A PRELIMINARY STUDY INVESTIGATING THE EFFECTS OF VITAMIN D PHOTOTHERAPY ON BLOOD GLUCOSE LEVELS IN A SAMPLE OF PRE-DIABETICS

by

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "A PRELIMINARY STUDY INVESTIGATING THE EFFECTS OF VITAMIN D PHOTOTHERAPY ON BLOOD GLUCOSE LEVELS IN A SAMPLE OF PRE-DIABETICS" by Noam D. Ami in partial fulfilment of the requirements for the degree of Master of Science.

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Abstract

Improved insulin sensitivity and glucose tolerance may be attributable to vitamin D sufficiency. An experimental design was created to investigate the use of phototherapy to increase serum vitamin D levels and improve circulating blood glucose levels in pre-diabetics. Seven participants with a pre-diabetic condition were randomly allocated to either 3 months of phototherapy or no therapy. OGTT, HbA1c, and serum vitamin D levels were assessed before and after treatment. In 2 treatment participants, pre-diabetes status was reduced from combined IFG/IGT to iIFG in one and iIGT in the second, while 1 control participant developed combined IFG/IGT and another, type 2 diabetes. HbA1c values improved in the phototherapy group but not in the controls. Phototherapy also increased serum vitamin D levels in the treatment group but not in the controls. This pilot study suggests that the experimental protocol was effective and it should be implemented in a larger sample size to confirm those trends.

List of Abbreviations and Symbols Used

DM2 – Type 2 Diabetes Mellitus
DM – Diabetes Mellitus
DM1 – Type 1 Diabetes Mellitus
DPP- Diabetes Prevention Program
IGT – Impaired Glucose Tolerance
IFG – Impaired Fasting Glycemia
HbA1c – Hemoglobin A1c
OGTT – Oral Glucose Tolerance Test
NGT – Normal Glucose Test
ALT – Alanine Aminotransferase
AST – Aspartate Aminotransferase
FBG – Fasting Blood Glucose
CDA – Canadian Diabetes Association
RDI – Recommended Daily Intake
UVB – Ultra-Violet B Light
UVA – Ultra-Violet A Light
7-DHC – 7-dehydrocholesterol
25(OH)D – 25-hydroxyvitamin D
$1,25(OH)_2D$ – 1,25-dihydroxyvitamin D
s-25(OH)D – Serum 25-hydroxyvitamin D
IU- International Unit
IOM – Institute of Medicine
PTH – Parathyroid Hormone
BMD – Bone Mineral Density
VDR – Vitamin D Receptor
SAD – Seasonal Affective Disorder
CDHA – Capital Health District Authority
HRM – Halifax Regional Municipality
CH – Capital Health
HR – Human Resources
REB – Research Ethics Board
BMI – Body Mass Index
WC – Waist Circumference
HIC – Hospitals In-Common
pmol/L– Picomoles per liter
µmol/L– Micromoles per liter
nmol/L– Nanomoles per liter

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Chapter 1: Introduction

Pre-diabetes is defined as a state in which blood glucose levels are elevated above normal but are not high enough for diagnosis of type 2 diabetes mellitus (DM2). It is therefore intermediate to euglycemia and clinically diagnosed DM2. Beneath the umbrella of pre-diabetes, there are two states of endocrine dysfunction that individuals may display: isolated impaired glucose tolerance (iIGT) and isolated impaired fasting glycemia (iIFG) (1). Individuals who are classified as having iIGT display elevated blood glucose levels postprandially, which is a dysfunction that is due in part to being both insulin resistant and hyperinsulinemic (1). Individuals with iIFG exhibit elevated fasting blood glucose levels, but normal glucose levels postprandially.

Although the etiology of each of these pre-diabetic states is not fully understood, the onset of these conditions typically arises in individuals who have a family history of type 2 diabetes, are overweight and over 45 years of age, or are overweight and under 45 years old with other high risk conditions such as cardiovascular disease, hypertension or hyperlipidemia (2). Other risk factors such as race and inactivity are contributing factors in the eventual diagnosis of pre-diabetes.

In response to the elevated blood glucose levels present in pre-diabetes, the beta-cells in the pancreas attempt to lower blood glucose levels by secreting more insulin into the bloodstream (4). The insulin travels to cells within the body, where it interacts with insulin receptors to cause an uptake of glucose into the cells. If hyperglycemia is chronic, the constant demand for more insulin places a strain on the beta-cells of the pancreas, which are responsible for blood glucose control, eventually lessening their ability to produce enough insulin to maintain proper circulating blood glucose levels (4).

Acute symptoms of hyperglycemia include extreme thirst, frequent urination, difficulty concentrating, fatigue, and weight loss (5). Complications resulting from DM2 can include microvascular damage (retinopathy, neuropathy and nephropathy) as well as macrovascular damage (myocardial infarction, stroke and peripheral artery disease) (5). DM2 requires medical intervention and puts a strain both on the finances and quality of life of the individual (6).

It is imperative that individuals who receive a diagnosis of pre-diabetes explore treatment options as soon as possible in an effort to manage the condition before it progresses to overt DM2. Individuals with pre-diabetes are more likely to develop DM2 if diagnosis and intervention are not in place early on in the disease progression (7). In a report on diabetes in Nova Scotia, admission rates to hospitals were 2.1 to 2.6 times higher in people with diabetes when compared to non-diabetics entering with similar conditions (8). Furthermore, those with diabetes were more likely to be hospitalized for a longer period of time than those without diabetes, with an average 1 to 2 days longer median stay (8). Over time, hospitalizations for complications associated with prolonged hyperglycemia have become a prevalent medical matter (6). Cardiovascular disease is the most common co-morbidity for which diabetics are admitted to hospital (5). In order to maintain the health of these individuals, physicians must prescribe medications to manage this condition, which puts a financial strain on the Canadian health care system as well as the individual.

Current methods of coping with pre-diabetes are both preventative and proactive in nature. Individuals with pre-diabetes are placed on diet and exercise regimens to delay or reduce the development of DM2 (9). Education is also of critical importance for pre-

diabetic individuals in order to learn, understand, and cope with their condition. Most hospitals in Canada have diabetes outpatient clinics that specialize in diabetes education and management. These clinics house diabetes educators (nurses, registered dieticians) who aid those with pre-diabetes in understanding and treating their specific condition. Participating in a diabetes education program often results in reduced hemoglobin A1c (HbA1c) values, a key measure of average blood glucose levels over time (10, 11).

The Diabetes Prevention Program (DPP), a 3 year clinical trial aimed at determining preventative measures for DM2, has shown that various interventions could help reduce the incidence of DM2 in high risk populations (12). This study, which focused on two different methods of DM2 prevention including lifestyle modification and the use of an anti-diabetic drug, showed that it is possible to prevent the development of DM2 by lowering the patient's weight or by increasing insulin sensitivity at the cellular level via anti-diabetic drug use (12).

Researchers have also examined alternate methods for preventing pre-diabetes and the onset of DM2, which could be implemented into a patient's daily routine. Recently, investigation into the use of vitamin D_3 as a primary intervention has been explored. Vitamin D is a steroid hormone that is synthesized by the body when the skin is exposed to UV radiation from the sun (13). Fortified foods and supplements are secondary sources from which vitamin D can be absorbed to increase serum vitamin D levels. Cade et al. (14) investigated the effect of vitamin D_3 supplementation on rats with IGT and found that supplementation with vitamin D_3 improved their blood glucose levels.

A few mechanisms of action have been proposed with regards to how vitamin D could affect changes in insulin sensitivity and glucose levels. Vitamin D has been shown

to be involved in inflammatory cytokine inhibition as well as in activation of insulin-signaling cascades, both of which could enhance insulin secretion as well as glucose tolerance. Currently, there is little information available on how effective vitamin D_3 is in reducing hyperglycemia and improving insulin sensitivity in individuals with pre-diabetes. The primary purpose of this study is to investigate whether the use of a phototherapy lamp for a period of 3 months in individuals with pre-diabetes will increase serum vitamin D levels and produce a beneficial change in their blood glucose levels.

Chapter 2: Literature Review

2.1 Homeostasis

Biological homeostasis relates to the ability of a body to self-regulate and maintain an internal equilibrium in response to both internal and external stimuli that cause fluctuations above or below the norm (15). In humans, there are several systems that regulate the body's internal workings. When changes above or below the body's normal range are detected, organs like the liver, kidneys, pancreas and the brain adapt and react to these fluctuations in an effort to return the levels back to normal (15).

The brain is the origin of the primary and most influential homeostatic regulators, which maintain the balance of the entire nervous system (15). Through control of the hypothalamus, the brain is able to sense and manipulate changes within the body by controlling the nervous system when homeostasis is challenged. This is accomplished through a series of negative and positive feedback systems that constantly check and recheck changes in values to ensure that they lie within healthy parameters. If the body is unable to maintain proper equilibrium, it is considered to be in homeostatic imbalance, and such an imbalance may cause disease, or even death.

2.2 Endocrine System

The endocrine system primarily maintains hormonal homeostasis. The pineal, pituitary, thyroid, thymus, adrenal and parathyroid glands, pancreas, ovaries in females and testes in males are the 8 key endocrine glands involved in hormonal homeostasis (16). Endocrine (through the bloodstream), autocrine (from one cell to itself), paracrine (from cell to cell), and juxtacrine (itself or other cells directly) are signaling methods that

allow the endocrine system the ability to modify hormone levels in the body. Endocrine signaling is systemic, resulting in a whole body effect and is the primary type of signaling used in hormonal regulation (16).

2.2.1 Hormonal Regulation

Many hormones are hypothalamically controlled (16). When a particular hormone is required, a cascade reaction occurs that begins at the hypothalamus. When the hypothalamus senses a fluctuation in a specific hormone, it secretes a primary hormone into the bloodstream. This hormone triggers a secondary endocrine gland to secrete a secondary hormone. This new hormone activates the final gland, or target gland, to secrete the intended hormone required at that time (16). Such cascades are examples of the hypothalamic-pituitary-adrenal (HPA), hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) axes (16), as illustrated in Figure 1A.

The directly activated metabolic pathway is able to sense changes in specific hormone levels without the requirement for hypothalamic activation, as seen in Figure 1B (16). When a hormone concentration drops, a positive feedback loop is activated, which directly increases the secretion of the target hormone. If there is an excess of a certain hormone, a negative feedback is activated, lowering or shutting down production of that hormone. The combination of positive and negative feedback loops allows for hormonal homeostasis to be achieved and maintained (16). Endocrine diseases like diabetes occur due in part to dysfunctions in these feedback systems which, in turn, cause dysregulation, inappropriate responses to certain hormones, or changes in certain endocrine glands. They may also occur due to dysfunction at the insulin receptor level or as a result of an

insulin secretory defect.

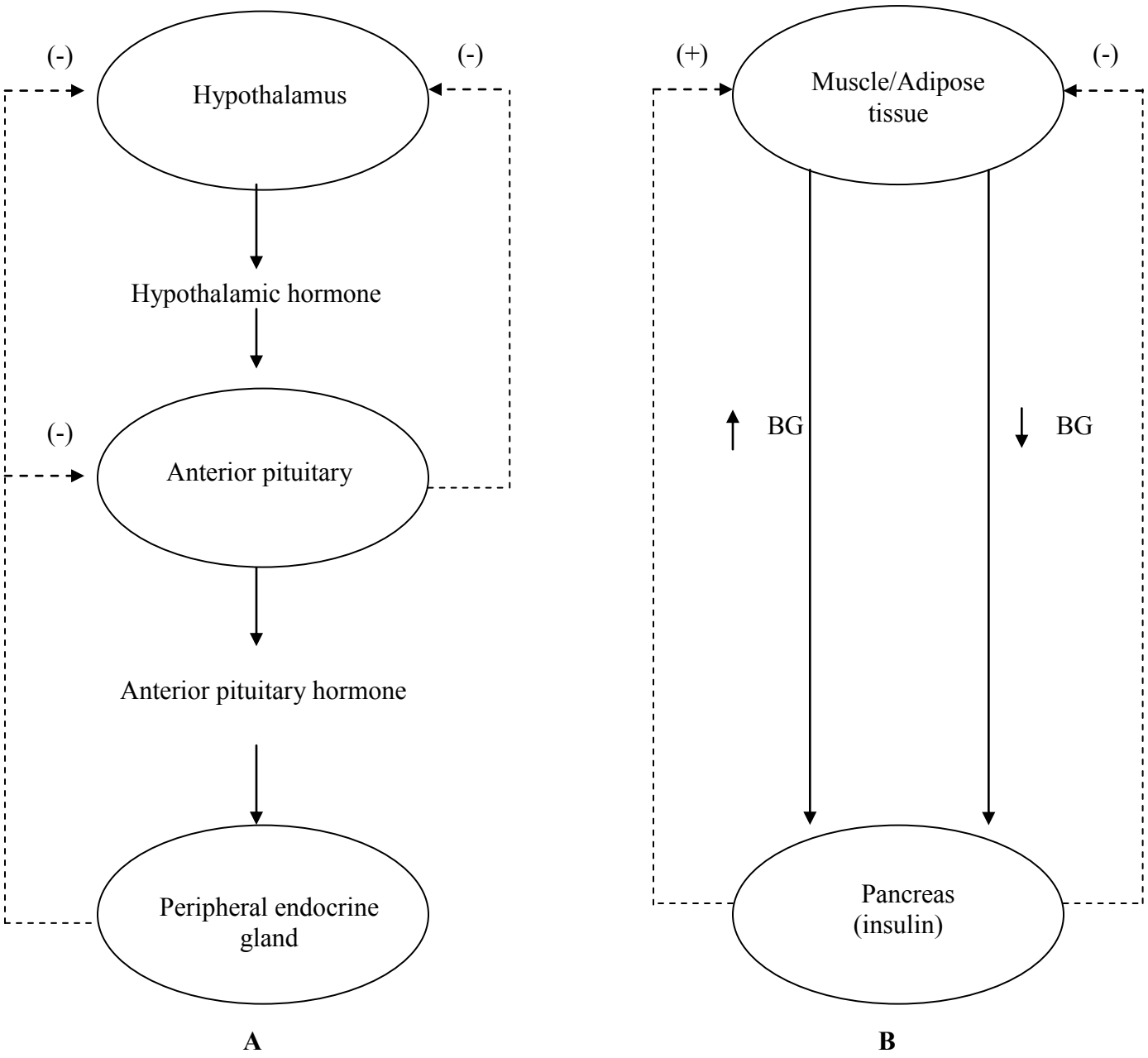


Figure 1. Example of a hypothalamic-pituitary-adrenal axis (1A) and of directed metabolic pathways, blood glucose concentration (BG) used as an example (1B)

2.3 Pancreas

The pancreas is one of the 8 primary endocrine glands and has both exocrine and endocrine functions. The exocrine function of the pancreas deals primarily with digestion by secreting digestive enzymes directly into the small intestine to aid in nutrient breakdown (16).

The pancreas is considered a directly activated endocrine gland; therefore, it does not require hypothalamic intervention for secretion of its hormones. The endocrine portion of the pancreas is integral to blood glucose control via insulin and glucagon production and secretion (16). Within the pancreas lie the islets of Langerhans, which contain both alpha and beta-cells. Alpha-cells are responsible for glucagon secretion and are stimulated when blood glucose levels drop below normal values (16). These cells replenish circulating levels of glucose by promoting glycogen breakdown in both muscle and liver tissues, as well as initiating gluconeogenesis (16). The beta-cells of the islets are responsible for insulin secretion, and are activated when blood glucose levels rise above normal values (16). These cells drive glucose out of the circulatory system and into liver and muscle tissue for storage as glycogen (16) in addition to being used as an intermediate fuel.

A few significant diseases affect the pancreas and its ability to function as an exocrine and endocrine gland. One of these is diabetes mellitus, a disease where there could be dysfunction in the insulin secreting cells within the pancreas, in the case of type 1 diabetes, or a lack of sensitivity to insulin of the receptors located in muscle and adipose tissue as found in DM2.

2.4 Diabetes Mellitus

Diabetes mellitus (DM) is a metabolic disease marked by elevated blood glucose levels due to dysfunction of insulin secreting cells in the pancreas or due to lack of sensitivity to insulin at the cells. Individuals with DM are unable to control elevated blood glucose levels, which leads to hyperglycemia. According to the Canadian Diabetes Association, the normal fasting blood glucose range is between 4.0 to 6.0 mmol/L (2). Postprandial blood glucose levels can range from 5.0 to 8.0 mmol/L, and should fall to within normal fasting levels within 2 hours of eating (2, 17, 18). If blood glucose levels do not lie within these ranges, pre-diabetes or DM is a possible diagnosis; however, determining which type of disorder is present requires further testing. The two forms of pre-diabetes and three types of DM all have their own distinct pathologies and etiologies (17-19).

