e). Staff will not provide additional support to the patient for treatment; however, protocols for increased depression have been delineated (3b). Finally, it is possible that if depression improves, self-management may not. Therefore, the next planned study would be a randomized trial to see if treating depression with vitamin D prior to diabetes self-management education could enhance learning and self-management outcomes.

7. Timeline. We have proposed a timeline of four years with the enrollment of 180 in the treatment arms and follow-up of all women completed by Year 4. For year 1, time is committed for start up, enrollment and start of intervention. We plan to enroll 50 subjects into the treatment arms in year 1. For years 2 and 3 we will plan to enroll 65 each year into the treatment arms. We will complete intervention delivery by the third month of year 4 with follow-ups complete by the sixth month of that year. Final data analysis and reports will be completed by the end of Year 4.

VI. PROTECTION OF HUMAN SUBJECTS.

A. Risks to the Subjects

(1) Human Subjects Involvement and Characteristics. We will enroll a total of 360women to allow for attrition and screen failure over the course of the study (our pilot study had an attrition of 8%). Women 21 and older with a depression score of CES-D $\geq$ 16 and/or taking antidepressant medication with a CES-D depression score of $\geq$12 and/or CES-D score average of $\geq$16 of phone screen and face to face screen,, and type 2 diabetes who are under the care of a health care provider will be recruited. They must also have a vitamin D level of less than 32 ng/ml verified by laboratory testing.

Participants will be excluded if they have: 1) current alcohol or substance abuse disorders, 2) a history of bipolar depression or any other psychotic disorder, 3) debilitating chronic illness (e.g., cancer, multiple sclerosis), 4) severe complications of diabetes (e.g., blindness or amputation) since these will impact depression, 5) malabsorption disorders (e.g., crohn's disease, celiac sprue, gastric bypass) since this impacts vitamin D absorption (122), 6) Elevated serum calcium since vitamin D may increase serum calcium (123). 7) Taking St. John's Wort and not willing to stop for 3 weeks prior to enrollment) (124), 8) Use of vitamin D supplements in past 2 months and unwillingness to discontinue for 1 month prior to study 9) Pregnant or planning a pregnancy. 10) Baseline systolic blood pressure greater than 160 mmHG or diastolic greater than 100 mmHG. Having active treatment for depression (e.g., antidepressant therapy) will not be exclusion criteria if the patient has been under treatment for six weeks or more. Similar criteria were used for our pilot.

These exclusion criteria will be assessed over the phone verified at the baseline enrollment visit. In addition at baseline enrollment, the participant's depression will be verified using the diagnostic interview schedule. If during that interview the patient screens positive for self-harm, they will be excluded from participation (see Protection Against Risks, B2).

All eligible subjects will be randomized into either the treatment group (vitamin D 50,000 IUs) or the comparison group (5000 IUs). They will be requested to make 4 study visits for data collection over a period of 6 months (baseline data, prescription pick up 10 days later, 3 month data collection and 6 month data collection). Women will also be called at months 2, 4 and 9 months (as noted under 3.c) to assess for any issues related to the study supplement as well as to assess for depressive symptoms.

(2) Sources of Data. We will obtain questionnaire and interview data from all study subjects at baseline, 2, 3 6 and 9 months. Other data collected will consist of the following: a) a phlebotomy draw for blood specimens (one tube of 5 ml for Quest labs and one tube of 5 ml for immune markers) will be drawn from the participant at baseline, 3 and 6 months; and b) body measurements from the participant's physical exam (BP, height, weight) at baseline, 3 and 6 months; c) Body composition scan using dual-x-ray absorptiometry (DXA) scans at baseline, d) Urinary specimens (8 tablespoons) at study visits. All medical data will be obtained from the subject. Access to medical records is not being requested.

(3) Potential Risks. The potential risk to the research participants includes hemotoma at the site of the venipuncture when the blood is drawn at baseline, 3 and 6 months. Blood will be drawn by the staff that has been trained to do this, so risk of bleeding and hematoma will be minimized.

DXA will be used to measure body composition (which includes bone, lean, and fat mass). Prior to DXA, female participants will undergo a pregnancy test. Pregnant women will be excluded from the study due to the radiation. The radiation exposure with a DXA scan is about 12microSieverts, which is significantly less than the radiation absorbed by a passenger on a roundtrip transcontinental flight (about 60 microSieverts). The scan will take approximately 10 minutes to perform.

