University of Alberta

Vitamin D Supplementation and Bone Health in Adults with Diabetic Nephropathy: Preliminary Findings from a Randomized Controlled Trial.

by

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Abstract:

Vitamin D (vitD) is a nutrient of concern in Canada particularly for those with diabetes and chronic kidney disease (CKD). Participants (18-80y) with diabetes and CKD were randomized to receive either 50mcg/day (n=33) or 1,000mcg/month (n=30) $vitD_3$ for 6-months. Variables studied included: anthropometric/demographic data, routine clinical blood work, serum/plasma 25(OH)D, $1,25(OH)_2D$, PTH, bone turnover markers (BTM), and vitD intake. No significant differences in clinical characteristics between groups or study visits were observed ($p>0.05$). Adherence to daily and monthly supplementation was 93% and 100%, respectively. Mean 25(OH)D and $1,25(OH)_2D$ concentrations increased in both groups after 6-months; but significant increases in 25(OH)D were seen only in the daily group (78.4 to 95.1nmol/L; $p=0.01$). There were no significant differences between groups over 6-months in PTH or BTM ($p>0.05$). This suggests that both once daily and once monthly vitD supplementation strategies are equally effective at influencing vitD status despite differences in adherence.

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Other Publications Related to Master's Degree Course Work:

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List of Abbreviations

AE	Adverse event
ALP	Alkaline phosphatase
AHS	Alberta Health Services
BAP	Bone-specific alkaline phosphatase
BMD	Bone mineral density
BMI	Body mass index
CaP	Calcium-phosphorous product
CCHS	Canadian Community Healthy Survey
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
cm^2	Centimeter squared
CRU	Clinical Research Unit
CV	Coefficient of variance
CVD	Cardiovascular disease
d	Day
DIN	Drug Identification Number
dL	Decalitre
DNPC	Diabetic Nephropathy Prevention Clinic
DRI	Dietary reference intake
DSMB	Drug Safety Monitoring Board
DXA	Dual energy x-ray absorptiometry
EAR	Estimated average requirement
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
eGFR	Estimated glomerular filtration rate
e.g.	Example
ESRD	End-stage renal disease
FBG	Fasting blood glucose
FGF-23	Fibroblast growth factor-23
g	Gram
GFR	Glomerular filtration rate

GNH	Grey Nun's Community Hospital
HbA1c	Hemoglobin A1c
HREB	Human Research Ethics Board
i.e.	Illustrated example
IU	International units
IOM	Institute of Medicine
K/DOQI	Kidney Disease Outcomes Quality Initiative
kg	Kilogram
L	Litre
Log	Logarithmically transformed
L1-4	Lumbar spine vertebrae 1-4
m	Month
m^2	Meter squared
mcg	Micrograms
min	Minute
mL	Millilitre
mg	Milligram
mmol/L	Millimol per litre
mRNA	Messenger ribonucleic acid
MVM	Multivitamin and mineral
NARP	Northern Alberta Renal Program
NECHC	Northeast Community Health Centre
NHANES	National Health And Nutrition Examination Survey
nm	Nanometer
nmol/L	Nanomol per litre
NPN	Natural Product Number
NTx	N-telopeptide of type 1 collagen
pmol/L	Picomol per litre
PST	Plasma separation tubes
PTH	Parathyroid hormone
PYD	Pyridinoline
QoL	Quality of life
RBG	Random blood glucose
RCT	Randomized controlled trial

RD	Registered Dietitian
RDA	Recommended dietary allowance
RIC	Renal Insufficiency Clinic
RN	Registered Nurse
RPM	Revolutions per minute
SAE	Serious adverse event
SAS	Statistical Analysis Software
SD	Standard deviation
SE	Standard error
SST	Serum separating tubes
T1D	Type 1 diabetes
T2D	Type 2 diabetes
UAH	University of Alberta Hospital
UL	Upper limit
U/L	Unit per litre
UAE	Urinary albumin excretion
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet
VDBP	Vitamin D binding protein
VDR	Vitamin D receptor
VDRa	Vitamin D receptor analogues
vs.	Versus
$1,25(OH)_2D$	1,25-dihydroxyvitamin D; calcitriol
25(OH)D	25-hydroxyvitamin D; calcidiol
α	Alpha
β	Beta

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Diabetes is a leading cause of morbidity and mortality in North America impacting millions of individuals, and growing in prevalence (1-3). Both type 1 (T1D) and type 2 diabetes (T2D) are associated with severe complications that may impair quality of life (QoL) and increase risk of death. Disturbances in micro- and macrovascular functionality increase risk of developing both kidney disease and cardiovascular disease (CVD), the primary cause of mortality in this population (4-8). Diabetes is associated with reduced bone mineral density (BMD) and increased fracture risk, which has a negative impact on QoL through reduced mobility and independence, and increased risk of debilitating illness and mortality (3,9-13). Individuals with diabetes and chronic kidney disease (CKD), or diabetic nephropathy, are especially susceptible to poor bone health due to impaired vitamin and mineral metabolism, specifically calcium, phosphorus and vitamin D (14,15). Inflammation, oxidative stress, insulin resistance and hyperglycemia are additional components of the milieu that contributes to poor bone health in this population; vitamin D is implicated in these processes and a sufficient vitamin D status may reduce the severity of these complications (3,10,12,16,17).

There is evidence to suggest that vitamin D insufficiency is an independent risk factor for both T1D and T2D (3,10,11). This is of particular concern in diabetic patients who develop CKD since the reduced capacity to hydroxylate vitamin D to its active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) or calcitriol, will further increase their risk for vitamin D deficiency and poor bone health. Within the general North American population 16-52% have suboptimal vitamin D status (<75nmol/L) (18,19). The prevalence of suboptimal vitamin D status increases to 86% in the diabetic population, and those with concurrent kidney disease are 1.78-fold more likely to be vitamin D deficient (4). Certain ethnic groups (e.g. African Americans, Hispanics) are at increased risk for diabetic nephropathy, thus compounding their risk of vitamin D deficiency along with their reduced potential for cutaneous synthesis due to increased melanin content (4,18). By the time CKD patients reach dialysis, approximately 75% have metabolic bone disease (20).

Individuals living in northern communities are also at particular risk for vitamin D insufficiency due to reduced cutaneous synthesis as the result of limited sunlight exposure, further increasing their risk for low BMD and fragility fractures (18,19,21-23). According to the 2009 Canadian Community Health Survey (CCHS), approximately 22% of the general population aged 50 and older had inadequate vitamin D status (25(OH)D <50nmol/L) and 11.6% had a diagnosis of osteoporosis (1). Risk for vitamin D inadequacy in this population is further increased by CKD. Canadian data indicates that 93% of patients with stage 3-4 CKD (primarily caused by diabetes) have insufficient vitamin D status (<75nmol/L) while 41% are deficient (<37.5nmol/L) (21).

Although supplementation with vitamin D can increase serum vitamin D status and improve bone health, the optimal dose required for individuals with diabetic nephropathy is unknown, particularly for those living in northern climates. Another factor that might influence overall vitamin D status includes adherence to supplementation regimens. Patient adherence/compliance to daily oral vitamin D supplementation is poor; only 69% of patients are compliant with vitamin D supplementation in osteoporosis management and adherence is said to decrease as time progresses (24). This may be related to the silent nature of bone disease and limited tangible outcomes of non-adherence until a fracture is sustained (25). Furthermore, the presence of a number of concurrent chronic diseases and additional therapies for these can largely impact adherence to supplementation, which has been shown to decrease by 20% with each additional chronic disease (26). Determining the optimal strategy for vitamin D supplementation in adults with diabetic nephropathy is critical to ensure optimal vitamin D health and mitigate metabolic bone disease in this high-risk population.

1.2 Vitamin D

Vitamin D supplementation recommendations have an ambiguous history. Cod liver oil was used in the prevention of rickets since the late 1700's, yet not until the 1920's did scientists discover that a specific component of this oil actually prevented and cured rickets (27). The original dietary reference intake (DRI) for vitamin D of 10mcg/day (400 International Units (IU)) was based on the vitamin D content of one teaspoon of cod liver

oil (27). However, unlike other nutrients, recommendations for vitamin D are not entirely based on food sources as there are very few dietary options available (e.g. fish, liver and fortified dairy products), but rather the recommendations are designed to compensate for a deficiency of sunlight (28,29). Some argue that optimal vitamin D status should be based on physiological concentrations of vitamin D produced via cutaneous synthesis (27,29). In this case, 100-250mcg/d (4,000-10,000IU/d) could be recommended to reflect the vitamin D status of outdoor workers in the United States of America (USA) (27,29).

As recent as four decades ago, individuals presenting without radiological or clinical symptoms of rickets or osteomalacia were believed to have adequate vitamin D status (27). Since then serum measures of 25-hydroxyvitamin D (25(OH)D) have been well established as an objective and quantitative measure of vitamin D status, reflecting both dietary intake and cutaneous synthesis (30-33). Hydroxylated in the liver, free 25(OH)D has a half-life of approximately 2 months, and when bound to vitamin D binding protein (VDBP) this may increase to 3 months (32-35). Serum 25(OH)D is less biologically active than $1,25(OH)_2D$, which is produced primarily in the kidneys but can also be produced in other tissues with 1-α-hydroxylase activity (e.g. breast, pancreas, prostate and colon tissue) (32-34). Optimal serum 25(OH)D concentrations are a source of great controversy and have led to the use of different cut-off values to define the spectrum of vitamin D status. In healthy adults, 75nmol/L is believed by most researchers and clinicians to be sufficient for bone health and ≥100nmol/L may have added health benefits, yet disagreement exists regarding the cut-offs that should be used to define vitamin D deficiency (25-50nmol/L) (4,17,18,20,21,24,34,36). In November 2010, the Institute of Medicine (IOM) released new DRI values for vitamin D based on available data from observational and longitudinal research using a framework of health/disease indicators and risk assessment (37). The recommended dietary allowance (RDA) for adults aged 18-70 years is now 15mcg/d (600IU/d) and 20mcg/d (800IU/d) for those over 70 years, with an upper limit (UL) of 100mcg/d (4,000IU/d) for both age groups (37,38). However, the estimated average requirement (EAR) was set at 10mcg/d (400IU/d) for all age groups; a level perceived to result in a 25(OH)D of 50nmol/L, which the IOM states is adequate for bone health in ***healthy*** individuals (1,37). What level of 25(OH)D and vitamin D intake is

required in high-risk individuals (e.g. diabetes and CKD) remains unclear. Observational and interventional studies on vitamin D intake and/or status in individuals with diabetes, CKD, and/or living in northern latitudes are highlighted in ***Appendix 1, Table A1.1.***

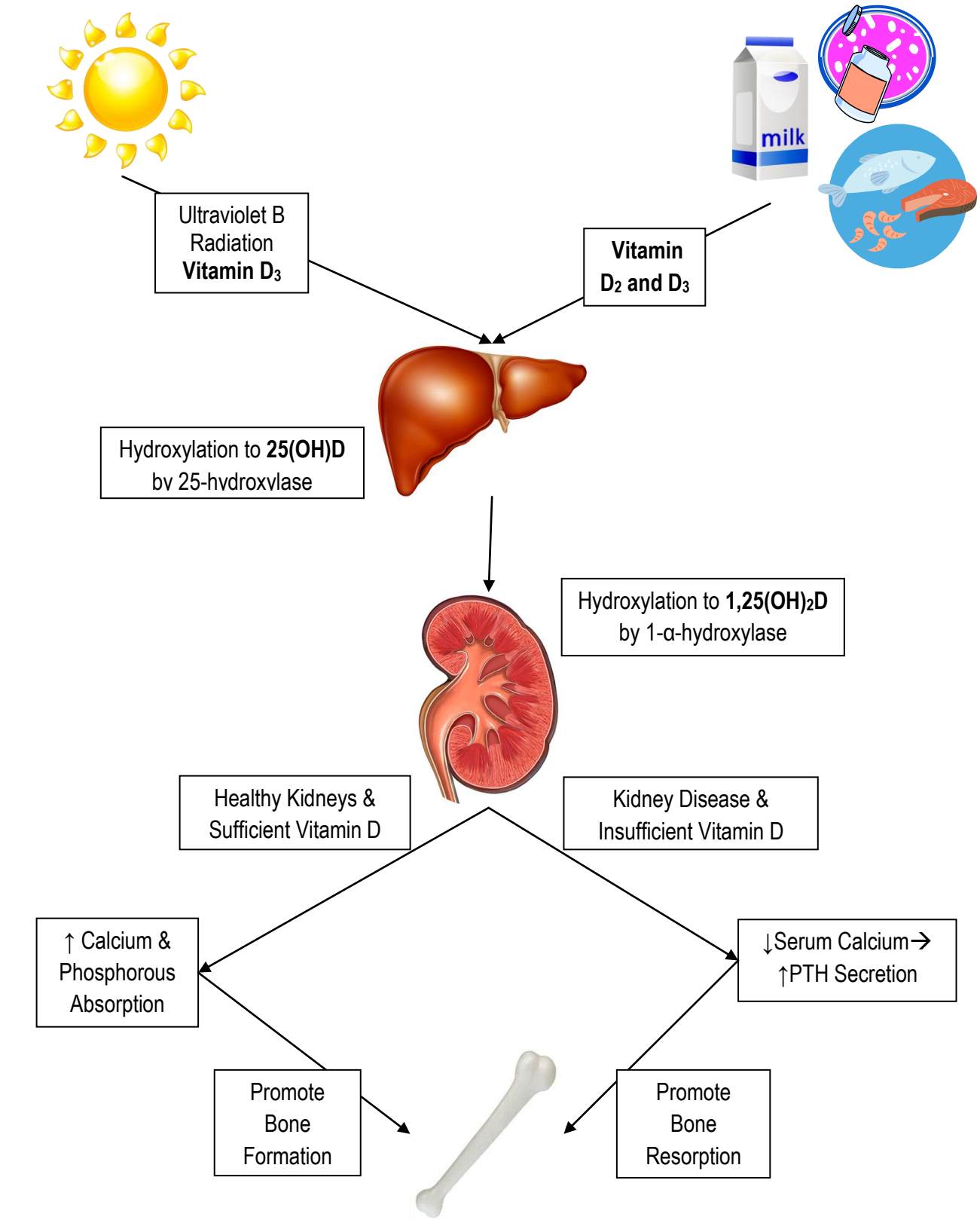

Figure 1.1: Vitamin D Metabolism

1.2.1 Dietary Intake of Vitamin D

Dietary intake of vitamin D is suboptimal in North America despite the availability of an increasing number of fortified products. In 2011, the United States Department of Agriculture (USDA) released the 2007-2008 National Health And Nutrition Examination Survey (NHANES) with detailed information on vitamin D intake from diet and supplements (39). The average (standard error) intake of males and females 20 years and older was 5.0mcg/d (0.22) and 3.8mcg/d (0.13) from diet alone, and 3.9mcg/d (1.08) and 6.0mcg/d (0.48) from supplements alone, respectively (39). Approximately 22% of males and 29% of females over 20 years used vitamin D supplements, and only in supplement users did the average total intake of vitamin D meet/exceed the RDA: 22.5mcg/d (4.73) and 22.6mcg/d (1.26), for males and females respectively (39). Average vitamin D intake was found to decline as family income declined, an important factor to consider in adherence to dietary and therapeutic recommendations (39).

According to data from the 2004 Canadian Community Health Survey Cycle 2.2 (CCHS 2.2), which focused on nutrition, dietary intake of vitamin D was also low in Canadians (40). The average dietary intake of vitamin D in males and females 19 years and older was 5.8-6.7mcg/d and 5.1-6.1mcg/d, respectively (40). The highest intake was observed in males 51-70 years at 7.0mcg/d and the lowest intake was 5.1mcg/d, which was the average intake of all adult women (19-70 years) (40). Only 8% of females and 20% of males met the EAR of 10mcg/d through dietary sources (40). Interestingly, dietary vitamin D intake did not vary significantly between individuals reporting a chronic disease (5.9±0.2mcg/d) and those who did not (5.8±0.1mcg/d; $p>0.05$) (40).

Milk products comprise the primary dietary source of vitamin D in the typical North American diet, however fortification of these products varies widely (40). In Canada milk is required to be fortified with vitamin D so that an individual would receive 7.5-10mcg (300-400IU) in a “reasonable daily intake”; this has been reported to translate to 2mcg/100mL of milk, which is twice the content in American fortified milk (40,41). However some researchers have found a large deviation in vitamin D content of sampled milk, whereby as much as 66% of the samples contained less vitamin D than labelled (31,42).

Information about vitamin D intake of individuals with diabetic nephropathy is lacking in the literature. However, the high prevalence of vitamin D insufficiency and deficiency provides reason to believe that total vitamin D intake is suboptimal (4,12,21,43). Individuals with diabetes and nephropathy are more likely to have a lower vitamin D status than those with either diabetes or nephropathy alone, as well as healthy individuals (12,16,43). Dietary restriction of milk products in CKD due to high phosphorus and potassium content limits consumption of the most common dietary source of vitamin D, thereby further increasing their risk for suboptimal vitamin D status. A phosphorus restriction of 800-1,000mg/d is often recommended when serum phosphorus levels exceed 1.49mmol/L or PTH exceeds 7.7pmol/L in CKD stage 3-5 (44). To put this into practical terms, 250mL (1cup) of fluid milk contains approximately 232mg of phosphorus, 366mg of potassium, 290mg of calcium and 2.4-5.0mcg of vitamin D_3; therefore fluid milk intake is often restricted to 125mL (1/2cup) in those requiring a phosphorus/potassium restriction (Food Processor SQL, v.10.8, ESHA Research, Salem, Oregon, USA). Phosphorus restriction has been suggested to have a beneficial effect on $1,25(OH)_2D$ production in the kidney by limiting further kidney damage (e.g. reduction of renal mass and subsequently 1α-hydrolylase), rather than directly related to phosphorus levels (44).

1.2.2 Dermal and Oral Sources of Vitamin D

It has been suggested that vitamin D is equally efficacious from both cutaneous and oral (dietary/pharmaceutical) sources, yet this must be interpreted cautiously as oral vitamin D preparations vary in efficacy (32,33).

Cutaneous synthesis of vitamin D requires ultraviolet B (UVB) radiation in the wavelength range of 290-315nm (45). Pro-vitamin D (7-dehydrocholesterol) is converted into pre-vitamin D in the skin and transported via systemic circulation bound to VDBP to the liver where it is metabolized to 25(OH)D by 25-hydroxylase (***Figure 1.1***) (31,33,34). Efficiency of cutaneous synthesis depends on environmental and lifestyle factors. Ultraviolet B exposure varies with latitude, ambient/environmental exposure (cloud cover), personal ambient exposure (time of day and year, protective clothing), and anatomical distribution of UV exposure (sitting vs. standing) (45). Sunlight exposure questionnaires

can be used to estimate UVB exposure and resulting vitamin D production, however these are most commonly validated for skin cancer or other disease risk (45,46). No gold standard exists to validate these questionnaires, however personal UV dosimetry is the most common methodology used (45). Personal factors known to reduce vitamin D synthesis include covering of skin (e.g. tight weave clothing, hats, sunscreen), increased age (elderly have 50% less 7-dehydrocholesterol), and increased melanin content of skin (melanin absorbs UV and prevents radiation of 7-dehydrocholesterol) (24,33,45).

Latitude is perhaps one of the most well studied variants in cutaneous vitamin D synthesis, as it reflects both environmental UVB availability and individual cutaneous synthesis. Individuals residing at a latitude >40° north or >40° south of the equator are capable of negligible vitamin D synthesis during winter months (31). Studies have found a high prevalence of vitamin D insufficiency (18-86%) in healthy individuals residing in "northern" countries, such as Canada, Germany, Finland and Switzerland (9,18,21,27,33,47). In Canada (approximate latitude 53°30' North), 63% of the population have 25(OH)D <80nmol/L in the summer, and this prevalence increases to 86% during the winter (21). A decline in 25(OH)D during winter months has been associated with a clinically significant decline in BMD (47). The effect of lifelong seasonal fluctuations in 25(OH)D may be detrimental and impair the activity of 1α-hydroxylase and 25-hydroxylase through continuous attempts to adapt to suboptimal levels (32).

Orally acquired vitamin D (e.g. from foods and supplements) is packaged in chylomicrons and lipoproteins, then transported to the liver via the lymphatic system for metabolism into 25(OH)D (31,33). There are two common forms of oral vitamin D available: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Synthetic production of vitamin D_3 is similar to the physiological process of cutaneous synthesis as it involves radiation of cholesterol, while production of vitamin D_2 involves irradiation of ergosterol from ergot and results in a less stable product (30). Although absorption of both forms appears to be equivalent, their bioactivity is not (36). In 1950, scientists discovered that vitamin D_3 is approximately 4-times more active than vitamin D_2 (30). Vitamin D_3 elicits a stronger response as measured by 25(OH)D in several animal models (e.g. 10-fold in birds and 2 to 3-fold in monkeys), and in human trials where 1.7 to 10-fold more vitamin D_2 is

needed to effect 25(OH)D to the same degree as vitamin D_3 (30). Oral administration of a single 1,250mcg (50,000IU) dose of vitamin D_3 or vitamin D_2 causes a comparable increase in 25(OH)D in the first 3 days post-supplementation (30). Yet by day 14, serum 25(OH)D in those supplemented with vitamin D_2 returns to baseline while concentrations from vitamin D_3 supplementation reach their peak (30). By day 28, 25(OH)D concentrations drop below baseline with vitamin D_2 while 25(OH)D from vitamin D_3 remains above baseline (30). This may be explained by vitamin D_3 having a stronger affinity for hepatic 25-hydroxylase, VDBP, and the vitamin D receptor (VDR), as well as a reduced propensity for degradation (30). Mitochondrial hydroxylation of vitamin D_3 is 3-fold faster than vitamin D_2, and certain cytochromes (e.g. CYP27A1) only 25-hydroxylate vitamin D_3 (30). Upon 24-hydroxylation of 25(OH)D and $1,25(OH)_2D$ in the kidney, the $24,25(OH)_2D$ and $1,24,25(OH)_3D$ metabolites of vitamin D_2 are deactivated yet the vitamin D_3 metabolites can still bind to the VDR (30). As a result of its reduced affinity for VDBP, vitamin D_2 may increase toxicity risk as a result of more biologically available $25(OH)D_2$ and $1,25(OH)_2D_2$ (30). In most cases, milk and over-the-counter supplements contain vitamin D_3 while non-hormone (e.g. non-calcitriol) vitamin D prescriptions often contain vitamin D_2 (27). Interestingly, vitamin D toxicity is more often iatrogenically derived by long term use of vitamin D_2 prescriptions in cases such as hypothyroidism and osteoporosis, compared to vitamin D_3 sources (27,33).

1.2.3 Vitamin D Toxicity

Chronic daily intake of 1,250-2,500mcg (50,000-100,000IU) of vitamin D_2 or D_3 can cause toxicity in adults (48). Acute hypervitaminosis D rarely presents with symptoms, however chronic hypervitaminosis D generally manifests as hypercalcemia (>2.75mmol/L), as vitamin D increases intestinal absorption of calcium (48). Hypercalcemia can cause generalized symptoms such as nausea, headache and fatigue, as well as more severe symptoms including renal dysfunction, pancreatitis and seizures (48). According to North American regulatory boards, 100mcg/d (4,000IU/d) is the UL of vitamin D that adults should consume, yet daily doses as high as 250mcg (10,000IU) have been proven safe as they have not resulted in serum calcium concentrations indicative of intoxication (24,36). A

serum 25(OH)D of 220nmol/L is generally regarded as safe, with hypercalcemia unlikely to occur until 25(OH)D reaches a non-physiologically high concentration of >700nmol/L (18,24,29,33,36). In a study on healthy northern-dwelling men, 250mcg/d (10,000IU/d) vitamin D_3 for 20 weeks resulted in a serum 25(OH)D of 220nmol/L without increasing serum calcium or causing significant adverse events (36).

Both 25(OH)D and $1,25(OH)_2D$ have been implicated in mechanisms of vitamin D toxicity. VDBP has a capacity of approximately 4,700nmol/L vitamin D metabolites; once VDBP is saturated, free 25(OH)D stimulates 1α-hydroxylase (29). The resulting increase in $1,25(OH)_2D$ leads to increased intestinal calcium absorption which could lead to hypercalcemia (29). Furthermore, chronic up-regulation of 1α-hydroxylase during vitamin D deprivation may impair down-regulation of $1,25(OH)_2D$ production during 25(OH)D abundance (29). Excessive 25(OH)D may also have a direct effect on toxicity risk through binding with the VDR, activating calcium transport channel1 and calbindin D_{9k} to increase calcium absorption, and/or stimulating $1,25(OH)_2D$ production in other tissues (31,33). Homeostatic controls exist to prevent vitamin D toxicity: the liver can reduce the 25-hydroxylation of vitamin D by reducing 25-hydroxylase concentrations and can also increase the catabolism and excretion of 25(OH)D via bile, additional pathways throughout the body can increase vitamin D catabolism through side chain cleavage and oxidation (30,33).

The risk for vitamin D toxicity in diabetic nephropathy is almost entirely related to hypercalcemia (>2.75mmol/L), which can increase risk for vascular calcification, a significant concern in this population due to increased risk for cardiovascular mortality (16,44,49,50). However, vitamin D deficiency has also been associated with increased risk for coronary artery calcification; therefore vascular homeostasis requires obtaining vitamin D sufficiency while avoiding toxicity (49). Although commonly associated with cardiovascular outcomes, vascular calcification can lead to mineral deposition and soft tissue calcification in other organs including the lungs, skin (e.g. calciphylaxis and skin necrosis) and kidney (49). In a retrospective autopsy examination of vascular calcification in pediatric end-stage renal disease (ESRD), n=72/120 had soft tissue calcification, including 29 patients with calcinosis of the kidney and 15 of these exhibited focal mineral

deposits within the renal tubules (51). According to stepwise logistic regression analysis, risk of soft tissue calcification was associated with vitamin D therapy, whereby severity of risk increased with use of vitamin D receptor activating analogs (VDRa) compared to vitamin D_2 or D_3 (51). Unfortunately, the doses used in this study were not available. Chronic high-dose vitamin D supplementation can also lead to over-suppression of PTH and adynamic bone disease, therefore caution and avoidance of over-supplementation is critical (20,49). Attaining a 25(OH)D concentration indicative of hypervitaminosis D (e.g. >220-700nmol/L) due to vitamin D supplementation (<1,250-2,500mcg/d or <50,000-100,000IU/d) is rare, however the risk for increasing serum calcium and phosphorus concentrations (through increased intestinal absorption) is a more relevant concern and the negative implications this could have on vascular calcification requires careful monitoring of serum calcium, phosphorus and PTH (44).