Type 1 Diabetes (DM1) is an auto-immune disease that is characterized by a destruction of insulin-secreting beta-cells (2). Once known as juvenile diabetes, this form of diabetes tends to occur in infancy or childhood. Rare cases commence over the age of 30, and are now known as latent autoimmune diabetes in adults (LADA) (20). Due to the destruction of their insulin secreting cells, Type 1 diabetics require exogenous insulin in order to maintain proper blood glucose levels (2). This can be administered via daily injections of slow and fast acting insulin, or with an insulin pump (21). There is currently no known cure for this disease; however, recent research has taken greater steps towards a cure, including the use of islet cell transplantation (22). These transplants involve isolating insulin secreting beta-cells from cadaveric donor pancreases and implanting them into the portal circulation of patients with type 1 diabetes. With a combination of

immunosuppressant drugs, the newly implanted insulin secreting cells are able to secrete insulin with a reduced threat of being destroyed again by the immune system, allowing the patient to reduce their need for exogenous insulin (22). Thirty-one percent of participants who have undergone this treatment were successfully diabetes-free for approximately 2 years. Research is still being conducted with the hopes of a permanent cure in the future (22).

Gestational diabetes is glucose intolerance that occurs during pregnancy due to certain hormonal changes that are considered to be diabetogenic (19). This form of diabetes only occurs in about 2 to 5 % of pregnancies (23). Current literature postulates that the drastic hormonal changes that occur during pregnancy cause an increased resistance to insulin (17, 18). This form of diabetes is temporary but has been known to increase the risk of developing DM2 later in life for both the mother and the fetus, with about 40 % of these women developing DM2 later in life (24). Studies have shown that children of mothers with gestational diabetes have an increased risk of developing either DM1 or DM2 later on in life (25). Women are treated for gestational diabetes with diet, exercise and insulin rather than medication due to the possible effects medication may have on the fetus (26).

DM2 is characterized by both a reduction in insulin secretion and sensitivity, which can be due to genetic factors, weight gain, sedentary lifestyle, or any combination of the above, which result in insulin resistance (17-19) and accounts for over 90 % of all diagnosed cases of diabetes in Canada (24). Once considered adult onset diabetes, DM2 was thought to be an unusual diagnosis in adults under the age of 40 years; however, recent increases in childhood obesity, and greater inactivity and overeating in all ages are

causing a reduction in the age of onset (24). With proper nutrition and sufficient exercise, DM2 can be prevented, delayed, or occasionally reversed if already diagnosed (12, 28).

The Diabetes Prevention Program (DPP) investigated how intensive lifestyle modification, which included diet and exercise versus anti-diabetic drugs, would cause regression in those with pre-diabetes to normal glucose regulation (12, 28). In this study, participants received either intensive lifestyle modification, or metformin or placebo and no lifestyle modification and were tested for fasting glucose, 2-hour post glucose load, insulin secretion and weight loss (12, 28). They noted that with lifestyle modification, there was a 58 % reduction in the development of diabetes versus the placebo group, mainly due to weight loss (12, 28). In a follow-up to the study 10 years later, those in the metformin group reduced their incidence of diabetes to that of the lifestyle modification group; however, lifestyle modification still showed the greatest reduction in DM2 incidence (12, 28).

The use of both pharmacological agents and bariatric surgery has also been associated with prevention of the onset of DM2 (12). The DPP study showed that use of metformin, an anti-diabetic drug, reduced diabetes risk by 31 % as compared to a group that received a placebo (12, 29). The STOP-NIDDM study, which investigated the effects of the anti-diabetic drug acarbose on prevention of DM2 found that routine ingestion of acarbose was associated with increased reversion of participants with iIFG to normal glucose tolerance (12, 30). Furthermore, these drugs were found to be even more effective in obese individuals, with those with a BMI of greater than 35 kg/m^2 showing a 53 % reduction in diabetes incidence (12, 30). Bariatric surgery has also been shown to have positive effects on diabetes prevention. Patients who underwent bariatric surgery

had a reduction in the risk of diabetes by 86 % at 2 years after the surgery and 75 % after 10 years (12, 31). Moreover, 73 % of patients who underwent gastric banding showed remission of existing diabetes versus 13 % who did not undergo gastric banding (12, 31).

Men are more often diagnosed with diabetes than women, with 6.3 % of males and 5.4 % of females being diagnosed with diabetes as of 2008 (32). Nova Scotia has been identified as the province with the largest prevalence of DM, with almost 9 % of the population so diagnosed (8).

Pre-diabetes typically precedes DM2. Two forms of pre-diabetes are common: Impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT). These forms of pre-diabetes tend to occur more often separately, and are therefore called "isolated" states, which are referred to as iIFG and iIGT. However, sometimes they co-occur; such co-occurrence indicates a greater risk for the development of DM2 (2). iIFG is typically caused by poor insulin secretion and higher than normal glucose output from the liver whereas iIGT is caused by insulin insensitivity, though either condition could be a result of any of those dysfunctions. In many cases, both iIFG and iIGT can worsen and evolve into DM2 due to lack of proper diagnosis and treatment (27), but can sometimes be prevented or reversed using the preventative measures noted in the DPP trials (12, 27, 29,).

2.4.1 Impaired Glucose Tolerance (iIGT)

Impaired glucose tolerance (iIGT) is determined by elevated glucose levels in serum 2 hours after the intake of a 75 gram (g) bolus of glucose solution. It is considered to be the most prevalent and dangerous pre-diabetic state and a primary precursor to DM2

(27). An individual with iIGT will demonstrate elevated blood glucose levels postprandially, with a very slow return into the healthy glycemic range (18). During a standard 2-hour oral glucose tolerance test (OGTT) of 75 g glucose, iIGT will result in elevated blood glucose levels that range from 7.8 to 11.0 mmol/L 2-hours post oral glucose load (8); however, fasting blood glucose levels, typically measured before the OGTT, remain within the healthy range (Figure 2A) (34). Women usually have higher rates of iIGT than do men, although the prevalence of IGT tends to increase uniformly across both sexes as they age (27). Diagnosis of iIGT is the most accurate predictor for later diagnosis of DM2 (27). It is estimated that as of 2002 over 4 million people in Canada are currently living with IGT, with most cases undiagnosed (34).

The etiology of iIGT is unclear but similar to that of DM2. iIGT patients tend have a family history of DM2, as well as obesity or another high-risk condition like high triglycerides or blood pressure (27). In many cases poor lifestyle habits and inactivity are also noted in individuals with diagnosed iIGT (27).

Sixty percent of individuals with iIGT will develop DM2 within 5 years of their diagnosis if an intervention is not in place early on. (27). If not treated, prolonged hyperglycemia places a strain on the beta-cells to produce more insulin. Over time this reduces the effectiveness of the insulin to reduce blood glucose levels, leading to hyperinsulinemia, which over time is associated with beta-cell loss and eventually DM2 (35). Continued hyperglycemia can lead to microvascular damage (retinopathy, neuropathy and nephropathy) as well as macrovascular damage (myocardial infarction, stroke and peripheral artery disease) (5, 27). This causes a reduction in quality of life and is a burden on the medical system to provide adequate medical care. In 2010, diabetes

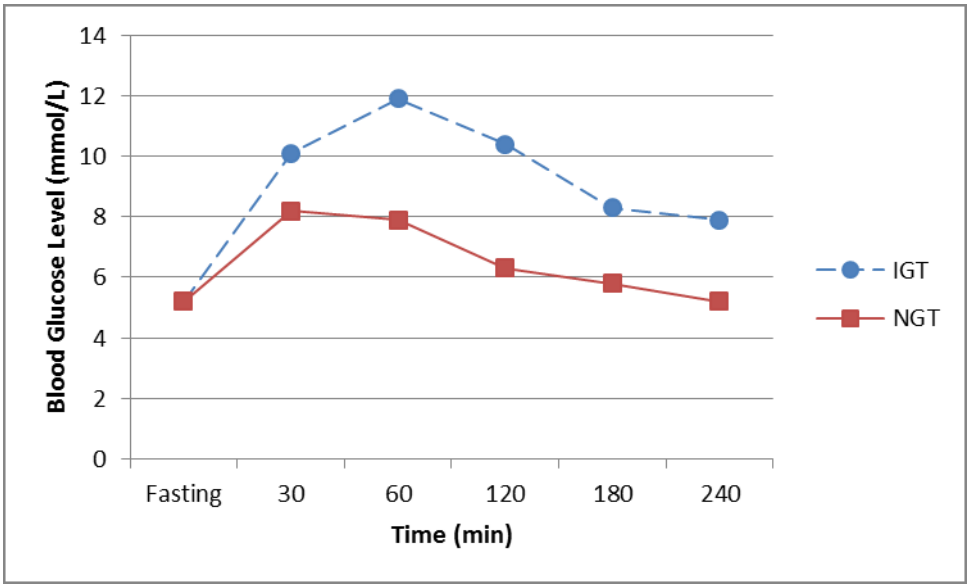
care was expected to cost the government of Canada about 12.2 billion dollars, an increase of 5.2 billion dollars since 2000 (36). Direct costs (insulin, needles, oral drugs) were expected to account for 3.5 % of public healthcare spending in Canada (32). Current treatment for iIGT is limited to diet and exercise. In most cases, anti-diabetic medications are not first line therapies prescribed to pre-diabetics, because lifestyle modifications are considered the best method of delaying the onset of DM2 (37, 38).

2.4.2 Impaired Fasting Glycaemia (iIFG)

Impaired fasting glycemia (iIFG) is a condition where an individual's blood glucose levels are elevated during a fasting period, but glucose clearance after ingestion of a glucose load is considered normal (Figure 2B) (2, 17, 18). Clinically, to be classified as iIFG, fasting blood glucose levels must lie between 6.1 mmol/L and 6.9 mmol/L in a fasted state, with normal to only slightly elevated blood glucose levels after the tolerance test has been completed, which would fall below 7.8 mmol/L (2). Diagnosis of iIFG tends to occur predominantly during middle age, with more cases being diagnosed in males then females (27). iIFG is a predictor of a future diagnosis of DM2 but not as strong a predictor as iIGT (27). iIFG is less common than iIGT. Though the numbers are not as large as compared to those with iIGT, individuals with iIFG still have a high likelihood of developing DM2, with literature estimating between 5 to 8 % of people with iIFG developing DM2 over a span of about 6 to 10 years (27). Akin to iIGT, iIFG is treated using lifestyle modification (27).

2.4.3 Combined IFG and IGT

In some cases, individuals may exhibit blood glucose behavior coinciding with both IFG and IGT. In these cases, fasting blood glucose levels range between 6.1 mmol/L to 6.9 mmol/L, and 2-hour post OGTT values range from 7.8 mmol/L to 11.0 mmol/L (Figure 2C) (2). People who have combined IFG plus IGT have a higher risk for both diabetes and cardiovascular disease (2). As with individuals with DM2, first-line treatment involves lifestyle modification.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12
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Time (min)
IGT
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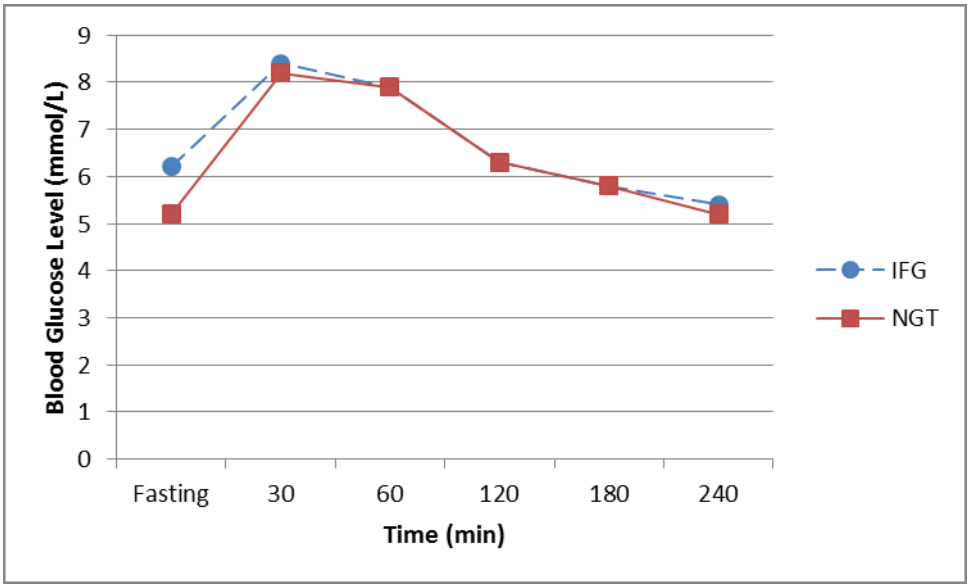
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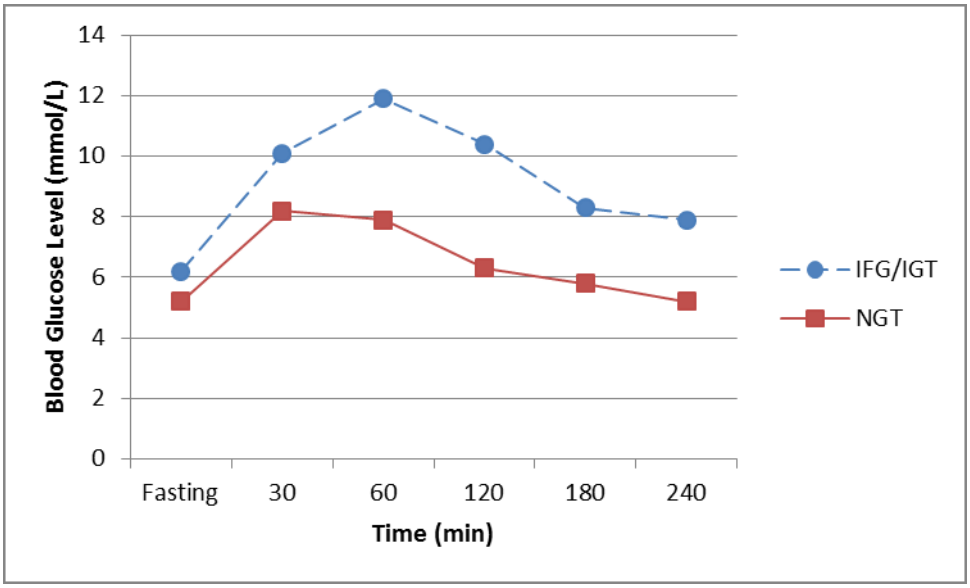

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Figure 2. Graphical representations comparing a normal glucose test (NGT) to three pre-diabetes conditions during a 2 hour OGTT. 2A: iIGT; 2B; iIFG; 2C combined IFG/IGT

2.5 Pathology of Pre-Diabetes

The etiologies of iIGT and iIFG are not fully understood; however, theories have been generated based on research on DM2. Galli et al. (39) reported that certain genetic markers were associated with the development of DM2. They found that the Niddm1 gene was involved in fluctuating blood glucose levels, and was responsible for variations in blood glucose levels over a 60-minute period. Furthermore, Niddm1 is also involved in body weight and plays an integral part in weight gain over time (39). The Niddm2 (chromosome 2) and Niddm3 (chromosome 3) genes were also found to be involved with glucose tolerance (39). These two genes appeared to affect both fasting and postprandial glucose levels (39).

Research on a proposed "thrifty gene" has suggested that there may be additional evidence of an association between genetics and pre-diabetes and DM2. The thrifty gene hypothesis proposes that environmental factors are the dominant cause for DM2 (40). According to the thrifty gene hypothesis, poor fetal and neonatal nutrition reduces the development of beta-cells within the pancreas, increasing the risk for dysfunction and insulin resistance (40). Once in adulthood, a more positive caloric balance causes a strain on the already compromised beta-cells resulting in cell exhaustion and eventual death (40). Poor fetal growth was also connected to increased insulin resistance (40). A study completed in Helsinki hypothesized that insulin resistance was linked to thinness at birth and throughout childhood followed by a dramatic shift in body weight towards obesity in adulthood (41). The explanation reported was that due to a lack of weight early in life, beta-cell creation was reduced. According to this theory, a dramatic weight gain later in life increases the requirement of insulin and taxed those cells to exhaustion or death (41).

These changes may be associated with an increase in pre-diabetes and subsequently more diagnoses of DM2 (41).

The thrifty gene is a contentious theory that has been debated for many years. Those who challenge the thrifty gene hypothesis point out that in many populations where obesity is prominent today have no history of famine or lack of food. Furthermore, some have pointed out that if a thrifty gene were to have existed, then diabetes would have been prevalent in populations long before the disease was identified. The theory is continually being researched and refined, but is not fully accepted as a reason for incidences of diabetes.

2.6 Screening and Diagnosis of Pre-Diabetes

Pre-diabetes is a condition that tends to be undiagnosed. It is estimated that in Canada, 3 to 5 % of the adult population are currently living with undiagnosed DM2, with a diagnosis only coming 5 to 12 years after hyperglycemia had developed (42). Bertram et al. (43) performed a meta-analysis to determine the average duration of pre-diabetes before developing overt DM2. After a literature review of 2,578 articles, they determined that, on average, in people over 30 years of age, men remain in the pre-diabetic stage for 8.5 years while women remain pre-diabetic for 10.5 years (43).