There is the potential that patients may become depressed over the course of the study. If patients demonstrate significant increases in the depression scores (i.e., an increase of 30% or more), either Drs. Halaris or Mumby will be consulted, and a determination will be made regarding contact with the primary care provider and a referral to a mental health care provider (HIPPA signed, see protection again risks). This will be done for those in treatment and control group at all points of contact (phone or face to face). If during the course of the study patients begin any medication for mood (e.g., depression or anxiety), this information will be recorded. If the patient begins therapy during the course of the study, the therapist will be contacted to ensure that the study does not conflict with the patient's current treatment protocol.

Hypercalcemia is a potential side effect of persons taking higher doses of vitamin D and this lab will be monitored to ensure that it is not present at time of enrollment and does not increase over the course of the study by the endocrinologist. Most recent evidence demonstrates that hypercalcemia may not be a significant side effect associated with vitamin D (49). Vitamin D levels will be measured at baseline to ensure that the level is less than 32 ng/ml. It will also be monitored over the course of the study to ensure that the levels do not increase or that there are symptoms of toxicity. Safety procedures are listed (see protection against risks).

To decrease the possibility of hypoglycemia from fasting, patients will be asked to make sure that they eat a protein before bedtime and bring their morning diabetes medications to take with the breakfast that is provided. For persons taking insulin, they will be informed that if they take insulin in the evening, to contact their health care provider about the dose that they should take when fasting for morning blood draws. They will also be informed to bring their morning medications to their data collection visits to take with their breakfast.

B. Adequacy of Protection Against Risks.

(1) Recruitment. We will actively recruit women from the LUMC endocrinology and general medicine clinics as well as diabetes centers in the surrounding communities as delineated in the recruitment section of the grant. We have received support letters for access to these sites (see letters of support). We have been very successful in our previous work. Institutional Review Board approved flyers will be available at these sites so that nurses may distribute the information to prospective study participants. Local newspapers to inform prospective participants about the research study will also be utilized. We will recruit racial/ethnic groups consistent with previous work and participating institutions (white-60%, nonwhite-40%).

(2) Protection Against Risks.
As state previously, for protection against the risk of bleeding and hemotoma, all phlebotomy staff will have been trained. For the DXA scan, the personnel will have been trained (per the NIH study of Dr. Durazo, co-investigator) on the use of the machine and procedures regarding radiation. The consent states that in the event that the

study participant demonstrates or verbalizes significant worsening of depression or suicidal ideation during the study, their health care provider will be informed. Written permission to contact the health care provider (HIPPA form) will be obtained at time of enrollment.

At baseline enrollment, the computerized diagnostic interview schedule will be administered to verify depression. During that interview, the patient is asked about self-harm. If active suicidal ideation is reported, the suicidal assessment form is completed. In addition, if the patient answers question #9 on the PhQ-9 depression tool with a response other than "Not at all", the suicidal assessment form will also be completed and similar procedures followed if active suicidal ideation.

If during this time the nurse identifies any patient as having suicidal ideation and being at moderate to high risk for self-harm that requires urgent evaluation and intervention, s/he will page Drs. Halaris or Mumby. The women will be evaluated by either Drs. Halaris, Mumby or psychiatry resident, who will confirm the treatment plan with the psychiatry attending on service that day. If the patient has active suicidal ideation and is deemed to be in need of immediate psychiatric care, a member of the research team will escort the patient to the emergency room. She will be seen by the psychiatry resident on call and reported to the psychiatry attending to confirm a treatment plan. Drs. Halaris or Mumby will follow-up with the delineated treatment plan as this will be important for communication with the patient's health care provider as well as for the integrity of the study. These individuals will not be eligible for participation. At baseline enrollment, both the treatment and control will receive a list of resources for mental health services developed by the clinical psychologist (as with our previous study), and will not be prevented from getting help if needed. In our previous depression study this occurred infrequently (<3%) over the duration of the study (i.e., 1 woman started an antidepressant and 1 woman restarted therapy as she has seen a therapist in the past).