1.2.4. *Interactions with Nutrients, Hormones and Bone Health*

Vitamin D plays a key role in intestinal absorption of calcium and phosphorus. Low serum calcium concentrations stimulate PTH secretion to increase production of $1,25(OH)_2D$, thus enhancing intestinal absorption of calcium and phosphorus (***Figure 1.1***). Intestinal calcium absorption peaks at 75-80nmol/L 25(OH)D (24,29,32). If serum $1,25(OH)_2D$ is inadequate, calcium and phosphorus are released from bone stores to maintain serum concentrations. Vitamin D and calcium work closely to promote bone health; without adequate calcium status vitamin D supplementation has a limited ability to protect bone (31).

Vitamin D, both $1,25(OH)_2D$ and 25(OH)D, impacts hormone secretion and enzyme activity. In northern climates where serum 25(OH)D typically declines during winter months, transient increases in PTH and markers of bone turnover, such as serum bone-specific alkaline phosphatase (BAP) and urinary pyridinoline (PYD), are observed (47). Maintaining serum 25(OH)D at sufficient summer levels (88±20nmol/L) has been shown to prevent seasonal fluctuations in PTH and bone turnover markers, and prevent bone loss (47). Researchers have suggested that 75-110nmol/L 25(OH)D may be required to suppress secondary hyperparathyroidism in otherwise healthy individuals (21,31,34).

Vitamin D also affects insulin response to glucose stimulation through several pathways (11). Pancreatic β-cells contain extracellular VDR and intracellular 1α-hydroxylase (11). Insulin secretion is a calcium-dependent reaction and vitamin D modulates calcium flux through β-cells (11). Vitamin D increases insulin receptor expression, yet inadequate calcium in the intracellular cytosolic pool can reduce glucose transporter-4 activity leading to peripheral insulin resistance (11). Lastly, vitamin D directly impacts cytokine production and activity thus reducing inflammation and its detrimental effect on β-cells (11,16). Therefore suboptimal vitamin D status likely plays a role in the development of diabetes, particularly in those with impaired glucose tolerance (11). Attaining adequate vitamin D status (e.g. through supplementation) may play a key role in ameliorating variables in the diabetic milieu that contribute to poor bone health, including inflammation, hyperglycemia and insulin resistance (3,10,11).

1.3 Diabetic Nephropathy

Diabetic nephropathy affects approximately 30% of individuals with T1D and 40% of those with T2D (16). At diagnosis of T2D 7% of individuals already have microalbuminuria, and 17% of those with T1D develop microalbuminuria by year 5 post-diagnoses (6). Several measures can be used to diagnose nephropathy, including: urinary albumin-to-creatinine ratio ≥30mg/g; proteinuria >0.5g/24hours or a spot urine sample >430mg/L (100% sensitivity, 82-93% specificity); and macroalbuminuria ≥300mg/24hours or a spot urine sample >300mg/g (4,6). Presence of microalbuminuria is predicative of developing diabetic nephropathy and can be diagnosed with a random spot urine test (100% sensitivity, 80% specificity); this should be confirmed by 2 out of 3 positive tests over a 3-6 month period due to large day-to-day variability (6). Individuals may have a low glomerular filtration rate (GFR; <60mL/min/1.73m^2) despite normal urinary albumin excretion (UAE), which is more common in diabetics with retinopathy, thus complicating nephropathy diagnoses (6). (***Table 1.1***)

Risk factors for developing diabetic nephropathy include sustained hyperglycemia, hypertension, dyslipidemia, genetic predisposition, puberty, and race/ethnicity (e.g. Asian, African, Mexican and Native American) (4,6). Diabetes is the most significant risk factor for

morbidity and mortality in CKD, and as such prevention of diabetes and management of glycemia and insulinemia are of upmost importance (7,8,12,16,52). According to the USA Renal Data System, diabetic nephropathy is the leading cause of ESRD with a prevalence of 54% (16). Mortality from diabetic nephropathy is 2-fold greater (20% yearly) than from CKD alone, and those with diabetic nephropathy and severe CVD are 5-fold more likely to die from complications than to progress to ESRD (39% vs. 7% mortality, respectively) (16).

Table 1.1: Chronic Kidney Disease Stages

Stage	Glomerular Filtration Rate ($mL/min/1.73m^2$)	Description
1	≥90	Kidney damage with normal or high GFR
2	60-89	Kidney damage with mildly reduced GFR
3	30-59	Moderately reduced GFR
4	15-29	Severely reduced GFR
5	<15 (or dialysis)	Kidney Failure

Adapted from the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) (44).

1.4 Impact of Diabetes and Kidney Disease on Vitamin D and Bone Health

Diabetes is associated with increased risk for poor bone health, suboptimal vitamin D status and CKD (12,16). Hyperglycemia and oxidative stress reduce PTH and $1,25(OH)_2D$ responses, PTH secretion, VDR activity, osteoblast function and bone formation markers (osteocalcin), and may reduce bone's buffering capacity resulting in increased serum calcium and phosphorus (12). Poor glycemic control can also cause hypercalciuria, increasing risk for low BMD (16). Suboptimal vitamin D status can increase vascular calcification and may contribute to CKD progression (4,5). Vitamin D has renoprotective properties in the renin-angiotensin system and VDR pathways that attenuate proteinuria, glomerulosclerosis and podocyte hypertropthy (4).

While diabetes predisposes individuals to poor vitamin D status and bone health, CKD compounds these issues independent of dietary differences (16,17). Uremia can impair vitamin D metabolism by reducing synthesis of cholecalciferol from 7-dehydrocholesterol, and reduced renal mass and tubulointerstitial injury leads to less 1α-

hydroxylase in proximal tubular cells (5,16). There are numerous hematological changes across the stages of CKD (***Table 1.1***) that contribute to and reflect osteodystrophy.

According to data from a cross-section of diabetic participants in the 2001-2006 National Health And Nutrition Examination Survey (NHANES), 48.9% had 25(OH)D <50nmol/L (deficiency) and 36.6% had 50-75nmol/L (insufficiency) (4). Individuals with diabetic nephropathy (excluding those with macroabluminuria) were 1.78-fold more likely to have vitamin D deficiency vs. diabetics without nephropathy (4). In a study conducted in Japanese pre-dialysis patients with and without diabetes (sex, age and GFR-matched), those with diabetic nephropathy were found to have significantly elevated concentrations of corrected calcium, phosphorus and calcium-phosphorus product (CaP) and significantly lower concentrations of 25(OH)D and $1,25(OH)_2D$ ($p<0.05$) (12). Phosphorus and CaP were inversely associated with GFR, while calcium, 25(OH)D and $1,25(OH)_2D$ were positively associated with GFR (12). The association between vitamin D insufficiency and reduced GFR extends beyond its involvement in $1,25(OH)_2D$ activation. The prevalence of low 25(OH)D has been found to increase from 71% to 83% between stage 3 and 4 CKD (5). Although concentrations of phosphaturic hormones, such as PTH and fibroblast growth factor-23 (FGF-23), were similar in both CKD stages, multivariate analysis controlling for phosphorus and GFR revealed a significant independent association for low FGF-23 and diabetic nephropathy ($p<0.001$) (12). Low FGF-23 may be caused by reduced osteocyte density and/or function in diabetic nephropathy as FGF-23 has a short half-life (46-58 minutes), indicating reduced production (12). Furthermore, proteinuria ≥ 3+ was more common in diabetic nephropathy and was significantly associated with declining GFR ($p<0.0001$) (12). Although 25(OH)D can be lost with VDBP in proteinuria, low 25(OH)D remained significantly associated with diabetic nephropathy after controlling for proteinuria ($p<0.001$) (12). In some clinical practices, differences in dietary protein restriction exist early in CKD for those with and without diabetes and should be considered for potential confounding of results, particularly those pertaining to phosphorus (12).

1.4.1 *Assessing Bone Health in Diabetic Nephropathy*

There are two kinds of bone: 1) cortical bone, which accounts for 80% of skeletal mass and is made of densely compacted tissue forming the outer shell of bones and provides protection and supports locomotion; and 2) trabecular bone, which accounts for 20% of skeletal mass and 80% of bone surface, located at the ends of long-bones and inner surface of flat short and irregular bones (e.g. vertebrae) (25). Trabecular bone has an increased turnover rate compared to cortical bone and acts as reservoir for calcium and phosphorus in mineral homeostasis; cortical bone is only involved in severe/prolonged mineral deficits (25). Bone histology (double labelled tetracycline) is the gold standard for assessing bone quantity and quantity, however this is highly invasive and expensive (25,53). Therefore Dual-energy X-ray Absorptiometry (DXA) is favored as a less invasive gold standard for measuring bone mineral density (BMD; g/cm^2) (25,53). DXA BMD values provide a static measure of bone health (typically focused on the lumbar spine, total hip and femoral neck), and are generally measured no more than once per year (25,54,55).This is a result of the risk-to-benefit analysis of radiation exposure vs. expected changes in BMD, which are unlikely to exceed 3% (considered to reflect real biological changes) within 1-2 years (25,54,55). Conversely, bone turnover markers are the dynamic reflection of bone remodeling (25). Careful interpretation is required due to the vast intra- and inter-individual variability of bone turnover markers based on time of day, menstrual cycle, urine vs. serum sample, fasting vs. fed, age and season (25). Bone remodelling is a balance of resorption and formation which renews approximately 10% of the skeleton per year (25% of trabecular mass vs. 3% of cortical) (25). Bone resorption (break down) is regulated by osteoclasts and can be measured by bone turnover markers such as N-telopeptide of type 1 collagen (NTx) (25). Bone formation is regulated by osteoblasts and can be measured by serum osteocalcin or bone-specific alkaline phosphatase (BAP) (25). However, other biochemical parameters, including phosphaturic hormones (e.g. PTH and FGF-23), can also be useful in evaluating bone health.

PTH is often used as a marker of bone health and is preferred over serum calcium and phosphorus aberrations as PTH changes become evident sooner at a GFR of $60mL/min/1.73m^2$ (stage 3 CKD) (20). However, serum calcitriol concentrations begin to

decline as early as stage 2 CKD, and serum FGF-23 concentrations increase before PTH concentrations (5). Elevated FGF-23 suppresses 1α-hydroxylase activity and expression of PTH mRNA, thus delaying the serum increase in PTH (5). FGF-23 may also suppress bone turnover by impairing osteoblast differentiation and maturation of the bone matrix (5). These hematological changes can begin very early so that mild forms of metabolic bone disease can be observed as early as stage 2 CKD (20). The role of FGF-23 in the bone-kidney axis makes it a good marker to observe the relationship between vitamin D status and bone health in diabetic nephropathy and may be a superior biomarker over PTH, which has an increased lag time and propensity for bone resistance in CKD (53,56,57).

1.5 Vitamin D Supplementation in Diabetic Nephropathy

In healthy adults, 75nmol/L is believed by most researchers and clinicians to be a sufficient 25(OH)D concentration for bone health, however great disagreement exists regarding values for deficiency which range from 25-50nmol/L (4,17,18,20,21,24,34,36). It is unknown what level of vitamin D supplementation will ameliorate or improve suboptimal vitamin D status in patients with diabetic nephropathy or contribute to improved bone health, particularly for those living in northern communities (21,58). A recent study in patients with stage 3-4 CKD (primarily a result of diabetes) in northern Alberta demonstrated that daily oral supplementation of vitamin D_3 (25mcg/d or 1,000 IU/d) for 3 months resulted in a mean increase in serum 25(OH)D of 25nmol/L, to a level of 67±26nmol/L post-supplementation (21). Although these increases in serum vitamin D levels appear promising, they were not accompanied by any significant changes in serum levels of PTH (15.88±8.99pmol/L vs. 14.30±7.94pmol/L, p=0.12), which is of particular importance to overall bone health (21,58). Hyperparathyroidism causes cardiovascular, immunological, haematological and metabolic changes, which contribute to the milieu responsible for impaired bone health (e.g. inflammation and calcium homeostasis) (5). Prolonged hyperparathyroidism can lead to calcitriol resistance of the parathyroid glands, thus making it difficult for supplemental vitamin D to exert a positive effect on bones (15). PTH increases when serum 25(OH)D is <75nmol/L, however vitamin D supplementation of 50-125mcg/d (2,000-5,000IU/d) may maintain 25(OH)D at 75-100nmol/L, depending on

age and baseline 25(OH)D status (21,34,59). Studies in CKD have shown that serum 25(OH)D ≥100nmol/L is needed to reduce serum PTH to <7.15pmol/L; a level that is needed to optimize bone health (21,33). Prior to initiating vitamin D supplementation and every 3 months thereafter, serum phosphorus and corrected calcium should be measured and medications adjusted or supplementation discontinued if corrected calcium is >2.75mmol/L, phosphorus is >1.76mmol/L, and CaP product is >55mg^2/dL^2 (5,20,48).

1.5.1 *Form of Vitamin D Used in Supplementation*

There is controversy regarding what form of vitamin D should be supplemented particularly in those with CKD, such as ergocalciferol (D_2), cholecalciferol (D_3) or vitamin D receptor activating analogs (VDRa; e.g. calcitriol, paricalcitol, doxercalciferol), and their associated risks for toxicity (5,24,44,60,61). In 2003, the Kidney Disease Outcomes Quality Initiative (K/DOQI) released clinical practice guidelines for bone metabolism and disease in CKD due to the growing evidence and concern regarding aberrations in mineral metabolism in CKD and its detrimental implications not only on bone health, but also on soft tissue calcification (44). Based on available knowledge and expert opinion at the time, the K/DOQI recommended supplementation with vitamin D_2 or vitamin D sterols (e.g. VDRa) based on an algorithm of serum PTH, calcium, phosphorus and 25(OH)D concentrations (44). For example, they suggested initiating vitamin D_2 supplementation in stage 3 CKD when 25(OH)D was 12-37nmol/L using 1,250mcg/week (50,000IU/week) for 4 weeks, then continuing with 1,250mcg/month (50,000IU/m) (44). If 25(OH)D was 40-75nmol/L, then 1,250mcg/m (50,000IU/m) of supplemental vitamin D_2 was recommended (44). There is no clear reason for why the recommendation to supplement with vitamin D_2 vs. D_3 was made. Vitamin D_2 has traditionally been the form used in pharmaceutical preparations, however vitamin D_2 is not necessarily believed to be any more efficient or effective than vitamin D_3 for treating hypovitaminosis D in CKD (30,36,44). Many vitamin D experts believe that vitamin D_3 is superior to vitamin D_2 as this is the form produced naturally through cutaneous synthesis, and for the multitude of reasons mentioned above (see *Dermal and Oral Sources of Vitamin D*) (27,30,47).

The primary indication for supplementation with a VDRa, such as calcitriol (0.25 mcg/d), in CKD is hyperparathyroidism in the presence of optimal 25(OH)D concentrations (>75nmol/L), where it is provided in effort to reduce serum PTH and calcium concentrations ((5,44,49). Of note, not all VDRa behave the same; they have differential effects on vascular calcification, mineral metabolism and bone resorption (49). VDRa supplementation should not be used in individuals with rapidly declining kidney function, poor medication adherence, hypercalcemia (>2.37mmol/L) or hyperphosphatemia (>1.49mmol/L) (5,44). Supplementation with VDRa requires especially careful monitoring as it is already biologically active (vs. vitamin D_2 or D_3 which require further hydroxylation for biological activity), and presents the greatest risk for vitamin D toxicity by direct (e.g. VDR mediated cellular functions) and indirect (e.g. calcium, phosphorus, PTH) effects (49,50). When choosing the form of supplemental vitamin D in CKD, careful attention should be paid to biochemical profile of the individual, and changes to therapy made accordingly (44).

1.5.2 *Efficacy of Vitamin D Supplementation*

Clinical trials examining vitamin D supplementation in health and in disease are summarized in ***Appendix 1, Section 1, Table A1.1.*** The efficacy of vitamin D supplementation for treatment of suboptimal vitamin D status depends on numerous variables, including severity of vitamin D insufficiency/deficiency, age, concurrent disease, polypharmacy, medication interactions, individual coping skills and health related QoL, and compliance/adherence.

The presence of multiple chronic conditions, each with its own required therapy, is associated with reduced adherence to therapeutic calcium and/or vitamin D supplementation; with each additional chronic disease adherence is reduced by 20% (26). Compliance with therapies for bone health (like other asymptomatic conditions) is a major challenge (24,26,62). Despite its impact on functional ability and independence, only 50-69% of individuals prescribed osteoporosis medications (e.g. bisphosphonates, vitamin D, calcium) comply to them regularly (e.g. take 80% of the time), and only 25-35% are compliant for more than one year (24,26,62). The socioeconomic impact of metabolic bone

disease is significant, particularly in those with chronic disease (25). Considering the increased risk of fracture, reduced QoL and increased mortality that accompanies bone disease, promoting awareness and screening for risk factors, and encouraging therapy adherence is critical in this population.

Adherence is believed to improve when patients are provided adequate information on their condition and on the rationale for their medication/supplement; when instructions are provided verbally and in writing; when frequency of delivery is reduced; and when they are informed of their progress (e.g. results of DXA scans and bone turnover markers) (26,62). This suggests that current modes of vitamin D supplementation, particularly low dose daily administration (15-25mcg/d or 600-1,000IU/d), may be ineffective at optimizing vitamin D status. Higher daily doses (>25mcg/d or >1,000IU/d) or the use of high dose, less frequent modes of administration (monthly vs. daily) need to be explored to ensure adequacy of overall vitamin D status, particularly in those populations at high risk for vitamin D insufficiency and suboptimal bone health (e.g. diabetic nephropathy).

1.6 Conclusion

Suboptimal vitamin D status is a critical concern for individuals with chronic diabetes and kidney disease. Not only is vitamin D associated with the development of these chronic diseases, it has also been associated with their progression. Individuals with diabetic nephropathy are at increased risk for suboptimal vitamin D status, therefore increasing their risk for poor BMD and fragility fractures, which has a significant impact on morbidity and mortality. There is a great deal of controversy regarding vitamin D supplementation and serum 25(OH)D concentrations required for bone health and other health benefits, particularly in individuals with diabetic nephropathy and those living in northern climates. Adherence to supplementation strategies continues to be a challenge in this population for myriad of reasons. Development of evidence-based clinical practice guidelines for promoting optimal vitamin D status and preventing metabolic bone disease are needed and warrant prospective clinical research in this area.

CHAPTER 2: RATIONALE, HYPOTHESES AND OBJECTIVES

2.1 Rationale

Suboptimal vitamin D status (25(OH)D <75nmol/L) is associated with the development and progression of both diabetes and chronic kidney disease (CKD) (11). Within the general North American population 16-52% have suboptimal vitamin D status; the prevalence of vitamin D insufficiency increases to 86% in the diabetic population and those with concurrent kidney disease are 1.78-fold more likely to be vitamin D deficient (4,18,19). Patients living in northern communities such as Alberta are at particular risk for vitamin D insufficiency due to limited sunlight exposure, further increasing their risk for low bone mineral density (BMD) and fragility fractures (18,21-23,58,63,64). Canadian data indicate that 93% of patients with stage 3-4 CKD (primarily caused by diabetes) have insufficient vitamin D status (<75nmol/L) while 41% are deficient (<37.5nmol/L) (21). By the time CKD patients reach dialysis, approximately 75% have metabolic bone disease (20).

Unlike other nutrients, recommendations for vitamin D are not based on food sources as there are very few dietary options available (e.g. fish, liver, fortified dairy products), but rather are designed to compensate for a deficiency of sunlight (28,29). This places the individual living in northern climates at particular risk for inadequate vitamin D status, especially in the winter months when sunlight exposure is unlikely to contribute to overall vitamin D status. Most evidence suggests that when vitamin D requirements are met, it is with a combination of dietary vitamin D and a supplement, as it is uncommon for Canadians to meet their vitamin D needs by diet alone (65-67). Patients with diabetic nephropathy are at increased risk for poor dietary intake of vitamin D due to restrictions on vitamin D rich foods/beverages (e.g. dairy based products) as these products also have a high carbohydrate, phosphorus and/or potassium content.

It is unknown what level of vitamin D supplementation will ameliorate or improve suboptimal vitamin D status in patients with diabetic nephropathy or contribute to improved bone health, particularly for those living in northern Alberta (21,58,63). A recent study in patients with stage 3-4 CKD (primarily the result of diabetes) in northern Alberta demonstrated that daily oral supplementation of vitamin D_3 (25mcg/d or 1,000IU/d) for 3 months resulted in a mean increase in serum 25(OH)D of 25nmol/L (67±26nmol/L post-

supplementation), however this was not accompanied by any significant changes in serum levels of parathyroid hormone (PTH; 15.88±8.99pmol/L vs. 14.30±7.94pmol/L, P=0.12), which is of particular importance to overall bone health (21,58,64). Studies in CKD have shown that serum 25(OH)D ≥100nmol/L is needed to reduce serum PTH to <7.15pmol/L; a level that is needed to optimize bone health (68). The suggested vitamin D_3 doses (50mcg/d or 2,000IU/d vs. 1,000mcg/month or 40,000IU/m) in this study are expected to achieve a serum 25(OH)D of 100nmol/L (29,34).

Evolving literature suggests that adherence to daily vitamin D supplementation may be an important factor influencing vitamin D status, and compliance with therapies for bone health (like other asymptomatic conditions) is a major challenge (24,26,62). Chronic diseases, such as poor bone health, as well as suboptimal vitamin D status have been associated with reduced quality of life (QoL) (7,13,62,69). Yet despite its impact on functional ability and independence, only 50-69% of individuals prescribed osteoporosis medications (e.g. bisphosphonates, vitamin D, calcium) comply to them regularly (e.g. take 80% of the time), and only 25-35% are compliant for more than one year (24,26,62). This suggests that current modes of vitamin D supplementation, particularly low dose daily administration (<25mcg/d or <1,000IU/d), may be ineffective at optimizing vitamin D status. Higher daily doses (>25mcg/d or >1,000IU/d) or the use of high dose, less frequent modes of administration (monthly vs. daily) need to be explored to ensure adequacy of overall vitamin D status, particularly in those populations at high risk for vitamin D insufficiency and suboptimal bone health (e.g. diabetic nephropathy).

This study will contribute valuable information regarding two different approaches to high dose vitamin D_3 supplementation (50mcg daily vs. 1,000mcg monthly) on vitamin D status. More specifically, we examined the impact of these two dosing strategies on changes in serum vitamin D status (25(OH)D and $1,25(OH)_2D$) and changes in bone health (e.g. PTH and markers of bone resorption and formation), and compared adherence between the two dosing strategies. Results from this study will fill a gap in the literature and help contribute to clinical practice guidelines regarding recommendations for vitamin D supplementation in adults with chronic diabetes and kidney disease.

2.2 Hypotheses for RCT

1. Vitamin D_3 supplementation (50mcg/d and 1,000mcg/m) for six months will result in significantly improved overall vitamin D status and improved markers of bone health in adult patients with diabetic nephropathy. Serum 25(OH)D will increase by a minimum of 25nmol/L or to an average concentration of 100nmol/L. Markers of bone resorption will decrease and markers of bone formation will increase after six months of vitamin D_3 supplementation when compared to baseline levels.
2. Monthly dosing of vitamin D_3 (1,000mcg/m) over six months will result in improved patient adherence and satisfaction with vitamin D_3 supplementation when compared to daily dosing of vitamin D_3 (50mcg/d). This will improve vitamin D status and bone health parameters, which will result in an increased quality of life.

The focus of this thesis will be on the preliminary findings pertaining to the first 63 patients enrolled into the Randomized Controlled Trial (RCT). Results and discussion will be specifically related to hypothesis 1 and objectives 1 and 2a only.

2.3 Overall Objectives for RCT

The objectives of this RCT are two-fold:

1. Examine the impact of two approaches to oral high dose vitamin D_3 supplementation (50mcg/d vs. 1,000mcg/m for six months) on overall vitamin D status (25(OH)D and $1,25(OH)_2D$) and markers of bone turnover (BAP, osteocalcin, NTx and FGF-23) in adult patients with diabetic nephropathy. Interrelationships between BMD and vitamin D status prior to supplementation in adults with diabetic nephropathy will also be examined.

2a. Examine daily vs. monthly vitamin D_3 supplementation strategies in regards to adherence in adults with diabetic nephropathy.

2b. Examine daily vs. monthly vitamin D_3 supplementation strategies in regards to patient satisfaction with the supplementation strategies and quality of life in adult patients with diabetic nephropathy.

CHAPTER 3: METHODS

3.1 Methods/Design

Study Design

This randomized, controlled, open-label pilot study compared the effectiveness of two vitamin D_3 dosing strategies (monthly vs. daily) on vitamin D status and markers of bone health in adults with diabetes and nephropathy over a 6 month period. Participants acted as their own controls. We block-randomized participants (n=30/block; http://www.randomizer.org) to one of the two vitamin D_3 strategies: once monthly (1,000mcg/m; n=60) or once daily (50mcg/d; n=60) vitamin D_3 supplementation. Both vitamin D supplements contain vitamin D_3 (cholecalciferol) in gel capsule form; Jamieson Natural Sources® Vitamin D 25mcg Softgel (NPN 80017530), and EURO-Pharm International Canada Inc.® EURO D 250mcg (DIN 02253178). The two dosing regimens lasted for 6 months; 2 capsules of 25mcg vitamin D_3 daily (total dose = 50mcg/d), or 4 capsules of 250mcg vitamin D_3 at the end of each month (total dose = 1,000mcg/m). The daily dose was selected based on findings that although patients with stage 3-4 CKD in northern Alberta who were supplemented with 25mcg/d vitamin D_3 had increased serum 25(OH)D levels (by approximately 25nmol/L), there was no significant change in serum PTH suggesting a higher dose is required to optimize bone health (21,58). Thus a daily dose of 50mcg of vitamin D_3 was chosen. A monthly dose of 1,000mcg vitamin D_3 was chosen to achieve equivalent supplementation to daily dosing assuming an adherence rate of 69%, and with the goal of obtaining a serum 25(OH)D concentration of 100nmol/L (24). Severe reactions (e.g. toxicity) were unlikely to occur with the supplementation doses in this trial, particularly as no adverse effects have been observed at much larger doses (e.g. 1,250mcg/d for 10 days) (35,48,73). (see *Safety Variables and Analysis* section below)

This study was approved by the Human Research Ethics Board at the University of Alberta, has received a “No Objection Letter” from Health Canada, and is a registered clinical trial (NCT01476501). The study was monitored by a Drug and Safety Monitoring Board (DSMB) with annual safety reports submitted as per Health Canada protocol.