Considering the duration of time between euglycemia and DM2, a diagnosis of pre-diabetes at some point during that time could be expected. Karve et al. (44) estimated the prevalence, diagnosis and treatment of iIFG and iIGT in a non-diabetic population. After completing fasting glucose and OGTT blood tests, they reported that 34.6 % of a pool of 1,547 non-diabetic subjects actually fell within the category of being pre-diabetic

(19.4 % IFG, 5.4 % IGT and 9.8 % combine IFG/IGT), but only 4.8 % of those people indicated that they received a formal diagnosis of pre-diabetes from their physician (44). Benjamin et al. (45) analyzed data from the Third National Health and Nutrition Estimation Survey (NHANES) and reported that in a population of overweight American adults with pre-diabetes, 17.1 % had iIGT, 11.6 % had iIFG and 5.6 % had both.

Screening for pre-diabetes involves blood tests that assess the amount of glucose circulating through the bloodstream. Current methods of detecting pre-diabetes include a fasting blood glucose test, HbA1c test and the OGTT, which is often considered to be the gold standard, or any combination of these three tests.

2.6.1 Fasting Blood Glucose

For detection of pre-diabetes using fasting blood glucose, the potential pre-diabetic individual would have blood drawn after fasting for a minimum of 8 hours. If a fasting blood glucose level greater than or equal to 6.1 mmol/L is detected in two fasting blood glucose tests done on two separate occasions, then a diagnosis of iIFG can be made (2). Zhou et al. (46) compared the accuracy of the fasting blood glucose test and the HbA1c to the OGTT for determining pre-diabetes. After participants had completed a fasting glucose test, HbA1c and OGTT, the results of the fasting glucose test were closer than the HbA1c to the results of the OGTT. Less than 30 % of subjects with newly diagnosed diabetes could be identified via the HbA1c test (46).

2.6.2 Hemoglobin A1c

Physiologically, glucose molecules easily react with hemoglobin in the blood, creating glycated hemoglobin. This allows for glucose to readily travel throughout the bloodstream to get to cells and tissues that require it. In individuals with pre-diabetes and DM, there is an excess of glycated hemoglobin circulating through the system compared to euglycemic individuals. Because red blood cells last an average of 120 days, an HbA1c test assesses the average blood glucose level over a 3 month period.

According to the Canadian Diabetes Association, the target range for a healthy HbA1c level spans 4 to 6 % (2). Anything between 6 to 7 % can be considered unhealthy, while a value greater than 7 % is categorized as high risk of complications (2). Zhou et al. (47) compared the accuracy of the HbA1c test to that of the OGTT in people with possible pre-diabetes. The results revealed that the HbA1c test is accurate in detecting newly diagnosed DM; however, when compared with the results of the OGTT, the HbA1c test is less accurate in predicting pre-diabetes (47). When given a blood glucose cutoff of 5.7 mmol/L, the sensitivity of the HbA1c test to detect pre-diabetes was only 59.4 % while specificity of the HbA1c tests was 73.9 % for properly diagnosing pre-diabetes (47). Mann et al. (48) also compared the effectiveness of the HbA1c test against the fasting blood glucose test with respect to pre-diabetes diagnosis. Like Zhou et al., Mann showed that the HbA1c test diagnosed fewer people with pre-diabetes (12.6 % of non-diabetics tested) than did the fasting blood glucose test (28.2 % of non-diabetics tested) (48).

2.6.3 Oral Glucose Tolerance Test

The OGTT exam is considered the gold standard for detecting pre-diabetes. This exam requires a patient to fast for 8 hours and then drink a sugary, glucose-rich beverage. Prior to initiation of an OGTT, a blood sample is taken to ascertain fasting glucose levels, and a second blood sample is taken 2 hours post ingestion to determine glucose clearance from the bloodstream. A fasting glucose level greater than 6.1 mmol/L and/or a 2-hour post value between 7.8 to 11.0 mmol/L will identify a patient as pre-diabetic (2). Depending on the value of the fasting blood test, iIFG could be identified, while the value from the post-glucose ingestion sample determines iIGT.

DM2 can be an expensive disease. Screening for pre-diabetes may be beneficial in early diagnosis of the disease, thereby reducing the future costs associated with DM2 if intervention begins early. Chatterjee et al (49) compared the overall cost of screening patients for pre-diabetes and diabetes versus the cost with no prior screening in the United States. When screening tests for diabetes (fasting blood glucose, OGTT) were completed, the overall cost of diabetes management was reduced compared to those who had no screening over a 3-year period (49). This was due in part to the ability to begin treatment of the condition early on, further delaying the progression to DM2.

2.6.4 Liver and Kidney Function

Diabetes can cause deleterious effects to several different systems in the body due to the increase in serum glucose. When diabetes is not controlled, the consistently elevated glucose levels begin to affect the functioning of other organs, causing secondary complications as a result of poor glucose management. Physicians often assess the

function of other organ systems in order to determine the presence of dysfunction as a result of elevated blood glucose levels. Organs like the heart, liver and kidney and eyes are often assessed for function in diabetics due to the prevalence of micro and macrovascular complications that can result from poor blood glucose management.

To assess liver and kidney function, the alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine tests are often used. Elevated ALT levels in the blood may indicate possible liver damage, hepatitis, or other liver related diseases (50). Though the correlation between diabetes and liver dysfunction is not fully understood, research has shown that an increase in insulin resistance may cause non-alcoholic fatty liver disease (51, 52). AST is a secondary liver function test, similar to that of ALT; however, AST can be found in several other tissues, such as the heart, kidneys, and skeletal muscle. These two tests are usually done in combination and elevation in either can warrant further testing for liver disease.

Creatinine sampling is done to examine any possible effect that diabetes has on renal functioning. Creatinine is a product of the breakdown of creatine phosphate in the muscle, which is a primary process in rapid ATP production during anaerobic work. Creatinine is filtered by the kidneys, with little to no resorption of creatinine back into the system (53). Renal dysfunction is usually manifested in elevated creatinine being found in the circulation, and is associated with a dysfunction in glomerular filtration rate (54). Diabetic nephropathy is a progressive degeneration of the blood vessels in the kidney, causing deficits in filtration rates, which may indicate diabetic angiopathy (55). In the case of diabetic angiopathy, the blood vessels in the kidney slowly degenerate due to chronic blood glucose dysregulation (56). Pericytes, or Rouget cells, are connective tissue

cells located within small blood vessels, which help support the blood vessel. When there is an excessive amount of glucose in the bloodstream, it can cause apoptosis in these cells, causing a reduction in blood vessel integrity (57). A lack of pericyte cells can cause creatinine to leak back into the bloodstream. Therefore, serum creatinine levels can be used to assess the degree of kidney function.

2.6.5 Other Blood Glucose Tests

Other, more direct measures of diabetes, including glucose concentration (glucose AC), insulin, and C-peptide concentration are also measured. Glucose Ante Cibum (AC), or glucose pre-meal, is a simple blood test that assesses the concentration of glucose in the blood stream before ingestion of food. This is similar to the fasting blood glucose (FBG) test, which measures blood glucose concentration after a fasting period. Insulin, the primary hormone involved in blood glucose maintenance, is sampled to determine the concentration of insulin in the bloodstream. Hyperinsulinemia may indicate insulin resistance, a sign of pre-diabetes and DM2. This can be assessed by serum insulin levels or by completing a hyperinsulinemic euglycemic clamp, considered the gold standard in assessing serum insulin levels. C-peptide is a marker for pancreatic insulin secretion (58). This test assesses the ability of the beta-cells of the pancreas to secrete insulin in response to elevated blood glucose levels (59). Due to increased insulin resistance in pre-diabetes, the C-peptide test may be elevated, thereby reflecting an increase in insulin production which may lead to eventual beta-cell damage and/or dysfunction (59).

These tests are interrelated and tend to be done in unison; together they provide a picture of a person's glycemic status and are important in determining pre-diabetes or

DM2: elevated blood glucose, insulin resistance, and beta-cell dysfunction. A change in any or all values out of the healthy range could indicate diabetes.

2.7 Treatment

The development of pre-diabetes is heavily dependent on lifestyle habits, genetics, or a combination of both. Obesity, inactivity and unhealthy dietary choices are the primary factors involved in the development of pre-diabetes. Therefore, exercise, diet, and diabetes education are the three primary interventions used for treatment of the condition, with medication only given in cases where lifestyle modification proves ineffective. If diet and exercise improvements can be implemented in the early stages of pre-diabetes, it is possible to slow the progression to DM2 (60).

2.7.1 Exercise

Exercise can improve quality of life and prevent many diseases and conditions such as cardiovascular disease, cancer, hypertension and diabetes (60). Both acute and chronic exercise exerts positive influences on the prevention of pre-diabetes. Acute exercise can decrease plasma blood glucose levels after only one session of moderate to heavy exercise (61). Improvements from acute bouts of exercise can persist hours after the exercise has been completed, manifesting in longer periods of time at lower blood glucose levels due to the increased uptake of glucose by muscle (62).

Chronic exercise improves metabolic control and allows for better blood glucose control (61), further increasing the benefits acquired from acute exercise. Moreover, increased amounts of chronic exercise improve triglyceride levels, and decrease obesity

in many pre-diabetic individuals (63). The reduction in fat mass and increase in lean body mass that exercise provides results in a reduction in insulin resistance and an increase in insulin sensitivity in the cells (64, 65). This correlates with a decrease in the amount of insulin required to initiate glucose uptake and greater uptake of glucose by cells. Loimaala et al. (66) found a similar result in their study of long term endurance and strength training on metabolic control in individuals with DM2. Long term endurance and strength training caused a significant improvement in HbA1c versus the control group; they reported a drop from 8.2 % to 7.6 % in the exercisers and an increase from 8.0 % to 8.3 % in the controls (66). Prolonged decreases in glucose levels have been attributed to increased glucose transport and insulin sensitivity (67, 68).

Muscle myokines have been viewed as an endocrine organ and may be influential in blood glucose management. As a result of muscle contraction due to exercise, there is an increase in myokines within the circulation (69). An increase in myokines, specifically IL-6, appears to induce an anti-inflammatory effect as well as an endocrine effect, further increasing glucose uptake by cells (69). TNF-alpha, a cytokine involved in induction of inflammation, has shown to be linked to insulin sensitivity (70). An increase in IL-6 myokine activity has been shown to result in a reduction in the inflammatory effect of TNF-α, allowing for greater glucose uptake (71).

Exercise is a primary intervention for pre-diabetes; however, neither resistance type exercises nor aerobic or anaerobic training is considered the “best” form of training to reduce pre-diabetes. Snowling and colleagues (72) determined that both resistance and aerobic styles of training reduced HbA1c levels in individuals with risk factors for DM2. Sillanpaa et al. (73) investigated the effects of endurance, strength, and strength plus

endurance training on metabolic risk factors in healthy 40 to 65 year old men. Changes in serum glucose and insulin levels were found not to be different in any of the groups, but the endurance activity group showed decreases in serum fasting insulin levels (73). If such a change in fasting insulin levels is noted in healthy adults, then implementation of physical training could provide greater positive effects in pre-diabetic individuals.

2.7.2 Diet

According to the Canadian Diabetes Association, an estimated 80 to 90 % of people who have DM2 are overweight or obese (2), in comparison to the euglycemic population where 33 % are considered overweight and 15 % to be obese (74). Marshall et al. (75) showed that a diet high in saturated fats significantly increased the chances of progression from pre-diabetes to DM2. Subjects who ingested 43.4 % of their total caloric intake from fat developed DM2 compared to those who ate only 38.9 % fat, who reverted back to normal glucose tolerance (75). Therefore, individuals with pre-diabetes are encouraged to limit their intake of foods with saturated fat (76). It is recommended that individuals at risk for developing DM2 eat a wide variety of healthy foods to aid in weight reduction (76). Garg et al. (77) found that a diet high in glucose and other simple carbohydrates caused a worsening in glycemic control as well as worsening hyperinsulinaemia in those with DM2. Sarkkinen et al. (78) observed that a diet rich in monounsaturated fatty acids improved both serum lipid levels and glucose metabolism in those with pre-diabetes. Others found that high intakes of saturated fats and trans-fatty acids caused an increase in postprandial insulin and glucose levels (79). High protein intake can also be helpful in improving glycemic levels in individuals with pre-diabetes.

When a predominantly carbohydrate diet was altered to include greater amounts of protein, a drop in weight as well as an increase in insulin sensitivity was observed (80).

Weight loss is a key factor in reducing the probability of acquiring DM2. Even modest weight loss can result in quite a change in glucose concentration. Heilbronn et al. (81) found a 6 % reduction in glucose levels after placing individuals with IGT on a regimented diet. This change was due in part to an increase in insulin sensitivity and a reduction in insulin resistance (81). A concomitant 12 % drop in free-fatty acids also occurred, which led to a further decrease in insulin resistance (81).

2.7.3 Medication

The Canadian Diabetes Association (CDA) recommends that individuals with pre-diabetes not be given medication unless it is in the best interest of the patient due to elevated blood glucose levels or they have reached type 2 diabetic levels (2). The CDA also suggests that diet and exercise be the preferred means of intervention. However, when remedial exercise and diet regimens either cannot be implemented or do not prove efficacious, medication may lower hyperglycemia and insulin resistance in pre-diabetic individuals. Drugs like acarbose and metformin are used for type 2 diabetics to control high blood glucose levels, but may be prescribed to those with pre-diabetes.

Acarbose, an alpha-glucosidase inhibitor, may be prescribed to type 2 diabetics for elevated blood glucose levels. Arcabose controls high blood glucose levels by slowing carbohydrate digestion, which limits the speed at which glucose enters the bloodstream (82). When individuals with iIGT are given acarbose, they demonstrate a decrease in

postprandial glucose and insulin levels. One study showed that steady-state plasma glucose was lower than in a control group (82).

Metformin is the most often prescribed anti-diabetic drug on the market today. Metformin works by reducing the amount of glucose created by the liver, and increases insulin sensitivity (83). Scheen et al. (84) demonstrated that administration of metformin to individuals with iIGT rapidly improved insulin sensitivity and fasting insulin level. A meta-analysis done by Lilly et al. (85) determined that both low and high dosages of metformin prescribed to pre-diabetics reduced the likelihood of converting from pre-diabetes to DM2 after 3-years of treatment.

Perreault et al. (86) analyzed data from the DPP program, which investigated various interventions and their effect on incidence of diabetes. Perrault investigated how intensive lifestyle modification, which included diet and exercise, versus anti-diabetic drugs would cause regression in those with pre-diabetes to normal glucose regulation (86). In this study, participants received either intensive lifestyle modification, metformin or placebo and no lifestyle modification and were tested for fasting glucose, 2-hour post glucose load, insulin secretion and weight loss (86). Regression to normal glucose regulation was found to occur in those who had intensive lifestyle modification and weight loss whereas the use of metformin showed only a non-significant trend (86). Moreover, every 1 kg of weight loss resulted in a 16 % reduction in diabetes risk (86). This study demonstrated the effectiveness of diet and exercise as a primary treatment method for pre-diabetes, with medication not required until DM2 has been diagnosed.

2.7.4 Education

Education, with regards to chronic medical conditions, is pivotal in patient care. When patients with chronic diseases were given education and counseling relevant to their disease, they demonstrated a greater behaviour change towards disease prevention (83). Similar positive changes in behaviors towards disease prevention can occur when patients with diabetes are exposed to greater diabetes education.

In Canada, diabetes education programs are available in most hospitals. In Nova Scotia, diabetes education programs and events are provided by the District Health Authorities with provincial guidance from the Diabetes Care Program of Nova Scotia (8). This program is provincially funded to provide guidelines, access to knowledge, education, and research in the pursuit of improved care and quality of life for those with diabetes (8). The Diabetes Care Program employs nurses, dieticians, and diabetes educators in order to educate patients in all aspects of diabetes management. In 2008, the Nova Scotia Diabetes Care Program reported that patients who took part in its programs achieved a 61 % reduction in blood lipid levels and HbA1c levels versus those who did not partake in a diabetes management program (8).

Similar results have been found in other studies that employed diabetes education as a primary intervention. A meta-analysis reported by Norris et al (10) looked to ascertain the value of self-management education for adults with DM2. Their results demonstrated that such education reduced HbA1c levels by 0.76 % relative to those who received no education, which was reduced by 0.73 % 1 to 3 months after initial follow-up. Furthermore, a reduction of an additional 0.26 % was noted after four or more months and a further 1 % for every additional 23.6 hours of contact between the patient and

diabetes educators (10). When investigating the effect of group education versus one-on-one education, Trento et al. (11) concluded that group education produced a greater change in HbA1c levels than one-on-one consultations after two years. In groups of 9 to 10 diabetic individuals, HbA1c levels remained constant, while those in one-on-one counseling had an increase in HbA1c (11). In addition, the group members showed greater diabetes knowledge and had better quality of life and positive health attitudes (11).

As noted, DM is treated with various forms of lifestyle changes and medications. Current research is investigating how sufficiency in vitamins and minerals may help in the treatment of these diseases. Vitamin D, in particular, is a vitamin of interest to many researchers based on its potential use in other chronic diseases like cardiovascular disease and cancer. It is possible that vitamin D may be potentially beneficial in the treatment of DM as well.

2.8 Vitamin D

Vitamin D is a fat-soluble vitamin with hormone-like qualities (88). It maintains calcium levels via manipulation of intestinal and renal absorption and excretion (13, 88). However, recent research indicates that vitamin D may have a role in the possible treatment for many diseases such as multiple sclerosis, osteoporosis, cancer and diabetes (89). With respect to pre-diabetes, Baynes et al. (90) reported a correlation between the severity of insulin resistance, glucose intolerance and vitamin D deficiency in individuals with iIGT. It is therefore possible that vitamin D supplementation may exert a positive effect on pre-diabetes.