During the course of the study, Dr. Halaris, psychiatrist and co-investigator or Dr. Mumby, psychologist and co-investigator, will work with the research staff and consult as necessary. During any time where the nurse identifies any patient as being at moderate to high risk for self harm or with significant worsening of depression (a 30% increase in the CES-D), Drs. Halaris or Mumby will be contacted and procedures previously described followed. Subjects will be withdrawn if there is significant worsening of depression per Drs. Halaris or Mumby in consultation with their primary care provider. Similarly, for persons who drop out, we will assess the reason for drop out and the extent to which they require further treatment in consultation with Dr. Halaris or Mumby and their primary care provider. These protocols were followed for the pilot study and there were no adverse events.

In terms of the treatment intervention, those assigned the comparison group of 5000 IUs placebo will have a dose that approximates that recommended by the IOM, to ensure that they have adequate intake which is consistent with other vitamin D trials. For those who receive the treatment dose, published reports suggest that 50,000 IU per day of vitamin D (note that the proposed study dose is 50,000 IU per week) can increase vitamin D levels to more than 150 ng/dl and cause hypercalcemia (35). The current dose for this study is one capsule of 50,000 IU per week which equates to about 7000 IU per day.

The dose at which the Vitamin D is being administered has minimal side effects, which may include gastrointestinal symptoms of constipation, stomach upset, and loss of appetite. Symptoms associated with vitamin D toxicity include bone pain, nausea, and vomiting (35, 120). We will be monitoring patient labs and for these symptoms at all study visits (baseline, 3, 6 months). Telephone interview (months 2, 4) will be used when patients are not seen for these assessments as with our pilot study (see procedures).

The vitamin D levels will be sent to the endocrinologist who will monitor the labs for the study, and inform the PI if the supplement needs to be stopped. This will be done when a study subject has either: (1) hypercalcemia or (2) vitamin D level of greater than 100 ng/ml with normal serum calcium or symptoms of vitamin D previously listed that may be indicative of possible toxicity (35). Patients will be requested to return for a serum level of vitamin D within one month (since the half life is approximately two weeks) of stopping therapy (120) and then again at the end of the study.

Patients will be allowed to continue their preferred calcium intake but will be discouraged from taking more than 1200 mg total per day (to be consistent with the IOM guidelines, 97) as per the recommendation of our consultant, Dr. Pittas (see biosketch) who is an expert in vitamin D and calcium intake This is consistent with our pilot study.

Protection against potential for hypoglycemia at data collection will be taken as previously described. It is also possible that as women become less depressed, they may engage in better self-care behaviors and improve their glycemic control, with potential for hypoglycemia.

Because the urine is being examined for bacteria in a research laboratory, we will not be able to provide the test results to the patient. However, if they have symptoms of urinary tract infection, they will be advised to speak to their healthcare provider.

C. Data Safety Monitoring Plan.

A monitoring plan has been established to ensure that the data remain confidential and that the participants are not experiencing any harm from the intervention, or experiencing significant increases in depression. A committee to ensure that the plan is followed will be formed. The committee will meet twice a year to assess the activities of the study and any concerns or problems that may have occurred. Members of the data safety monitoring board will include individuals who have demonstrated expertise in areas related to the project and have agreed to serve in this capacity. Patrick Lustman, PhD, is a Professor and psychologist in the School of Medicine at Washington University in St. Louis, MO. He has extensive experience in the assessment of depression in obese individuals as well as those with type 2 diabetes. He has conducted numerous clinical trials using psychotherapy as well as medication therapy for these individuals, and has published extensively in this area. He also has more than 20 years of NIH funding to support his work. Dr. Michael Holick, PhD, MD is a Professor of Medicine, Physiology, and Biophysics at Boston University School of Medicine. Dr. Holick is known nationally for his program of research in vitamin D. He has NIH funded studies examining vitamin D and its effect in different populations. He is an expert on vitamin D dosing, regimen, and serum measurement. He is nationally and internationally known for his work on vitamin D. Dr. Grazia Allepo, Endocrinologist at Northwestern Hospital with expertise in diabetes will be a member. Finally, Dr. Patsy Marie Brannon Professor of Nutritional Sciences, Cornell University & Director of the Cornell Dietetic Internship Research who has expertise on nutrition and development including vitamin D, maternal and child nutrition, diet-gene regulation of the placenta and pancreas is willing to serve. Dr. Brannon is a Member of the Steering Committee on the International Vitamin D Standardization Project (2010 to present), Member of the IOM Committee on the DRI's for Calcium and Vitamin D (2009-2010), member of Technical Expert Panel for the Systematic Evidence Based Review on "The Relationships of Vitamin D and Calcium Intakes to Nutrient Status Indicators and Health Outcomes.", Tufts Evidence Based Practice Center, 2008-2009, Co-chair of the Federal Interagency Working Group on Vitamin D (2006-2008). At the data and safety meetings, they will review the research protocol, plans for data collection and safety monitoring and be responsible for noting adherence to the following:

(1) The Progress of Study and Safety of Participants: The Data Safety Monitoring Plan (attached) has been modified to be congruent with the study protocol. Since there is no upper limit for HBA1c, an increase in HBA1c of 3% or more will be monitored. The following data will be examined to evaluate safety issues: adverse events, attrition rates, reasons for attrition, and rates of adherence to treatment intervention at all contact points. In order to provide a level of protection to all study subjects, the following will be specifically included for monitoring and follow-up: (1) number (if any) of patients who had suicidal ideation during the course of the study, and adherence to safety protocol (2) number (if any) of patients who have experienced a 30% increase in the CES-D score and recommendations by Dr. Halaris for follow-up and referral (3) number (if any) of patients who began medication for mental health or sought additional therapy.
(4) number (if any) patients who had hypercalcemia or vitamin D levels greater than 100 ng/dl or symptoms of vitamin D toxicity and recommendations by Dr. Emanuele.

(2) Compliance with the reporting of adverse events: Determining whether all adverse events and/or unanticipated problems were reported in writing to Loyola University Medical Center within 48 hours of discovery of the incident.

(3) Data Accuracy and Protocol Compliance: Evaluating records for the training of personnel and other protocol documents as needed. All staff personnel will be required to have human subjects training, HIPPA training, and training on all study protocols (Appendix). Dr. Penckofer will have weekly meetings with the project staff and co-investigators. She will also train the staff personnel regarding all procedures (phone as well as in-person protocol), and meet with them as they facilitate the recruitment of study participants and schedule them for data collection. Training will also be provided for the research nurse on the use of the C-DIS. Dr. Penckofer will observe phone as well as on-site screening to ensure that the protocol is being followed, and provide feedback. At baseline, three and six month data collection points, the following will be done by Dr. Penckofer and the staff to ensure protocol compliance: 1) reviewing the informed consent and HIPPA documents; 2) checking questionnaire booklets for missing data prior to study subjects leaving the data collection site; 3) running standards for the hemoglobin A1c prior to subject testing in order to ensure the accuracy of the lab test. Dr. Penckofer will obtain reproducibility data from off-site labs during the times that study specimens are processed.

D. Potential Benefits of the Proposed Research to the Subjects and Others.
At this time, preliminary data from the pilot study suggests that vitamin D may be beneficial for improvement of depressive symptoms. However, given the need for a randomized clinical trial to determine whether the pilot data will substantiate these findings, at this time it is unknown if women will benefit from participating in the study. To our knowledge, there are no studies that have examined whether treating persons with diabetes for depressive symptoms using vitamin D supplementation. Therefore, our study will serve as a new and important contribution to our understanding of whether improvement of their nutritional state by vitamin D supplementation can be effective in improving both mental and physical outcomes. Practice initiated interventions to improve the quality of care for depression management in patients with diabetes may also substantially increase the welfare of these patients, particularly as it relates to their own self-management, however, this has not been well studied either.

E. Importance of the Knowledge to be Gained. Given the high cost of treating depression, its prevalence in persons with diabetes, particularly women, studying the benefit of vitamin D supplementation for this group has significant impact. With an improvement in depression, there may be an improvement in self-care behaviors which is also being examined and has not been well studied relative to depression. Finally, studying vitamin D supplementation for the effects on blood pressure also has important clinical implications as research examining this outcome is limited. In clinical studies that have explored vitamin D and mood outcomes (other than Jorde et al.) the amount of vitamin D has been low, the duration of supplementation short, and blood levels were not measured (23). The proposed study is unique in that the amount of vitamin D to be administered is currently what is recommended for obese individuals (34), thus the translation of study findings to practice is significant. The most important benefit of vitamin D supplementation if effective, is its low cost (as low as $3 per month) and affordability to the general public. Given its low cost, association with minimal side effects (36), that 1 in 10 Americans now take antidepressants which can have negative effects on weight and metabolic control (100), that depression is higher in women than men (7) especially in diabetes, this study can benefit the general public.