Participants

Patients were recruited from Northern Alberta Renal Program (NARP) clinics at Alberta Health Services (AHS) in Edmonton, Alberta. This is a multidisciplinary program (endocrinologists, nephrologists, registered nurses (RN), registered dietitians (RD), pharmacists and social workers) that provides care to over 1,500 patients with diabetic nephropathy in northern Alberta. Sixty adults with diabetes and nephropathy per vitamin D_3 supplementation group will be recruited into this RCT (n=120); **this thesis focuses on the first half of the participants who enrolled in and completed the RCT (e.g. n=30 per supplement strategy)**. Potential participants were approached by a member of the clinical team (e.g. RD or RN) and asked if a research team member could discuss this study with them. If verbal consent was provided, then a research team member would contact the patient, explain the study to them and determine their eligibility for participation in this RCT; if eligible and agreed by the patient then informed consent was signed and the baseline study appointment was booked. Subsequent study visits (e.g. 3 month and 6 month follow-up appointments) were booked via telephone calls made approximately 2 months later to follow-up with the participants and address any questions or concerns they may have had about their supplement strategy. Patient eligibility for this RCT was determined upon information available in the medical chart at time of screening. Inclusion /exclusion criteria are as follows:

Inclusion Criteria

1) Adult (18-80 years) patients diagnosed with diabetes and stage 1-4 CKD (Glomerular Filtration Rate (GFR) ≥90-15 mL/min/1.73m^2) (52).

Exclusion Criteria

1) Patients with co-morbid conditions known to affect vitamin D metabolism including gastrointestinal, liver, rheumatoid or bone disorders (e.g. hyperthyroidism, untreated celiac disease, cancer, Paget's disease, sarcoidosis, malabsorption). Individuals with severe, permanent vision impairment were excluded as this could preclude them from reading supplement labels accurately and safely. Pregnant women were excluded as Dual-energy X-ray Absorptiometry

(DXA) scans are not recommended during pregnancy. Patients weighing >136kg were excluded as the DXA table could not accommodate this weight.

2) Patients on drug therapy known to interfere with vitamin D (e.g. oral glucocorticoids, cholestyramine, colestipol, mineral oil, Orlistat, digoxin).

3) Patients with stage 5 CKD (GFR <15mL/min/1.73m^2), receiving dialysis or on a kidney transplant list.

4) Patients with pre-existing hypercalcemia (>2.75mmol/L), hyperphosphatemia (>2.0mmol/L), severe secondary hyperparathyroidism (PTH >600pg/mL), and/or serum 25(OH)D >200nmol/L.

5) Patients with serum 25(OH)D <37.5nmol/L to control for correction of vitamin D deficiency (21).

Sample Size

The sample size for the entire RCT (n=120) will enable us to detect a mean difference of 25nmol/L in serum 25(OH)D from baseline levels in each group (α=0.05 and β=0.8) with an additional 15% to account for potential subject attrition (21,58). The goal sample size for this thesis was recruitment and completion of 60 participants. At the time of thesis completion, a total of 63 participants were recruited into the RCT. Another graduate student is currently responsible for the recruitment and completion of the remaining 57 participants required for this RCT. Recent evidence has shown that a mean increase of 25nmol/L 25(OH)D with 25mcg/d supplemental vitamin D_3 was insufficient to promote 25(OH)D in excess of 100nmol/L; the concentration needed to promote reductions in serum PTH towards normal, thus having a beneficial impact on markers of bone health (21,33). Therefore, we chose to increase the vitamin D_3 dose to ensure serum 25(OH)D would increase by 25-50nmol/L.

Research Plan for Data Collection

Assessment of vitamin D status, bone health and lifestyle factors (diet, physical activity, sunlight exposure, QoL) was performed during study visits at the Clinical Research Unit (CRU) at the University of Alberta at baseline, 3 and 6 months post study enrolment

(*Figure 3.1*). Participants were given a 3 month supply of their respective vitamin D_3 supplementation strategy at baseline and again at the 3 month study visit. They were asked to return their vitamin D_3 vials/pill containers to the study investigators at the 3 and 6 month study visits. At baseline, information was collected on patient demographics and anthropometrics, including: age, gender, ethnicity, medications/supplements, insulin regime, height, weight (Health o meter Professional model 597KL, Pelstar LLC, Alsip, IL, USA) and BMI. Changes in these variables between appointments were documented (e.g. medications, height, weight). Bone mineral density (BMD) was measured at baseline using DXA; a validated tool to assess BMD (General Electric LUNAR Prodigy, version 10.5, Madison, WI, USA). Whole body scans as well as site specific scans of the lumbar spine (L1-L4) and left total hip (including femoral neck) were conducted. The precision error, expressed as a percentage coefficient of variation (%CV), for the Lunar DXA located in the CRU are as follows: whole body BMD 0.7%, lumbar spine 0.9% and total hip 1.2%.

Lifestyle factors were assessed at baseline, 3 and 6 months using validated tools (46,73-78). These included: 1) 3-day food records to assess vitamin D and calcium intake and other dietary factors known to influence vitamin D and bone health (phosphorus, carbohydrates, protein, caffeine). Dietary intake was analyzed using the Food Processor Database (Food Processor SQL, v.10.8, ESHA Research, Salem, Oregon, USA); 2) Weight-bearing physical activity records; 3) Sunlight exposure questionnaires; 4) Health related QoL (SF-36) questionnaire; and 5) Adherence and acceptance survey (46,73-78). Adherence to vitamin D_3 supplementation was also be assessed by pill counting at 3 and 6 months and in monthly follow-up calls by the research team.

Of the abovementioned lifestyle factors, this Thesis focuses on dietary intake of vitamin D and calcium at baseline, 3 and 6 months, and on vitamin D_3 supplement adherence as determined by pill counts at 3 and 6 months. The remaining secondary outcome variables are the focus of subsequent analysis and will be presented in the final results of the overall RCT, not in this current Thesis.

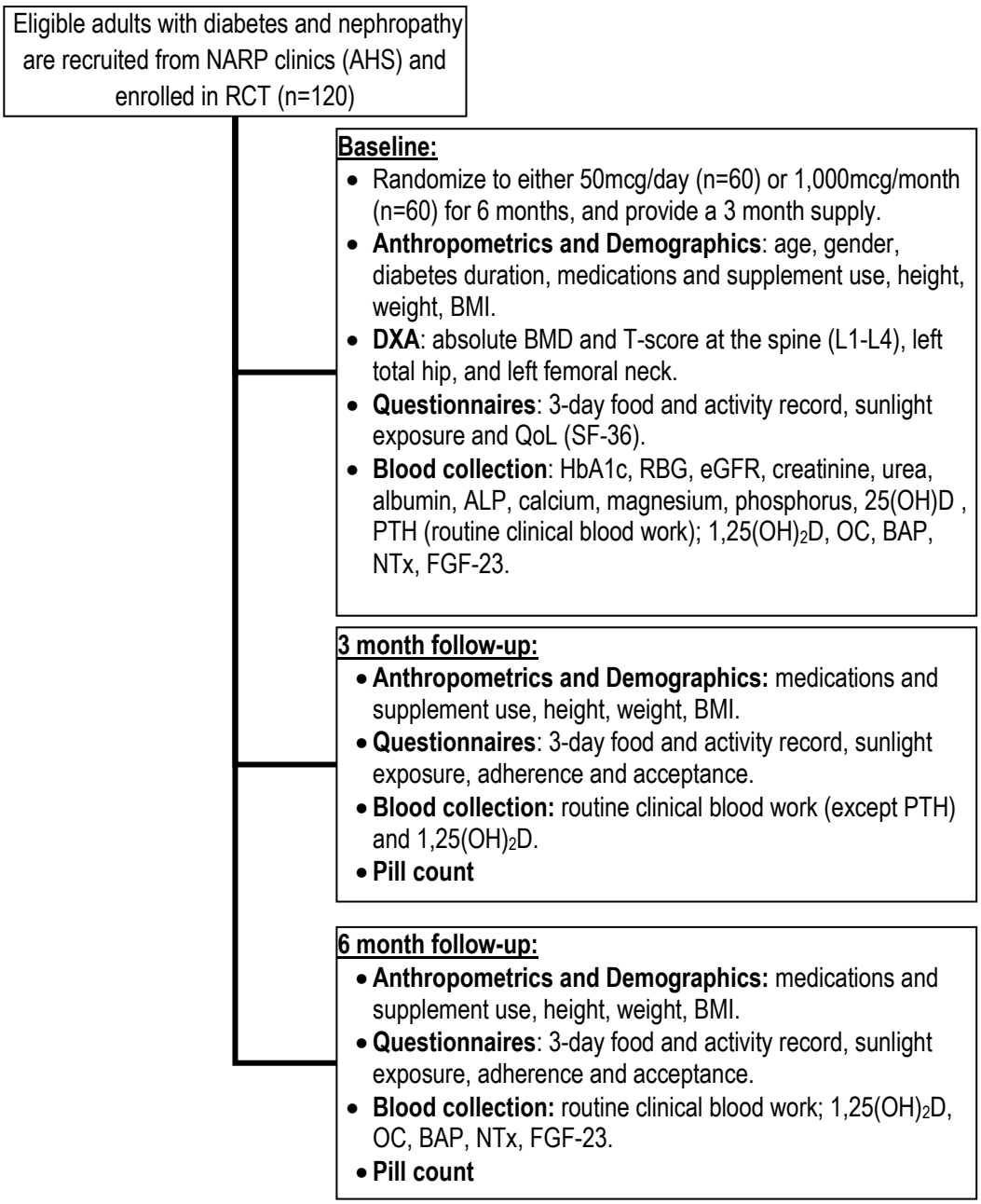

Figure 3.1. Study Design[1,2]

[1] Sample size shown for entire RCT. This Thesis focuses on first half of sample size (n=63).
[2] Abbreviations: Northern Alberta Renal Program (NARP); Alberta Health Services (AHS); randomized controlled trial (RCT); micrograms (mcg); Body mass index (BMI); Dual-energy X-ray absorptiometry (DXA); Bone mineral density (BMD); Hemoglobin A1c (HbA1c); random blood glucose (RBG); estimated glomerular filtration rate (eGFR); 25-hydroxy vitamin D (25(OH)D); 1,25-dihydroxy vitamin D ($1,25(OH)_2D$); alkaline phosphatase (ALP); osteocalcin (OC); bone-specific alkaline phosphatase (BAP); N-telopeptide of type 1 collagen (NTx); fibroblast growth factor-23 (FGF-23).

Laboratory Investigations

To avoid risk of hypoglycemic events due to variations in appointment availability (e.g. insulin regimen incompatibility with fasting and later appointment times), random serum/plasma samples for measurement of routine clinical blood work and study blood work were collected. Blood samples were collected onsite at each research visit by a trained phlebotomist using validated techniques and tubes (SST gel for serum, and lithium heparin PST gel and EDTA for plasma). Once collected, blood samples were immediately held at 2-8°C until processing by the research team or provincial laboratory system. Patients routinely receive clinical blood work to assess their glycemic control, kidney function and overall health, including: estimated GFR (eGFR), fasting/random blood glucose (FBG/RBG), urea, creatinine, hemoglobin A1c (HbA1c), calcium, albumin, phosphorus, magnesium, 25(OH)D and PTH. These variables were collected at all 3 study visits (except for PTH) along with $1,25(OH)_2D$ status. Serum PTH and bone turnover markers were collected at baseline and 6 month follow-up study visits. Routine clinical blood work, $1,25(OH)_2D$ and PTH were measured by validated, specific and sensitive methodologies used by the provincial laboratory system. The provincial laboratory system reports eGFR up to 60mL/min/1.73m^2; therefore eGFR was also calculated by the research team using validated online equations (Modification of Diet in Renal Disease (MDRD) study group and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) group) provided by K/DOQI (http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm).

Bone turnover markers were analyzed by the research team using standardized commercial ELISA kits. After blood collection, EDTA plasma and clotted serum samples (e.g. SST gel) were kept for approximately 30-60 minutes at 2-8°C and then centrifuged at 2,500 RPM at 4°C for 10minutes (CR4.22 centrifuge, Jouan, Winchester, VA, USA). Recovered serum and plasma were aliquoted into clean micro-tubes according to volumes required for each specific assay to be tested in duplicate. Samples were then stored frozen at -80°C until ELISA testing, and prepared according to the manufacturer instructions for each commercial ELISA kit: serum intact osteocalcin (OC; MicroVue Osteocalcin EIA Kit, Quidel, San Diego, CA, USA), serum bone-specific alkaline phosphatase (BAP; MicroVue BAP EIA Kit, Quidel, San Diego, CA, USA), serum N-telopeptide of type 1 collagen (NTx;

Osteomark NTx Serum, Wampole Laboratories, Princeton, NJ, USA), and plasma intact fibroblast growth factor-23 (FGF-23; Human Intact FGF-23 ELISA Kit, Immunotopics Inc, San Clemente, CA, USA). The intra-assay (a) and inter-assay (b) coefficient of variance (CV) for these commercial kits were as follows: OC a) 4.8-10.0%, b) 4.8-9.8%; BAP a) 3.9-5.8%, b) 5.0-7.6%; NTx a) 4.6-13.99%, b) 6.9-13.99%; and FGF-23 a) 2.6-4.4%, b) 6.1-6.5%.

***Outcome Measurements** (**Figure 3.1**)*

Primary outcome variables:

• **Serum 25(OH)D and 1,25(OH)2D:** Serum 25(OH)D is considered the most reliable measure of vitamin D status as it accounts for cutaneous and dietary sources of vitamin D (32); we expected both supplementation strategies to increase serum 25(OH)D by *at least* 25-50nmol/L or to a mean serum concentration of approximately 100nmol/L. $1,25(OH)_2D$ was measured to determine concentration of active vitamin D in participants and explore the relationship of active vitamin D with 25(OH)D levels and with bone health. Vitamin D levels were measured by validated, specific and sensitive methodologies used by the provincial laboratory system. The ratio of $1,25(OH)_2D$ (product) to 25(OH)D (precursor) was calculated as a surrogate measure of 1α-hydroxylase activity (product to precursor ratio).

• **Bone Health:** Bone health was assessed by measurement of BMD and bone turnover markers. BMD was measured using DXA (non-invasive gold standard) to characterize bone health of participants at baseline (General Electric LUNAR Prodigy, version 10.5, Madison, WI, USA; %CV: whole body BMD 0.7%, lumbar spine 0.9% and total hip 1.2%). Bone turnover markers were measured at baseline and at 6 months; bone resorption: N-telopeptide type 1 collagen (NTx); bone-kidney axis: fibroblast growth factor-23 (FGF-23); and bone formation: bone-specific alkaline phosphatase (BAP) and osteocalcin (OC) (78). Serum PTH was measured at baseline and 6 months to assess how serum PTH concentrations changed with our vitamin D supplementation strategies and the association between this and bone turnover markers.

Secondary outcome variables in this Thesis:

• **Participant adherence:** Adherence to the dosing regimens was assessed by counting the remaining vitamin D_3 capsules in the returned vials (at 3 and 6 month visits).

• **Dietary intake:** 3-day food records (2 weekdays, 1 weekend day), a validated tool for diet analysis, were completed by participants at baseline, 3 and 6 months (74,75). Responses were verified by a RD on the research team and analyzed for macro- and micronutrient intake (e.g. calcium, vitamin D, phosphorus, carbohydrates, protein and caffeine) using Food Processor (SQL v10.8 ESHA Research). In cases where a 3-day food record could not be completed, a 24-hr recall of a "typical day" was performed by the RD.

• **Seasonal affects:** Seasonal variations were accounted for and assessed in terms of potential impacts on vitamin D status.

Safety Variables and Analysis

Severe reactions were unlikely to occur in relation to the supplementation doses in this trial, particularly as no adverse effects have been observed at much larger doses (24,35,48). Prolonged daily doses of 1,250-2,500mcg (50,000-100,000IU) vitamin D can lead to hypervitaminosis D, which can cause hypercalcemia (48). Acute toxicity is rare, yet chronic excessive intake may cause hypercalcemic symptoms such as "anorexia, nausea, vomiting, fatigue, confusion, headache, weakness, renal impairment, arrhythmias, hypertension, calcification of soft tissue and hyperphosphatemia" (48). Adverse events with vitamin D_3 supplementation are unlikely to occur in adults with diabetes and nephropathy as this analogue is believed to be much safer than supplementation with active vitamin D analogues or vitamin D_2 (5,27,30,35). Vitamin D binding protein has a stronger affinity for vitamin D_3 than D_2, which may increase toxicity risk as a result of more biologically available $25(OH)D_2$ and $1,25(OH)_2D_2$ (30). Supplementation with active vitamin D analogues may be useful for individuals with severely diminished renal function and capacity for active hydroxylation, and is primarily used to treat severe hyperparathyroidism. However, providing already active vitamin D results in bypassing the homeostatic controls that are in place to prevent vitamin D toxicity (e.g. reducing 25-hydroxylation and

increasing catabolism and excretion of 25(OH)D via bile), which in turn can increase risk for vitamin D toxicity (30,33). Serum phosphate (>2.0mmol/L), calcium-phosphorous product (CaP; >4.4mmol^2/L^2) and magnesium (>1.0mmol/L) were all monitored as part of routine clinical care for variation from healthy ranges (48).

A data safety monitoring board (DSMB) was assembled consisting of experts in nutrition and vitamin metabolism, diabetic nephropathy, and biomedical statistics. They received annual reports regarding patient recruitment, interim analysis and reports regarding any safety issues/adverse events. All adverse events (AE) were documented. AE were identified as related to the study protocol (e.g. anything directly related to the vitamin D supplementation intervention, blood collection or other examinations in the study protocol) or not related to the study protocol. Serious adverse events (SAE) are significant health/safety issues that are not expected to occur as a result of this study protocol (e.g. acute renal failure, death). The responsible physician, University HREB, and DSMB were notified of all AE; in the case of a SAE, the former were all notified as well as Health Canada.

Concomitant Medication: Participants were asked to continue taking their normal medications as advised by their physician, e.g. insulin, oral hypoglycaemic agents, anti-hypertensives, statins, diuretics, phosphate binders, potassium lowering agents, anemia treatment, Replavite, and/or proton-pump inhibitors. Concomitant medications were reviewed at each visit to ensure no contra-indicated medications were being used. Patients could not participate in this study if they were on medications listed in point 2 of the exclusion criteria. Participants were asked to discontinue all vitamin/mineral supplements containing calcium and/or vitamin D, unless prescribed for therapeutic treatment (e.g. calcium carbonate-containing antacids for treatment of hyperphosphatemia).

Rescue Medication & Risk Management: Serum phosphate (>2.0mmol/L), CaP product (>4.4mmol^2/L^2) and magnesium (>1.0mmol/L) were all monitored as part of routine clinical care for variation from healthy ranges (29). If vitamin D toxicity was to occur, the participant would discontinue taking the vitamin D_3 supplement and appropriate health care providers would be notified. Serum and urine electrolytes, renal function, electrocardiogram, and fluid balance would be monitored and maintained (76). If

necessary, the following measures may have been taken to enhance excretion/metabolism of the vitamin D: IV administration of furosemide; corticosteroid, bisphosphonate or calcitonin therapy; hemo- or peritoneal dialysis (48). If hypersensitivity to vitamin D_3 occurred that included anaphylaxis, then appropriate treatment with epinephrine and ventilation would be provided as needed. Adverse events were reported immediately to the responsible physician or the on-call endocrinologist/nephrologist was notified as per standard clinical care, and also the DSMB and Health Canada were notified. Participants were taken to the University of Alberta Hospital, AHS if they required immediate medical treatment.

Premature Withdrawal: Participants could voluntarily withdraw from the trial at any time without any negative consequences to their clinical care. Any and all reasons for participant drop out were documented. Any individual demonstrating clinical signs and symptoms of vitamin D toxicity and/or an SAE related to the study protocol would: 1) have vitamin D_3 supplementation discontinued, 2) be notified to the responsible physician for treatment of toxicity, 3) be notified to the DSMB and Health Canada, and 4) patient participation in the study would be discontinued.

Statistical Analysis

Participants could withdraw from the study at any time should they so wished. Interim data analysis was performed on the data collected prior to study exit. Data was analyzed on an intention-to-treat basis as well as a per-protocol basis; **however this thesis focused on the results of intent-to-treat analysis due to limited sample size at this point in the RCT**. Analyses were performed using Microsoft Excel 2010 and Statistical Analysis Software (SAS; version 9.3 SAS Institute, Cary, NC, USA). Statistical significance was determined at $p<0.05$. Continuous variables were expressed as mean, median, ranges, and standard error (SE) or standard deviation (SD).

The differences between dosing type (daily vs. monthly) over the intervention period was assessed by repeated measures analysis of variance, followed by a post-hoc pair-wise t-test with Bonferroni corrections to assess for within and between group comparisons. Potential risk factors for the development of vitamin D insufficiency and poor

bone health in adults with diabetic nephropathy include bone turnover, vitamin D intake, vitamin D status (serum levels of 25(OH)D and 1, 25$(OH)_2$D), age, gender, ethnicity, severity of kidney disease, and diabetic management/control.

Continuous and categorical variables (e.g. 25(OH)D, lab parameters, BMD T-scores, adherence percentages) were quantitatively analyzed. Bivariate and univariate analysis was done to assess the potential effect of these variables on vitamin D status and bone health (bone turnover markers). Variables shown to be associated with a poor vitamin D status were assessed using multinomial logistic regression models to assess risk for development of vitamin D deficiency and poor bone health. Regression analysis was also done to assess correlations between biochemical parameters (PTH, 25(OH)D, 1,25$(OH)_2$D, calcium, phosphorus, magnesium, albumin, eGFR, RBG) and bone turnover markers. Analysis of variance was performed to assess for significant differences in vitamin D status and bone turnover markers over the intervention period in both groups. Regression analysis was also performed with serum 25(OH)D and plasma PTH as categorical variables; e.g. 25(OH)D sorted by greater/less than 75nmol/L and plasma PTH sorted by greater/less than 7.15pmol/L, the optimal concentrations for bone health (21,33). Where necessary, vitamin D was adjusted for potential confounding variables (e.g. age, gender, ethnicity, disease severity) using an analysis of co-variance. In the event that supplementation to the daily vitamin D dosing regimen was greater or less than 69%, the variation in compliance was accounted for in an analysis of co-variance. For variables demonstrating skewed distributions a logarithmic transformation was used to normalize the data.

Data Management and Validation

Ongoing, real-time data entry was utilised to improve accuracy. Food records and questionnaires were entered only by individuals trained for entry of food records or questionnaires. Prior to data entry, these individuals underwent ethics training and education related to protecting participant information (confidentiality) and health, including online University of Alberta and National Institute of Health ethics training and testing, criminal record checks, and ensuring up-to-date immunizations. Source data was coded

and kept in a locked filing cabinet within the Clinical Research Unit, University of Alberta. Electronic files were encrypted and kept in a password protected computer according to University of Alberta (Faculty of Medicine) encryption policy (79).

The electronic data was audited in a timely manner to ensure any discrepancies were addressed and that the potential for future discrepancies were reduced. Discrepant results were compared with source records, and amendments were made to the electronic records as necessary. Data audits were conducted by volunteers and graduate students within the Principle Investigator's research group, trained for the specific data items (e.g. questionnaires, food records, biochemical analysis). All data entry was cross verified by one trained volunteer/graduate student, along with the primary graduate students involved in the project.

3.2 MSc candidate's role in RCT

This study was designed by the PI (Dr. Diana Mager, PhD RD) and graduate student (Stephanie Schwindt, RD). This involved all aspects of regulatory and ethics approvals (e.g. Health Canada and the Human Research Ethics Board at the University of Alberta), as well as the operational and administrative approvals for Alberta Health Services. The role of the graduate student also included participation in grant writing (KFOC) as a co-investigator and assisting with vitamin D_3 supplement choice. Establishing research relationships with the clinical teams and study participants and determining the best methods for approaching potential participants in consultation with the clinical teams was another important role of the graduate student (SS). A total of 63 participants were recruited in this first phase of the study by the graduate student; all study visits associated with these first 63 participants were completed by the graduate student (SS). Another important role also included following results of study blood work and communicating results to clinical team members (MD, RD and RN).

The graduate student (SS) also trained all volunteers, other graduate students (PL/MH) involved in phase II of the project, conducted all laboratory analysis of the bone turnover markers related to the first 63 patients enrolled in the study, and performed much of the statistics (under the supervision of the PI) related to the first 63 participants enrolled

in the study. And lastly, the role of the graduate student (SS) also included preliminary dissemination of RCT results in both poster (e.g. Alberta Diabetes Institute Research Day) and oral presentations at a variety of local, national and international conferences (e.g. A.S.P.E.N and Canadian Nutrition Society Annual Meeting) related to: 1) preliminary results of the RCT, and 2) results of other research activity (related to MSc course work).

CHAPTER 4: RESULTS

4.1 Thesis Chapter Objective

This Thesis chapter will present preliminary findings of the first 63 subjects who enrolled in and completed this randomized controlled trial (RCT; including drop-outs), focusing on objectives 1 and 2a of the overall RCT objectives. Refer to chapter 3 for a detailed description of the methods utilized for this study.

Monthly verses Daily Vitamin D_3 Supplementation

For the purposes of the results section, all subjects randomly allocated to daily dosing of vitamin D_3 (50mcg/d) will be referred to as 'daily'. Subject's randomly allocated to monthly dosing of vitamin D_3 (1,000mcg/m) will be referred to as 'monthly'.

4.2 Description of Subjects and Protocol Outcomes

4.2.1 Subject Selection, Recruitment and Participation

Screening, recruitment and participation of subjects can be found in ***Figure 4.1***. All subjects were recruited from the Diabetic Nephropathy Prevention Clinic (DNPC; Northeast Community Health Centre and Grey Nun's Community Hospital sites) and the Renal Insufficiency Clinic (RIC; University of Alberta Hospital and Grey Nun's Community Hospital sites), both part of the Northern Alberta Renal Program (NARP), Alberta Health Services (AHS). A referral criterion for the DNPC is a diagnosis of diabetes as well as hypertension and/or albuminuria, conversely the patient referral criteria for the RIC is a glomerular filtration rate (GFR) ≤30mL/min/1.73m^2. A total of 791 patient charts were screened by members of the research team. Of these, 324 met study criteria and 202 were informed about the study by members of the clinical team (e.g. RN). Only patients who provided prior consent to the health care team were approached by the research team. Of

these, 66 patients (33%) agreed to participate, 63 attended the baseline appointment (n=33 daily, n=30 monthly), 61 attended the 3 month follow-up (n=33 daily, n=28 monthly), and 59 completed all 6 months of the study protocol (n=32 daily, n=27 monthly).