2.8.1 Accumulation of Vitamin D

Vitamin D may be obtained by ingestion of fortified food or via cutaneous photoconversion of pre-vitamin D_2 in the skin. Both pathways can produce enough vitamin D for healthy living; however, supplementation of vitamin D_3 may be required in order to reach the daily recommended intake (DRI) in people who lack adequate sunlight or do not consume fortified foods. Some varieties of fish are the only foods that are naturally rich in vitamin D_3; therefore, the most abundant source of vitamin D, aside from sunlight, is fish oils (13). In many developed countries, foods such as milk, cereals and margarines are enriched with vitamin D in an attempt to counteract vitamin D insufficiency and deficiency (13). These foods are fortified with vitamin D_2 or vitamin D_3 during the manufacturing process.

Cutaneous conversion of 7-dehydrocholesterol to vitamin D_3 is generated through a photochemical conversion triggered by ultraviolet B (UVB) radiation absorbed by the skin (91). UVB radiation, between the wavelengths 270 to 300 nanometers (nm), is ideal for vitamin D_3 synthesis by the skin (92). Once UVB rays interact with the skin, a cascade of reactions is put in motion that converts previtamin D_3 into active 1,25-dihydroxyvitamin D_3, cholecalciferol (91).

2.8.2 Conversion from UVB Light to Vitamin D_3

Prior to exposure to sunlight, inactive 7-dehydrocholesterol (7-DHC) can be found within skin cells. When exposed to UVB light, 7-DHC becomes pre-vitamin D_3 (91). Pre-vitamin D_3 then immediately isomerizes to become cholecalciferol, or vitamin D_3 (88). Cholecalciferol and vitamin D_2 travel toward the liver (88). Hepatocytes within

the liver produce 25-hydroxylase, an enzyme that adds a hydroxyl group (OH) to cholecalciferol and vitamin D_2 to create 25-hydroxyvitamin D [25(OH)D], also known as calcidiol, a second form of vitamin D that then travels towards the kidneys (93). Additionally, calcidiol may be stored in the liver for later use in its inactive form, or can be transported to the kidney via α-globulin to be further refined to active vitamin D_3 (94).

Within the kidneys, calcidiol travels through the proximal tubule of the kidney where it is hydroxylated to become calcitriol. This process is achieved when calcidiol is acted upon by 25-hydroxyvitamin D 1-α-hydroxylase, which converts it from an inactive vitamin D to the active 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] (95, 96). The interaction of calcidiol and the renal enzyme to become active vitamin D_3 is based on requirement of the body for active vitamin D, which is contingent on the concentration of parathyroid hormone in the system. The greater amounts of parathyroid hormone within the bloodstream increase the amount of 25-hydroxyvitamin D 1-α hydroxylase present, which in turn causes an increase in active vitamin D_3 to be created and transported within the bloodstream.

Inactive forms of vitamin D can be stored in fat tissue for up to 2 months, and are activated only when is necessary (88). Vitamin D toxicity is possible from oral supplementation but not due to sun exposure. It is estimated that an acute overdose of vitamin D would require ingestion of over 600,000 IU of vitamin D in a day or 1,680,000 IU in a week (97). Moreover, the body has a naturally occurring protective mechanism against vitamin D toxicity whereby excessive vitamin D is broken down and excreted as a waste product (98, 99). Figure 3 is a visual representation of the metabolic conversion of 7-DHC to active vitamin D_3.

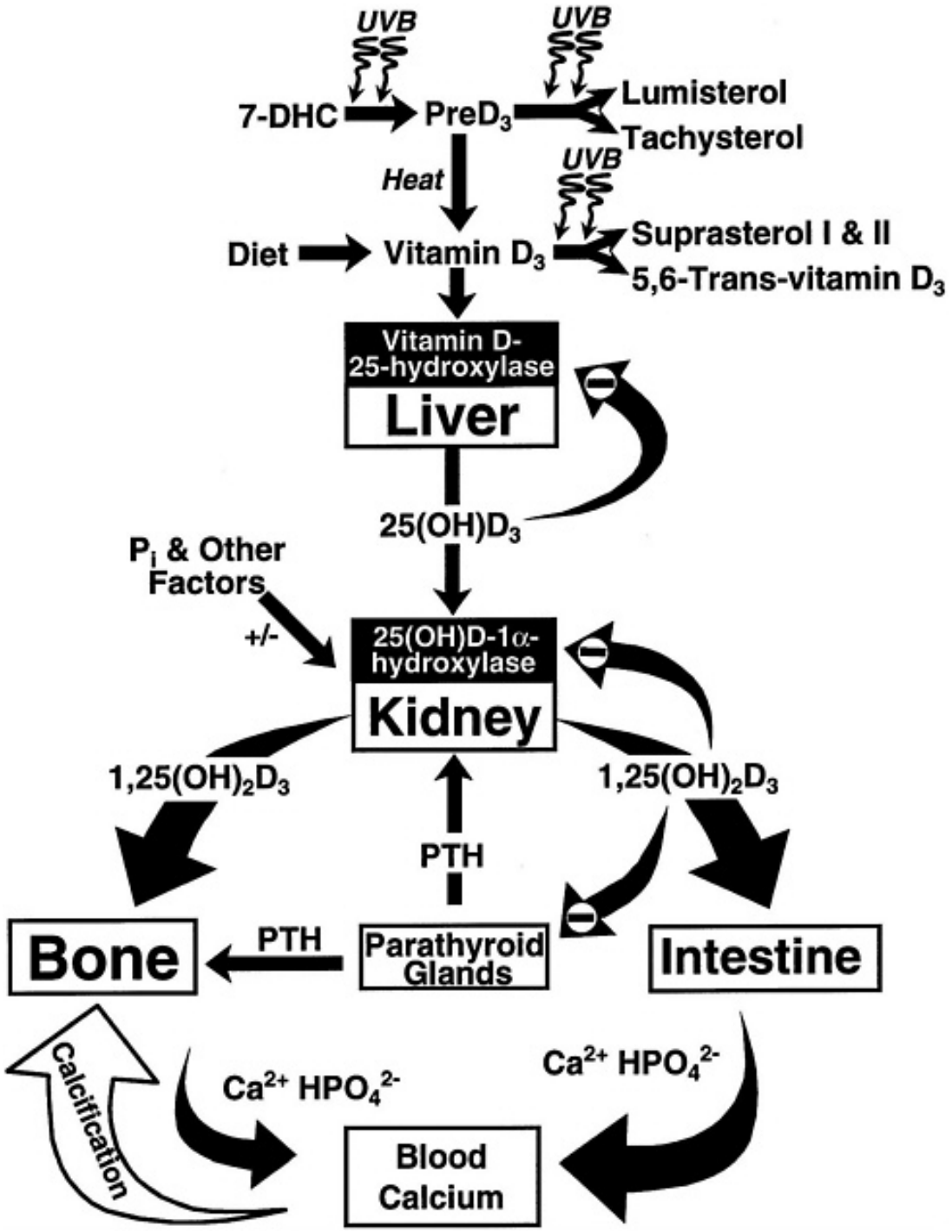

Figure 3. Illustration of the metabolism of vitamin D from interaction of UVB light from the sun/ingestion of supplementation to active vitamin D. Image was adapted from Holick, et al. (1996).

2.8.3 Function of Vitamin D_3

Vitamin D has myriad of functions within the body. These include maintenance of serum calcium and phosphorus levels, neuromuscular effects, healthy cellular growth and regulation, cardiovascular effects, as well as immunomodulatory effects (100).

In order to maintain serum calcium levels in the body, vitamin D interacts with receptors in the small intestine. Through calbindin D, a calcium binding protein, vitamin D facilitates calcium movement through the cytoplasm, thereby increasing the concentration of serum calcium (13). A lack of calbindin D would reduce calcium absorption within the intestines. If there is reduction in circulating calcium in the body, vitamin D interacts with osteoblasts in the bone to produce calcium via the RANKL/RANK interaction (101). When signaled, vitamin D increases the production of RANKL, which bonds to RANK, a receptor found on pre-osteoclastic cells (101). Once the two interact, pre-osteoclasts mature to become active osteoclast cells, which break down bone to release calcium (101). In addition, vitamin D also increases proteins that are essential for bone formation and resorption such as osteocalcin, osteonectin and osteoprotegerin (101).

Parathyroid hormone (PTH) secretion is also affected by changes in vitamin D and calcium levels. Vitamin D can attach to a vitamin D receptor (VDR) on PTH chief cells causing a decrease in PTH synthesis and secretion (101). Secretion of PTH is inversely correlated to the amount of calcium in the system (102) and is therefore dependent on vitamin D level fluctuations in response to changes in calcium levels.

2.9 Vitamin D Deficiency

Accruing adequate vitamin D requires a combination of adequate sunlight with intake of vitamin D from diet. A person's serum vitamin D level is determined by measuring the serum 25(OH) D concentration within the blood (96). To date, there is no set definition to what levels of vitamin D are considered ideal for optimal health, with many experts in the field differing on their definitions. The recently published Canadian Health Survey of vitamin D status in Canadians relied on the definition created by the Institute of Medicine (IOM) (103). The IOM specified that serum vitamin D below 27.5 nmol/L is associated with vitamin D deficiency sufficient to cause rickets in children and osteomalacia in adults (103). Those with a concentration below 37.5 nmol/L are at risk for inadequate bone health, but a level above 75 nmol/L is considered the desirable level for optimal health. On the other hand, a value greater than 375 nmol/L is nearing toxicity (103).

Being vitamin D deficient or insufficient can cause a variety of negative effects on the body, such as on the skeletal system. Vitamin D insufficiency causes intestinal calcium absorption to increase through the release of greater amounts of PTH (13). Continued vitamin D insufficiency can lead to vitamin D deficiency, which will eventually cause weakening in bones and can progress towards diseases such as rickets and osteomalacia along with an increased risk of cardiovascular disease, cancer and diabetes (104, 105).

In general, exposure to sunlight in combination with ingesting fortified foods and supplements are the best methods of avoiding vitamin D deficiency. During the spring and summer months or in areas where sun exposure is strong year round, exposure to

sunlight via the hands and face for a minimum of 20 minutes has been found to be adequate to satisfy the vitamin D needs of the body for the day (106). However, in most cases, the population still lacks sufficient vitamin D levels to be considered healthy. This can be attributed to several circumstances, including living above 42 degrees latitudes, hours of sunlight during the fall and winter seasons, being elderly, and skin pigmentation related to ethnicity.

2.9.1 Geography

Varying latitudes on Earth differ in the amount and strength of UV sunlight that hits the ground at varying times of the year. Lines of latitude denote changes of the angle of the Earth, with 0 degrees latitude represented by the equator, up to 90 degrees at the poles of the Earth. At increasing latitude, both in the northerly and southerly directions from the equator, the amount of sunlight available over the year fluctuates. This change in latitude away from the equator in conjunction with the Earth's changing zenith angle causes dramatic effects to both weather and sunlight patterns, particularly in how sunlight hits the Earth (88, 107). This causes the characteristic seasonal changes that are seen when summer becomes winter and the change in the amount of daylight during the day (105).

People who live at latitudes greater than 42 degrees are unable to adequately use the sun as a source of vitamin D during certain parts of the year (105). In Canada, the 42 degree latitude line crosses through the southern tip of Ontario; therefore, the major percentage of the Canadian population live above 42 degrees latitude, creating a high risk of vitamin D deficiency for a large portion of the year. Research has emphasized the

requirement for people living at these latitudes to ingest fortified foods and supplements to counteract the reduction in UV radiation during these times of the year. Kimlin et al. (108) corroborated this idea by measuring the surface UV radiation at various latitudes over the course of a year. Throughout a calendar year, people living at 0 degrees latitude sustain a high level of UV radiation, and therefore can achieve vitamin D sufficiency year round, if they can expose their skin to the sun. However, at 90 degrees latitude there is no opportunity to receive adequate UV radiation to synthesize any vitamin D for most of the year (108). Living at 40 degrees latitude allows for adequate vitamin D synthesis throughout the summer months, with vitamin D status in June through August even exceeding levels found at 0 degrees latitude during the same months (108). However, during subsequent months, UVB exposure trails off to below levels sufficient to synthesize adequate vitamin D, with no vitamin D synthesis occurring in December and January (108).

Webb et al. (109) investigated the uptake of vitamin D via sunlight in people who lived in Edmonton versus those in Los Angeles and Puerto Rico. They reported that Edmontonians were unable to convert 7-DHC to active vitamin D_3 during the months of November to February, whereas in June there was considerable conversion (109). This was in contrast to those living in Los Angeles and Puerto Rico where active vitamin D was cutaneously stimulated during these months (109). Furthermore, when the amount of cutaneous vitamin D production measured in Los Angeles, at 34 degrees north, was compared to the amount produced in Puerto Rico, which is 16 degrees north, there was less conversion in Los Angeles than Puerto Rico (109), indicating that a difference in vitamin D production can also occur in two areas where sun exposure is year round.

In 2008, Genuis et al. (110) investigated the clinical implications of vitamin D status in areas at higher latitudes, specifically looking at Edmonton, which is located at 53 degrees latitude. A total of 1,433 patients from 3 medical offices around Edmonton had blood drawn for vitamin D analysis taken between June of 2001 and March of 2007. 16.75 % of all participants tested were considered deficient in vitamin D, with serum levels less than 40 nmol/L. Additionally, 51.5 % were considered insufficient in vitamin D, with serum levels under 80 nmol/L (110). The latitude at which the subject pool lived is one of the primary determinants of their lack of vitamin D (111).

In a similar study, Rucker et al. (111) investigated the incidence of vitamin D insufficiency in a population situated in Calgary, which lies at 51 degrees latitude. Blood was drawn for serum vitamin D analysis from 188 people during the 1999 calendar year. For his study, Rucker's definition of vitamin D insufficiency was less than 40 nmol/L, unlike most studies where less than 80 nmol/L was considered insufficient (111). Using their tailored reference range, it was determined that 34 % of the participants were vitamin D insufficient (111). However, when insufficiency was set at 80 nmol/L, 97 % of his population would have been vitamin D insufficient at one point during the study (111). In both studies the importance of supplemental vitamin D to counteract low UV radiation was recommended (110, 111).

With changes in latitude come changes in seasons. Winters include less sunlight and lower temperatures requiring adaptation to the amount of time exposed to the environment and clothing worn. This combination of less UV radiation during winter months and the reduced exposure to the sun due to cold temperature causes a further reduction in the ability to synthesize vitamin D from the sun. During the fall and winter

seasons, Genuis et al. (110) reported that in his study investigating vitamin D levels in Edmontonians, 50 % of the population had serum vitamin D levels that were insufficient, ranging from 40 to 80 nmol/L. Similarly, Kimlin (108) illustrated that as the latitude increased, vitamin D synthesis is reduced.

Interestingly, even in areas of year-round sun, vitamin D deficiency has been noted. A study from southern Florida found that even though there was adequate sunlight year-round, there was a 14 % decrease in vitamin D in the subjects during the winter months (112). In these areas, although sunlight is steady year-round, excessive heat and humidity may have deterred people from being outside too long (105). Similar studies have been done in areas such as the Middle East where sun exposure and strong UVB radiation is year round, and have shown that even people in those areas can be vitamin D deficient. Guzel et al. (113) looked at the vitamin D levels and bone mineral density (BMD) in 30 veiled and 30 Western clothed women living in Turkey. Using serum vitamin D and BMD of the spine, Guzel was able to determine that veiled women had significantly lower vitamin D levels than the aged-matched controls, and a significantly lower BMD in the lumbar spine (113). Islam et al. (114) found similar results in his study looking at vitamin D status in three classes of Bangladeshi women, non-veiled women, veiled women, and non-veiled diabetic women, Islam et al. determined that 78, 83 and 76 % respectively had vitamin D insufficiency, with serum vitamin D levels below 40 nmol/L (114). In these countries, both the cultural norm to be covered up in public and high temperatures year round caused vitamin D deficiency in areas where it would not be expected to occur.

Interestingly, The Canadian Human Activity Pattern Survey (CHAPS) showed that during a typical day, Canadians spends an average of 88.6 % of their time indoors and only 6.1 % outdoors during a given day (115). Especially during the winter months, such a large amount of time spent indoors would not facilitate synthesis of vitamin D.

2.9.2 Age

The ability to synthesize vitamin D from the sun diminishes with age (105). This is due, in part, to physiological changes in the skin and its ability to synthesize vitamin D from UVB radiation. As we age, skin becomes thinner and frailer due to the inability of keratinocytes to differentiate into functional skin cells. There is also a slowing of the formation of neutral lipids that contributes to barrier function (116-118). This slowing causes the traditional dry and pale looking skin associated with aging (118). Skin changes are also caused by excessive UV irradiation. In this case, increased UV exposure causes alteration to the collagen and matrix of the skin cells and connective tissue, resulting in a deleterious change to the integrity of the skin (118).