Inclusion of Women and Minorities Depression occurs more often in women with diabetes than men with diabetes, and over 25% of women with diabetes have depression (7). It has been reported that depression is associated with poorer diet, physical activity and medication adherence for women with diabetes (6, 72-76). Having both diabetes and depression is associated with a significantly increased risk for complications, including cardiac mortality which occurs more often in women with type 2 diabetes (53). The Women's Health Initiative (WHI) reported that low vitamin D intake was associated with significant depressive symptoms (27). Preliminary data has suggested it has s potential benefit for depression treatment for women (33). The Nurses' Health Study reported that women were twice as likely to develop hypertension if vitamin D levels were less than 15 ng/ml (83). Pfeifer and colleagues (91) reported a 7% decrease in systolic blood pressure in

vitamin D deficient women following 800 IUs of D_3 after 6 weeks. Thus, there is evidence to suggest that targeting women at this time is justified.

Since women with diabetes have low vitamin D and high levels of depression, this group is being studied as efficacy is more likely to be demonstrated. If results are significant, men could also be examined (although they don't have as high risk for depression or the same body fat content as women which contributes to their low vitamin D) to assess for gender differences on dosing and impact on health outcomes. The investigator will recruit almost half minority (40%), which is consistent with the proportion that currently seeks care at the primary sites of data collection.

Inclusion of Children
It would not be reasonable to include children (less than 21) since the dose proposed for this study has only been tested in several adult populations. Given the recent increase in childhood obesity and type 2 diabetes, it may be possible to develop a study for vitamin D supplementation in the future for this age group.

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Appendix 1 for Recruitment

RECRUITMENT UPDATES 1/07/2014

a. Distribution of approved flyers and information at professional meetings (e.g., Diabetes Nurse Educator meets January 16th and Dr. Penckofer is speaker).

b. Presentation by our Loyola approved research volunteer Sana Rizvi to the Illinois Institute of Technology IIT) Muslim Student Association (MSA) about the Sunshine 2 study with flyer distribution to attendees. Sana has contacted the MSA president (Adnan Qureshi) and has gotten approval to inform attendees about the study. There has been little to no research on vitamin D in this population.

c. Meetings with churches in area to distribute approved materials. Dr. Penckofer had pervious support from Nurse Carolyn Johnson from Health Ministry group at Broadview Baptist Church for the pilot study. We also request to use our approved flyer and information for recruitment, meetings or communications with other churches in the community.

d. Facebook site to inform subjects about Sunshine Study which will be monitored by study nurse (sample attached). Potential subjects will be directed to contact study personnel.

e. Creation of Loyola Website for SUNSHINE 2 study through the offices of Ron Price and Greg Klitz. This office has built in shell templates available for use. For our study, we will follow the www.D2Dstudy.org where our consultant, Dr. Pittas who is the PI has given us permission. Participants will be provided information about the study and informed to contact study personnel. Content for the proposed site is attached.

f. Linking Sunshine 2 study to other websites (e.g., the American Diabetes Association of Chicago, American Association of Diabetes Nurse Educators, etc...).

RECRUITMENT UPDATES 2/3/14

This is for the flyer (which has been approved by marketing)

1. Major community events (e.g., health fairs)
2. Schools with approval of principal (e.g., we have a school based health center at Proviso and are seeking approval to leave at health center since parents come into site)
3. Grocery bulletin boards (with approval of store manager, e.g., Whole Foods is checking to see if we can post on their community bulletin board)
4. Social service agencies (e.g., Lawndale community health center and other centers such as WIC where dietetics students do their internships)
5. Diabetes clinics (e.g., private clinics from MD offices if approved).
6. Community restaurants (e.g., Panera has agreed to post flyer on community bulletin board)

RECRUITMENT UPDATES 5/7/2014

The addition of 3 family medicine physicians (Tony Pangan, Norma Lopez and Jessica McIntyre) who have agreed to have their diabetes patients sent recruitment letter using EPIC database (Attached).