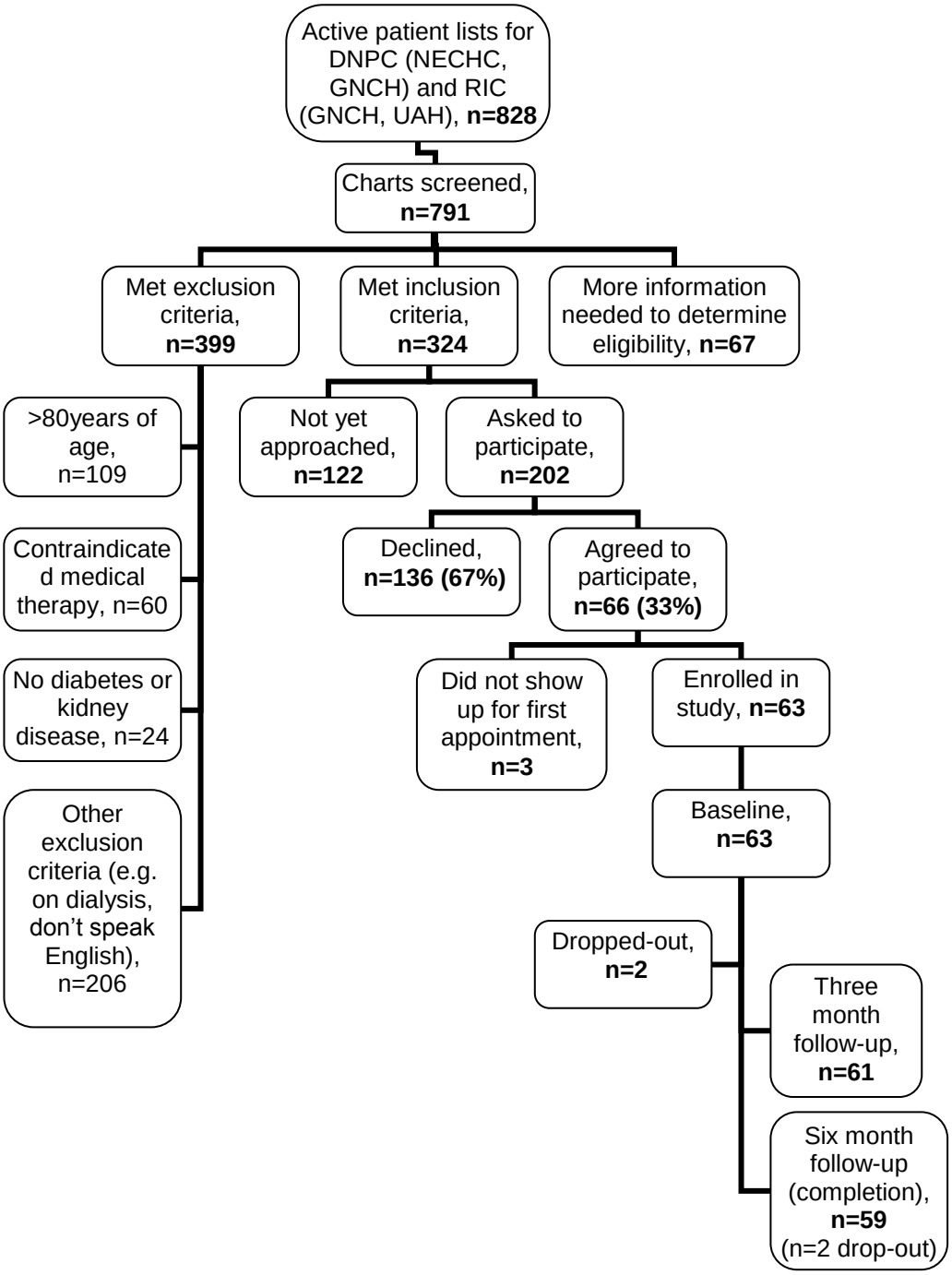

Figure 4.1. Recruitment Flow Chart

4.2.2 Subject Attrition and Safety Outcomes

Of the two subjects who did not complete the 3 month follow-up, one was discontinued from the study during the baseline appointment due to a non-study protocol related adverse event (AE) that occurred during the dual energy x-ray absorptiometry (DXA) scan and the other subject dropped out stating time constraints; both subjects had been randomized into the monthly supplement group. The two subjects who completed the 3 month but not the 6 month follow-up appointments were discontinued from the study due to a non-study protocol related serious AE (SAE; both had fatal cardiovascular accidents); one subject had been randomized into the daily group and one into the monthly group. Following the 6 month follow-up appointment, abnormal lab results collected at the final appointment from one subject (monthly) indicated acute on chronic renal failure; appropriate actions were taken to ensure proper care and treatment (see *Study Protocol: Safety Variables and Analysis*). This SAE was deemed not to be related to study protocol by the qualified investigator, but rather to a change in health status due to coinciding rapid decline in renal function unrelated to vitamin D_3 supplementation (infection). The SAE became apparent upon completion of the last study day (6 months) during examination of pertinent laboratory blood work. As this subject had already completed the entire intervention, they were included in study analysis. All SAEs were notified to the appropriate regulatory agencies, responsible caregiver, and qualified investigator (see *Study Protocol: Safety Variables and Analysis*).

There were a total of ten AEs (including those stated above), and none were related to the study protocol: hypoglycemic events at the appointment (n=1), falls (n=2), serious infection (n=1), calcified mass identified in DXA scan (n=1) at baseline prior to

initiation of RCT vitamin D_3 supplementation, severe hyphophosphatemia (n=1), query panic attack (n=1), acute on chronic renal failure (n=1), and death (n=2).

4.2.3 Compliance to Protocol Supplement Changes: Use of Non-prescribed/ Prescribed Alternative Multivitamin Preparations (not protocol related).

All subjects were asked to discontinue any other supplemental source of vitamin D and/or calcium, unless prescribed by a medical professional (e.g. calcium carbonate for phosphorous binding). Data regarding the use of supplemental vitamin D_3 and the mean dose prior to starting this study are illustrated in ***Table 4.1***. Use of calcium supplementation prior to study enrollment was similar between groups (n=16 daily, n=13 monthly) as were elemental doses taken (Daily: 282±412(0-1500)mg/d vs. Monthly: 228±375(0-1200)mg/d; p=0.51). Those subjects who did not discontinue their personal vitamin D_3 supplement as requested did not vary for baseline characteristics from the rest of the study group, nor did their change in vitamin D status vary significantly from the rest of the study group over the course of the intervention, nor did they experience any adverse sequelae (e.g. n=3/4 were within 1.5 SD and n=1/4 was within 2 SD from mean 25(OH)D percent change).

Baseline-3 Months

Between the baseline and 3 month follow-up appointment, n=1 subject in each the daily (female) and monthly (male) groups were still taking a multivitamin and mineral (MVM) preparation containing vitamin D_3: 10 vs. 2.5mcg/d vitamin D_3, respectively (p=0.93). Of note, the daily subject discontinued taking their MVM 1.5 months after starting the study (removed from blister-pack). During this same baseline to 3 month period, n=10

(daily) vs. n=2 (monthly) subjects continued to take a calcium supplement (Daily: 182±365(0-1500)mcg/d vs. Monthly: 39±189(0-1000)mcg/d; p=0.09).

3-6 Months

Between the 3 and 6 month follow-up appointments, n=1 (daily) subject (20mcg/d) and n=2 (monthly) subjects (2.5-12.5mcg/d) took a MVM containing vitamin D_3 (p=0.98). During this same 3 to 6 month period, n=7 daily vs. n=5 monthly subjects were taking supplemental calcium (Daily: 148±340(0-1500)mg/d vs. Monthly: 48±121(0-500)mg/d; p=0.24). The main reasons subjects indicated for taking a calcium supplement and/or a MVM containing vitamin D_3 and calcium despite being asked to stop (other than the permitted calcium carbonate for binding of phosphorous) were 1) forgetting that they had been asked to not take any additional supplemental vitamin D_3 and/or calcium during the study duration, and 2) suggestions from their family physician to take a MVM for general health. Additional information on supplement/MVM use can be found in ***Appendix 1, Section 2A***.

4.3 Baseline Results: Anthropometric, Demographic, Laboratory and Bone Health Parameters

4.3.1 Anthropometric and Demographic Characteristics:

Baseline clinical characteristics of our study population can be found in ***Table 4.1***. There were no significant differences in anthropometric (age, weight, height, BMI) or demographic (gender, duration of diabetes, season of recruitment, medication and supplement use) variables between subjects in the daily vs. monthly supplementation groups over the 6 month supplementation period (p>0.05). All subjects had type 2

diabetes (T2D), except for two who had type 1 diabetes (T1D); one of each were randomized into the daily and monthly group. Of note, the majority (82% daily, 90% monthly) of the subjects were previously taking vitamin D_3 supplements (daily=26.9±17.8mcg/d, monthly=29.4±15.6mcg/d; p=0.55) as part of their regular clinical care regimen. Only one subject was previously taking a vitamin D_2 supplement (2.5mcg/d) prior to starting this study.

Table 4.1. Baseline Clinical Characteristics [1]

		Daily (n=33)	Monthly (n=30)	P-value [3]
Males, n(%)		19 (58)	20 (67)	0.12
Age, years		64.5 ± 6.9 (46.3-78.0)	63.9 ± 10.3 (31.5-78.9)	0.47
Weight, kg		94.2 ± 21.5 (60.0-135.4)	94.3 ± 18.7 (49.1-135.7)	0.84
Height, cm		167.2 ± 11.6 (146.4-190.4)	167.5 ± 7.9 (151.7-182.9)	0.85
BMI, kg/cm^2		33.5 ± 5.7 (24.4-44.1)	33.7 ± 6.6 (17.3-45.9)	0.92
Diabetes duration, years		14.9 ± 9.1 (3.0-36.0)	15.6 ± 9.6 (2.0-37.0)	0.60
Oral Hypoglycemic Agent use, n(%)		25 (76)	23 (77)	0.93
Insulin use, units/kg		0.50 ± 0.66 (0.00-3.10)	0.40 ± 0.42 (0.00-1.54)	0.48
Medication count, n [2]		12 ± 3 (6-20)	11 ± 4 (4-21)	0.57
Previous Vitamin D_3 Supplement Use, n (%)		26 (79)	27 (90)	0.39
Vitamin D_3 Supplementation, mcg/d		26.9 ± 17.8 (0.0-50.0)	29.4 ± 15.6 (0.0-50.0)	0.55
Season at Baseline, n (%):	Jan-Mar	8 (24)	11 (37)	0.88
	Apr-Jun	15 (45)	12 (40)	
	Jul-Sep	9 (27)	4 (13)	
	Oct-Dec	1 (3)	3 (10)	

[1] Values are mean ± SD (range).

[2] Medication count refers to the number of different prescription medications and supplements (oral, inhalable and injectable) that a subject is taking.

[3] No significant differences between groups.

4.3.2 Routine Clinical Blood Work at Baseline:

Baseline results of routine clinical blood work for markers of glycemic control, renal function, and nutritional and bone health can be found in ***Table 4.2***. There were n=20 subjects with HbA1c ≥7% in both the daily (61%) and monthly (67%) groups. According to eGFR calculations using the CKD-EPI equation (http://www.kidney.org), daily and monthly subjects were categorized into CKD stages at baseline (***Figure 4.2***)

One daily subject had an elevated serum calcium concentration (>2.60mmol/L) while n=2 had a low corrected serum calcium concentration (<2.10mmol/L); there were no abnormal calcium results in the monthly group's lab work at baseline. One monthly subject had a low serum phosphorous concentration (<0.80mmol/L), while n=1 daily and n=1 monthly subject each had an elevated serum phosphorous concentration (>1.45mmol/L). Only n=1 daily subject had an elevated calcium-to-phosphorous product (4.5mmol^2/L^2) specifically related to an elevated serum phosphorous (2.3mmol/L) and low corrected serum calcium (1.96mmol/L) and 25(OH)D (12nmol/L). The elevated serum phosphorous was not evident at time of screening (< 3 months prior to baseline appointment) and was clinically assessed to be related to poor dietary and calcium carbonate adherence and dealt with by the clinical team thusly. No serum calcium or phosphorous concentrations exceeded maximum concentrations (except the phosphorous mentioned above, which occurred after study entry) set for safety in this clinical study (see *Study Protocol: Safety Variables and Analysis*).

Table 4.2. Routine Clinical Blood Work [1,2]

	Daily			Monthly		
	Baseline (n=33)	**3months (n=33)**	**6months (n=32)**	**Baseline (n=30)**	**3months (n=28)**	**6months (n=27)**
HbA1c, %	7.4 ± 1.4 (4.8-11.7)	7.5 ± 1.2 (5.1-11.1)	7.2 ± 1.1 (4.4-9.5) *	7.8 ± 1.7 (5.8-12.5)	8.0 ± 1.8 (5.9-12.9)	8.2 ± 2.0 (5.9-12.5) *
RBG, mmol/L	8.8 ± 3.6 (2.6-17.8)	8.5 ± 3.6 (4.1-18.7)	8.9 ± 3.1 (3.7-15.6)	8.5 ± 3.9 (4.4-23.4) §	9.4 ± 4.0 (2.7-17.6)	10.9 ± 5.1 (4.3-20.8) §
Creatinine, umol/L	137 ± 69 (50-353)	141 ± 68 (50-321)	143 ± 70 (38-294)	136 ± 74 (60-318)	140 ± 80 (68-309)	167 ± 173 (66-932)
Urea, mmol/L	10.9 ± 6.6 (4.0-31.3)	10.6 ± 6.4 (3.1-28.1)	11.0 ± 6.8 (3.4-29.0)	9.3 ± 7.6 (2.6-35.9)	10.0 ± 8.2 (2.6-32.4)	10.9 ± 11.0 (3.1-55.8)
eGFR, ml/min/1.73m^2	54 ± 29 (15-109)	52 ± 28 (16-103)	52 ± 29 (18-109)	56 ± 26 (17-109)	56 ± 25 (17-99)	53 ± 25 (5-104)
Albumin, g/L	41 ± 5 (22-46)	40 ± 5 (20-47)	40 ± 5 (20-45)	42 ± 3 (38-49)	42 ± 3 (37-50)	41 ± 4 (33-48)
Calcium, mmol/L	2.35 ± 0.14 (1.96-2.62)	2.34 ± 0.11 (2.13-2.66)	2.34 ± 0.12 (2.00-2.52)	2.36 ± 0.10 (2.17-2.56)	2.34 ± 0.12 (2.10-2.54)	2.33 ± 0.11 (2.08-2.55)
Phosphorous, mmol/L	1.15 ± 0.27 (0.85-2.30)	1.13 ± 0.24 (0.60-1.84)	1.13 ± 0.20 (0.72-1.69)	1.15 ± 0.20 (0.78-1.59)	1.12 ± 0.24 (0.71-1.75)	1.15 ± 0.35 (0.48-2.54)
Magnesium, mmol/L	0.80 ± 0.15 (0.50-1.17)	0.79 ± 0.15 (0.51-1.22)	0.79 ± 0.14 (0.54-1.14)	0.78 ± 0.09 (0.61-1.07)	0.80 ± 0.09 (0.61-0.97)	0.80 ± 0.14 (0.62-1.33)
ALP, U/L	76 ± 35 (12-179)	75 ± 37 (11-182)	72 ± 32 (12-171)	76 ± 24 (41-151)	79 ± 26 (43-143)	75 26 (42-141)

[1] Values are mean ± SD (range). Values with the same superscripts represent significant differences between vitamin D supplement doses and/or visits ($p<0.05$). * p=0.02, § p=0.02.

[2] Sample size varied within each laboratory parameter analysed so that sample size did not always equal 33 for daily or 30 for monthly.

Abbreviations: Hemoglobin A1c (HbA1c); Random Blood Glucose (RBG); estimated Glomerular Filtration Rate (eGFR; http://www.kidney.org); Alkaline phosphatase (ALP).

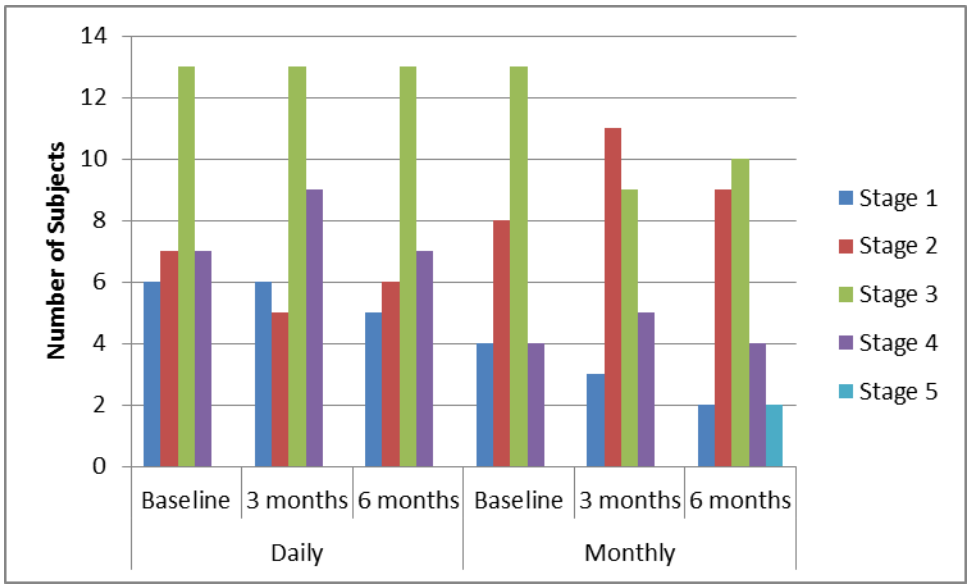

Figure 4.2. Proportion of Subjects with Varying Stages of Chronic Kidney Disease at Baseline, 3 and 6 Months

The number of daily and monthly subjects at baseline, 3 months and 6 months with stage 1 through 5 chronic kidney disease are indicated by the vertical bars.

4.3.3 *Blood Work Related to the Study Protocol:*

Hypomagnesemia (0.54-0.69mmol/L) was equally common in both groups (n=5 daily and monthly), but hypermagnesemia (1.01-1.17mmol/L) was more common in the baseline values of the daily group (n=4 daily vs. n=1 monthly); abnormal results were notified to the clinical care team and treated as per clinical care guidelines. While all baseline serum ALP concentrations where normal in the monthly group, there were n=2 subjects with high values (>130U/L) and n=1 with a low value (<30U/L) in the daily group. Baseline serum PTH concentrations were found to be slightly low (<1.4pmol/L) in n=2 daily vs. n=1 monthly subjects, while elevated PTH (>6.8pmol/L) was observed in n=14 daily vs. n=6 monthly subjects at baseline.

4.3.4 Bone Mineral Density (BMD) as measured by Dual Energy X-ray Absorptiometry (DXA):

Baseline DXA results were available in n=18 daily vs. n=24 monthly subjects (***Table 4.3***). The remaining 21 subjects did not have DXA performed at the baseline appointment because of either 1) having already received a DXA scan within the previous year, or 2) scheduling conflicts between subject availability and DXA technician availability. Low BMD was defined by the lowest T-score measurement between the lumbar spine (L1-L4), left total hip and left femoral neck, and categorized based on gender and menopause status (54). An insufficient sample size precluded the possibility of performing statistical analysis on this data, therefore this data is presented for descriptive purposes only. Abnormal T-scores were observed in n=3 subjects in both the daily (17%) and monthly (13%) groups. In the daily group there were n=3 post-menopausal women with osteopenia, and in the monthly group there were n=2 postmenopausal women with osteopenia and n=1 male (T1D for 29 years) with reduced BMD. There were n=8 daily vs. n=13 monthly subjects with a positive fracture history, however only n=1 constituted a fragility fracture in each the daily and monthly groups. One post-menopausal subject in each the daily and monthly groups were taking a bisphosphonate (>6 months duration); unfortunately BMD data was not measured for either subject.

When treated as a categorical variable (less/greater than 75nmol/L) and/or continuous variable, 25(OH)D did not show any significant interrelationships with BMD ($p>0.05$). No significant relationships were observed in multivariate analysis of BMD and vitamin D status (25(OH)D and $1,25(OH)_2D$).

Table 4.3. Bone Mineral Density (BMD) by Dual Energy X-Ray Absorptiometry (DXA) at Baseline [1]

	n	Daily	n	Monthly	P-value
Total Body Absolute BMD, g/cm^3	18	1.246 ± 0.108	23	1.221 ± 0.114	0.47
Total Body T-score	18	0.7 ± 1.1	24	0.3 ± 1.4	0.44
Spine Absolute BMD, g/cm^3	22	1.271 ± 0.208	25	1.237 ± 0.226	0.59
Spine T-score (L1-L4)	22	0.6 ± 1.67	24	0.1 ± 1.6	0.32
Total Hip Absolute BMD, g/cm^3	22	1.052 ± 0.129	24	1.018 ± 0.153	0.43
Total Hip T-score	22	-0.0 ± 0.9	24	-0.4 ± 1.2	0.31
Femoral Neck Absolute BMD, g/cm^3	22	0.973 ± 0.113	24	0.932 ± 0.149	0.30
Femoral Neck T-score	22	-0.5 ± 0.8	24	-0.8 ± 1.1	0.25

[1] Values are mean ± SD.

4.3.5 Vitamin D Status:

I) 25-hydroxyvitamin D (25(OH)D)

Baseline markers of vitamin D status can be found in ***Table 4.4***. At baseline, the mean 25(OH)D concentrations were not significantly different in the daily (78.4±30.5(17.0-147.0)nmol/L) vs. monthly (89.6±30.1(22.0-169.0)nmol/L) groups (p=0.11). Subjects were categorized according to commonly used cut-off levels for 25(OH)D deficiency (<50nmol/L), insufficiency (50-74nmol/L) and sufficiency (≥75nmol/L) (4,17,18,20,21,24,34,36). Statistical analysis could not be performed due to insufficient sample size to detect differences (e.g. n<5 per cell), therefore results based on these categories are presented for descriptive purposes (***Figure 4.3***).

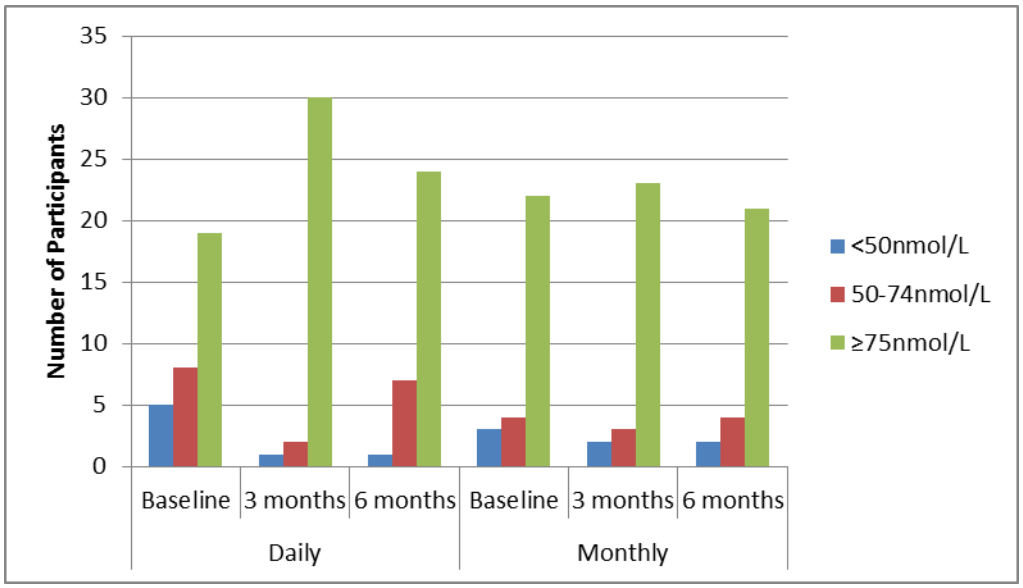

Figure 4.3 Proportion of Subjects with Vitamin D Deficiency (25(OH)D <50nmol/L), Insufficiency (50-74nmol/L), and Sufficiency (≥75nmol/L) at Baseline, 3 and 6 Months.

II) 1,25-dihydroxyvitamin D ($1,25(OH)_2D$)

There was no significant difference in baseline $1,25(OH)_2D$ concentrations between daily (83.2±41.7(12.0-158.0)pmol/L) and monthly (82.2±43.7(24.0-221.0)pmol/L) groups (p=0.93). The majority of subjects in both daily and monthly groups had normal $1,25(OH)_2D$ concentrations (43-168pmol/L) at baseline (n=27 daily vs. n=23 monthly). There were n=4 daily subjects with low $1,25(OH)_2D$ at baseline compared to n=5 subjects in the monthly group. Most of these subjects had Stage 3 (n=1 daily vs. n=3 monthly) or Stage 4 (n=2 daily vs. n=2 monthly) CKD, yet n=1 daily subject had Stage 2 CKD. There was only n=1 monthly subject with elevated $1,25(OH)_2D$ at baseline (Stage 1 CKD), and none from the daily group.

III) Product to Precursor Ratio

The ratio of 25(OH)D to $1,25(OH)_2D$ was very similar between daily (1.15±0.77(0.31-3.52)) and monthly (1.03±0.67(0.23-3.20)) groups (p=0.47). This ratio was calculated as a surrogate marker of renal 1α-hydroxylase activity. As previously mentioned (***Table 4.1***), there were no significant differences in baseline demographic variables that contribute to vitamin D status, such as season of recruitment, prior use of vitamin D_3 supplements and supplementation dose, or level of renal dysfunction (p>0.05).

Table 4.4. Vitamin D Concentrations at Baseline, 3 and 6 Months in Adults with Diabetic Nephropathy Supplemented with Daily (50mcg/day) or Monthly (1,000mcg/month) Vitamin D_3 [1]

	Daily (n=33)			Monthly (n=30)		
	Baseline	**3months**	**6months**	**Baseline**	**3months**	**6months**
25(OH)D, nmol/L	78.4 ± 30.5 (17.0-147.0)* §	94.0 ± 21.4 (24.0-131.0)*	95.1 ± 25.9 (22.0-139.0) §	89.6 ± 30.1 (22.0-169.0)	90.3 ± 26.1 (23.0-136.0)	94.2 ± 27.0 (36.0-147.0)
1, $25(OH)_2D$, pmol/L	83.2 ± 41.7 (12.0-158.0)	93.8 ± 41.7 (9.0-185.0)	102.5 ± 48.4 (30.0-218.0)	82.2 ± 43.7 (24.0-221.0)	83.3 ± 42.4 (29.0-191.0)	80.3 ± 43.9 (9.0-213.0)
1,25(OH)2D : 25(OH)D ratio	1.15 ± 0.77 (0.31-3.52)	1.07 ± 0.63 (0.14-3.29)	1.14 ± 0.58 (0.30-2.40)	1.03 ± 0.67 (0.23-3.20)	1.01 ± 0.62 (0.37-2.55)	0.93 ± 0.65 (0.10-3.14)

[1] Values are mean ± SD (range). Values with the same superscripts represent significant differences between vitamin D supplement doses and/or visits ($p<0.05$). * $p=0.02$, § $p=0.01$.