MacLaughlin et al. (117) subjected various skin samples representing various ages from 8 to 92 years old to UV light and tested for previtamin D_3 content. When comparing 8 and 18 year skin samples to 77 and 82 year samples, it was determined that the samples from the older subjects were unable to synthesize as much vitamin D compared to those taken from the younger counterparts when exposed to UV rays (117). Holick (100) found that uptake of vitamin D in aged skin could decrease by up to 75 % by the age of 70 due to the physiological changes to the skin as a result of aging. Van der Wielen et al. (119) validated those findings when he investigated the serum vitamin D levels in elderly

participants in Europe. Of 824 elderly participants from various European countries, 36 % of men and 47 % of women were found to have serum vitamin D levels less than 30 nmol/L (119).

Decreases in vitamin D production in the elderly may also be a result of changes in the amount of VDRs within the body. As we age, the amount of VDRs decreases. Bischoff-Ferrari et al. (120) investigated the expression of VDRs in human muscle tissue and age. They noted that specimens of older aged muscle tissue displayed a significant decrease in VDR expression versus a younger counterpart (120). Similar results were found by Horst et al. (121) who investigated the concentration of VDRs in bone and the intestines of rats. A reduction in both intestinal and bone VDRs were found in elderly rats as compared with younger ones (121). A reduction in VDRs would cause disruptions in the ability for vitamin D to affect PTH synthesis and secretion and therefore calcium regulation.

Other aspects of aging are causes for reduction in vitamin D synthesis. Many elderly people do not go outside enough to get exposure to the sun or eat many foods that are fortified with vitamin D (107, 117). In a study investigating the adherence to vitamin D supplementation after hip fracture, it was found that 75 % of patients who were advised to begin vitamin D supplementation did not adhere to the advice given after a 3 month follow up (122). Milk, a product fortified with vitamin D in North America, is consumed less by the elderly due to the increased prevalence of lactose intolerance in this population (123). Furthermore, intestinal malabsorption, a condition that impairs the body's ability to absorb nutrients from foods, tends to be more prevalent in older adults

and could affect the ability of vital nutrients, like vitamin D, to be fully absorbed into the system (117).

2.9.3 Skin Pigmentation

Skin colour, or pigmentation, is a factor in vitamin D synthesis. Pigmentation of the skin is caused by melanin found within the skin (124). When exposed to UVB radiation, DNA within the skin is damaged, which causes an increase in melanin (125). The amount of melanin in the skin determines the amount of UV photons that can be absorbed by the skin, because it acts as a photoprotectant, absorbing harmful UV rays and converting it to heat, acting like a natural sun block (124, 126, 127). Therefore, individuals with lighter skin are known to have higher levels of vitamin D than those with darker skin, but are also at greater risk for skin cancer (124, 126).

Matsuoka et al. (126) performed a study investigating the effect of darker skin pigmentation on vitamin D synthesis. They exposed skin with different pigmentation levels to UV light, and reported that individuals with darker skin pigmentation, especially those of black and Indian descent, synthesized less vitamin D from the sun in comparison to subjects with lighter skin, even though both groups had similar concentrations of VDRs and serum vitamin D (126). Clemens et al. (128) observed that it required 6 times the dosage of UVB radiation to exhibit the same amount of vitamin D synthesis in a Black participant compared to a Caucasian participant. Consequently, populations of people with darker skin would require more time in the sun to achieve the same serum vitamin D levels than those who are of lighter skin.

2.10 Vitamin D via Diet

Accumulation of vitamin D can come from sources aside from sun exposure. This is especially important in geographical areas that lack constant sun exposure, which will aid in maintaining adequate serum vitamin D levels during times where sun exposure is unavailable. Therefore, accrual of vitamin D can be achieved via ingestion. This includes eating foods that are naturally rich in vitamin D and by taking vitamin D supplements.

Vitamin D in foods can come in two forms: ergocalciferol, which is vitamin D_2, or cholecalciferol, which is vitamin D_3. Vitamin D_2 is a form of vitamin D that is synthesized from plants (129). This form of vitamin D was produced in the early 1920s by irradiating foods using UV light (129). Vitamin D_3 is synthesized from lanolin, which is found in sheep wool, and is created by irradiating 7-dehydrocholesterol to become vitamin D (96). Originally, vitamin D_2 was used in many pharmaceuticals, but recent research has indicated that vitamin D_2 is less bioavailable than its counterpart. Vitamin D_3 has been shown to be 1.7 times more potent than vitamin D_2 in raising serum vitamin D levels (130). Many products are now using vitamin D_3 for fortification and supplementation of vitamin D (129).

2.10.1 Natural Sources of Vitamin D_3

Vitamin D_3 is naturally present in very few foods. In most cases, consumption of vitamin D_3 from diet usually comes from ingesting foods that have been fortified with vitamin D_3 during the manufacturing process. However, eggs, beef liver and certain species of cold water fish do contain vitamin D_3 (130). Fatty fishes, which include salmon, mackerel, tuna, sardines, and eel, contain vitamin D_3 in their flesh (13). These

fish, when cooked, can yield various amounts of vitamin D_3, with a 3.5 oz piece yielding around 400 IU (13). Egg yolks and beef liver also contain vitamin D, but in smaller amounts. Cod liver oil and halibut liver oil contain large amounts of vitamin D, and can be purchased over the counter as a liquid or in pill form as well.

Mushrooms contain naturally occurring vitamin D_2 (130). In order to increase the vitamin D_2 found naturally in mushrooms, they undergo a UV irradiation process during manufacturing (130). This process enhances the amount of vitamin D_2 that can be attained from mushrooms that have been irradiated.

2.10.2 Vitamin D Fortification and Enrichment

When a vitamin or mineral is lacking in the population, foods can either be fortified or enriched in order to compensate for the low level of that particular vitamin/mineral. Enrichment of a product is the process of replenishing a nutrient that was lost during a manufacturing process. An example of this would be enriched flour in bread. Fortification of foods is the process of adding a nutrient into a food that would not naturally contain that nutrient, such as adding vitamin D to margarine.

In order to help achieve better vitamin D status in the population, many products are fortified with vitamin D_2 or D_3. In Canada and other countries, products like milk and margarine are fortified with vitamin D. Other products like cereal, cheese, yogurts, and juices are also fortified with vitamin D (131). Most products are fortified with vitamin D_3, due to its greater potency to increase serum vitamin D levels versus vitamin D_2. However, because vitamin D_3 is often synthesized from animal products, products that are geared towards vegans and people who do not ingest animal products are usually

fortified with vitamin D_2, which is synthesized from plants (130). Notable examples are rice and soy milks, which may be fortified with vitamin D_2.

2.10.3 Vitamin D Supplementation

Vitamins and minerals can either be essential or non-essential. Those vitamins that are essential are required to be ingested via diet because the body cannot synthesize them naturally. While the DRI for certain nutrients is easy to reach, and sometimes exceed, other nutrients are more difficult and it may be necessary to supplement with an orally ingested vitamin/mineral supplement or through a multi-vitamin. These supplements, ingested daily, complement the diet in order to achieve the DRI for the vitamins and minerals that are essential in the diet.

Vitamin and mineral supplementation standards and regulations are controlled by Health Canada under the *Natural Health Products Regulation* of 2004 (132). Under these regulations, products like vitamin supplements undergo regulation of their manufacturing and retailing of the product (132). Health Canada regularly inspects production facilities in order to maintain a high standard of production and make sure that every supplement has met approval for sale to the public (132). Items such as acceptable substances, proper dosage, packaging, and labeling are scrutinized to ensure that the product is safe for over-the-counter sale (132). Products that have met the criteria of the Natural Health Products Regulation bear a Drug Identification Number (DIN) or a Natural Product Number (NPN) and thus are deemed safe for use on a daily basis (Figure 4) (132).

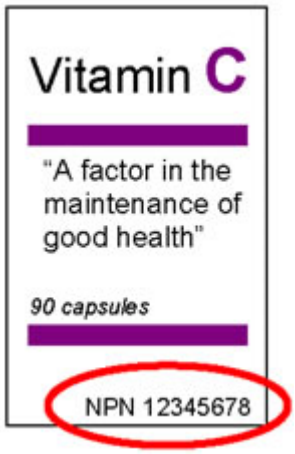

Figure 4. Example of a Natural Product Number (NPN) (132)

Vitamin D supplementation can be taken orally in two forms: in a stand-alone form or as part of a multi-vitamin or in combination with other minerals such as calcium. Vitamin D supplements can range from 400 IU to 1,000 IU per pill, whereas the amount of vitamin D in a multi-vitamin is usually 200 IU to 400 IU. Individuals may also ingest fish oil pills, which contain vitamin D; however, fish oil pills have come under scrutiny for toxic pollutants (133).

Current recommendations on the amount of vitamin D supplementation are contested. In Canada and the United States, the amount of vitamin D recommended for ingestion via supplement differs by age bracket. Health Canada suggests that from one year of age to 70, the DRI is 600 IU/day of vitamin D (134). The DRI for people over 70 years of age is 800 IU/day (134). The National Institute of Health (USA) suggests that in areas where winters have fewer hours of sunlight per day, people may require higher dosages of vitamin D, such as up to 1,000 IU/day. For women who are pregnant it has been recommended that they ingest 600 IU/day during gestation (134).

Though Health Canada and similar associations have set the DRI between 600 to 800 IU/day, there has been debate as to whether those values are sufficient to provide adequate vitamin D in order to reach sufficient serum blood values (99, 130). The IOM has stated that there is no additional risk to ingesting over the DRI for vitamin D, as long as they do not reach the tolerable upper intake level (UL), in order to deter people from over ingesting vitamin D and becoming vitamin D toxic. Health Canada believes that adults should not ingest more than 4,000 IU/day (99, 130). However, Heaney (135) discussed the possibility that ingestion of even greater amounts of vitamin D can still be considered safe. In a controlled metabolic study, dosages of 50,000 IU/day from 1 to 5 months showed no signs of toxicity (135). Furthermore, no cases of vitamin D toxicity were found in a study where it was reported that participants ingested up to 30,000 IU/day for several months, and no intoxication occurred when serum vitamin D levels reached 500 nmol/L (135). In many cases, an UL is put in place in order to have a comfortable margin of safety and to stay away from values that may cause toxicity.

2.11 Vitamin D Lamps

Vitamin D is best synthesized from UVB light from the sun, but obtaining this sunlight is difficult when sun exposure may be lacking due to location or season. Tanning beds, sunlamps, and UVB lamps have been an alternate method of acquiring vitamin D when acquisition from the sun is more difficult. Due to their ease of use, portability (in the case of sunlamps) and convenience, these methods of vitamin D accumulation are becoming more prevalent, especially in the younger population. Lim et al. (106) noted that when adolescents in Minnesota were polled about their tanning bed use, 37 % of high

school girls and 11 % of high school boys indicated that they used tanning beds with some even reporting that they had received a burn from one or blistering and rashes as a result of over exposure. Even though vitamin D_3 can be attained from tanning bed use, these beds do emit both UVA and UVB wavelengths. Prolonged exposure to these wavelengths, especially UVA wavelengths have been known to cause skin cancer (106).

The use of sun lamps has been shown to improve the quality of life in some individuals. In many cases sunlamps have been used to counteract seasonal affective disorder (SAD). SAD is considered a mood disorder that occurs during the winter months where individuals become more depressed (136). This disorder has been linked to the lack of sunlight and the amount of time that is spent indoors during colder winter months (136). A lack of serotonin, which is a hormone related to depression, may be the chief cause of SAD (137). Lack of vitamin D_3 has also been attributed to the increase in SAD during the winter months as a result of reduced daylight hours and exposure to sunlight (138). Bertone-Johnson et al. (139) systematically reviewed studies that investigated vitamin D levels and depression. Several randomized trials indicated that high doses of supplemental vitamin D may contribute to improvements in mild depressive states, but at this time they were unable to conclude that vitamin D status related to the occurrence of depression (139). In a similar study, Murphy et al. (140) investigated the effect of vitamin D in mood disorders among women and determined that low vitamin D levels were correlated with a higher incidence of mood disorders (140). However, Howland et al. (141) reviewed studies investigating vitamin D and depression and concluded that current evidence does not demonstrate that vitamin D deficiency causes or is a risk for depression

and that vitamin D may not be an effective therapy to counteract depression (141). With differing results, more research in the area of vitamin D and mood disorders is required.

The use of tanning beds and sunlamps for increasing vitamin D has come under scrutiny due to some adverse effects it may have. Melanoma, a type of skin cancer, has been shown to be prevalent in individuals who spend considerable time using tanning beds (106). This is due to tanning beds using both UVB and UVA rays, which spans 280-400 nm on the light spectrum, the wavelength range of light most associated with skin cancer (142). An increase in cancer has been noted because of the use of tanning beds by adolescents and younger adults, who are often not educated on proper usage of the lamp or safety regarding length of exposure under the lamp (106). In an extensive review of the topic, Lim et al. (106) commented that it is generally accepted that individuals who choose to use tanning beds for both tanning and vitamin D limit their usage to 20 minutes every couple of days.

Current vitamin D lamps on the market, such as SolRx series (Barrie, Canada) use only the UVB bandwidth of light. These new brands of vitamin D lamps do not have the harmful UVA rays associated with skin cancer, lowering the risk of inducing cancer as a result of using the lamps that have both UVA and UVB light. These lamps are also advantageous for those individuals who wish to acquire vitamin D but have a malabsorption disorder. Malabsorption disorders affect the ability to absorb nutrients from foods in the gastrointestinal tract. Therefore, accrual of vitamin D from fortified foods or supplement would be reduced in this population. Since vitamin D lamps work on the concept of attaining vitamin D from exposed skin to UVB light, accrual of vitamin D

would not be affected by the malabsorption disorder, allowing serum vitamin D to be increased.

2.12 Vitamin D Analysis

For analysis of serum 25-hydroxyvitamin D samples, a chemiluminescence immunoassay from Diasorin Liason is commonly used. This test is a competitive chemiluminescence immunoassay, meaning that the antigen of the unknown sample competes with a known antigen to bind with antibodies. The amount of known antigen bound to the antibodies is then measured. The result of the binding will be inversely proportional to the concentration of the unknown sample. For testing of 25-hydroxyvitamin D, an initial incubation period causes dissociation of 25(OH) vitamin D from its binding protein and then binds to vitamin D antibodies (143). A reagent is added to initiate a flash chemiluminescent reaction. This light reaction is measured as relative light units (RLI) and is inversely proportional to the amount of 25(OH) vitamin D found within the sample (143). This sampling method could be used to measure concentrations of either vitamin D_2 or vitamin D_3 depending on the requirements of the research.

Vitamin D samples can be measured using various different methods including radioimmunoassay or chemiluminescence, as described above. The sensitivity and the reliability of each sampling method are important in order to consistently achieve accurate and precise measures for each sample tested. In a review of the literature, the Diasorian Liason chemiluminscence immunoassay had been rigorously tested against other vitamin D sampling assays to determine the most accurate and sensitive method of vitamin D sampling. In all reviews of testing methods, the Diasorian Liason

chemiluminscence method had been shown to be an accurate and precise assay for vitamin D sampling (143-145). Sujishi et al (143) examined the chemiluminescence assay by testing its precision and accuracy by completing the assay for 9 separate samples. His results showed a very low coefficient of variation for serum reliability, between 3.1 to 5.5 %, and a reproducibility of between 6.9 to 12.7 % (143). Ersefeld et al. (144) reported similar accuracies when testing the assay, with an inter-assay imprecision of less than 20 % and when compared with radioimmunoassay had a close correlation as well as similar equivalence in vitamin D range (144).

2.13 Vitamin D and Diabetes

Vitamin D has been studied in relation to several chronic diseases such as cardiovascular disease and cancer. A possible relationship between vitamin D and DM has also been investigated with several theories having emerged about the function of vitamin D in association with obesity, genetics and its effect on insulin secretion and blood glucose control.

Pre-diabetes and DM2 are highly associated with obesity. In Canada, as of 2005, 2 of 3 Canadian adults, as well as 1 of 3 children were considered overweight or obese (2, 146). In 2004, 3.7 % of adults with a BMI of 25.0 to 29.9 kg/m^2 (overweight), and 11 % who's BMI was greater than or equal to $30kg/m^2$ (obese) had diabetes (147). Increased vitamin D insufficiency has been noted with increased obesity, prompting the hypothesis that vitamin D insufficiency may be associated with obesity and the subsequent diagnosis of DM2 (148-150). Current explanations for reduced serum vitamin D in obese people suggest that vitamin D is less bioavailable because body fat sequesters vitamin D and

removes it from circulation (148, 151). To assess whether obesity alters the ability of the body to produce vitamin D_3 or absorb vitamin D_2, Wortsman et al. (152) conducted a study where obese subjects were paired with lean control subjects and were randomized to either receive whole-body UVB radiation or an oral supplement of vitamin D_2 over a 24-hour period. The obese subjects had lower serum vitamin D levels versus the age-matched control subjects, and the increases in serum vitamin D as a result of treatment were lower in obese subjects (152). Furthermore, the study found that BMI inversely correlated with serum vitamin D concentration after irradiation (152).

