NFS498 Proposal Outline

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| Requirements  * 5 pages, double spaced, excluding references * Research area & why it is important * Research question * Methods/approach to solving research question |

Diabetes rates in Canada have almost doubled over the past decade and will continue to rise. In 2010, the prevalence of diabetes across 216 countries was found to be 6.4%, and is expected to increase to 7.7% by 2030 (1). While there are many complications associated with type 2 diabetes mellitus (T2DM), diabetic nephropathy (DN) is one of the major causes of morbidity and mortality. DN results in end stage renal disease (ESRD), which can only be treated by kidney transplant or dialysis (2,3).

One of the complications observed in patients with diabetes is low vitamin D status. 7-dehydrocholesterol from sunlight or cholecalciferol from diet is the precursor to active vitamin D (4). One hydroxyl group is added by 25-hydroxylase in the liver to form 25-hydroxyvitamin D3 (25(OH)D), and another hydroxyl group is added by 1α-hydroxylase in the kidneys to form active 1,25-dihydroxyvitamin D3 (1,25(OH)2D) (4,5). Active 1,25(OH)2D is very unstable, and must be bound to carrier proteins (5). In circulation, 85-90% of 1,25(OH)2D is tightly bound to vitamin D binding protein (DBP), and a small percentage is carried by albumin; less than 1% of vitamin D exists in an unbound form (6,7).

Many studies have demonstrated a link between vitamin D and diabetes, but it is unclear whether vitamin D deficiency is a cause or consequence of the disease. Additionally, vitamin D deficiency in diabetes is independently associated with the presence of DN (8). Low vitamin D levels predisposes individuals to both type 1 and type 2 diabetes (4). These observations were confirmed in animal models, which demonstrated that pancreatic insulin secretion is inhibition by vitamin D deficiency (9). Vitamin D deficiency has also been shown to impair insulin synthesis and secretion in humans and animal studies of diabetes, suggesting a role in the development of T2DM.

The mechanism by which vitamin D deficiency impairs insulin response to glucose is linked to alterations in the calcium-dependent signal transduction pathways (10). Injection of 1,25(OH)2D significantly increases β-cell cytosolic Ca2+ levels, though it is unclear whether the increase in Ca2+ is due to an influx of external Ca2+ via voltage-dependent Ca2+ channels or the mobilisation of Ca2+ from the endoplasmic reticulum. Calcium promotes the exocytosis of insulin from islet cells. Other proposed mechanisms suggest involvement of the phosphoinositide and protein kinase A pathways (11).

Alternatively, low vitamin D levels may also be caused by diabetes pathophysiology. The first detectable stage of DN is microalbuminuria (12), and one of the proteins detected in the urine is the DBP (13,14). Excretion of DBP–which carries the majority of 25OHD–could explain the low vitamin D status observed in individuals with diabetes. DBP is also involved in the activation of 25(OH)D to 1,25(OH)2D. The DBP carries 25(OH)D into the proximal tubules of the kidney via reabsorption by megalin (15). 1α-hydroxylase then converts 25(OH)D to the activated form. In addition, DBP acts as a potential urinary biomarker for DN (14). However, there has only been a limited number of studies examining the role that this protein plays in the vitamin D status of individuals with diabetes.

**Literature Review**

A study conducted by Thrailkill et al. (13) in 115 subjects with type 1 diabetes (T1DM) and 55 age-matched controls showed that there is exaggerated urinary loss of DBP in T1DM subjects compared to the control group, particularly in association with poorer glycemic control and albuminuria. Multivariate analysis showed significant positive correlations between urinary DBP with microalbuminuria (β = 1.312), glycosylated hemoglobin (β = 0.208), average capillary glucose (β = 0.931), and serum 1,25(OH)2D concentrations (β = 0.607). The study suggests that exaggerated urinary loss of DBP in T1DM could be contributing to the low vitamin D levels observed in this disease.

Other studies support the finding that there is higher levels of urinary DBP in the disease state compared to the control. Tian et al. (14) found that DBP was higher in diabetic patients with nephropathy and micro- or macroalbuminuria compared to healthy controls or diabetic patients with normoalbuminuria. Blanton et al. (16) conducted a retrospective, cross-sectional analysis of DBP levels from 472 subjects (203 with T1DM, 116 relatives of those with T1DM, and 153 age-matched controls). They found that serum DBP levels were highest in control subjects, intermediate in relatives, and lowest in those with T1DM. However, serum DBP levels were not associated with serum vitamin D levels, suggesting that perhaps urinary DBP would be a poor predictor of vitamin D status in diabetes. Other studies have also supported the idea that polymorphisms in DBP could predict risk to T2DM. A meta-analysis conducted by Wang et al. demonstrated that this association was observed in Asian populations, but not found in Caucasians (17).

Anderson et al. proposed that low vitamin status in diabetic rats could be caused by problems with renal reabsorption of the DBP (18). Moreover, a digestion-resistant starch diet prevented urinary excretion of DBP in rats with T1DM (19), indicating a potential pathway between glucose and DBP elimination. Further study by Koh et al. investigated whether feeding resistant starch could similarly prevent vitamin D loss in diabetic rats. It was found that rats fed the control diet had 89% and 97% higher urinary excretion of 25(OH)D and 1,25(OH)2D respectively. Serum 25(OH)D levels were also 31% in those on the control diet. Histopathologic examinations of the kidneys revealed that the resistant starch diet attenuated diabetes-mediated damage by 21%. This suggests that starch digestion plays a role in loss of DBP.

### Gaps in knowledge

There is controversy about the quality of DBP as a biomarker for diabetes and kidney damage. While some studies indicate that urinary or polymorphisms in DBP precedes diabetes (16,17), other studies suggest low vitamin D status could be due to elimination of the binding protein (13,15,18,19). More study is needed in order to conclude the role that DBP plays in vitamin D status and the pathophysiology of diabetes.

## **Hypothesis**

The objectives of this project are to examine the association of urinary vitamin D binding protein concentrations with type 2 diabetes and its underlying characteristic, including hyperglycemia and kidney dysfunction in a longitudinal cohort study. The hypothesis is that patients who develop T2DM would have lower serum concentrations of 25(OH)D and higher levels of urinary DBP. These finding would implicate that low levels of vitamin D in individuals with T2DM is partly due to loss through DBP in the urine. In application, DBP could be offered as a novel marker for vitamin D status in addition to serum 25(OH)D levels. Measuring DBP easy and non-invasive, and analysis would be quick and inexpensive.

# **Design**

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| * What is going to be done? * Why are we using this approach? |

# **Experimental work & data collection**

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| * What is the analytical procedure being used? * What are the steps taken to ensure reliability, precision, and accuracy? * N=? * Animals/humans? * Time frame for sample acquisition and analysis? |

* PROMISE cohort: subjects are at high risk for T2DM
* Longitudinal study with follow-ups every 3 years (9 year mark)

# **Data Analysis**

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| * Method used to process data |

Statistical analysis will be performed using SAS (version 9.3; SAS Institute Inc., Cary, NC) and R statistical software (version…).

# **Time Allocation**

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| October 2015 | Receive urinary data |
| 13 November 2015 | Submit proposal + presentation |
| October 2015 – February 2016 | Data analysis |
| February 2016 – March 2016 | Write final manuscript |
| April 2016 | Submit manuscript + presentation |

References

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