[2] Sample size varied within each laboratory parameter analysed so that sample size did not always equal 33 for daily or 30 for monthly.

4.4 Effect of Vitamin D Supplementation: 3 Month and 6 Month Results

4.4.1 Glycemic and Renal Function Parameters

Changes in routine clinical blood work between baseline, 3 and 6 months can be found in ***Table 4.2***. The only significantly different results between the two groups were a higher HbA1c and RBG at 6 months in the monthly group (HbA1c Daily: 7.2±1.1(4.4-9.5)% vs. Monthly: 8.2±2.0(5.9-12.5)%, p=0.02; RBG Daily: 8.9±3.1(3.7-15.6)mmol/L vs. Monthly: 10.9±5.1(4.3-20.8)mmol/L, p=0.05). Only RBG in the monthly group increased significantly over time from the 3 month to 6 month visits (9.4±4.0(2.7-17.6)mmol/L to 10.9±5.1(4.3-20.8)mmol/L; p=0.02). There were no other significant differences in routine clinical blood work between the two groups or within each group at any of the 3 study visits (p>0.05).

Indices of renal function remained consistent in each supplement group throughout the follow-up period; uremia (>8.0mmol/L) was evident at 3 months in n=19 daily and n=11 monthly subjects, and at 6months in n=20 daily and n=12 monthly subjects (p=0.99). The number of subjects in the different CKD Stages also remained consistent between 3 months and 6 months (p>0.05) (***Figure 4.2***).

4.4.2 Vitamin D Status:

Absolute changes in all markers of vitamin D status throughout this 6 month supplementation study can be found in ***Table 4.4***. There were no significant differences in 25(OH)D concentrations between the daily vs. monthly vitamin D_3 supplementation strategies at baseline, 3 months or 6 months (p=0.11, p=0.60 and p=0.90, respectively). However, there was a trend towards a significantly higher $1,25(OH)_2D$ at 6 months in the

daily vs. monthly group (Daily: 102.5±48.4(30.0-218.0)pmol/L vs. Monthly: 80.3±43.9(9.0-213.0) pmol/L; p=0.06). A post-hoc power calculation indicated a power of 0.58 for 25(OH)D with the current sample size.

I) 25-hydroxyvitamin D (25(OH)D)

In the daily group, there was a significant increase in 25(OH)D between baseline and 3 months (78.4±30.5(17.0-147.0)nmol/L vs. 94.0±21.4(24.-131.0)nmol/L; p=0.02) and baseline and 6 months (78.4±30.5(17.0-147.0)nmol/L vs. 95.1±25.9(22.0-139.0)nmol/L; p=0.01). In the monthly group, 25(OH)D did not increase significantly between the 3 time-points (Baseline: 89.6±30.1(22.-169.0)nmol/L, 3months: 90.3±26.1(23.0-136.0)nmol/L, 6months: 94.2±27.0(36.0-147.0)nmol/L; p>0.05). Results for the percent change in 25(OH)D between baseline and 3 months, 3 months and 6 months, and baseline and 6 months can be viewed in ***Figure 4.4***.

The same vitamin D status cut-off points mentioned at baseline were also assessed for 3 and 6 months; like at baseline, statistical analysis could not be performed to compare these changes due to small sample sizes (e.g. n<5 per cell). (***Figure 4.3***)

II) 1,25-dihydroxyvitamin D (1,25(OH)$_2$D)

Concentrations of 1,25(OH)$_2$D did not change significantly between baseline, 3 and 6 months in either the daily (83.2±41.7(12.0-158.0)pmol/L, 93.8±41.7(9.0-41.7)pmol/L and 102.5±4.4(30.0-218.0)pmol/L, respectively; p>0.05) or in the monthly group (82.2±43.7(24.0-221.0)pmol/L, 83.3±42.4(29.0-191.0)pmol/L and 94.2±27.0(36.0-147.0)pmol/L; p>0.05) (***Table 4.4***).

The majority of subjects in both daily and monthly groups had normal 1,25(OH)$_2$D concentrations (43-168pmol/L) at baseline (n=27 daily, n=23 monthly), 3 months (n=28

daily, n=22 monthly) and 6 months (n=25, n=21). There were n=4 daily vs. n=3 monthly subjects with low 1,25$(OH)_2$D at 3 months, and n=2 daily vs. n=5 monthly subjects with low 1,25$(OH)_2$D at 6 months; only one individual subject was consistently low at all three time-points in each group. There were no significant differences in the number of subjects with normal/abnormal 1,25$(OH)_2$D between dose type or study visits ($p>0.05$). Most of these subjects had Stage 3 (n=5) or 4 (n=6) CKD. However, one monthly subject with Stage 2 CKD had low 1,25$(OH)_2$D at 3 months, and two subjects on monthly vitamin D_3 supplementation with low 1,25$(OH)_2$D were in Stage 5 CKD according to the 6 month follow-up blood work. These Stage 5 CKD subjects were not discontinued from the study as they had already completed the 6 month visit when the blood work was analyzed. None of these subjects were on dialysis. The appropriate people were notified of these reductions in renal status and appropriate medical care was provided (see *Study Protocol: Safety Variables and Analysis*). A few subjects had elevated 1,25$(OH)_2$D (>168pmol/L) at 3 months (n=1 daily, n=3 monthly) and 6months (n=3 daily, n=1 monthly); all were in Stage 1 (n=4 daily, n=1 monthly) and 2 (n=3 monthly) CKD.

II) Product to Precursor Ratio

There were no significant differences in 25(OH)D-to-1,25$(OH)_2$D ratios between groups and/or between visits ($p>0.05$) (***Table 4.4***).

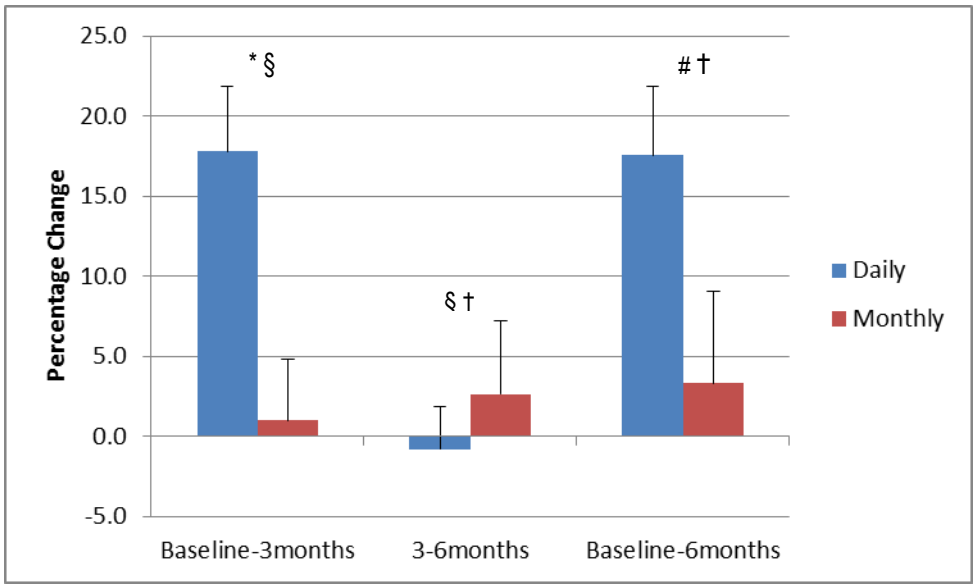

Figure 4.4. Percent Change in 25(OH)D Concentrations Over the 6 Months of Vitamin D_3 Supplementation [1]

[1] Values are mean ± SE. Significant differences ($p<0.05$) between different subject supplement groups and/or study visits are as follows:
* Daily baseline-3months vs. Monthly baseline-3months ($p<0.01$)
# Daily baseline-6months vs. Monthly baseline-6months ($p=0.02$)
§ Daily baseline-3months vs. 3-6months ($p<0.01$)
† Daily 3-6months vs. baseline-6months ($p<0.01$)

4.4.3 Adherence to Vitamin D_3 Supplementation Strategies:

Adherence to the monthly vitamin D_3 supplementation strategy was 100% between both baseline to 3 months and baseline to 6 months. Adherence to the daily vitamin D_3 supplementation strategy was 92±10 (49-100)% between baseline and 3 months, and 93±8 (67-99)% between baseline and 6 months ($p=0.87$). This was greater than the projected 69% (24). There was a significant difference in overall (baseline to 6 months) compliance between the two groups ($p<0.0001$; $r^2 =0.3222$).

The effective dose consumed by each group was determined by multiplying adherence by their cumulative doses; e.g. the total vitamin D_3 supplied from baseline to 6 months for Daily: 9,120mcg/ 6 months vs. Monthly: 6,000mcg/ 6 months. Despite having a

significantly higher adherence rate in the monthly group, the effective dose taken from baseline to 6 months was significantly higher in the daily group (Daily: 738,435±291,0990 (0-902,880)mcg/ 6 months vs. Monthly: 600,000±0mcg/ 6 months; p=0.02, r^2=0.065). When serum levels of 25(OH)D and 1,25$(OH)_2$D at the different time points of study were adjusted for differences in effective dose between the two groups, no significant differences in concentrations were found (p=0.53 and p=0.53).

4.4.4 Parathyroid Hormone and Bone Turnover Markers:

Bone turnover markers and PTH were measured at baseline and 6 months (***Table 4.5***). No significant differences were observed between or within groups between baseline assessment and after 6 months of vitamin D_3 supplementation (p>0.05). At the 6 month visit, elevated PTH (>6.8pmo/L) was observed in n=9 daily (vs. n=14 at baseline) and n=7 monthly (vs. n=8 at baseline) subjects. PTH was also assessed according to the concentration believed to be optimal for bone health (<7.15pmo/L) (21,33). At baseline n=13 daily and n=4 monthly subjects had a plasma PTH >7.15pmol/L, and at 6 months n=9 daily and n=6 monthly subjects had PTH >7.15pmol/L. No subjects had a low PTH (<1.4pmol/L) at the 6 month visit. Both groups experienced a non-significant increased percentage change in osteocalcin (OC) concentrations (p>0.05). In contrast, both groups experienced a non-significant reduction in the percentage changes for bone-specific alkaline phosphatase (BAP), fibroblast growth factor-23 (FGF-23), and PTH concentrations (p>0.05).

The anticipated “normal” range for BAP in this population was 16-44U/L (81,82). At baseline, n=5 daily vs. n=7 monthly subjects had elevated BAP concentrations (>44U/L)

and n=2 vs. n=0 monthly had low BAP concentrations (<16U/L) (p=0.41). At 6 months, n=5 daily vs. n=6 monthly subjects had elevated BAP and n=1 vs. n=0 monthly had low BAP (p=0.52). The majority of daily and monthly subjects had OC concentrations within the anticipated range of 2.3-30.6ng/ml (72,81,83), however n=2 daily and n=1 monthly were elevated at baseline and n=3 daily and n=0 monthly were elevated at 6 months (insufficient sample size for statistical determination).

The anticipated "normal" range (based on a review of the literature for this population) for FGF-23 concentration was 18-97 pg/ml (12,57,84,85). At baseline, there were n=2 daily vs. n=1 monthly subjects with elevated FGF-23, and n=15 daily vs. n=17 monthly subjects with low FGF-23 (p=0.25). At 6 months, there were n=1 daily vs. n=0 monthly subjects with elevated FGF-23, and n=22 daily vs. n=18 monthly subjects with low FGF-23 (p=0.89).

Logarithmic transformation of serum/plasma concentrations of bone turnover markers (OC, BAP, FGF-23) and PTH were performed and re-analysis of interrelationships with dose and visit showed no significant differences over the intervention period, with the exception of FGF-23 which showed a reduction in concentrations after 6 months of vitamin D_3 supplementation.

Overall, significant interrelationships were observed between logarithmic transformed FGF-23 (log FGF-23) and markers of renal function. There was a significant inverse relationship between log FGF-23 and eGFR ($p<0.0001$; $r^2=0.1896$), and between log FGF-23 and serum phosphorous ($p<0.0001$; $r^2=0.1711$), but neither were related to dose type or study visit ($p>0.05$). A significant positive relationship was also found between

log FGF-23 and serum log PTH ($p<0.0001$; $r^2=0.2136$), which did not differ between dose types but was significantly greater at the 6 month visit ($p=0.02$).

Table 4.5. Bone Turnover Markers at Baseline and 6 Months [1]

	Daily			Monthly		
	Baseline (n=33)	6months (n=32)	% change	Baseline (n=29)	6months (n=27)	% change
BAP, U/L	36.6 (7.4 – 106.8)	34.0 (6.2 – 114.8)	10 (-52 – 64)	36.9 (16.4 – 97.7)	34.9 (17.8 – 70.1)	4 (-57 – 29)
OC, ng/ml	9.4 (2.9 – 46.6)	8.8 (3.0 – 59.0)	29 (-45 – 249)	7.3 (4.4 – 39.9)	8.7 (3.9 – 29.9)	21 (-12 – 97)
FGF-23, pg/ml	1.2 (5.3 – 178.4)	12.4 (1.2 – 144.6)	-20 (-87 – 163)	15.2 (7.8 – 97.9)	7.9 (1.2 – 89.1)	-41 (-86 – 346)
PTH, pmol/L	4.8 (1.0 – 31.9)	5.3 (1.4 – 26.7)	0 (-63 – 90)	5.4 (1.3 – 24.3)	4.1 (1.4 – 36.8)	8 (-41 – 88)

[1] Values are median (range). There were no significant differences between groups or study visits.

Abbreviations: Bone specific alkaline phosphatase (BAP); Osteocalcin (OC); Fibroblast Growth Factor-23 (FGF-23); Parathyroid Hormone (PTH).

4.4.5 Other Laboratory Variables:

There were no significant changes in serum calcium, phosphorous, magnesium, albumin or ALP concentrations between the two supplementation strategy groups, nor within either group between the baseline, 3 and 6 month study visits (p>0.05) (***Table 4.2***).

Baseline-3 Months

The number of subjects with abnormal serum concentrations of calcium, phosphorous and magnesium at the 3 month follow-up visits are as follows; no levels exceeded the upper limit for this clinical study (see *Study Protocol: Safety Variables and Analysis)*: Elevated serum calcium (>2.60mmo/L): n=1 daily; Elevated serum phosphorous (>1.45mmol/L): n=2 daily vs. n=2 monthly; Low serum phosphorous (<0.80mmol/L): n=2 daily vs. n=2 monthly; Elevated serum magnesium (>1.0mmol/L): n=4 daily; Low serum magnesium (<0.7mmol/L): n=7 daily vs. n=4 monthly.

Baseline-6 Months

The number of subjects with abnormal serum concentrations of calcium, phosphorous, and magnesium at the 6 month follow-up visit are as follows; again, no levels exceeded the upper limit for this clinical study (see *Study Protocol: Safety Variables and Analysis)*: Low serum calcium (<2.10mmol/L): n=1 daily; Elevated serum phosphorous (>1.45mmol/L): n=1 daily vs. n=2 monthly; Low serum phosphorous (<0.8mmol/L): n=1 daily vs. n=2 monthly; Elevated calcium-to-phosphorous product: n=1 monthly ($5.3 mmol^2/L^2$; see section on SAE above); Elevated serum magnesium (>1.0mmol/L): n=2 daily vs. n=2 monthly; Low serum magnesium (<0.7mol/L): n=7 daily vs. n=5 monthly.

Serum albumin concentrations remained consistently low (<35 g/L) in n=2 daily subjects throughout the study (eGFR 34-42 and 66-79 mL/min/1.73m^2), while only n=2 monthly subjects experienced hypoalbuminemia at the 6 month visit (eGFR 5 and 16 mL/min/1.73m^2). Serum ALP was low (<30U/L) at 3 months in n=1 daily subject, and elevated (>130U/L) in n=4 daily and n=2 monthly subjects. At 6 months, serum ALP was low in n=1 daily subject, and elevated in n=2 daily and n=2 monthly subjects.

4.5 Interrelationships between Vitamin D Status and Other Variables

Multivariate analysis was conducted to assess interrelationships between the primary outcome variables (markers of vitamin D status and bone health), and their relationships to other variables (e.g. study visit, supplementation dose (daily vs. monthly), renal function, diabetes duration, PTH). According to multivariate analysis, season did not have a significant effect on vitamin D status at any time point in the study (p>0.05).

I) 25-hdroxyvitamin D (25(OH)D)

No significant relationships were observed between 25(OH)D and serum levels of log PTH (p=0.42) or study visit (p=0.38). However a significant inverse relationship was observed between serum concentrations of 25(OH)D and log PTH in the daily group only (p=0.04). A significant positive relationship was also observed between 25(OH)D and diabetes duration (p<0.01). Significant inverse relationships between serum log OC and log BAP were found with serum 25(OH)D in the daily group only (p=0.05 and p=0.04, respectively). No other significant interrelationships were found between 25(OH)D and the variables of study.

Serum 25(OH)D analysis by quartiles (Q) resulted in the following concentrations: Q1: 17-75nmol/L; Q2: 76-92nmol/L; Q3: 93-107nmol/L; and Q4: 107-169nmol/L. Seventy-five percent of the subjects had a 25(OH)D >75nmol/L, the optimal concentration for bone health (4,17,18,20,21,24,34,36), at some point in the study; and 50% had a 25(OH)D above the median concentration of 93nmol/L. Both 25(OH)D and PTH were analyzed as categorical variables using the cut-offs for vitamin D (<75nmol/L or ≥75nmol/L) and PTH (<7.15pmol/L or ≥7.15pmol/L) associated with optimal bone health (4,17,18,20,21,24,33,34,36). A trend towards a significant inverse relationship was found between 25(OH)D ≥75nmol/L (categorical variable) and PTH as a continuous variable ($p=0.05$), yet study visit (e.g. baseline and 6 months) had no significant effect ($p=0.23$). When 25(OH)D and PTH were both defined as categorical variables, a significant inverse relationship between these variables ($p=0.004$) was found, although effects of duration of supplementation were not significant ($p=0.18$). Moreover, the number of subjects with PTH >7.15pmol/L did not change significantly between baseline and 6 months when categorized by optimal 25(OH)D (>75nmol/L; n=10 baseline vs. n=8 6months; $p=0.45$) or suboptimal 25(OH)D (<75noml/L; n=7 baseline and 6 months; $p=0.28$). According to this same 25(OH)D categorisation, a significant inverse relationship was found between suboptimal 25(OH)D (<75nmol/L) and OC ($p=0.006$). However, no significant relationship was found between 25(OH)D and OC over time, or between 25(OH)D and BAP or FGF-23 ($p>0.05$).

Serum 25(OH)D was also sorted based on the median concentration of 93nmol/L, and multivariate analysis performed between bone turnover markers. No significant relationships between 25(OH)D and bone turnover markers or PTH were found ($p>0.05$).

II) *1,25-dihydroxyvitamin D ($1,25(OH)_2D$)*

According to multivariate analysis, a significant inverse relationship was observed between $1,25(OH)_2D$ and log PTH ($p=0.01$); this was only significant for the daily group ($p=0.01$) and there was no relationship to visit ($p=0.52$). In addition, a significant positive relationship was observed between $1,25(OH)_2D$ and eGFR in both groups ($p<0.0001$). In the monthly group, a significant inverse relationship was found between diabetes duration and $1,25(OH)_2D$ ($p=0.009$). There were no significant relationships between $1,25(OH)_2D$ and study visit ($p>0.05$).

Significant relationships were found between $1,25(OH)_2D$ and logarithmically transformed markers of bone turnover. There was a significant inverse relationship between $1,25(OH)_2D$ and log OC ($p=0.002$) which was present in the daily group only ($p=0.03$), and not related to study visit ($p=0.79$). A significant inverse relationship was also found between $1,25(OH)_2D$ and log FGF-23 ($p=0.003$), which was not related to dose type ($p=0.19$) or study visit ($p=0.31$). Log BAP had a significant inverse relationship to $1,25(OH)_2D$ in the daily group only ($p=0.007$), and was not related to study visit ($p>0.05$).

The median $1,25(OH)_2D$ concentration was 79.5pmol/L. Multivariate analysis was conducted with $1,25(OH)_2D$ as a categorical variable (less/greater than the median), and with bone turnover markers and PTH as continuous variables. No relationship was observed between $1,25(OH)_2D$ and BAP ($p=0.92$) between doses ($p=0.30$) or study visits ($p=0.18$). Significant inverse relationships were found for $1,25(OH)_2D$ with OC ($p=0.02$) and with FGF-23 ($p=0.004$), however this was not significantly related to visit or dose type ($p>0.05$). There was only a trend towards an inverse relationship between $1,25(OH)_2D$ and PTH ($p=0.09$), with no relationship to visit ($p=0.19$) or dose type ($p=0.36$).

4.6 Dietary Intake

Dietary intake was assessed at baseline, 3 and 6 months in the daily and monthly supplement groups; results can be found in ***Table 4.6***. There were no significant differences in average macronutrient or micronutrient intake.

The majority of the subjects' diets were inadequate for vitamin D intake. Statistical analysis could not be performed due to insufficient sample size to detect differences (e.g. n<5 per cell), therefore results based on these categories are presented for descriptive purposes. At baseline, only n=1 daily subject and n=0 monthly subject met the recommended dietary allowance (RDA) for vitamin D (≤70 years: 15mcg/d, >70 years: 20mcg/d). At 3 months, n=2 daily and n=1 monthly subjects met the vitamin D RDA through diet alone, and the same could be said at 6 months for only n=1 daily and n=2 monthly subjects. The estimated average requirement (EAR) for vitamin D intake is 10mcg/d; this is the intake believed to support a serum 25(OH)D of 50nmol/L (1,37). The number of subjects who met the EAR for vitamin D intake through diet alone was as follows: baseline n=2 daily vs. n=1 monthly; 3 months n=3 daily vs. n=1 monthly; and 6 months n=1 daily vs. n=2 monthly subjects. Dietary intake of vitamin D was a minor contributor to overall vitamin D intake: 8% of daily subjects' and 11% of monthly subjects' total vitamin D intake.

Dietary intake of vitamin D was not normally distributed (skewness=2); therefore this data was logarithmically transformed (log) and univariate analysis conducted with markers of vitamin D status and bone turnover. No significant relationships were found between log dietary intake of vitamin D and 25(OH)D (p=0.87), $1,25(OH)_2D$ (p=0.28), log BAP (p=0.99) or log OC (p=0.08). Significant inverse relationships were observed between

log dietary intake of vitamin D and log PTH ($p=0.0001$; $r^2=0.12$) and FGF-23 ($p=0.005$; $r^2=0.06$).

Table 4.6. Dietary Intake of Adults with Diabetic Nephropathy at Baseline and at 3 and 6 Months after Participation in RCT [1]

	Daily			Monthly		
	Baseline (n=33)	**3months (n=33)**	**6months (n=32)**	**Baseline (n=30)**	**3months (n=28)**	**6months (n=27)**
Kilocalories, kcal/d	1681 ± 536	1695 ± 507	1566 ± 499	1669 ± 520	1710 ± 591	1828 ± 601
Carbohydrate, g/d	200 ± 60	200 ± 67	182 ± 61	200 ± 54	204 ± 69	212 ± 71
Protein, g/d	76.1 ± 28.4	75.1 ± 25.5	73.1 ± 28.8	74.6 ± 26.2	75.3 ± 31.4	79.2 ± 30.0
Fat, g/d	64.6 ± 27.8	66.7 ± 29.4	61.3 ± 26.0	63.8 ± 31.6	67.5 ± 31.7	72.7 ± 28.7
Vitamin D, mcg/d	3.9 ± 3.1	5.0 ± 4.3	4.1 ± 3.0	3.8 ± 2.8	4.0 ± 3.4	4.9 ± 4.9
Calcium, mg/d	697 ± 327	739 ± 278	667 ± 302	613 ± 290	669 ± 332	661 ± 269
Magnesium, mg/d	282 ± 128	304 ± 110	254 ± 99	283 ± 139	271 ± 103	273 ± 105
Phosphorous, mg/d	1121 ± 563	1213 ± 411	1062 ± 398	1088 ± 437	1114 ± 429	1131 ± 434
Sodium, mg/d	2701 ± 1144	2560 ± 1299	2359 ± 1095	2749 ± 144	2788 ± 1261	2555 ± 996
Caffeine, mg/d	172 ± 138	173 ± 122	192 ± 137	214 ± 157	227 ± 189	220 ± 160

[1] Values are mean ± SD. There were no significant differences between vitamin D supplement doses and/or visits ($p<0.05$). There was a trend towards greater daily intake of kilocalories ($p=0.07$) and carbohydrates ($p=0.08$) in the monthly group vs. the daily group at the 6 month follow-up appointment. There was also a trend towards a higher dietary intake of magnesium in the daily group at 3 months vs. baseline ($p=0.08$)

CHAPTER 5: DISCUSSION

5.1 Thesis Chapter Objective

This Thesis chapter will discuss the preliminary findings of the first 63 subjects who enrolled in and completed this Randomized Controlled Trial (RCT; including drop-outs), focusing on hypothesis 1 and objectives 1 and 2a of the overall RCT. Refer to chapter 3 for a detailed description of the methods utilized for this study, and chapter 4 for the preliminary results of the first 63 subjects enrolled in this RCT.

5.2 Summary of Major Findings

Overall, no significant differences were observed in anthropometric and demographic variables, routine clinical blood work or dietary intake between subjects in the two supplementation strategy groups (daily: 50mcg/d or 2,000IU/d vs. monthly: 1,000mcg/m or 40,000IU/m) or during the 6 month study period ($p>0.05$). In terms of markers of vitamin D status, the only significant difference observed was an increase in serum 25(OH)D in the daily group from baseline to 3 months ($p=0.02$) and baseline to 6 months ($p=0.01$). Average serum 25(OH)D concentration increased by approximately 12.2nmol/L between baseline and 6 months to a concentration of 95.9nmol/L. Therefore, at this preliminary point of analysis, the data indicates that the hypothesis that vitamin D_3 supplementation with 50mcg/d or 1,000mcg/m would increase serum 25(OH)D by 25nmol/L to a mean concentration of 100nmol/L has not been supported . There were no other significant differences in markers of vitamin D status between dose types or study visits ($p>0.05$). Although adherence to the monthly supplement (100%) was significantly greater than the daily supplement (93%) ($p<0.0001$), correction for differences in the effective vitamin D_3 dose delivered resulted in no changes in vitamin D concentrations ($p=0.53$).