At the genetic level, vitamin D is linked to a vitamin D-dependent calcium-binding protein, calbindin-D_{28k} (153). This binding protein is found on beta-cells and is pivotal for protection of the cells from cytokine mediated cell death (154). A lack of vitamin D in the system inhibits the effectiveness of this binding protein, thus causing a rise in beta-cell exhaustion and death. The ApaI Restriction Fragment Length Polymorphism (RFLP) is known to be involved in insulin secretion as a response to change in glucose levels (155). More recently, a vitamin D receptor has been linked to ApaI RFLP, which may be related to an increase in glucose intolerance, further suggesting that a reduction in serum vitamin D levels would cause greater glucose intolerance (155). Furthermore, various polymorphisms of vitamin D receptors have been associated with susceptibility to obesity (155). The presence of the TT genotype, Taq1 SNP, or bb genotype of Bsm1 SNP was found to be correlated with greater body weight by 9 kg and a 30 % increase in obesity in type 2 diabetics (155). These results suggest that a person who is deficient in vitamin D may be predisposed to having increased

numbers of adipocytes, further increasing the odds that they will become obese and develop DM2 (155).

In a clinical study completed by Chiu et al. (156), 126 healthy subjects were assessed for how serum vitamin D levels correlated with insulin sensitivity and beta-cell function. They observed that those subjects who displayed low serum vitamin D levels also had lower insulin sensitivity (156). Furthermore, they reported that subjects with higher vitamin D levels had more robust insulin responses as a result of beta-cell activation during an OGTT (157). Gedik et al. (157) performed a study on 4 females with vitamin D deficiency and 10 controls where alpha and beta-cell function were assessed in response to varying levels of vitamin D. The 4 experimental subjects underwent vitamin D therapy, which consisted of 2,000 IU of vitamin D ingested daily for a 6-month period, while the control subjects received no therapy. An OGTT as well as an insulin tolerance test were conducted pre- and post-treatment to determine if vitamin D had caused any change in either alpha or beta-cell function (157). Insulin secretion was significantly lower before vitamin D treatment but increased after vitamin D treatment, though it was still lower than the control group (157). No change was found in OGTT results in response to vitamin D treatment (157).

Baynes et al. (90) also investigated the relationship between vitamin D and OGTT results and reported an inverse relationship between OGTT results and vitamin D status. Vitamin D_3 deficiency also seems to have an effect on beta-cell functioning. Increased vitamin D deficiency places a greater strain on beta-cells to secrete more insulin due in large part to increased insulin resistance (158). These results are echoed in a review completed by Teegarden et al. (159). In their review of various studies, they concluded

that vitamin D status was inversely associated with diabetes, and that vitamin D_3 improves insulin sensitivity in both glucose intolerant and euglycemic subjects (159).

2.14 Vitamin D and the Reduction of Pre-diabetes

Epidemiological studies have determined that in trials where supplementation of vitamin D were given to those with vitamin D deficiency, an improvement in glucose tolerance was noted (95). Chiu et al. (156) determined that vitamin D could be a major factor in reducing beta-cell strain and increasing insulin sensitivity in iIGT and reported that if serum vitamin D levels were increased by as little as 10 to 30 ng/mL (25 to 75 nmol/L), improvements in insulin sensitivity could improve by 60 % (156). This is a greater improvement than that reported in a study by Inzucchi et al. (160), which showed an improvement in insulin sensitivity of 54 % using troglitazone and 13 % with metformin.

The mechanism with which vitamin D affects insulin resistance and glucose tolerance has yet to be confirmed, but two theories have postulated how changes in vitamin D levels might affect this. The first theory involves vitamin D's ability to suppress the inflammatory response (161). Obesity and insulin resistance are associated with an increase in proinflammatory cytokines, specifically TNF-α and IL-6, which in higher serum concentrations can promote insulin resistance (161). Vitamin D, on the other hand, has anti-inflammatory actions in higher concentrations and therefore could be responsible for greater suppression of TNF-α and IL-6 cytokines, reducing insulin resistance (161-163). In patients with congestive heart failure, Schleithoff et al. (162) demonstrated that those who were allocated to receiving vitamin D supplementation

showed significantly higher concentrations of anti-inflammatory cytokines compared to those who received no supplementation. Muller et al. (163) found similar inhibitory effects on proinflammatory cytokines where higher vitamin D levels resulted in greater inhibition in the production of IL-6 and TNF-α.

The second theory postulates that vitamin D influences insulin resistance with its regulation of the insulin signaling cascade (161). Research has identified a vitamin D response element on the human insulin receptor gene promoter (161). In vitro treatment with vitamin D_3 showed increased transcription of the insulin receptor gene, along with improved insulin-dependent glucose transport (161, 164, 165). Maestro et al. (164) showed that treatment with vitamin D for 24 hours increased insulin responsiveness for glucose transport in a dose-dependent manner. In a follow up study, Maestro et al. (165) found that vitamin D caused transcriptional activation of human insulin receptor gene, causing a greater response to insulin in terms of glucose oxidation. Vitamin D also appears to stimulate glucose oxidation by regulating phosphatidylinositol 3-kinase activity, an enzyme noted in insulin insensitivity (165). Understanding how vitamin D is connected to changes in insulin sensitivity and glucose tolerance could be advantageous in counteracting the effects of high blood glucose levels associated with pre-diabetes and diabetes. Further research in this area is required.

2.15 Gaps in the Literature

Research into vitamin D and pre-diabetes is just beginning. Few studies have investigated the effects of vitamin D on decreased insulin sensitivity or glucose intolerance that are associated with pre-diabetes. Furthermore, current studies

investigating vitamin D and pre-diabetes have come to different conclusions regarding its effectiveness. However, potential beneficial effects of vitamin D on blood glucose levels in patients with DM2 have been reported. Therefore, vitamin D treatments might benefit those with pre-diabetes. It is possible that studying the effects of vitamin D on pre-diabetes may lead to an intervention that would aid in decreasing the risk and progression of pre-diabetes towards DM2, or better still, reverse either iIGT or iIFG. This study aims to investigate the ability of vitamin D accrued from phototherapy to improve blood glucose levels in pre-diabetics.

Chapter 3: Protocol Development

3.1 Recruitment Setup

Recruitment for this study required the cooperation of various departments and staff within the Capital District Health Authority (CDHA) in the Halifax Regional Municipality (HRM) of Nova Scotia. First, approval for the study was required from the Capital Health (CH) Research Ethics Board (REB). Upon obtaining this approval, further methods to conduct the trial were developed. Clinical studies that are not related to pharmaceuticals require different protocols than those commonly conducted in CH. Navigating how to conduct this study within the framework of both CH and an academic unit of Dalhousie not normally affiliated with Dalhousie Medicine required considerable time and effort. In this chapter, practical details related to the development of the study's methodology and data collection are chronicled, detailed and explained.

Participants were recruited mostly via poster and informational leaflets provided by the research coordinator to various CH venues around the HRM. In order to display recruitment posters and leaflets throughout CH venues, these materials needed to be pre-approved by CH Human Resources (HR). There are 14 available display cases within the QEII hospital and any advertisement posted is displayed for only one week before being taken down. Therefore, the research coordinator was required to provide a sufficient number of posters in order to have one displayed in each of the display cases for the amount of weeks that he wanted it to be displayed. For example, if the research coordinator wished for the posters to be displayed for a 4-week period, it was required that 56 posters, in 4 sets of 14, be handed in to CH HR. Any subsequent advertising would be required to go through the same protocol. Posters were also displayed in

Diabetes Centers throughout the HRM; these were posted by the research coordinator within the centers with consent from the staff of each particular branch. The branches that received posters were the Cobequid Health Center in Lower Sackville, Bayers Road Health Center at Bayers Road, Dartmouth General Hospital in Dartmouth and the Victoria General Hospital in Halifax, Nova Scotia.

Diabetes-related information sessions are also held in various other venues in the HRM, such as large grocery stores; these sessions vary in focus from cooking classes to exercise classes. The research coordinator contacted Sobeys grocery retailers in the HRM and requested that posters and leaflets for the study be displayed in the diabetes education classes that are held in their grocery stores. The Nova Scotia branch of Diabetes Canada was also contacted with respect to poster advertising. They agreed to display a poster at the main branch of Diabetes Nova Scotia. The Dalplex at Dalhousie University, which houses the Department of Kinesiology and the headquarters for the study, as well as serving as a recreation center in Halifax, also displayed posters near the change rooms. Posters were also displayed in Stairs House, which houses the School of Health and Human Performance.

3.1.1 Diabetes Education Sessions

In unison with poster advertising, the research coordinator attended pre-diabetes education sessions throughout the HRM. Pre-diabetes sessions are put on by CH every couple of months throughout the year. In order to attend pre-diabetes sessions, it was required that the research coordinator receive approval from the diabetes session coordinator before attending. The research coordinator was contacted via email and given

dates and times where it would be acceptable to come and provide informational leaflets, as well as give a short oral presentation explaining the study to any potential participants.

Pre-diabetes sessions run in sets of two. Session one is an introductory session, where pre-diabetic individuals are introduced to their condition and have a lengthy class about how to cope day-to-day, including explanations of proper nutrition and exercise. Session two occurs 3 months after the first session, and is a follow up with diabetes nurses and dieticians regarding each individual's progress in dealing with their condition. The research coordinator was advised that attending session one would be most beneficial due to both the nature of the session, and the propensity for individuals to not attend the second session. If session one was attended, it was not required for the research coordinator to attend session two. Once a session had been chosen, the diabetes educator for that session would be emailed to inform him or her about the expected presence of the research coordinator at that session. A time where he would be able to meet and discuss the study with the potential participants would then be set.

Initially, the research coordinator was unaware if he was attending a session one or two. To remedy this issue the research coordinator phoned the secretaries at the diabetes centers and asked them to tell him which dates on their calendars were reserved for session ones.

Upon arriving at a pre-diabetes session, the research coordinator was required to sign in with the receptionist and wait until the diabetes educator was available. The point in the session when the research coordinator would be allowed to speak with the participants in the session was determined at the diabetes educator's discretion. In most instances, the research coordinator was allowed to discuss the study early in the session,

before the mid-session break, though this varied based on the preference of each educator. An early time was usually requested because it allowed the research coordinator to return to the session during the mid-session break and have an additional opportunity to discuss the study in further detail with any interested potential participants. The educators tended to allow a 5 to 10 minute oral presentation about the study during the pre-diabetes session. During this time, the study leaflets were handed out and discussed. The research coordinator explained the reason for attending the session, the background of the study and how being a part of the study may be beneficial to pre-diabetics. If time allowed, a small question and answer period would also take place.

If individuals expressed interest in being a part of the study, the research coordinator would discuss the first step for inclusion into the study during the mid-session break or at any time where a one-on-one discussion could be facilitated. This study employs a pre-study questionnaire, usually completed over the telephone when a potential participant contacts the research coordinator. However, since these participants were in contact with the research coordinator at that time, the questionnaire could be completed in person, allowing for the first step in the inclusion process to be completed. Having the ability to complete one step of the inclusion criteria at this point increased the ability to recruit participants. Normally, it would be the responsibility of the interested individual to contact the research coordinator at a later time. However, in many cases, when a participant expressed interest in being a part of the study, they would subsequently fail to pursue their interest after the session had been completed. This could be due to a busy schedule or discontinued interest after the fact. In addition, diabetes educators and dietitians met with pre-diabetic individuals outside of group sessions.

These educators appeared very willing to hand out leaflets or give information to those whom they felt might be interested in taking part in the study. It is unknown if this method of recruitment was pursued by the educators.

Problems faced at participant recruitment revolved around the length of the study and the location of the therapy. A number of interested individuals displayed hesitance to enter the study process with the knowledge that the therapy group would be required to remain in the study for a 3 month period and to attend therapy sessions 3 times a week during that period. In many cases, the individuals attending the pre-diabetes sessions expressed that they had very busy lives and were cautious about devoting such a large amount of time to being part of the study, if randomized to the therapy group. The research coordinator attempted to explain that the 3 times a week sessions were flexible and that the schedule of sessions would be based on their availability. It was emphasized that 3 times a week was ideal, but that it would not be a major problem if a session was to be missed; however, participants did need to show some consistency in order to achieve the desired results.

The location of the therapy caused further issues with many potential participants. Pre-diabetes sessions were conducted in various areas around the HRM, from within Halifax to Dartmouth to Lower Sackville. In many cases, individuals would need to drive in from the Annapolis Valley and other areas outside of the downtown Halifax area. This factor caused difficulty for those who were living outside of the immediate area. In many cases, individuals chose not to participate due to their inability or inflexibility with arriving at the therapy sessions. The research coordinator emphasized that allocation to the treatment group was random and that it was possible that those who lived further

away from the treatment center had an equal chance of being allocated to either the control group or the treatment group. He then asserted that interest in the study should not be dissuaded by the possibility of being allocated to the therapy group and not the control group. Unfortunately, many would not attempt to enter the study due to the attendance protocol. The research coordinator found that most interested participants, and those who ended up being a part of the therapy group, were recruited from diabetes centers located within peninsular Halifax. This included the Diabetes Center at Bayers Road, as well as posters displayed in the QEII hospital and around the Dalplex. Another issue that may have negatively affected recruitment was the lack of compensation for participating in the study, particularly if a drive from outside the region was required. In the future, greater focus on recruitment within the Halifax peninsula seems expedient. Further interest was also generated from a newspaper article and a television story that aired on local Nova Scotian television.

3.1.2 Participant Contact

Potential participants could contact the study coordinator either via the phone number found on the posters and leaflets that were displayed or via email. A telephone located in the Bone Lab, room 213G, of the Dalplex at Dalhousie University was equipped with a voice mail recording, which gave detailed instructions on leaving a message for the research coordinator so he could contact them. Emails were directed to the research coordinator's university email. The research coordinator was not allowed to "cold call" potential participants; all contact was initiated by the prospective participant. However, once contact was initiated, the research coordinator often found it difficult to

establish further contact with many potential participants. Additionally, several participants completed the first sections of the inclusion process but were unable to be contacted later on with further instructions on completing the entrance into the study despite repeated attempts.

3.2 Participant Inclusion into the Study

If an interested participant completed the pre-study questionnaire and met the first set of inclusion criteria, a meeting was set up to discuss and sign the consent form. At this time they also received the first requisition for blood work. The time between the initial contact with a participant and the consent meeting varied. Initial phone calls were made or emails were sent out a few days after the pre-study questionnaire was completed. This phone call or email was made to ensure that the individual was still interested in continuing with the study. Once the individual agreed to continue with the study, an email was sent out to request a meeting date for them to arrive at the Dalplex. To allow them additional time to read the consent forms, the document was attached to the email.

If the primary phone call or email did not receive a response, a second email or phone call was made roughly 2 weeks after the initial one. Subsequent contact was made every 5 days for a period of 1 to 2 weeks, until the point where either successful contact was made or it was determined that the individual had lost interest and did not wish to be contacted again. In some instances, interested individuals began the process but had to leave due to unforeseen circumstances, such as family issues. In such cases, the research coordinator expressed his regret and inquired if, in the future, they would be interested in being contacted again about potential participation in the study. If they agreed to future

contact, the research coordinator made an attempt to re-contact those individuals after a couple of months. However, in cases where a person had accepted future contact, none accepted a second invitation to enter the study.

3.2.1 Consent Session

The consent process took between 30 and 60 minutes to complete, depending on the diligence with which the individual read and understood the consent form prior to arriving. The research coordinator expressed in emails how willing he was to answer any question they may have regarding any part of the study, and that they would be asked to discuss the study to determine how well they read and understood the forms. Consent was done in the Bone Lab at the Dalplex, which is located within the Kinesiology Suites. Acquiring access to the Kinesiology Suites requires an individual to have a membership card that is swiped at turnstiles when entering the facility. All Dalplex employees at the front desk were aware that participants would be arriving for a research study and would need access to the Kinesiology Suites in the Dalplex. In most cases, the research coordinator met the participants at the front doors and escorted them into the Dalplex.

Once in the Kinesiology Suites, the consent process occurred in the back of the Bone Lab. In all cases, the individuals had read and understood the consent form in sufficient detail. When prompted to answer a *Consent Comprehension Form*, the individuals showed considerable understanding of what the consent form explained. At this point the consent form was signed. Few questions were asked regarding the nature of the study, with any inquiries revolving around the vitamin D therapy lamp and its ability to enhance vitamin D levels and any potential side-effects. After the completion of the

consent signing, a participant was invited to look at the vitamin D therapy lamp. If a participant was interested, a demonstration was performed for them. Two questionnaires were then administered to the participant.

3.2.2 Accrual and Distribution of Blood Requisition Forms

Blood work was a required component of this study. Setting up the systems needed to send participants for blood draws, for the samples to be sent to be both local and Ontario labs for analyses, for the results to be returned to study personnel and for the funds for these tests to be allocated as needed required extensive interaction with CH Blood Services, Research Services and the Finance Department. Before any blood work requisition forms could be given to participants, a research bank account needed to be created in Financial Services. At first, there was a little funding in an already established lab account to sustain the costs of all the blood work required for this study. With the help of one of the physicians on the research coordinator's thesis committee, enough funding to cover the costs of all blood work required for this study was successfully obtained from Sanofi-Aventis.