Appendix 2 Protocal for Putonti Lab at Lakeshore Campus:

Nanosep Tube Preparation

Materials Required: Nanosep Tube, molecular grade Water, 70% Ethanol, 1% BSA in PBS, microcentrifuge

1. Pipet into the Nanosep Tube 500 µL of 70% Ethanol and centrifuge the Column at 14,000g for 10 minutes
    a. Discard the flow-through - May need to use a pipet to remove all of it
    b. When centrifuging during any of these steps be sure not to over-centrifuge as this will cause the membrane to dry out
2. Pipet in 500 µL of PCR Water and centrifuge at 14,000g for 7 minutes
    a. Remove the flow-through - May need to use a pipet to remove all of it
3. Pipet in 500 µL of Bovine Serum Albumin (BSA) and leave for at least an hour at room temperature
    a. The BSA serves as the passivation step so that the membrane is not overly sticky and prevent the capture of non-phage materials
    b. Do not centrifuge the BSA
    c. This is a potential pause point if one cannot do the whole preparation in one day - Can be left overnight at room temperature
4. Pipet out BSA from the membrane
    a. Be sure not to scratch the membrane when pipetting (can tilt to limit contact)
5. Wash the membrane with PCR Water
    a. When washing use approximately 50 µL of PCR water
    b. Hold the column at an angle and dispense the water at the top of the membrane and remove at the bottom - Repeat until the water begins to appear cloudy
    c. Be careful not to scratch the membrane
6. Add another 500 µL of PCR Water to the Column and centrifuge at 14,000g for 5 minutes
    a. Remove the flow-through - May need to use a pipet to remove all of it
7. If not using the column within 20 minutes, add 100 µL of PCR Water to the Column and store at 4° C. Try to use refrigerated columns within 1 week.
    a. This layer of water will prevent the membrane from drying out before use

Concentration Protocol

Materials Required: Prepared Nanosep Spin Column, urine sample, PBS, microcentrifuge

1. Add 1 mL of the Urine Sample to a microcentrifuge tube and centrifuge at 10,000g for 10 minutes
    a. This will cause a pellet to form at the bottom of the tube - The pellet includes the bacteria and eukaryotic cells that were in the urine sample
2. Transfer the supernatant to a new microcentrifuge tube
    a. Discard the tube with the pellet
3. Remove the 100 µL of water from the membrane of the Nanosep Spin Column and add 500 µL of the Urine sample supernatant to the membrane
4. Centrifuge the Nanosep Tube initially at 1,000g for 7 minutes
    a. Continue to spin the Nanosep tube at 1,000g for decreasing amounts of time until approximately 50 µL remain above the membrane
        i. 50 µL above the membrane appears almost as if there is no liquid left
        ii. It is difficult to determine 50 µL by eye so the use of a 50 µL pipet is recommended in order to keep track of how much liquid is left above the membrane

iii. If processing multiple samples at once, some will finish before others. Have additional tubes and sterile media available to prepare blanks for re-balancing the centrifuge.

5. After the 50 µL have been collected, transfer it to a new microcentrifuge tube and add the remaining 500 µL of the Urine Sample Supernatant to the Nanosep Tube
    a. Be sure to remove the flow-through
6. Repeat Step 4 for the second volume of the Urine Sample Supernatant
7. Discard the flow-through

Optional Steps 8-10:

8. Add the 50 µL from the first round of spinning to the 50 µL from the second round of spinning in the Nanosep tube
    a. After this step you should have approximately 100 µL of concentrated phage in the Nanosep tube
9. Add 400 µL PBS to the 100 µL of concentrated phage
10. Spin the PBS and phage at 1,000g for 5 minutes
    a. Keep spinning at 1,000g for shorter amounts of time until approximately 50 µL of liquid remain above the membrane
11. Add 1 mL of PBS to the pellet that was kept from Step 1 and spin at 10,000g for ten minutes
12. Discard the supernatant and add the 50 µL from Step 10 to the pellet
13. Use 20 µL of PBS to wash the Nanosep membrane
    a. Use the same washing technique shown in Step 5 of the Nanosep Preparation
14. After washing the membrane, add the 20 µL to the pellet