No significant differences were observed in markers of bone health and turnover (parathyroid hormone (PTH), osteocalcin (OC), fibroblast growth factor-23 (FGF-23) and bone-specific alkaline phosphatase (BAP)) between the two dose types or study visits ($p>0.05$). However, significant interrelationships were observed in the daily group only

between markers of vitamin D status (25(OH)D and $1,25(OH)_2D$) and markers of bone health (PTH, OC, BAP and FGF-23; $p<0.05$).

5.3 Vitamin D Status: 25-hydroxyvitamin D (25(OH)D)

The prevalence of suboptimal vitamin D status (25(OH)D <75nmol/L) has been reported as up to 52% of the general North American population, up to 86% in the diabetic population, and up to 93% in adult patients with chronic kidney disease (CKD; primary etiology diabetes) (4,18,19,21). However, the average serum 25(OH)D of subjects in this study at baseline was higher than anticipated; 83.7nmol/L for the overall group. Furthermore, suboptimal vitamin D status (<75nmol/L) was observed in only 33% (n=20/61) of the subjects at baseline compared to the 52-93% prevalence which was expected in this population of northern dwelling patients with diabetes and CKD (4,18,19,21). One of the major reasons for these differences was that the majority of the subjects enrolled into this study were previously taking an average of 28mcg/d vitamin D_3 prior to study enrolment. This was particularly evident in patients enrolled from the DNPC clinics, where routine vitamin D_3 supplementation is a part of clinical care. Although, the prevalence of vitamin D sufficiency observed in these subjects at baseline was higher than expected; it was not associated with season or dietary intake of vitamin D ($p>0.05$), and is most likely the result of pre-existing use of vitamin D_3 supplements. A previous vitamin D_3 intervention study in adults with diabetes and CKD in Edmonton found the prevalence of 25(OH)D insufficiency (<75nmol/L) to be 100% at baseline and 63% after 3 months of supplementation with 25mcg/d vitamin D_3 (21). Hence, the rationale for vitamin D_3 supplementation in this study was to increase the vitamin D_3 dosing to ensure adequacy of overall status. After 6 months of this RCT vitamin D_3 supplementation protocol, the prevalence of serum 25(OH)D <75nmol/L decreased from 33% to 22%; which suggests that for the majority of the population this level of dosing may be sufficient to ensure serum 25(OH)D >75nmol/L.

5.3.1 Potential Confounding Variables for Serum 25(OH)D

No significant relationships were observed between serum 25(OH)D and season of study visit (blood draw), effective vitamin D_3 dose (e.g. dose actually taken), or dietary intake of vitamin D ($p>0.05$).

I) *Time between Vitamin D Supplement Ingestion and Blood Sampling*

Subjects were asked to not take their vitamin D_3 supplement on the day of the study appointments and blood draws; however this request was not always abided by in the daily supplement group (n=15). Appointments with subjects randomly allocated to the daily supplement were scheduled as close to 90 days (as per the number of vitamin D_3 pills provided) as possible. Conversely, appointments with the monthly group were scheduled approximately 14 days after they were to take their final monthly vitamin D_3 dose. This was determined an appropriate time-point as a previous study found that a single high oral dose of vitamin D_3 (1,250mcg) resulted in 25(OH)D concentration peaking after 14 days and declining to baseline after 28 days (30). Therefore by waiting approximately 14 days from administration of the 1,000mcg of vitamin D_3, a more accurate and consistent picture of how a single high dose would affect serum 25(OH)D should be possible. The average number of days between when the last vitamin D_3 dose was taken and the blood work collected was very close to the planned schedule; 3 month visit: daily=1 day vs. monthly=15 days; and 6 month visit: daily=2 days vs. monthly=13 days. Therefore it can by inferred that the serum 25(OH)D results are accurate/consistent on a dose-time basis.

II) *Recruitment of Subjects from Local Clinics*

All study participants were recruited from the Diabetic Nephropathy Prevention Clinic (DNPC) and the Renal Insufficiency Clinic (RIC) located in Edmonton, Alberta. Both clinics consist of multidisciplinary teams including nephrologists, endocrinologists, registered nurses, registered dietitians and pharmacists, who strive to optimize their patients' health and prevent renal decline and other diabetic complications (e.g. cardiovascular and neuropathy) (7,8). Although only 33% of the DNPC/RIC patients approached about this RCT agreed to participate, these participants were very dedicated

to their role in this RCT. This is evidenced by the low attrition rate (only 4 drop-outs = 6%) and high rate of adherence to the vitamin D_3 supplementation strategies (93% daily and 100% monthly). This high rate of subject retention and adherence is a major strength of this study and a testament to the hard work and positive relationships developed between the graduate student and the subjects and clinical team. The primary reasons for patients to decline participation were a lack of time, transportation issues, or too many other health issues/appointments to dedicate 6 months to this RCT.

While it is believed that overall the patients who participated in this study represent the general DNPC/RIC population, it is recognized that a natural and unintentional selection bias may have occurred. The possibility exists that individuals who agreed to participate in this RCT may be more dedicated to improving their health, as evidenced by voluntary participation in extra clinical appointments and supplement/pill requirements; and these individuals may therefore be more compliant to supplementation recommendations.

The majority of the subjects came from the DNPC, where the aim is to prevent/limit diabetic complications, including CKD. Therefore, subjects from the DNPC can generally be viewed as individuals who are taking a proactive approach to managing their diabetes and renal health in order to prevent further complications. The presence of this 'self-care' in conjunction with proactive care from the clinical team has a major impact on improving clinical parameters associated with diabetic complications (7,8). Furthermore, this proactive care model can impact adherence to supplementation by encouraging self-care and creating a positive approach to their medical care (e.g. following medication and dietary recommendations) (7,8). The aim of the RIC is to delay the progression of CKD, and to provide education on dialysis and transplant options when renal decline can no longer be managed by medications or lifestyle. Individuals who attend the RIC generally have worse health, particularly renal function as the clinical referral criteria for this program is a GFR ≤ 30 mL/min/1.73m^2. Although not the case for all, this poor renal status may be the result of an inability or indifference towards preserving renal function and preventing further decline. Worse physical health and/or the presence of additional chronic diseases can have a major impact on adherence to vitamin D supplementation (26). Research has shown that adherence to bone health therapies is only 50-69% (24,26). Moreover,

adherence is reduced by 20% with each additional chronic disease an individual has and if they are on >6 other medications (26). Pre-scheduled routine clinical appointments, a positive rapport between clinician and patient, and sharing/explaining patient's results from bone health analyses are all reported to increase the likelihood of continued adherence to bone health therapies (e.g. vitamin D) beyond 6-12 months; otherwise 23-35% will discontinue supplementation after 6 months (26,62,86).

III) *Clinical Characteristics:*

There were no significant differences between the subjects randomized to the daily vs. monthly vitamin D_3 supplementation strategies in regards to baseline anthropometric (height, weight) and demographic (diabetes duration, medication and insulin use) variables. Furthermore, there were no significant changes in these variables over the 6 month supplementation duration. One very important baseline characteristic to note is the high prevalence of previous vitamin D_3 supplementation in both supplement strategy groups. Seventy-eight percent (n=49/63) of the subjects were recruited from the DNPC where vitamin D_3 supplementation (25mcg/d) and 25(OH)D monitoring is part of routine clinical care. While some subjects recruited from RIC were previously taking a vitamin D_3 supplement (n=6/14), vitamin D_3 supplementation is not routinely recommended, nor is 25(OH)D monitored, in RIC as a part their clinical guidelines/protocol. Therefore, previous vitamin D_3 supplementation was much more common in subjects recruited from the DNPC (n=48/49; average 33±13mcg/d) vs. those recruited from the RIC (n=6/14; average 11±16mcg/d, p=0.0002). However, this did not appear to have a significant impact on differences in baseline 25(OH)D (DNPC: 89±23(25-147)nmol/L vs. RIC: 67±44(17-169)nmol/L; p=0.10). Moreover, no significant differences in previous vitamin D_3 supplement use/dose or baseline vitamin D status (25(OH)D and $1,25(OH)_2D$) were found between subjects randomly assigned to the daily vs. monthly supplement groups.

Body composition, particularly excess adiposity, plays a role in vitamin D status by sequestering vitamin D in adipose tissue (87). The average body mass index (BMI) of the subjects in this study was 34kg/m^2, indicating a very high propensity for excess adiposity.

However, BMI was the same in both dose groups and at all study visits. Therefore the potential impact of excess adiposity sequestering vitamin D was consistent between the two dose groups and throughout the study duration.

5.4 Vitamin D Status: 1,25-dihydroxyvitamin D ($1,25(OH)_2D$)

The active form of vitamin D, $1,25(OH)_2D$ (calcitriol), plays an important role in many skeletal and non-skeletal (e.g. immune responses and insulin secretion/sensitivity) functions (87). Yet the most well-known roles of $1,25(OH)_2D$ are in mineral homeostasis (calcium and phosphorous intestinal absorption) and bone health (regulation of PTH, osteoblasts and FGF-23) (87). There were no significant differences in $1,25(OH)_2D$ concentrations between the daily and monthly groups or between study visits ($p>0.05$). However, there was a non-significant increase in $1,25(OH)_2D$ concentrations from baseline to 3 months to 6 months in the daily group, whereas the monthly group experienced a slight decline in $1,25(OH)_2D$ concentration. At baseline, serum $1,25(OH)_2D$ was low (<43pmol/L) in 9 subjects. Although $1,25(OH)_2D$ concentration is strongly influenced by 25(OH)D status, 5 of these 9 subjects had sufficient 25(OH)D concentrations (>75nmol/L). At the 3 and 6 month visits, 7 subjects had low serum $1,25(OH)_2D$ concentrations; yet 4 of these subjects had a sufficient 25(OH)D concentration. Lower renal function (stage 3-4 CKD) was common in subjects with low $1,25(OH)_2D$ and a significant contributing factor.

Unfortunately the half-life of $1,25(OH)_2D$ is only 6 hours, making it a challenging marker of vitamin D status and the kidneys' ability to activate 25(OH)D clinically (48,88). Of note, there was no significant difference in fasting hours (e.g. time between last meal/snack and blood collection; average 4-6 hours) between the daily and monthly groups ($p>0.05$). We analyzed the ratio of 25(OH)D to $1,25(OH)_2D$ in an effort to capture the proportion of vitamin D activated (e.g. precursor to product ratio). Although there were no significant differences between the daily and monthly supplement groups or between study visits (always a positive value), the 25(OH)D to $1,25(OH)_2D$ ratio was slightly higher in the daily vs. the monthly group at all three visits. Therefore, as a result of the consistently higher $1,25(OH)_2D$ concentrations and product to precursor ratios, the daily

vitamin D_3 supplementation strategy may be more beneficial for vitamin D activation to $1,25(OH)_2D$, although statistically there was no difference between the two groups.

5.5 Bone Health

5.5.1 Bone Mineral Density at Baseline

Poor bone mineral density (BMD) is associated with an increased fracture risk, which has a negative impact on QoL through reduced mobility and independence, and increased risk of debilitating illness and mortality (3,9-13). According to the 2009 Canadian Community Health Survey, approximately 11.6% of the general population aged 50 and older had a diagnosis of osteoporosis, which is defined as a BMD T-score < -2.5 when measured by Dual-energy X-ray Absorptiometry (DXA) (1,54). Individuals with diabetes and CKD are especially susceptible to poor bone health due to impaired vitamin and mineral metabolism, inflammation, oxidative stress, insulin resistance and hyperglycemia (14,15)(3,10,12,16,17). Furthermore, it has been estimated that by the time CKD patients reach dialysis, approximately 75% have metabolic bone disease (20).

Detailed DXA analysis is beyond the scope of focus for this Master's Thesis, therefore the BMD results are discussed for descriptive purposes. According to the DXA scans conducted at baseline, BMD was normal in the majority of subjects. Of the 42 subjects with DXA results, 6 subjects (14%) were found to have low BMD T-scores; 5 of whom were post-menopausal females with osteopenia and one male with low BMD and a 29 year history of type 1 diabetes. No subjects were diagnosed with osteoporosis and only one subject had been diagnosed with a fragility fracture prior to study enrollment.

BMD testing by DXA is a non-invasive and useful clinical tool to identify low BMD and assess fracture risk in the general population (54,89). Yet it is a static reflection of bone health and impacted by a lifetime of various lifestyle factors including dietary intake and weight-bearing physical activity. Body weight also impacts BMD status; 40 of the 42 subjects with DXA results were classified as overweight or obese based on their baseline BMI (89,90). A significant positive correlation between BMI and BMD has been reported, which may relate to mechanical stimulation of osteoblasts (bone formation) (91).

Biochemical abnormalities (e.g. serum calcium, phosphorus, vitamin D and PTH) that are common in CKD, particularly advanced stages, can complicate DXA results and interpretation (88). These biochemical abnormalities can result in abnormal bone quality (turnover, mineralization and volume) even when BMD appears to be normal, thereby providing an inaccurate picture of bone health in CKD (88). Bone biopsy is the gold standard for assessing bone quality; however it is quite invasive and expensive (88). Fortunately, serum markers of bone turnover have been found to correlate with findings from bone biopsies; e.g. positive correlation between BAP and bone formation, and between PTH and bone turnover (88). However interpretation of the relationship between PTH and bone turnover can be challenged by bone resistance to PTH (88). According to biopsies performed on adults with stage 3-5 CKD, only 16% had normal bone formation (e.g. normal mineralization and turnover); the majority had normal mineralization but high rates of turnover (osteitis fibrosa, 32%), abnormal mineralization with increased turnover (mixed bone disease, 20%), or low bone turnover with acellularity (adynamic bone disease, 18%) (88).

5.5.2 Markers of Bone Turnover

Serial measurement of bone turnover markers allows for exploration of dynamic bone remodeling, and has the added value as a tool for assessing adherence to bone health therapies, including vitamin D_3 supplementation (80). Careful interpretation of bone turnover markers is required due to the vast intra- and inter-individual variability based on time of day, menstrual cycle, urine vs. serum sample, fasting vs. fed, age and season (25). Analysis of bone turnover markers included in this Thesis involved markers of bone formation, osteocalcin (OC) and bone-specific alkaline phosphatase (BAP), and a marker of the bone-kidney axis, fibroblast growth factor-23 (FGF-23). Serum was also collected for analysis of the bone resorption marker N-telopeptide of type 1 collagen (NTx); however these results were are not available for inclusion in this Thesis. Although I did not find any significant differences in concentrations of OC, BAP, FGF-23 or PTH between supplement groups or over time, I also did not observe any detrimental effects (e.g. PTH suppression

or significantly up/down-regulated bone turnover) related to this vitamin D_3 supplementation protocol.

Most of the sampled subjects had serum OC and BAP within the expected concentration ranges. Furthermore, there was very little change in the proportion of subjects outside of the normal ranges between baseline and 6 months. In order to assess whether there was a net increase/decrease in BMD, information on the bone resorption (NTx) of these subjects is needed.

As could be expected, significant relationships were observed between logarithmically transformed FGF-23 (log FGF-23) and eGFR (inverse), log PTH and serum phosphorous (positive; $p<0.05$). This is not surprising as FGF-23 is a phosphaturic factor that is reported to increase before PTH concentrations (e.g. stage 3 CKD), making FGF-23 a particularly useful marker of bone health in less severe CKD stages (e.g. stage 1-2) (5,20). While elevated FGF-23 concentrations can be negative (e.g. suppress 1α-hydroxylase activity, impair osteoblast differentiation and maturation of the bone matrix), low FGF-23 concentrations can also be detrimental, as it may indicate reduced osteocyte density and/or function (5,12).

Although the sample size was too small to test statistically, the proportion of subjects with hyperparathyroidism (PTH >6.8pmol/L) reduced from 22 at baseline to 16 at 6 months. However, the PTH concentrations for most of these subjects were <7.15pmol/L (>7.15pmol/L in n=17 at baseline and n=15 at 6months), the concentration believed to be optimal for bone health (21,33). It is possible that a significant reduction in PTH was not observed as the average serum 25(OH)D concentration did not exceed 100nmol/L, the concentration believed to be required to reduce PTH to <7.15pmol/L, and because most subjects already had a PTH <7.15pmol/L at baseline (21,33). Further research needs to be done in order to elucidate the extent to which vitamin D supplementation (amount and duration) may be associated with beneficial reductions in serum PTH across the spectrum of CKD in patients with diabetes.

Results from multivariate analysis identified a significant inverse relationship between $1,25(OH)_2D$ and log OC, log BAP and log PTH in the daily group ($p<0.05$). The fact that this relationship was only observed in the daily group may be related to several

factors: 1) the short half-life of $1,25(OH)_2D$; 2) a more consistent supply of its precursor (vitamin D_3 to be synthesized into 25(OH)D) in the daily group; and/or 3) the significantly greater percentage change in 25(OH)D observed in the daily group (+18% daily vs. +3% monthly; p=0.02), thereby providing more substrate for $1,25(OH)_2D$ synthesis (14,29,49). However, serum concentration of $1,25(OH)_2D$ is primarily regulated by serum calcium and PTH concentrations, which were overall normal in both dose groups (38).

5.6 Dietary Intake and Routine Clinical Blood Work

Diet was not a significant contributor to overall vitamin D intake or status in these study subjects. This is likely related to a reduced intake of vitamin D fortified dairy products in individuals with diabetes and CKD due to restrictions on carbohydrate, potassium and phosphorous intake. The average dietary vitamin D intake in these subjects was only 3.8-5.0mcg/d, far below the recommended dietary allowance (RDA) of 15-20mcg/d.

Inadequate dietary intake of vitamin D is a common observation in the general Canadian population (40). Yet, the average dietary intake of vitamin D in these subjects was slightly less than what has been recently reported in Canada for the general population (5.1-6.7mcg/d) (40). The primary dietary source of vitamin D in Canadian diets is vitamin D fortified dairy products (e.g. fluid milk and yogurt) (40). However, individuals with diabetes and CKD may limit dairy consumption for several reasons. One such reason may be related to the carbohydrate content of dairy and impact on glycemic control. Another may be related to limiting intake of saturated fat (found in dairy products); many individuals reported a dislike for low fat (skim or 1%) milk and yogurt and would rather not eat these products at all if they couldn't have their higher milk-fat versions. However the most common reason for limited dairy intake, particularly in those with more advanced CKD (stage 3-4), is management of hyperkalemia and hyperphosphatemia as dairy products are a high source of both. In this patient cohort, the primary reasons for suboptimal dairy intake (e.g. vitamin D fortified milk) were due to prescribed reductions in saturated fat and potassium. No significant relationships were found between log dietary intake of vitamin D and markers of vitamin D status ($p>0.05$); congruent to reports by some researchers (33,65) but not all (92).

Markers of renal function (e.g. estimated glomerular filtration rate (eGFR)) remained consistent between the subjects in both supplement strategies over the duration of the study. This is an important finding as $1,25(OH)_2D$ concentrations were highly related to eGFR. While there were no significant differences in dietary intake between the two supplement groups or between study visits, an interesting trend was observed. The average daily kilocalorie (kcal/d) and carbohydrate (g/d) intake at 6 months was slightly greater in the monthly vs. daily group. This is an interesting trend considering the significantly higher HbA1c and RBG observed at 6 months in the monthly vs. daily group, and may be related to differences in glucose utilization and insulin sensitivity. The average serum 25(OH)D in these subjects was within the range reported to be beneficial for insulin sensitivity (80-110nmol/L) (87). Significant inverse relationships were observed between the baseline to 6 months percentage changes in HbA1c and 25(OH)D ($p=0.04$) and $1,25(OH)_2D$ ($p=0.03$). However, this study was not powered to evaluate insulin sensitivity, especially not in this preliminary analysis of the first half of the RCT sample size.

5.7 Strengths and Limitations

There are several strengths to this RCT, including the fact that vitamin D_3 (cholecalciferol) was used instead of vitamin D_2 (ergocalciferol), and it was provided as an oral supplement which appeals to the general public more than vitamin D injections. Moreover, this intervention was focused on isolated preparations of vitamin D_3 supplements, with no other changes to diet or lifestyle. This allowed me to ascertain the impact of vitamin D_3 supplementation alone on vitamin D status and bone health, as opposed to many other studies that combine vitamin D_3 with calcium supplementation. Previous research demonstrated that daily supplementation with 25mcg of vitamin D_3 is ineffective at attaining a serum 25(OH)D associated with improved PTH status and bone health in the same population (21). The vitamin D_3 dosing in this study (e.g. 50mcg/d) has been proposed by other researchers to be more effective at improving vitamin D status to a concentration associated with PTH concentrations optimal for bone health (e.g. 25(OH)D >100nmo/L and PTH <7.15pmol/L) (21,24). Unfortunately, at this stage of preliminary analysis I was unable to confirm this hypothesis due to insufficient power (0.58).

Another strength lies in the fact that participants were enrolled throughout the year, thus accounting for the potential effects of seasonal variations on serum vitamin D concentrations. Participants completed sunlight exposure and weight-bearing physical activity questionnaires at each appointment so that the potential impact of sunlight exposure during the different seasons as well as indoor and outdoor activity can be assessed for its potential contribution to overall vitamin D status and markers of bone health. In addition, dietary intake was analyzed using validated methodologies for its contribution to total vitamin D intake and status, as well as intake of other nutrients that are known to impact bone health (e.g. calcium, magnesium, phosphorus).

Bone health was assessed by multiple methods. BMD was assessed by DXA, the non-invasive gold standard, to characterize the study subjects' bone health at baseline. I also measured serum/plasma markers of bone turnover before and after supplementation, which allowed for assessing dynamic changes in bone health. I looked at several aspects of bone turnover, including measures of bone formation (BAP, OC) and resorption (NTx; to be analyzed after Thesis submission), as well an important marker of the kidney-bone axis and mineral homeostasis (FGF-23).

Lastly, for the greater RCT, details pertaining to co-morbidities, concomitant medication use, and health-related quality of life (QoL) were collected and will be analyzed for relationships with vitamin D status and bone health as well as adherence to the supplementation strategies. These factors are important to assess all the important factors that may contribute to overall vitamin D status and bone health in adults with diabetes and CKD. These last parameters were beyond the scope of focus for the present Thesis.

Despite my best efforts to conduct a well-controlled trial, there are some limitations in this RCT (focusing on the first half of the sample size). The possibility of a subject selection bias is quite likely. The majority of the subjects came from a clinic where recommending vitamin D_3 supplementation was part of routine clinical care (DNPC; 25mcg/d). Most likely, this had a large impact on the high prevalence of vitamin D sufficiency at baseline. Furthermore, the fact that most of these subjects were previously taking a vitamin D_3 supplement likely had an impact on their adherence and therefore the

high adherence rate we observed. However, there were some difficulties in assessing adherence, as not all subjects brought their vitamin D_3 pill bottles/vials to the 3 and 6 month study visits as requested (n=14 bottles). In these circumstances pill counts were based on subject's reports of the number of pills remaining. However, it is not likely that this resulted in an over estimation of adherence because the majority of the subjects did bring in their bottles. Overall this suggests that few deviations in the study protocol related to the total vitamin D_3 dosing occurred in this cohort.

Another limitation was that serum 25(OH)D, 1,25$(OH)_2$D and PTH concentrations were not always available. Some samples were determined to have exceeded the stability limit and be unsuitable for analysis, or were too lipemic (n=6) for analysis. This suggests that it might be important for future studies to obtain fasting blood work samples.

I was unable to measure BMD by DXA in all the subjects as several had already received a DXA within the previous year, or due to scheduling conflicts between the DXA technician and the subjects. Even so, DXA results were available for the majority of the subjects and no significant differences in BMD were likely present between those subjects who underwent a DXA scan and those who did not. And lastly, NTx was unable to be analyzed prior to the completion deadlines for this Thesis, and therefore could not be included in analysis. Serum NTx for the subjects in the first half of this RCT will be analyzed and included in the overall RCT results.

5.8 Clinical Implications

Despite a significant difference in adherence, I found no significant differences between once daily vs. once monthly vitamin D_3 supplementation on overall vitamin D status and bone health. Which supplementation strategy would be most beneficial would depend on the individual patient, their vitamin D status, severity of renal disease, and use of concomitant medications and supplements. Therefore recommendations should be based on a patient-to-patient basis. Importantly, no adverse events occurred that were related to the vitamin D_3 supplementation used in this RCT protocol.

Approximately 84% of our subjects were already taking a vitamin D_3 supplement prior to enrolling in this RCT, and the average dose was approximately 28mcg/d.

Considering the average serum 25(OH)D of all these subjects was 83.7nmol/L at baseline, this would suggest that 25mcg/d may be sufficient in most individuals with diabetes and stage 1-2 CKD to obtain a serum 25(OH)D >75nmol/L. However, individuals with more advanced CKD (stage 3-4) likely require higher supplementation to maintain a serum 25(OH)D >75nmol/L. Additional factors, such as increased age and reduced dietary vitamin D intake, sunlight exposure, vitamin D binding protein and hydroxylase enzyme activity can also impact an individual's requirements for vitamin D_3 supplementation in order to attain and maintain a serum 25(OH)D >75nmol/L.

According to the preliminary analysis of the first half of the subjects in this RCT, 6 months of supplementation with 50mcg/d or 1,000mcg/m of vitamin D_3 did not result in an average serum 25(OH)D of 100nmol/L. This may have been the reason why I did not observe any significant changes in markers of bone turnover or PTH. However, the average PTH concentration at 6 months was <7.15pmol/L in both groups, which is promising. Early initiation of vitamin D_3 supplementation and aiming for a serum 25(OH)D of approximately 100nmol/L may have beneficial impacts on serum PTH concentration (21,31,33). PTH has a propensity for bone resistance in CKD (15,53,56,57). Preventing sustained hyperparathyroidism in this population may help prevent PTH resistance and the need for treatment with active vitamin D analogs (e.g. Rocaltrol), which incur an increased risk for hypercalcemia (49,50).

Vitamin D is truly a nutrient of concern in Canada, particularly for those with diabetes and CKD, as a result of limited sunlight exposure, poor dietary intake of vitamin D rich foods, and dietary restriction of these same foods with therapeutic renal diets. As a result, supplementation with vitamin D_3 is needed to obtain and maintain vitamin D adequacy. Preliminary results suggest that 25-50mcg/d of vitamin D_3 results in a sufficient 25(OH)D concentration in most patients with diabetes and CKD. Furthermore, <100% adherence to 50mcg/d is not necessarily detrimental to overall vitamin D status and may be equally efficacious as a once monthly vitamin D_3 supplementation strategy (e.g. 1,000mcg/m).