Once an account was created with CH Finance, the research coordinator was required to connect the funding with Blood Services. In order to achieve this connection, the research coordinator utilized the services of the Endocrinology Research Group. This group oversees all the research conducted by the physicians in the Division of Endocrinology, including participant recruitment and data collection for their studies. The lead research coordinator for the Division of Endocrinology became the liaison between Blood Services and Finance Services. The role of the Research Group was to ensure that

Blood Services received payment for all blood work, whether the samples were sent to local or Ontario labs for analysis.

CH blood requisition forms were used to collect the blood required for analysis in the study. Blood requisition forms were picked up by the research coordinator from one of the physicians in the QEII Hospital's Division of Endocrinology. When received, the requisition forms were blank; each one was completed by the research coordinator when it was given to a participant. In order to access the funding for the study, the research account number was placed on the requisition form. This ensured that no participant was required to spend any money out of pocket for blood work. Participants were given an explanation of how each of the blood tests would be performed in so far as the explanation might be relevant to them. Completion of blood work is discussed in further detail in Chapter Five.

3.3 Blood Sampling

For this study, each sample of blood work required a different blood service to complete. For the preliminary blood test, an oral glucose tolerance test (OGTT) test was completed. This test was used for both inclusion criteria and for data analysis. The research coordinator let the participants know which blood services sites within Nova Scotia could perform this specific test. Furthermore, it had to be clearly explained that the test took 2 hours to complete. Many participants required shifting around work schedules to complete the test; however, no problems arose from this.

One specific test, the test for serum 25-hydroxyvtiamin D (s-25(OH)D), is not performed in the province of Nova Scotia. All samples that required testing for s-

25(OH)D were sent to Hospitals-In-Common (HIC), which is located in Toronto, Ontario. In order to attain the services of HIC, the research coordinator created a contract linking CH Research and Financial Services, and HIC. This contract indicated the type of tests that would be completed by HIC, the total number of samples that HIC would be expected to analyze and how the shipment of samples was to be done. It was then the job of the research coordinator to receive and submit bills from HIC to Finance Services for payment.

In order to receive, store and send samples for s-25(OH)D testing, the research coordinator, with the help of Endocrinology Research, contacted CH Research Services. Due to Nova Scotia's lack of capacity to test for s-25(OH)D, Blood Services needed to know that all samples to be tested for s-25(OH)D were to be sent to Ontario. A form was designed by the research coordinator in combination with Research Services detailing to Blood Services that samples to be tested for s-25(OH)D were to be sent directly to Research Services. Research Services kept all the samples frozen, until the research coordinator had accumulated enough samples that they could be shipped in a batch to HIC. HIC was chosen to perform the tests for s-25(OH)D because they had been used previously by members of the research committee. The research coordinator and Research Services formulated the vitamin D accession form to allow for smooth transition of vitamin D samples from Blood Services to HIC. HIC supplied biochemical boxes and dry ice to transport the samples to their laboratories for analysis.

Some problems arose with respect to completion of the blood work. It was very difficult to get the participants to complete the blood work in a timely fashion. When a participant completed the consent process, the first requisition was given to him or her in

order to complete the OGTT test. A participant could not continue in the study if this preliminary blood work was not completed. Many participants did not complete the test in a reasonable time, with the research coordinator having to remind the participants that this sample needed to be completed in order to be placed in either the control or treatment groups.

Communication difficulties occurred again with the second pre-treatment blood work. This blood work needed to be completed before treatment, or for those in the control group, before the 3-month period could begin. This caused some participants' study period to be longer than the 3 months that was expected.

At the end of the 3 month period it became increasingly more difficult to get in touch with control participants to have them return to receive their second blood requisition form. However, once contact was achieved, completion of the blood work was done in a timely fashion. Conversely, this issue was exacerbated in the therapy group. It was unknown to the research coordinator that Blood Services protocols specified that if a fasting blood glucose results exceeded 7.1 mmol/L then the OGTT test would not be completed. A couple of participants alerted the research coordinator about this problem and asked what would be required to remedy this issue. It was imperative that this test be completed for statistical and data analysis reasons. With the help of Research Services, a document was drafted instructing the CH phlebotomist in charge of these blood tests that the OGTT should be completed regardless of the fasting blood glucose values. Unfortunately, one participant refused to consent to a second test, while another did not complete the test in a time where it was considered adequate by the research coordinator to show the effect of the vitamin D therapy. In those cases, the values of change in OGTT

values could not be used for data analysis. Unfortunately, a solution to the problem of delayed completion of blood work has not been determined. It is up to the research coordinator to express the importance of punctuality in blood work completion in the hopes of getting proper data for each participant. Future participants should be given a timeline for completion or some type of completion date in order to have blood work done in a timely fashion.

3.4 Blood Work Results Acquisition

Acquisition of blood work results required communication between the research coordinator and various members of the Division of Endocrinology. Due to the nature of the blood work and doctor-patient confidentiality, the research coordinator was not allowed to be the primary recipient of any blood work documents related to the study. In order to receive the results of any blood work, either one of the physicians on the study committee, or their secretary, would print out the results that were relevant for data analysis. The research coordinator worked in tandem with the physician's secretary to receive confirmation of any blood work, if the physician was unavailable. The secretary would then keep a copy of the results for the physician so that he or she would have the opportunity to peruse the results when there was time available to do so.

Results from the s-25(OH)D tests were sent from HIC to the office of the research study's physician 4 to 6 weeks after the samples were shipped to Toronto. Results from regular blood work processed in Nova Scotia were available 24 hours after phlebotomy. The bill for vitamin D sampling also arrived at the Division of Endocrinology. In this case, it was the job of the research coordinator to receive the bill from the Division of

Endocrinology and transfer it to Endocrinology Research, who in turn gave it to Financial Services. It was imperative that the research coordinator update both Research Services and the Endocrinology secretary as to the dates of blood draws. This allowed for quicker and easier identification of lab results when they arrived.

The research coordinator received a monthly email from CH Financial Services regarding the balance of funds in the study's account. This email contained a Microsoft Excel file indicating the current balance in the account, including any recent transactions with any of the services used. It was necessary for the research coordinator to double-check the amounts deducted from the account with the amount of activity that occurred over that month (e.g. how many blood samples were drawn, vitamin D samples shipped).

3.5 Vitamin D Therapy

For those participants who were allocated to the therapy group, a strict treatment protocol was developed. If the procedure was not conducted correctly, possible harm may have occurred. This harm includes burning or other skin-related issues. Because the therapy lamp was located in a private room and the participants needed to remove most of their clothing, the research coordinator did not enter the room with any participant. The only time the research coordinator entered the room was to pre-set the phototherapy lamp with the proper time for that participant's phototherapy session. Therefore, each participant received detailed instructions before his or her initial vitamin D therapy lamp session on how to properly use the phototherapy lamp. This included knowing how to press the start button on the phototherapy lamp timer as well as correct practices while the machine was in operation, such as where and how to stand and use of the goggles.

The participants maintained the same procedure each time they came in for each therapy session. The research coordinator was present at each session for every participant that underwent vitamin D therapy, and was in the vicinity of the phototherapy room during all sessions in case a problem arose.

Participants were asked to attend phototherapy 3 times a week during the 3 month study period. The research coordinator met each participant at the front of the Dalplex and escorted him or her to the vitamin D lamp. At the completion of each week of therapy, the research coordinator and the participant discussed the following week's schedule. If any sessions needed to be rescheduled or missed, the participant was asked to let the research coordinator know before the next week's therapy sessions were to begin, if possible. In those cases, the research coordinator asked the participants to contact him. Participants were requested to maintain the 3 times a week for 3 month treatment schedule as closely as possible. However, in an instance where a session was to be missed, 2 sessions per week were considered acceptable. If a participant was missing an inordinate number of sessions, the research coordinator discussed changing the schedule in order to facilitate more arrivals. In most cases, the 3 times per week treatment protocol was adhered to by participants, with only a few missed sessions. All participants who began phototherapy completed the protocol.

The decision to utilize a vitamin D phototherapy lamp rather than vitamin D supplementation for the intervention was due to the documented success of a lamp therapy in increasing serum vitamin D as well as to avoid the complicated documentation process required by Health Canada for supplement use. Vitamin D lamp usage may be able to augment serum vitamin D levels the same, if not greater than supplementation,

and also avoids the risk of vitamin D toxicity that can occur with supra-supplemental dosages of vitamin D. Furthermore, when the protocol for this study was created, vitamin D supplementation was considered a drug according to Health Canada. In order to utilize supplements as an intervention, approval would require extensive documentation to Health Canada, which would have taken extensive preparation. It was the decision of the research team to utilize a phototherapy lamp, which has previously been approved by Health Canada due to the diligence of the manufacturer.

3.6 Summary

The study protocol and subsequent start of the study took considerable effort to navigate and complete. The research coordinator laid the groundwork for a successful and meaningful study to be completed with more participants. At the end of the research coordinator's tenure with the study, over 20 people had been recruited for the study, although only 7 had completed it as either an experimental or control participant. This small sample size precluded statistical analysis of data. The values achieved for this study were examined for positive or negative effects that may be conferred to individuals with pre-diabetes. Currently, participant recruitment is continuing. A new research coordinator will use the protocols and networks now in place in order to proceed with recruitment of the full complement of subjects for this study. The following sections reflect the outcome of the implementation of the protocol on the 7 participants who have completed the study at this time.

Chapter 4: Objectives and Hypotheses

4.1 Objective

Taking into consideration that vitamin D_3 has been demonstrated to be beneficial in manipulating blood glucose levels in individuals with DM2, the primary objective of this study was to create a methodology that would allow for study of how vitamin D phototherapy would affect blood glucose levels in pre-diabetic individuals. It was then necessary to perform a pilot study to determine if the methodology implemented would produce results in a sample population. It was the objectives of the pilot study to:

1. Determine if vitamin D phototherapy will increase serum vitamin D levels
2. Determine if narrowband phototherapy improves glucose tolerance in pre-diabetics

4.2 Hypotheses

In relation to the research objectives being tested, the following hypotheses were formulated:

1. Serum vitamin D levels will increase in response to vitamin D phototherapy
2. Glucose tolerance and fasting blood glucose levels will be improved in pre-diabetics who had vitamin D phototherapy.

Chapter 5: Methodology

5.1 Participants

5.1.1 Exclusion Criteria

Participants were recruited for this study if they had pre-diabetes, were between 20 and 70 years of age and were otherwise healthy. To be considered to be pre-diabetic, either fasting blood glucose levels were greater than or equal to 6.1 mmol/L but less than 7.0 mmol/L or 2-hour post OGTT levels were 7.8 to 11 mmol/L, as established by the Canadian Diabetes Association (2) (Table 1). To ensure that participants had either isolated impaired fasting glycemic (iIFG) or isolated impaired glucose tolerant (iIGT) condition, each participant underwent an oral glucose tolerance test (OGTT). If a potential participant had already completed that test within 3 months prior to signing consent, a second OGTT was not required; however, confirmation of the results from their family physician was required for documentation and subsequent inclusion into the study.

Participants were excluded if they had been recently admitted to hospital with diabetes-related ailments, had developed DM2, or were on medication associated with diabetes. Individuals were not to be on medication for other hormonal diseases, have any myopathy, hepatic dysfunction or be taking glucocorticoids during or in the 3 months prior to this study. Any participant taking medications known to affect blood glucose levels or insulin sensitivity were excluded due to the inability to determine whether changes in glucose levels or insulin levels occurred due solely to vitamin D therapy. Participants could have no known medical disorders that could affect vitamin D absorption. Furthermore, participants could not have any skin condition that could be

exacerbated by exposure to vitamin D lamp emissions. Females who were eligible to be part of the study could not be pregnant or expect to become pregnant during the study period, because hormonal changes that occur with pregnancy may alter blood glucose levels or even result in gestational diabetes (17-19). Anyone who had visited or was planning to visit a warm climate area, or who used tanning beds or UV lamps was excluded from this study, in order to better define changes in blood glucose and vitamin D levels that were due to the vitamin D lamp itself.

Table 1. Diagnostic levels of glucose for IFG and IGT according to the Canadian Diabetes Association (CDA) (2)

	FPG (mmol/L)		2hrPG in the 75-g OGTT (mmol/L)
IFG	6.1-6.9		NA
IFG (isolated)	6.1-6.9	and	<7.8
IGT (isolated)	<6.1	and	7.8-11.0
IFG plus IGT	6.1-7.9	and	7.8-11.0

IFG- Impaired Fasting Glycemia
IGT- Impaired Glucose Tolerance
FPG- Fasting Plasma Glucose
2hrPG- 2 hour Post Glucose
OGTT- Oral Glucose Tolerance Test

5.1.2 Recruitment of Participants

Study participants were primarily recruited from venues in the Halifax Regional Municipality (HRM). Recruitment posters (Appendix A) were placed in various Capital Health venues across the HRM, including QEII and Dartmouth hospitals, Capital Health Research Services building, Canadian Diabetes Association (Nova Scotia branch) and the

Dalplex of Dalhousie University. Additionally, the research coordinator attended several pre-diabetes informational sessions conducted at the QEII Diabetes Management Centers. At these sessions, the research coordinator delivered information about the study to session participants and handed out informational leaflets. These leaflets contained further information about the study and contact information for potential study participants to contact the research coordinator (Appendix B). Media coverage for the study occurred with a newspaper article was published in the Chronicle Herald (Halifax, Nova Scotia) on May 6, 2010, as well as a TV newscast on "Live At 5", on CTV, displaying the vitamin D lamp and information about the study.

5.2 Experimental Design

In order to determine the sample size needed to reject the null hypothesis, a sample size analysis was required. In order to determine an approximate study sample size that would allow us to reject the null hypothesis, a previous study that most resembled our study parameters was selected (166). The primary variables of this study were pre-diabetic women and serum vitamin D levels. A α of 0.05 and a power of 80 % were inputted into biostatistics software (PS- Power and Sample Size Calculations version 2.1.31) created by Plummer and Dupont (Vanderbilt University, Tennessee). This calculation estimated that 38 individuals would be required to reject the null hypothesis.

For this study, a 2 x 2 split plot design was employed. This study design allowed for analysis of the changes in both serum vitamin D and blood glucose levels between the treatment and control groups as well as pre- and post-treatment values within each group. Participants were randomly allocated to either the control or phototherapy group by a

randomized block design completed in Microsoft Excel. Participants were enrolled in this study on a rolling timeline. In total, data collection for this study occurred from January 2010 to November 2010.

The control group was required to do nothing with respect to the intervention in the study. They were asked to maintain their daily activities, and to try not to change any activity that they were already taking part in. If participants were previously taking vitamin D supplements, they were encouraged to continue taking the supplement throughout the study. Those who were active outside during the summer months were also asked to maintain their activity as per normal, even if it were to cause a skewing of the data due to elevated vitamin D levels.

The therapy participants were asked to arrive at the Dalplex at Dalhousie University 3 times per week for the 3-month study period to receive their vitamin D treatment (Figure 5).

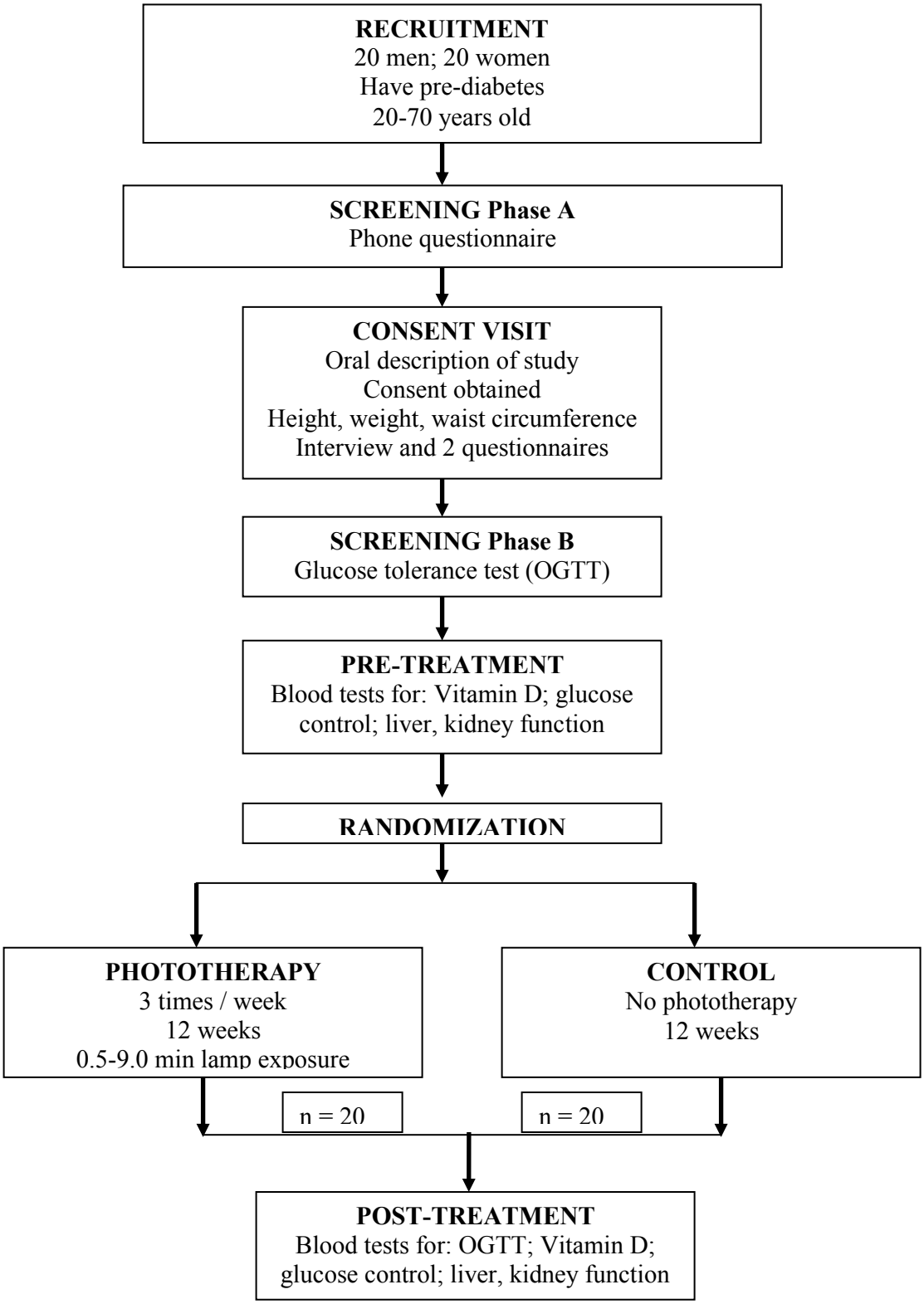

Figure 5. Experimental design proposed for the study

5.3 Pre-Screening

Initial contact with the research coordinator was made by prospective participants. Individuals who were interested in participating in the study called or emailed the research coordinator from the information provided on the posters and leaflets. Once the potential participants identified themselves and showed interest in the study, the research coordinator spoke with them about the study, using a *Preliminary Screening Telephone Script* (Appendix C). This was the first in a two-step process of identifying any possible exclusion criteria. This questionnaire took an average of 5 minutes to complete. Questions focused on the time of pre-diabetes diagnosis, current medications, tanning bed use, and travel plans. If the potential participant had any medication that was found to be a contraindication to sunlamp therapy, used tanning beds on a regular basis, or had planned to travel to a tropical or sunny location during the study period, they were immediately excluded from the study. If no exclusion criteria were evident, the participant was invited to meet with the research coordinator to discuss the study in person and to sign the consent form (Appendix D). To allow participants ample time to read the document, a copy of the consent form was emailed to each participant prior to arriving to the consent signing.