5.9 Future Directions:

The remaining 57 subjects are currently being recruited and enrolled into the RCT (n=24 at time of Thesis submission). Ongoing data collection and analysis consists of: markers of vitamin D status, markers of bone health, dietary intake, weight-bearing physical activity, and sunlight exposure. Once these remaining subjects have completed the 6 month study protocol, the study investigators hope to have sufficient power to determine the efficacy of daily (50mcg/d) vs. monthly (1,000mcg/m) vitamin D_3 supplementation.

In addition, data is being collected for hypothesis 2 and objective 2b regarding quality of life (QoL). This data consists of a validated health-related QoL questionnaire completed at baseline and after 6 months of the vitamin D_3 supplementation protocol, as well as documentation of comorbid conditions and concomitant medications/supplements. Analysis of this additional data will look at the impact of QoL on adherence to vitamin D_3 supplementation in this population.

Once subjects have completed the 6 months for this RCT study protocol they are being asked to participate in a separate cross-sectional observational study looking at dietary intake of vitamin D/K and calcium, vitamin D status (25(OH)D and $1,25(OH)_2D$) and bone health (PTH, bone turnover markers and DXA). This new study visit takes place approximately 6 months following their final 6 month appointment for the RCT. Together, data from these two studies will provide important information on changes in vitamin D_3 supplementation practices and status after active participation in a RCT, as well as one year changes in bone health (e.g. BMD via DXA).

Additional areas for future research related to vitamin D_3 supplementation and bone health in adults with diabetes and CKD could include: kinetic studies looking at various dosing strategies and the half-life of vitamin D metabolites; relationship between vitamin D status and inflammation and its impact on bone health; and/or the impact of vitamin D_3 supplementation on insulin secretion and sensitivity in adults with diabetes and CKD.

There is an overwhelming need for prospective RCTs looking at vitamin D_3 supplementation and its effects on vitamin D status and bone health in adults with diabetes

and CKD (14,49,57,61,88,93,94). Results gleamed from the completion of this RCT, and in conjunction with the new cross-sectional study, will hopefully contribute to this gap in the literature and to the development of clinical practice guidelines.

Appendix 1:

Table A1.1: Summary of Evidence from Observational and Interventional Studies on Vitamin D

Source (country)	Population	Sample size (male)	Baseline age, years	Vitamin D dose; study design and/or duration	Vitamin D measure; comparison/incidence	Other outcomes	Comments
Genius, 2009 (Canada)	3 clinics: ob/gyn, primary care, family medicine	N=1,433 (370)	<19, 19 to <30, 30 to <60, 60+	n/a; 5y	*25(OH)D concentration*; n=240 <40nmol/l, n=738 40-79nmol/l, n=455 80-250nmol/l; 68% insufficient	n/a	Significant correlation: fish/fish oil intake, vitamin D supplement use >400IU/d, >2cup/d milk, sun exposure
Rucker, 2009 (Canada)	CKD stage 3-5	N=128 (73)	C: 67±14, D_3: 71±11, (53-82)	Randomly assigned to 1,000IU/d D_3 vs. control; 3months	*25(OH)D concentration*; C: 40±14 to 67±26nmol/l, D_3: 54±24 to 56±26nmol/l	↓ PTH with D_3 supplement vs. control (p=0.02)	41% <37.5nmol/l and 93% <75nmol/l at least once during spring or fall. DM was the main cause of CKD.
Diaz, 2009 (USA)	Adults with DM (with/without nephropathy) from 2001-2006 NHANES	N=1,216 (582)	n=224 20-45y, n=992 >45y	n/a; cross-section, throughout yr and across country	*25(OH)D concentration*; 51.5nmol/l (49-54 nmol/l); 85.5% <75nmol/l; 90.4% <75nmol/l with nephropathy vs. 83.4% without (p=0.03)	n/a	↑ risk for vitamin D insufficiency in DM with nephropathy (OR=1.78) vs. without nephropathy (controlled for race, age, sex, obesity, hypertension, cholesterol, smoking, ACE/ARB use).
Wu, 2003 (New Zealand)	Out-patient (women), vs. In-patient (men and women)	N=32 (0) N=49 (6)	76±4 (67-84) 84±5 (69-94)	50,000IU/d D_3 x 10d; 17±7 (5-31)weeks 300,000IU D_3 once; 17weeks on average	*25(OH)D concentration*: 8±1mcg/l to 21±5mcg/l 7±4mcg/l to 25±11mcg/l	In-patient: ↑serum Ca (p<0.001), ↑PTH (p<0.05), ↓ALP (p<0.05) Out-patient: n/a	In-patient 25(OH)D maximum peak of 51mcg/l between day 13-21, then ↓to an average of 10mcg/l by day 90.
Meier, 2004 (Germany)	Healthy men and post-menopausal women	N=43 (14)	Men: 60.6±10.3 (34-75) Women: 54.1±10.8 (38-75)	500IU/d D_3 + 500mg/d Ca during Oct-Mar vs. control (C); 1yr observation, 1yr supplementation	*25(OH)D* concentrations; Baseline: VD: 30.1±11.4 vs. C: 30.8±9.3mcg/L. *25(OH)D change from yr 1 to yr 2:* VD: 35.1±8.1mcg/L (p=0.02) vs. C: 20.5±8.5mcg/L (p<0.001)	Supplement ↑ lumbar BMD, and ↓PTH, BSAP and DPD vs. previous winter (p<0.05)	Baseline (observation) seasonal peaks: 25(OH)D and $1,25(OH)_2D$= Sept/Aug, PTH=Feb, BSAP=Dec/Oct, PYP and DPD=Jan/Feb. Mean ↑ in lumbar (0.8%) and FN (0.1%) BMD with supplement.

Tanaka, 2009 (Japan)	Predialysis patients with and without DM (1:1 age, sex and eGFR-matched DM and controls)	N=224 (112)	66 (57-73)	n/a; cross-sectional observation throughout yr	*25(OH)D concentration (multiple linear regression):* DM<C (p<0.0001), DM interaction p=0.0048; *1,25(OH)$_2$D concentration (multiple linear regression):* DM<C (p=0.0419), DM interaction p=0.4278.	Significantly ↑ serum Ca and P0$_4$, proteinuria and CVD with DM; ↓FGF-23 with DM. No difference in PTH, BSAP, NTx or BMD.	25(OH)D was constant across CKD stages in controls, but in DM 25(OH)D ↓ as renal function ↓, relationship remained robust after adjusting for proteinuria. 1,25(OH)$_2$D significantly ↓ in DM, especially as function ↓, likely due to poor 25(OH)D status.
Patel, 2010 (USA)	Adults with early CKD (eGFR <60 ml/min/1.73m^2 at screening)	N=1661 (794)	Anemic: 71±11 Non-anemic: 69±11	n/a; cross-sectional design with study visit during peak sunlight season (June-October 2004)	*25(OH)D concentrations*: 26±13 vs. 31±12ng/ml (anemic vs. non-anemic; p<0.001) *1,25(OH)$_2$D concentration*: 24(15-33) vs. 32(22-45) pg/ml (anemic vs. non-anemic; p<0.001)	Prevalence of anemia (hemoglobin <13.5g/dl males, <12g/dl females): n=680 anemic, n=981 non-anemic	Linear relationship between hemoglobin and 25(OH)D (r=0.22, p<0.001) and 1,25(OH)$_2$D (r=0.26, p<0.001). 25(OH)D <25nmol/l ↑anemia risk by 2.8-fold, and 1,25(OH)2D <30pg/ml ↑ risk by 2-fold vs. highest vitamin D status.
Mehrotra, 2008 (USA)	NHANES 1988-1994: A)Deliberate oversampling of Black, Mexican, and elderly; B)Cohort with diabetes and nephropathy	N=15,828 (approx. 7,597) N=146 (75)	40.7 – 72.5 (non-CKD to stage 4 or 5 CKD, p<0.0001) 57.0±0.6	n/a; cross-section – association between CKD stages and 25(OH)D deficiency n/a; cross-section – effects of suboptimal 25(OH)D on BMD (1year follow-up)	*Adjusted odds ratio (OR) for 25(OH)D:* 25-50ng/ml OR=1.13-1.15, <15ng/ml OR=1.32-1.39; *Dietary vitamin D intake*: 230.8-346.8 IU/d across all CKD stages (p=0.3) *25(OH)D concentration*; n=55 <15ng/ml, n=66 15-30ng/ml, n=25 >30ng/ml	n/a PTH: r = -0.33, p<0.0001. BMD: Spine T-score<-1.0 in 64%	Those with CKD were 32% more likely to be deficient (controlled for variables associated with vitamin D intake and status and SES); relationship between low 25(OH)D and CKD not likely due to dietary intake. Unable to demonstrate relationship between 25(OH)D and bone health in adults with diabetes and nephropathy.
Janner, 2010 (Switzerland)	Adolescent out-patients with T1D	N= 129 (69)	11.6 (95% CI: 11.0-12.3)	n/a; cross-sectional, throughout yr: winter (Dec-Mar, n=44), spring (Mar-June, n=20), summer (June -Sep, n=34), autumn (Sept-Dec, n=31)	*25(OH)D concentration*: n=112 <75nmol/l, n=78 <50nmol/l; 90-98% <50nmol/l in autumn-spring vs. 68% in summer.	↑ALP if 25(OH)D <75nmol/l(p=0.03) ↑PTH (+2 SD for age) in n=3 <50nmol/l, n=2 <25nmol/l (n=8 iPTH <50pg/ml)	25(OH)D only exceeded 75nmol/l in July, Aug and Sept. Lower prevalence of ↑PTH than expected, queried if due to near optimal intake of calcium (mean calcium intake: 845mg/d)

Sanfelix-Genovés, 2009 (Spain)	Independent-living post-menopausal women treated for osteoporosis (pharmaceutical plus vitamin D and/or calcium)	N=630 (0)	64.1±8.7	Vitamin D and calcium dose and vitamin D status not specified or available; cross-sectional, observational, multi-site	*Adherence to therapeutic vitamin D and/or calcium supplement via physician administered "Haynes-Sackett" test*: 50% had good adherence (took >80% of the time)	Additional pathologies: 62.5% had >2. Additional medications: non-adherent = 3.2± 1.5, vs. adherent= 2.5±1.3	Factors that impacted adherence: good attitude to treatment (OR=11.7), no adverse effects (OR=3.3), concurrent treatments (OR=0.8, p=0.017), having diabetes (OR=0.83, p>0.05). Non-adherence ↑20% with each additional pathology with its own concurrent therapy/medication.
Stavroulo-poulos, 2008 (UK)	CKD stage 3-4	N=112 (67)	51 (26-65)	n/a; cross-sectional observational	*25(OH)D concentration*: 52±30nmol/l (median 47nmol/l); n=34 <12nmol/l, n=35 40-75nmol/l, n=20 >75nmol/l	↓PTH with ↑ 25(OH)D status. BMD (femur): indirect relation-ship via PTH.	Primary cause of CKD was diabetic nephropathy (34%). Participants recruited from a coronary artery calcification study (excluding those with heart disease)
Mangat, 2010 (UK)	Ambulatory pre-dialysis patients, stratified into groups based on CKD stage: A)stage 1-2, B)stage 3, C)stage 4	N=41 (18) N=59 (37) N=45 (19)	47±13 57±15 55±13	n/a; cross-sectional observational	*25(OH)D concentration;* 53.1±26.5 vs. 50±23.7 vs. 50.6±26.8nmol/l, respectively. *1,25(OH)$_2$D concentration;* 89±41.5 vs. 67.5±32.2 vs. 44±34.6pmol/l, respectively (p<0.05).	Concentrations of PTH, CRP and FGF-23 ↑with ↑CKD stage. 81% had DXA scan; FN BMD correlated with 1,25(OH)$_2$D	CKD etiology: 2.4, 15.3, and 17.8% of cases were attributed to diabetes in the 3 CKD stage groups, respectively. When corrected for confounding variables, FN BMD correlated with BMI (p=0.025), age (p=0.021) and BSAP (p=0.006).
Mitri, 2011 (USA)	Adults at risk for T2D or with early T2D (Calcium and Vitamin D for Diabetes Mellitus (CaDDM) trial).	N=92 (45); n=6 with T2D, n=86 at risk for T2D	57±1	2-by-2 factorial, double-masked, placebo-controlled, randomized (1:1) trial; 2,000IU/d D_3 with/without 800mg/d calcium vs. placebo with/without 800mg/d calcium;16 weeks, throughout yr	*25(OH)D concentration:* VD: 30.6±1.2ng/ml (↑6.3±1.0ng/ml; p<0.001) Placebo: 18.4±1.1ng/ml (↓6.3±1.0ng/ml) (p<0.001). *Supplement adherence [a]:* 89% D_3 and 85% calcium. *Baseline dietary intake:* 216 ±15IU/d vitamin D and 859±49mg/d calcium.	Supplement ↑ HbA1c (p=0.055) and AIRg (p=0.07) from baseline; no change in insulin secretion (p=0.16).	Isolated calcium supplementation had no effect. No cases of hypercalcemia or nephrolithiasis. Vitamin D increased disposition index (beta-cell function) 26% vs. a 14% reduction with placebo (p=0.011).
Gagnon, 2011 (Australia)	Non-institutionalized adults ≥25y (Australian Diabetes, Obesity and Lifestyle study)	N=5,200 with complete data from 1999 baseline and 2005 follow-up (2,355)	DM: 55.6± 11.9 No DM: 50.6±12.5 (p<0.001)	n/a; population-based prospective observational study	*Incident cases of DM:* N=199 (3.8%). *25(OH)D concentration*: DM 58±23nmol/l, vs. no DM 65±25nmol/l (p<0.001)	Dietary Ca and Mg intake: DM 881±260mg/d and 286(256-328) mg/d, no DM 932±263mg/d and 297(263-337)mg/d (p=0.03)	For each 25nmol/l ↑ 25(OH)D, Dm risk ↓ 29% (OR: 0.71; adjusted for age, ethnicity, waist circumference, smoking, physical activity, family history of DM, season and latitude). Ca intake was not associated with DM risk or insulin sensitivity in any of the models.

Wu, 2009 (Canada)	Young healthy adults of diverse ancestry (3 main ethnic groups)	N=105 (47)	20.7±2.0	n/a; Jan-Mar 2007	*25(OH)D concentration*: 42.8±16.5nmol/l *Vitamin D intake (mcg/d):* 7d diary: 4.3±3.6, FFQ-1: 7.8±5.4, FFQ-2: 6.6±6.0; ↑intake in European vs. other 2 groups (p<0.05).	Ca intake (mg/d): 7d diary 916±379, FFQ-1 1213±812, FFQ-2 1003±851	East Asian (n=27), European (n=31), and South Asian (n=32). Significant difference in melanin index and 25(OH)D (p<0.001), and vitamin D and Ca intake (p<0.05).
Poliquin, 2009 (Canada)	Adults ≥25yr participating in a longitudinal osteoporosis study (CaMos)	Women: N=6,539 Men: N=2,884	63.1±12.8 59.9±14.5	n/a; cross-sectional, observational, multi-site	*Total vitamin D intake*: Women: 5.6±5.9mcg/d, Men: 4.8±5.5mcg/d *Dietary vitamin D intake*: Women: 2.7±2.9mcg/d Men: 3.0±3.5mcg/d	Calcium intake: Women: 1038± 614mg/d, Men: 904±583mg/d	Dietary intake assessed by a trained interviewer with an abbreviated FFQ. Vitamin D intake assessed by consumption of fluid milk (2.5mcg/ 250ml) and supplements only. Likelihood of meeting DRI ↓with ↑age
Dalgård, 2010 (Faroe Islands)	Elderly Faroese people residing in fishing village (part of study on methylmercury exposure)	N=713; n=669 provided serum samples	72.4±1.2	n/a; cross-sectional, observational	*25(OH)D concentration*: Men: 20% <25nmol/l, 41% >50nmol/l, 7% >80nmol/l; Women: 17% <25nmol/l, 52% >50nmol/l, 14% >80nmol/l (x^2=9.4, p<0.03)	No significant difference in intake of fish (p=0.68) or whale blubber (p=0.21) on 25(OH)D.	Only in July and Sept was 25(OH)D >80nmol/l for 22-24%; 25(OH)D significantly ↑ in July-Sept vs. all other months (p<0.0001). Haddock and cod most commonly eaten; only 1mcg vitamin D/100g.
Anderson, 2010 (Canada)	Women stratified by age: 25-50yr, 51-70yr, 71-74yr	N=3,393 (0)	n=1,251 n=1,902 n=240		Dietary vitamin D intake: 5.3±3.4mcg/d; 54% also took supplements	FFQ modified for Canadian foods was 0.54mcg/d↑	Intake assessed by Block (1998) FFQ pre-and-post modification for food fortification with vitamin D in Canada. Likelihood of meeting DRI ↓with ↑age
McCullough, 2009 (USA)	Adults living in southeastern USA, who were participating in "calcium, vitamin D, and markers of adenomatous polyps" pilot study	N=91 (64) at baseline; N=85 at follow-up	Men: 58.4±6.7 Women: 61.7±8.4	randomized, double-blind, placebo-controlled 2x2 factorial clinical trial; A)800IU/d D_3, B)2,000IU/d D3 + 2000mg/d Ca; C)2,000mg/d Ca, or D)placebo; 6months	*Baseline 25(OH)D (nmol/l):* A)52.6±4.4, B)52.3±4.3, C)64.2±4.3, D)51.1±4.3 (p=0.12); n=21 deficient, n=54 insufficient, n=16 sufficient *Change in 25(OH)D (nmol/l; not adjusted):* A)21.1±5.9; B)19.0±6.7; C)-6.2±6.2, D)-6.3±5.6	Adherence to supplements (≥80%): 93% at 1month, 84% at 6months	25(OH)D status defined as: Deficient <37.5nmol/l, Insufficient 37.5 to <75nmol/l, Sufficient ≥75nmol/l. *Prevalence of 25(OH)D status' at 6m follow-up (*deficient, insufficient, sufficient): A)n=1, 7, 14; B)n=1, 12, 8; C)n=5, 11, 5; D)n=6, 15, 0
Yaturu, 2011 (USA)	Veterans living in Louisiana	n=2990 (2990)	68	n/a; Retrospective electronic chart review	*25(OH)D concentration:* 77% <30ng/ml and 47% <20ng/ml	35% had T2D, 38% had CKD, 45% had CKD and T2D	Those with T2D, CKD, or T2D and CKD had significantly lower 25(OH)D than those without T2D and/or CKD (p<0.05)

Kim, 2011 (England)	Adults with T2D and CKD (GFR 15-90 ml/min/1.73m^2)	n=63 (48) enrolled, n=49 treated (compliant) with vitamin D_3	69 ± 9.6	40,000IU/wk x 2m, then q monthly (deficient), or 40,000IU/m (insufficient); Open-label prospective observational study	*25(OH)D concentration:* baseline=18.4±9.8ng/ml (n=54 (86%) <29ng/ml), 2m=41.2±11.4ng/ml, 4m=39.7±12.8ng/ml; *1,25(OH)$_2$D concentration*: Baseline=23.9±13.8pg/ml, 2m=51.0±16.0pg/ml, 4m=42.8±23.8pg/ml	Strong correlation for urinary VDBP: creatinine ratio and uACR (R = 0.8720-0.8170, p<0.0001). Significant ↓ in uACR (p<0.05).	Significantly ↑ 25(OH)D in all patients treated for insufficiency/deficiency (p<0.05). Significantly ↑ 1,25(OH)$_2$D only at 4m in those with stage 3 CKD.

[a]: Adherence is defined as taking the vitamin D ≥80% of the time.

Definitions: DM, diabetes mellitus; C, control; VD, vitamin D intervention group; D_3, cholecalciferol; D_2, ergocalciferol; VDBP, vitamin D binding protein; uACR, urinary albumin to creatinine ratio; Ca, calcium; Mg, magnesium; FFQ, food frequency questionnaire; wk, week;

Appendix 2:

Health Canada / Santé Canada

Therapeutic Products Directorate
5th Floor, Holland Cross, Tower B
Address Locator# 3105A
OTTAWA, Ontario
K1A 0K9

Your file / Votre référence

Our file / Notre référence

10 August 2011

9427-G0890-67C

Diana R. Mager, PhD, RD
Assistant Professor, Clinical Nutrition
Department of Agricultural, Food and Nutritional Science
The Governors of the University of Alberta
4-126 Li Ka Shing Centre for Research Innovation
EDMONTON, Alberta
T6G 0K2
(780) 492-7687

No Objection Letter RE: Protocol # DIABETES VITAMIN D 20112012

Dear Dr. Mager:

I am pleased to inform you that the information and material to support your Clinical Trial Application for **VITAMIN D3**, control number **148625**, received on July 15, 2011, have been reviewed and we have no objection to your proposed study.

I would remind you of the necessity of complying with the *Food and Drug Regulations*, Division 5, in the sale of this product for clinical testing. In addition, the regulations impose record keeping responsibilities on those conducting clinical trials.

You are also reminded that all clinical trials should be conducted in compliance with the Therapeutic Products Directorate's *Guideline for Good Clinical Practice*.

Please note that for drugs marketed in Canada and in clinical trials, any serious and unexpected adverse drug reaction occurring inside or outside Canada should be reported to both MHPD and TPD until completion of the trial then the reports should be sent to MHPD only.

Should you have any questions concerning this letter, please contact the Office of Clinical Trials (613) 941-2132.

Yours sincerely,

K. Rakusan
for

Léo Bouthillier, Ph.D.
Manager - Clinical Trials Group II
Office of Clinical Trials

LB/kr

Canada

Health Canada / Santé Canada

Canada

Health Products and Food Branch
Direction générale des produits de santé et des aliments

Therapeutic Products Directorate

OUR MISSION: We contribute to the health of Canadians and to the effectiveness of the health care system by regulating pharmaceuticals and medical devices and by providing Canadians with access to information to make informed choices.

Direction des produits thérapeutiques

NOTRE MISSION : Nous contribuons à l'amélioration de la santé des Canadiens et à l'efficacité du système de soins de santé en réglementant les produits pharmaceutiques et les matériels médicaux et en offrant aux Canadiens un accès à l'information pour qu'ils puissent faire des choix éclairés

If you receive this fax in error, please advise the sender immediately.
Si vous recevez cette télécopie par erreur, veuillez en aviser immédiatement l'expéditeur.

TO/À
Name/Nom: Dr. Diana R. Mager Date: 10 August 2011
Organization/Organisme: The Governors of the University of Alberta
Tel./Tél.: 780-492-7687 Fax/Télécopieur: 780-492-0979
No. of Pages, including this page/Nbe pages, incleant cette page: 2

FROM/DE
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TITLE / TITRE
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Directorate / Direction
Manager - Clinical Trials Group I / Gestionnaire - Programme des essais cliniques Groupe I
Office of Clinical Trials / Bureau des essais cliniques
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DIRECTION DES PRODUITS THÉRAPEUTIQUES
Room / Pièce: 5073
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Location / Lieu: 1600 Scott Street / 1600 Rue Scott
Address Locator / Localisateur d'adresse: 3105A
City/Province / Ville/Province: OTTAWA, Ontario
Postal Code / Code postal: K1A 0K9
Website/site Web : http://www.hc-sc.gc.ca/dhp-mps/prodpharma/index_e.html/
http://www.hc-sc.gc.ca/dhp-mps/prodpharma/index_f.html

MESSAGE

IMPORTANT:

Before submitting an application please ensure that your CD is in a CD Jewel Case/hard plastic CD case. The case is required for storing the CD appropriately. / Avant de soumettre votre demande, veuillez vous assurer que votre CD est inséré dans un étui rigide en plastique. L'étui est nécessaire afin de conserver le CD en bon état.

Clinical Trials Manual/Manuel d'essais cliniques

http://www.hc-sc.gc.ca/dhp-mps/prodpharma/applic-demande/guide-ld/clini/index_e.html or /index_f.html

Release of Protocol Safety and Efficacy Assessment Template-Clinical Trial Application (PSEAT-CTA)/
Diffusion du Modèle d'évaluation de l'innocuité et de l'efficacité des protocoles - Demande d'essai clinique (MEIEP-DEC)

http://www.hc-sc.gc.ca/dhp-mps/prodpharma/update-miseajour/index_e.html or /index_f.html

Re-Approval Form

Date:	July 5, 2012
Principal Investigator:	Diana Mager
Study ID:	Pro00022639
Study Title:	Vitamin D Supplementation and Bone Health in Adults with Diabetic Nephropathy
Approval Expiry Date:	August 15, 2013
Sponsor/Funding Agency:	Kidney Foundation of Canada
Sponsor/Funding Agency	University of Alberta Start Up Funds NO31000542

The Health Research Ethics Board - Biomedical Panel has reviewed the renewal request and file for this project and found it to be acceptable within the limitations of human experimentation. We acknowledge that you have updated your study staff for this project.

The re-approval for the study as presented is valid for another year. It may be extended following completion of the annual renewal request. Beginning 45 days prior to expiration, you will receive notices that the study is about to expire. Once the study has expired you will have to resubmit. Any proposed changes to the study must be submitted to the HREB for approval prior to implementation.

All study-related documents should be retained so as to be available to the HREB on request. They should be kept for the duration of the project and for at least five years following study completion. In the case of clinical trials approved under Division 5 of the Food and Drug regulations of Health Canada, study records must be retained for 25 years.

Sincerely,

S.K.M. Kimber, MD, FRCPC
Chair, HREB Biomedical

Note: This correspondence includes an electronic signature (validation and approval via an online system).

Version 3
Readability: 8.3
July 4, 2011

U N I V E R S I T Y O F A L B E R T A

INFORMATION LETTER

Title of Project: Vitamin D Supplementation and Bone Health in Adults with Diabetic Nephropathy.

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Co-Investigator: Dr. Peter Senior, MBBS PhD Telephone: 780-407-8852
Study Coordinator: Stephanie Schwindt, MSc(c) RD Telephone: 780-901-8990

Purpose of this study

We are asking if you would like to take part in a research study that will help us learn about the best way to give vitamin D pills (daily vs. monthly) to adults with both diabetes and kidney disease (diabetic nephropathy). Vitamin D is made in our bodies and plays a big role in having strong healthy bones. When our kidneys don't work well the body is not able to make enough vitamin D for our bones. This is a big issue especially when people live in northern communities because a person might not get enough sunshine or eat enough vitamin D to meet the body's need for vitamin D. Taking vitamin D pills can help you meet your body's needs for vitamin D. Sometimes it is hard to remember to take a vitamin pill every day especially if you are taking other pills, so we want to study if taking a bigger dose once a month would be a better way to get vitamin D instead of every day. We will ask you to come to the Clinical Research Unit (CRU) at the University of Alberta 3 different times: when you first start the study, and then 3 and 6 months after you start the study.

Procedures of the study

1. Vitamin D Pills

You will be placed in 1 of 2 groups: 1) take low dose vitamin D pills by mouth each day (2,000 IU), or 2) take high dose vitamin D pills by mouth once a month (40,000 IU). Both doses are safe and you will get the same total dose of vitamin D over 1 month. The major difference is that you will either take pills every day (with a smaller amount of vitamin D), or you will take pills once per month with a larger amount of vitamin D. You will be asked to take one of the different types of vitamin D pill doses for 6 months. It is important that you take the pills in the way that we ask you to. Please bring your pill bottles and the survey about the type of vitamin D pills that you are taking to your study visits. Do not share your vitamin D pills with your family or friends.

2. Anthropometric Measures

We will measure your weight and height. This will happen during your study visits at the start of the study, and then at the visits at 3 and 6 months. This is part of routine clinical care.

3. Bone Health

We will also measure how healthy your bones are using a special machine called a DEXA (dual-energy x-ray absorptiometry). A bone density test is not painful or uncomfortable and will take about 20 minutes to perform. You will need to lie still for about 1-5 minutes for up to 3 times while the test is being done. The DEXA machine measures how dense your bones are and will help us know if your bones are healthy. We

will do this test at the CRU at the University of Alberta when you first start the study to learn how healthy your bones are at the start of the study. Having a DEXA scan will expose you to a very small amount of radiation. This is about the same amount that a person is exposed to when taking an airplane ride across the country, which meets national safety standards. We will ask you to sign a different consent form on the day of the DEXA scan. This form will ask you to confirm that you are not pregnant because it is not safe for pregnant woman and their babies to have a DEXA scan.

4. Food Intake

We will ask you to fill out a 3 day food record based on what you eat. This will help us to see how your diet affects the vitamin D in your blood and your bone health. For the 3 day food records we will ask you to write down what you have eaten for the 3 days (2 weekdays and 1 weekend day). It will take about 10 minutes to fill out the food record on each of the 3 days. We will provide you with a self-addressed stamped envelope so you can mail this back to the research team. We would like you to fill out the food record when you first start the study, and then after 3 and 6 months. This is in addition to routine care.

5. Physical Activity

We would like you to fill out a survey on the amount and type of physical activity you do. This will help us understand how the weight-bearing activity you do (like walking and running) might affect your bone health. We would like you to write down what types of physical activity you do every day (such as walking up and down stairs, walking to the bus stop, etc) on 3 different days (2 weekdays and 1 weekend day). You can do this at the same time you record your 3 day food record and use the same forms. These activity records should be done when you first start the study and then after 3 and 6 months. This is in addition to routine care.

6. Sun Light

We would like you to fill out a survey on the amount of time you spend in the sun and if you do things to protect yourself from the sun. For example, do you wear sunscreen, if so what kind and how often do you put it on? This will help us learn how the time you spend in the sunshine can affect your vitamin D levels and your bone health. It will only take 5-10 minutes to fill out and can be done at a study visit or at home. This is in addition to routine care.

7. Quality of Life

We would like you to fill out a survey when you enter the study and after 6 months that will help us understand your health related quality of life.

8. Blood work

Your doctor will order your regular blood work. You will be asked not to take any vitamin pills on the day of your blood work. This will give us a better idea of the vitamin D in your blood. We will not poke you for an extra blood test; we will take an extra half of a teaspoon of blood when you are having your normal blood work done. We will use this extra blood work to help us learn how well the 2 ways of taking vitamin D pills work and how this affects your vitamin D blood levels and bone health. The blood work will be done when you first start the study and then 3 and 6 months later.

9. Medical Records

We would also like to look at your medical records to find out about medications, blood work (for example, the amount of calcium, phosphorous and sugar that is in your blood) and results of other medical tests that were used to find out about your kidney health. This will help us learn how your body uses vitamin D.

Possible Benefits:

You may get better vitamin D levels in your blood and healthier bones.

Possible Risks:

The radiation dose from the bone scans is about 10μSv. This is about half the dose you receive from a chest X-ray, or about the same amount an adult gets during a flight across Canada. You should not have x-ray tests during pregnancy. The DEXA test will only be done on women that are not pregnant or trying to get pregnant.

There are no reports of harm caused by the vitamin D doses in this study. Long term use of 50,000 IU – 100,000 IU each day is not recommended, particularly if you take this amount every day for many months. These levels are much higher than you will be taking in the study. Taking too much vitamin D for your body may cause the following symptoms: weakness, nausea, headaches, dizziness, or vomiting. If any of these happen to you after starting this study please tell your health care provider and the research team.

Confidentiality:

We will not share any information in your personal health record with anyone. Any research data collected about you during this study will not identify you by name, only by a coded number. Your name will not be shared with anyone outside the research clinic and your name will not be in any reports published from this research.

For this study, the doctor or other members of the research team (dietitian graduate student) may need to access your personal health records for health information. He/she may also need to contact your family doctor and your other health care providers to obtain additional medical information. The health information collected as part of this study will be kept confidential unless release is required by law, and will be used only for the purpose of the research study. By signing the consent form you give permission to the study staff to access any personally identifiable health information which is under the custody of other health care professionals. This will only be done if it is thought to be necessary to carry out this research project.

The personal health information collected in this study may need to be checked by the Health Research Ethics Board (HREB) at the University of Alberta/Alberta Health Services. This may be necessary so the HREB can make sure that the data collected in the study is accurate.

By signing the consent form you give permission for the collection, use and sharing of information from your medical records for purpose of this research. In Canada, study information is required to be kept for 5 years. Even if you withdraw from the study, the medical information which is obtained from you the research will not be destroyed. You have a right to check your health records and request changes if your personal information is incorrect.

Voluntary Participation:
You are free to stop participating in the study at any time. No one will be upset and this will not affect the quality of medical care that you are provided. If there is any information that is gained from the study that may affect your choice to continue with this study, we will let you know right away.

Reimbursement of Expenses:
You will be given parking vouchers to cover the cost of your parking expenses.

Compensation for Injury*:*
If you become ill or injured as a result of taking part in this study, necessary medical treatment will be available at no additional cost to you. By signing this consent form you are not releasing the investigator(s) or institution(s) from their legal and professional responsibilities.

Do you have more questions?
You can ask your dietitian about anything you don't understand. You can also talk to Diana Mager or Peter Senior. Diana Mager's phone number is 492-7687. Peter Senior's telephone number is 407-3636. If you have any problems or concerns about any part of this study please call the Research Ethics Office at 780-492-2615. This office has no connection with the study researchers.

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CONSENT FORM

Title of Project: Vitamin D Supplementation and Bone Health in Adults with Diabetic Nephropathy.

Principal Investigator: Diana Mager, PhD RD **Phone Number:** 780-492-7687

Co-Investigator: Dr. Peter Senior, MBBS PhD **Phone Number:** 780-407-8852

	Yes	No
Do you understand that you have been asked to participate in a research study?	☐	☐
Have you read and received a copy of the attached Information Sheet?	☐	☐
Do you understand the benefits and risks involved for you by taking part in this research study?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Do you understand that you are free to withdraw from the study at any time, without having to give a reason and without affecting your future medical care?	☐	☐
Do you understand who will have access to your records, including personally identifiable health information?	☐	☐
Do you want the investigator(s) to inform your family doctor that you are participating in this research study?	☐	☐

If yes, doctor's name and phone number

_____

Who explained this study to you? _____

_____

Name _____

I agree to take part in this study: YES ☐ NO ☐

Signature _____ Date & Time _____

(Printed Name) _____

Signature of Witness _____ Date & Time _____

Signature of Investigator or Designee _____Date & Time _____

DXA Scan

Information Sheet

Test Background:
Dual Energy X-Ray Absorptiometry (DXA) is a simple test that provides a very accurate measurement of bone density, lean tissue mass, and total and regional body fat (ie. abdominal body fat). This test uses very low dose x-rays of two different levels to distinguish between bone and soft tissue.

DXA is a painless, non-invasive test. The test requires that you put on a hospital gown and lie on an x-ray bed. The scan takes about 5 minutes and is very low dose radiation (equivalent to approximately 1 day of natural background radiation). This dosage is 1000 times less than the limit for trivial exposure, and is classified as a negligible individual dose according to the standards of the National Council of Radiation Protection and Measurements.

Preparation for the Test:
No special preparation is necessary. Pregnant women and individuals who have recently undergone barium tests/exams (within 2 weeks), or who have had a nuclear medicine scan or been injected with an X-ray dye (within 1 week) cannot have a DXA scan. We ask that you do not wear anything metal (metal may affect bone density values). We will ask you to remove all jewellery.

PREGNANT WOMEN CANNOT PARTICIPATE IN A DXA SCAN. Prior to taking part in the scan, women will be asked to provide a urine sample to verify that they are not pregnant. The pregnancy test that we are using meets WHO guidelines for pregnancy testing, and can detect pregnancy within 1 week after conception. No pregnancy test is, however, 100% accurate, and there is always the possibility of an incorrect result. All results should be confirmed by your physician. You may choose not to undergo this test if you are pre-pubertal (no regular menstrual cycle), taking oral/injection contraceptives, post-menopausal (no menstrual cycle for $\geq$ 6 months), or if you have had a hysterectomy. All other women must undergo a pregnancy test.

Purpose and Time Commitment:
The purpose of the DXA scan is to assess body composition by quantifying bone, muscle, and fat mass. This information helps researchers to monitor changes in body composition over time. An experienced certified Medical X-Ray Technologist will be conducting the scan. The total time required to complete a total body scan is 20 minutes, including the time required to change into the gown, get positioned on the table and complete the scan. Women will be asked to provide a urine sample for a pregnancy test prior to the DXA scan, and thus the test may take up to 30 minutes.

Potential Benefits
After participating in this DXA scan, you will find out information about your body composition; that is – details about your lean body mass, fat mass and/or bone mass.

Potential Risks
The x-ray dose associated with a total body scan is very low and safe for repeated measurements. With the exception of pregnant women, there are no known risks associated with a DXA scan. The potential risks associated with radiation exposure to an unborn fetus are not known, and therefore we ask that you undergo a pregnancy test to verify that you are not pregnant. Having a DXA scan does not make it unsafe for you to have other x-rays taken in the near future.

Stopping the Test
You may ask the technologist to stop the test at any time without jeopardy to you.

Confidentiality
Your scan will be saved in our database using an identification number known only to the researcher for your study. The results of your scan will only be disclosed to the researcher for your study and will be saved in our database for one year.

DXA Scan
Consent Form

Consent*: (Please circle your answers)*

Sex **M** **F**

Females: Are you pregnant? **Yes** **No**

Females: Do you agree to undergo a pregnancy test? **Yes** **No**

If No, circle reason: Pre-pubertal (no regular menstrual cycle)
Taking oral/injection contraceptives
Post-menopausal (no menstrual cycle for ≥ 6 months)
Hysterectomy

Have you had a barium test/exam in the last 2 weeks? **Yes** **No**

Have you had a nuclear medicine scan or injection of an X-ray dye in the past week? **Yes** **No**

Have you read and received a copy of the Information Sheet? **Yes** **No**

Do you understand the benefits and risks involved in taking part in this test? **Yes** **No**

Have you had an opportunity to ask questions and discuss testing procedures? **Yes** **No**

Do you understand that you can stop the DXA testing at any time and that you do not have to say why? **Yes** **No**

Has confidentiality been explained to you? **Yes** **No**

_____ _____
Date **Date of Last Menstrual Period (If applicable)**

_____ _____
Name of Participant **Signature of Participant**

_____ _____
Name of Witness **Signature of Witness**

_____ _____
Name of Investigator **Signature of Investigator**

HUMAN NUTRITION RESEARCH UNIT

Mailing address: 4-126 Li Ka Shing Centre
Office: 2-021A Li Ka Shing Centre
Department of Agricultural, Food and Nutritional Science
University of Alberta
Edmonton, AB T6G 2P5
Tel: (780) 492-6668 Fax (780) 492-4320

DXA Bone Mineral Density Testing Patient Questionnaire

ID Number:_____ Date: _____

1. Your: Age: ____ Sex: Male Female

2. Have you had a barium X-ray in the last 2 weeks? Yes No
   Have you had a nuclear medicine scan or injection of an X-ray dye in the last week?
   Yes No

3. Have you had a recent weight change? Yes No
   If YES, tell us about it:
   _____

4. Have you ever broken a bone? Yes No

Bone broken	**Please describe how it happened**	**Age when this occurred**

5. Have you ever had surgery of the spine, hips, legs or arms? Yes No
   If YES, describe what type of surgery you had and which side was affected
   _____
   _____

6. Are you currently receiving, or have you previously taken ≥ 7.5 mg/d prednisone (cortisone) for more than 30 days?
   Yes, currently ____ Yes, previously _____ No ____

7. List any significant medical conditions that you have (ie Cancer, Diabetes,…):
   _____
   _____
   _____

8. Do you take any calcium supplements (including TUMS)? Yes No

9. Do you take any vitamin D supplements (including multivitamins and halibut liver oil)? Yes No

10. Are you currently receiving or have you previously received any of the following medications?

	Ever?	**Currently?**	**If current, how long?**
Medication for seizures or epilepsy			
Chemotherapy for cancer			
Medication for prostate cancer			
Medication to prevent organ transplant rejection			
Thyroid medication			

11. Have you been treated with any of the following medications?

Medication	**Ever?**	**Currently?**	**If current, how long?**
Hormone replacement therapy (Estrogen)			
Tamoxifen			
Raloxifene (Evista)			
Testosterone			
Etidronate (Didronel/Didrocal)			
Alendronate (Fosamax)			
Risedronate (Actonel)			
Intravenous pamidronate (Aredia)			
Clodronate (Bonefos, Ostac)			
Calcitonin (Miacalcin nasal spray)			
PTH (Forteo)			
Zoledronic acid (Zometa)			
Sodium fluoride (Fluotic)			

For women only…

12. Date of last menstrual period _____

13. Have you had your menopause (no menstrual cycle for ≥ 6 months)? Yes No
    If YES, at what age? _____

14. Have you had a hysterectomy? Yes No
    If YES, at what age? _____

15. Have you had both of your ovaries removed? Yes No
    If YES, at what age? _____

Technologist Notes – for office use only

Ht _____ Wt _____ 3R bags _____ Tall Block ____ Hip ROI moved _____

Med Block _____ L-spine profiles moved _____ Short block _____

Human Nutrition Research Unit

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Office: 2-021A Li Ka Shing Centre
Department of Agricultural, Food and Nutritional Science
University of Alberta
Edmonton, AB T6G 2P5
Tel: (780) 492-6668 Fax (780) 492-4320

DXA SCAN DATA SHEET

STUDY NAME _____ DATE _____

SUBJECT DETAILS:

ID NUMBER _____DATE OF BIRTH _____

Information related to ethnicity is requested because the expected BMD values are affected by ethnicity (also age and gender).

ETHNICITY (CIRCLE ONLY ONE) ASIAN BLACK HISPANIC WHITE OTHER

GENDER M F

Pregnancy Test (circle) Yes No Date of Last Menstrual Period _____

If no, circle reason:

Pre-pubertal Oral/Injection Contraceptives Post-menopausal Hysterectomy

May be waived only if subject is pre-pubertal (no regular menstrual cycle), taking oral/injection contraceptives, post-menopausal (no menstrual cycle for ≥ 6 mos), or has had a hysterectomy.

DO NOT WRITE BELOW THIS LINE

COPY OF WRITTEN INFORMED CONSENT OBTAINED _____

PREGNANCY TEST RESULTS (if applicable)_____

TECHNOLOGIST INITIALS _____ BMD or BODY COMPOSITION

HT _____ WT _____

POSITIONING NOTES

UNIVERSITY OF ALBERTA

Three-day Food and Activity Record

How to record what you eat:

- Write down everything that you eat and drink for 3 days. Include at least 1 weekend day (Saturday or Sunday).
- Refer to the following example below to help you complete the *Food and Activity Record.* Write down all the foods and drinks you consume. Include the amount eaten/drank, how the food was prepared (ex. Baked, fried, boiled, etc) and any added foods like sugar, cream, margarine, sauces and dressings. If you eat/drink something packaged, please write down the size of the package (ex. 500ml juice bottle) and the brand.
- Please write down any supplements you might take, what is in them and the brand name.
- Write down when you are active and for how long. Include all weight-bearing activities (when our feet and legs carry our weight) like walking, running, dancing, skiing and tennis. Please also complete the *Weight-bearing Physical Activity Questionnaire* after the *Food and Activity Record* example about the types of activities you like to do throughout the year.
- Eat and drink as you would normally during the recording period and do not change your physical activity habits. Remember that this form is not a test, but a tool to help you.
- Please mail the *Food and Activity Record* using the stamped self-addressed envelope provided.

Example:

It is best to measure your food using common household measuring cups and measuring spoons. Here are some ways that you can estimate the amount of food you eat when you cannot measure it:
3 oz meat = deck of cards
1 oz of cheese = size of a thumb
1 cup rice, cereal, pasta = size of a women's fist
Medium size of fruit = size of tennis ball
1 teaspoon peanut butter, sugar = size of a thumb tip

Name: Katie Smith **Date:** April 1 **Day of Week:** Monday

Time	Food/ Drink and Description	Amount Eaten	Activity
7:30	Cheerios®	1 cup (250 ml)	8:00 Walked 20 minutes to work
	1% Milk	½ cup (125 ml)	
	Coffee with cream and sugar (1 Tbsp each)	1 cup	
12:00	Peanut Butter and Jam Sandwich	1	
	(on white bread, 2 Tbsp each of regular peanut butter and jam)		
	Chocolate Chip Cookies (Dad's®)	2	
	Grapes	1 cup	
	Water	1 bottle (500ml)	
3:00	Apple	1 medium	4:30 Walked 20 minutes home from work
	Strawberry Yogurt (non-fat, Activia®)	1 container(100g)	
	Coffee with cream and sugar (1 Tbsp each)	1 cup	
5:30	Chicken Drumstick (dipped in Shake	1	
	and Bake, then baked in oven)		
	Fettuccini noodles in sauce (Sidekicks®)	½ cup (125 ml)	

	Carrot Sticks and Cucumber Slices	1 ½ cup	
	Ranch Dip (Kraft®)	1 Tbsp	
	Water	1 cup (250 ml)	
8:30	Homemade Blueberry Muffin (attached recipe)	1 small	7:00 Walked around mall for about 30 minutes

Weight-Bearing Physical Activity Questionnaire:

Please answer the following questions. Examples of activities are provided, and if there are any other activities you do please include them too.

1. How often do you do weight-bearing physical activity (activity where your feet and legs carry your body weight)? Examples: walking, running, tennis, dancing, skiing, etc.
   a) Less than once a month. How many days?
      _____
   b) Less than once a week. How many days?
      _____
   c) More than once a week. How many days?
      _____
   d) Daily?
      _____

2. Sometimes the activities we do depends on the time of year or season. What kind of activities do you do throughout the year? How often do you do them, and for how long each time?
   Example: Summer – play tennis for 1 hour a week, push lawn mower for 1 hour every 2 weeks
   Winter – ski for about 4 hours a day 4 times over the winter
   _____
   _____
   _____
   _____
   _____

3. Do you do these activities inside or outside?
   Example: Walk outside in the spring and summer, walk in malls in the winter.
   _____
   _____
   _____
   _____
   _____

4. Are there any other activities you do to keep active? If so, how often?
   Example: Tai Chi, yoga, lift weights
   _____
   _____
   _____
   _____
   _____

Three-Day Food and Activity Record: Day 1

Name: _____Date: _____Day of Week: _____

Time	Food/ Drink and Description	Amount Eaten	Activity

Three-Day Food and Activity Record: Day 2

Name: _____ Date: _____Day of Week: _____

Time	Food/ Drink and Description	Amount Eaten	Activity

Three-Day Food and Activity Record: Day 3

Name: _____ Date: _____Day of Week: _____

Time	Food/ Drink and Description	Amount Eaten	Activity

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Adherence and Acceptance of Monthly (40,000 IU) Vitamin D Supplement Strategy*

Please complete this survey before coming to your study visit at month 3 and 6 after starting the study. Please bring your vitamin D pill bottle to the study visit also.

How often did you take your vitamin D pills every month?

Every month 4 months 2 months 1 month

Please circle the number that best represents your answer to the questions below
(1) None of the time (2) Some of the time (1-2 times)
(3) Most of the time (3-5 times) (4) All of the time (6 times)

How often do you forget to take your vitamin D pills?
(1) (2) (3) (4)

How often do you decide not to take your vitamin D pills?
(1) (2) (3) (4)

How often do you miss taking your vitamin D pills when you are on holidays?
(1) (2) (3) (4)

How often do you miss taking your vitamin D pills in the summer?
(1) (2) (3) (4)

How often do you miss taking your vitamin D pills when you feel sick?
(1) (2) (3) (4)

How often do you take someone else's vitamin D pills?
(1) (2) (3) (4)

Please circle the number that best represents your answer to the questions below
(1) None of the time **(2) Some of the time (3-4 servings a week)**
(3) Most of the time (1 serving every day) **(4) All of the time (2-3 servings a day)**

How often do you eat dairy or calcium and vitamin D rich foods?
Serving sizes: 1 cup of milk or calcium/vitamin D fortified orange juice or soy beverage, 1.5 ounces (50 grams) cheese, or ¾ cup (175 grams) yogurt

(1) (2) (3) (4)

How often do you drink coffee?
Serving sizes: 1 serving = 1 cup (250ml or 8 ounces), 2 servings = 2 cups (500ml or 16 ounces), 3 servings = 3 cups (750ml or 24 ounces)

(1) (2) (3) (4)

What did you like the most about the vitamin D supplementation strategy you used?

What did you like least about the vitamin D supplementation strategy you used?

Overall, what did you think about the vitamin D supplementation strategy you used? Please provide any comments you may have.

*Adapted from Kim MT, et al. Development and Testing of the Hill-Bone Compliance to High Blood Pressure Therapy Scale. *Prog Cardiovasc Nurs* 2000; 15: 90-96.

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Adherence and Acceptance of Daily (2,000 IU) Vitamin D Supplement Strategy*

Please complete this survey before coming to your study visit at month 3 and 6 after starting the study. Please bring your vitamin D pill bottle to the study visit also.

How many days a week do you take your vitamin D pills?

7 days 5 days 3 days 2 days 1 day

Please circle the number that best represents your answer to the questions below
(1) None of the time (2) Some of the time (1-3 days a week)
(3) Most of the time (4-6 days a week) (4) All of the time (7 days a week)

How often do you forget to take your vitamin D pills?

(2) (2) (3) (4)

How often do you decide not to take your vitamin D pills?

(2) (2) (3) (4)

How often do you miss taking your vitamin D pills when you are on holidays?

(2) (2) (3) (4)

How often do you miss taking your vitamin D pills in the summer?

(2) (2) (3) (4)

How often do you miss taking your vitamin D pills when you feel sick?

(2) (2) (3) (4)

How often do you take someone else's vitamin D pills?

(2) (2) (3) (4)

Please circle the number that best represents your answer to the questions below
(1) None of the time **(2) Some of the time (3-4 servings a week)**
(3) Most of the time (1 serving every day) **(4) All of the time (2-3 servings a day)**

How often do you eat dairy or calcium and vitamin D rich foods?
Serving sizes: 1 cup of milk or calcium/vitamin D fortified orange juice or soy beverage, 1.5 ounces (50 grams) cheese, or ¾ cup (175 grams) yogurt

(2) (2) (3) (4)

How often do you drink coffee?
Serving sizes: 1 serving = 1 cup (250ml or 8 ounces), 2 servings = 2 cups (500ml or 16 ounces), 3 servings = 3 cups (750ml or 24 ounces)

(2) (2) (3) (4)

What did you like the most about the vitamin D supplementation strategy you used?

What did you like least about the vitamin D supplementation strategy you used?

Overall, what did you think about the vitamin D supplementation strategy you used? Please provide any comments you may have.

*Adapted from Kim MT, et al. Development and Testing of the Hill-Bone Compliance to High Blood Pressure Therapy Scale. *Prog Cardiovasc Nurs* 2000; 15: 90-96.

Subject Tracking Sheet

Patient/code: _____ Supplement Group: _____ Gender: ___ DOB: _____

Contact(s): _____
T1D/T2D: _____ Co-morbidities:_____

Screening: _____
Labs:

Date	A1c	RBG	Cr	Urea	GFR	ACR	Alb	Ca	Phos	Mg	PTH	25D	1,25D

Height: _____ Weight: _____ BMI: _____ (Date_____)

Supplements: _____

Medications: _____

Comments/Notes: _____

_____

Baseline: _____
Labs: FBG (meter): _____

A1c	RBG	Cr	Urea	GFR	ACR	Alb	Ca	Phos	Mg	PTH	25D	1,25D

BSALP	Osteocalcin	NTx	FGF-23

Height: _____ Weight: _____ BMI: _____

Supplements: _____

Medications:_____

	DXA scan	3day Diet Record	3day Activity Record	Sunlight	SF-36
Completed					
Analyzed					

Comments/Notes: _____

_____

Three Months: _____
Labs: FBG (meter): _____

A1c	RBG	Cr	Urea	GFR	ACR	Alb	Ca	Phos	Mg	PTH	25D	1,25D

Height: _____ Weight: _____ BMI: _____

Supplements: _____

Medications: _____

	3day Diet Record	3day Activity Record	Sunlight	Adherence	Pill Count
Completed					
Analyzed					

Comments/Notes: _____

_____

_____

<u>Six Months</u>: _____
Labs: FBG (meter): _____

A1c	RBG	Cr	Urea	GFR	ACR	Alb	Ca	Phos	Mg	PTH	25D	1,25D

BSALP	Osteocalcin	NTx	FGF-23

Height: _____ Weight: _____ BMI: _____

Supplements: _____

Medications:_____

	3day Diet Record	3day Activity Record	Sunlight	SF-36	Adherence	Pill Count
Completed						
Analyzed						

Comments/Notes: _____

_____

_____

Adverse Event Tracking **Participant code: _____**

AE No.	Date Started	Date Resolved	Description of Adverse Event	Type		Intensity			Relationship to Study					Treatment Required			Outcome			Action Taken		Initials
				Intermittent	Continuous	Mild	Moderate	Severe	None	Unlikely	Possible	Probable	Highly probable	None	Hospitalization	Medical therapy	Resolved	Recovered (S/S)	Worsened	None	Discontinued	

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