At the consent meeting, participants were asked a series of questions based on the Capital Health's research *Informed Consent Comprehension Questionnaire* (Appendix E). This short questionnaire was employed to ensure that each participant had read and understood the consent form in detail and was fully aware of the purpose of the research, the risks and benefits of the study and to address any questions had prior to signing the consent form. It was then clearly stated to the participant that they would be randomized

to either the control or vitamin D phototherapy group, that their participation in the study would last 3 months and that they could cease their participation at any time if they wished to do so.

5.4 Data Collection

After the consent form was signed, height (m), weight (kg), and waist circumference (cm) were measured and body mass index (BMI) (kg/m^2) was calculated. Additionally, the health, exercise and dietary questionnaire and the vitamin D and sun exposure questionnaire were completed by the participant during this session. Furthermore, a requisition form for the OGTT was given to the participants to take with them to the phlebotomy clinic. If the results of the OGTT indicated that a participant did have pre-diabetes, they were sent for a second blood draw, which included tests for diabetic markers as well as the serum vitamin D test. The remainder of data collection commenced after the participants had successfully met the inclusion criteria.

To facilitate confidentiality of the participants' records, a 3 digit numerical code was assigned to each participant at the time of informed consent. All participants were informed that they had full access to their own blood work results, and at their request those values would be faxed to their personal physician.

5.4.1 Health, Exercise, and Dietary Information Questionnaire

At the time of consent, the health, exercise and dietary information questionnaire was completed (Appendix F). This questionnaire was used to investigate the participant's current overall health, their exercise regimen, and current eating habits. Several questions

in the health section of the questionnaire centered on the participants' overall perceived health. Other questions documented all non-prescription and prescription drugs currently being taken. Questions on skin condition were also asked, including noting any skin condition they have/had, the severity of the condition, and any medications taken for it. Additionally, several diabetes-related questions were asked, including when and who made their diagnosis of pre-diabetes, if they monitor their blood glucose levels and if they required the use of insulin or oral hypoglycemic agents to counteract excessively high blood glucose levels. Physical activity data was then obtained from questions about the amount of physical activity the participant performed daily including the amount of walking done and any other activity additional to normal daily activity. If a participant did partake in these, they were then asked to detail the type of activity, the start and end date, and the amount of hours/week the activity was performed. If the individual was unable to recall specific dates then an estimated time was noted. Dietary intake of foods fortified with calcium and vitamin D were ascertained from questions centered around the amount of dairy products consumed on a daily basis, such as milk, cheese and yogurt. Information about juice, margarine and sodium intakes was also collected.

A 24-hour dietary recall was also administered to determine the dietary habits of a participant for an average day (Appendix G). Each participant was asked to recall his or her previous day's intake of food, both solids and liquids and where the meal was eaten. The research coordinator sat with the participant and assisted in recall by asking trigger questions to stimulate recall. Each section was completed from memory in as much detail as possible. Information from the 24-hour dietary recall was analyzed using EatRight Version 15.0, a nutritional analysis software program.

5.4.2 Vitamin D/Sun Exposure Questionnaire

The second questionnaire was the vitamin D and sun exposure questionnaire (Appendix H). This questionnaire investigated the amount of time the participant spent in the sun, including any vacations that the participant may have taken in the past year, and the amount of time they spent outside. Participants were asked to detail the amount of time they spend outside during a given day in the summer, and if they used any sunscreen. With regards to supplementation, the participant noted any multi-vitamin, vitamin D and calcium supplements they may have taken. In each case, the brand and dosage was noted. For assessment of the amount of vitamin D or calcium concentration within the supplement, the product websites or the actual product found within a local pharmacy were examined. In the case where the actual brand of supplement was unknown, or if the participant tended to buy what "was on sale" and never consistently bought the same brand, a baseline brand was used for analysis. In this case Life Brand supplement was used as the basis for intake analysis due to its availability and low cost. It consisted of 400 IU of vitamin D and 250 mg of calcium.

The questionnaires used in this study did not come from a validated source. Both of the questionnaires that were employed in this study were adapted from questionnaires used in a previous study by one of the members of the research team, which investigated both lifestyle and vitamin D/sun habits in another clinical population. Some of the questions were amended, while others were added, in order to assess the specific habits of this particular clinical population.

5.4.3 Blood Measurements

Blood phlebotomy and analyses were done through Capital Health blood services. Participants were informed that they could go to any of the Capital Health affiliated blood services site in order to have their blood work completed. The research coordinator instructed the participants as to which clinics were drop-in and which would require an appointment. It was at the participants' discretion where they chose to have their blood work completed. The only exception was Bayers Road blood clinic, where OGTTs are not performed; however, this location was acceptable for the secondary blood work. Participants were given Capital Health requisitions forms filled out with their name, date of birth, and sex. No health card number was required because all costs related to blood work were compensated for through the study research account. In order to receive the results of the blood work, one of the endocrinologists who was part of the study was identified as the consulting doctor.

All blood tests were completed after an 8-hour fast. Participants were allowed to drink water, but no other liquids or solids were to be ingested during that period. To determine baseline and post-intervention values for both vitamin D concentration and several diabetes-related blood assays, the blood tests were completed twice by the participants, both pre- and post-intervention.

5.4.3.1 Oral Glucose Tolerance Test

Each participant had an OGTT completed both pre- and post-intervention. The initial OGTT was foregone only when a participant had previously taken the test within 3 months prior to entering the study. In such a case, the participant was asked to provide

the results of that test to the research coordinator. A 300 mL glucose oral solution that contains 75 g of glucose (Ratiopharm, Germany) was consumed over a 5 minute period. Blood samples were taken immediately before ingestion, and 2 hours post ingestion of the glucose drink. For this study, the OGTT was used for two purposes. Participants who tested in either the euglycemic or DM2 ranges by the initial OGTT were excluded from the study. If a potential participant was found to have DM2, a physician from the study contacted that individual with further instructions about dealing with this condition. The secondary purpose was to determine if vitamin D caused a change in glucose clearance from pre- to post-intervention.

5.4.3.2 Hemoglobin A1c

It was hypothesized that there would be a change in the participants' blood glucose levels from pre- to post-intervention due to changes in serum vitamin D levels. In order to ascertain a value for the change in blood glucose concentration in the bloodstream, a specific type of blood test was required that would detect such a change. The hemoglobin A1c (HbA1c) test provides a 3-month average blood glucose levels and was an appropriate blood test to use in order to determine any change in blood glucose levels from pre- to post-treatment due to vitamin D supplementation. Participants had an HbA1c test pre-treatment to determine their current average blood glucose level, followed by a second HbA1c after the 3 month trial period. The aim was to determine if vitamin D therapy caused a change in average blood glucose levels in comparison to those who received no treatment.

5.4.3.3 Serum Vitamin D

In order to determine changes in vitamin D concentration, serum 25-hydroxyvitamin D was analyzed. Vitamin D blood samples were collected and batch shipped to Hospitals-In-Common Laboratory (HIC) (Toronto, Ontario) due to the lack of facilities to analyze vitamin D in the province of Nova Scotia. In order to send these samples, Capital Health blood services were requested not to accession the blood tests and have them sent to Capital Health research services. The samples were kept at this location in a deep freeze until it was time for them to be sent. Under frozen conditions, vitamin D samples can be kept for over a year before becoming unstable (167).

HIC utilizes a competitive chemiluminescence immunoassay to determine serum vitamin D in the blood samples. This type of vitamin D assay has been shown to be valid and accurate in the detection of serum vitamin D levels (143, 144), and was therefore appropriate for use in this study.

5.4.3.4 Liver and Kidney Function

DM2 can be associated with many secondary complications, including heart, renal and/or liver dysfunction. In all cases, completing various blood tests can assess any damage that might be occurring to these systems as a result of poor blood glucose management. For the purposes of this study, blood tests for renal and liver function were assessed. These tests included ALT and AST for liver function and creatinine for renal function.

These blood markers were tested concurrently with the other blood tests and were completed primarily to monitor if the diagnosis of pre-diabetes had caused any

deleterious effects on other systems. Tests were done pre- and post-intervention as well to determine if vitamin D may have caused any effect on serum concentration.

5.5 Vitamin D Lamp Exposure

Those participants who have been allocated to the vitamin D therapy group underwent 3 sessions of phototherapy a week for the 3 month trial period. When the participant arrived for their first session, an initial vitamin D therapy question was asked. This question was adapted from a similar question stated in the SolArc vitamin D lamp manual (Appendix I) (168). Participants were asked to rate their skin type based on how they react to prolonged sunlight. Responses could have ranged from "always burn, never tans" to "black" and their response determined both the initial treatment time and the rate at which time would increase for each ensuing treatment. For the subsequent weeks, a standardized log sheet was created to determine the amount of time that the individual would be standing in the lamp each session (Appendix J).

In order to achieve maximum exposure, participants were instructed that wearing minimal clothing while completing phototherapy sessions, with shorts and a tank top being the most ideal clothing. Once the participant had chosen the garments he or she would wear, it was requested that the same clothes be worn throughout the sessions. Participants were asked to stand 25 cm away from the vitamin D lamp for optimal exposure, indicated by a piece of tape placed on the ground for reference (Figure 6) (168). Exposure occurred to both the anterior and posterior of the participant, as displayed in Figure 7 below.

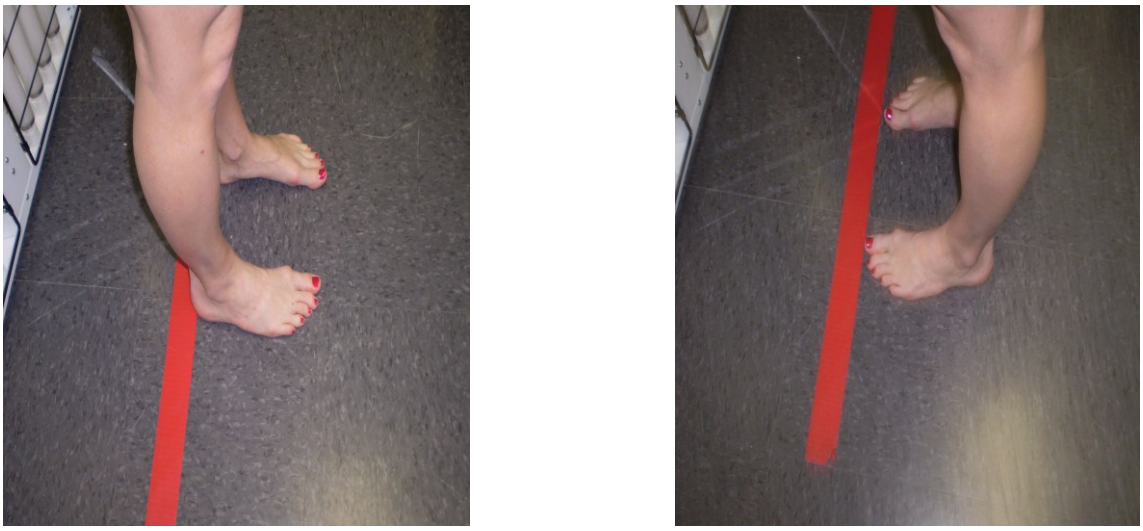

Figure 6. Feet position for exposure of the anterior and posterior surface of the body

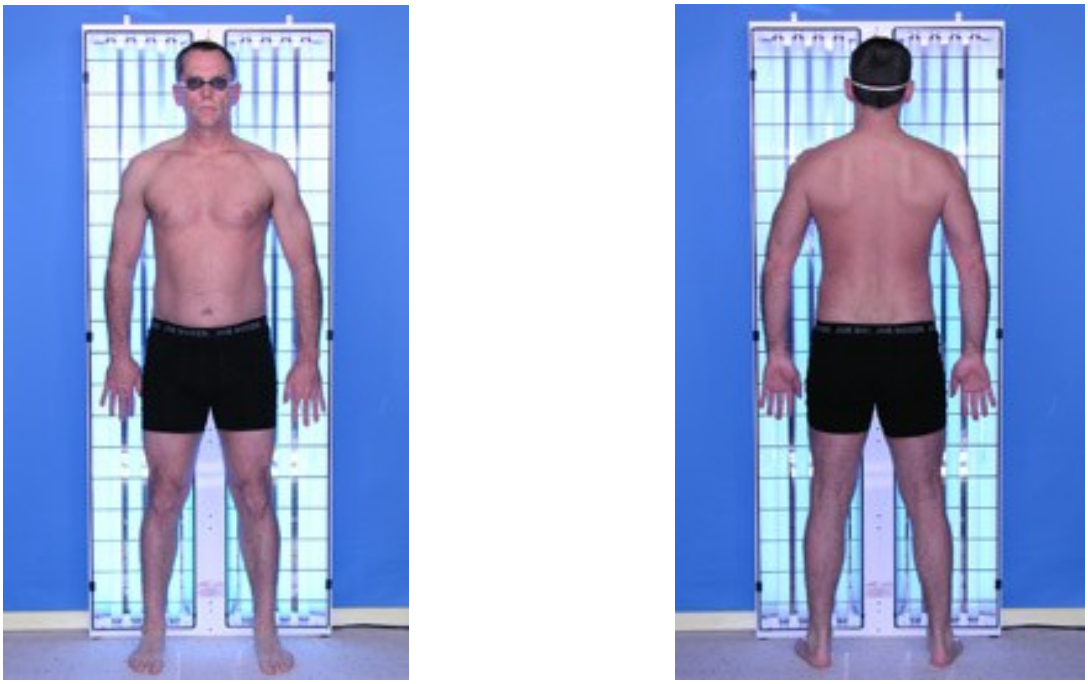

Figure 7. Positions for vitamin D exposure sessions (168)

Each session included an equal time of exposure to the anterior and posterior sides of the body. At every subsequent session the participants were asked how their skin looked or what it felt like 12 to 24 hours after the previous session. Responses to this question could range from “no or minimally noticeable effect” to “significant erythema (burns) edema/blisters” and adjustment of lamp exposure time reflected those responses. Time of exposure was to be only extended if the participants indicated that they had “no or minimally noticeable effect”, while the same time was maintained if they were to indicate that they had “light pink” as a response (168). Similarly, if a participant were to miss more than one session in succession, the previous time would be maintained. According to the SolRx instruction manual, if a participant indicated significant burn or blisters after treatment had occurred then session time would be skipped, reduced in time, or stopped all together (168).

5.6 Statistical Analysis

In order to gain statistical significance for this study, a minimum sample of 38 participants was required. Unfortunately, only 7 participants were successfully included in the study. Due to the small sample size attained by the end of the recruitment period, statistical tests were not completed. By completing statistical analysis with such a small population, the possibility of yielding a false-negative, or type II error, is more likely. This could, in turn, cause an inaccurate conclusion on effectiveness of vitamin D phototherapy on blood glucose levels to be made. All results were, therefore, analyzed based on categorical and normative values for each variable. Once a large enough sample size is attained, paired sample t-tests could be performed in order to determine if the

change in serum vitamin D levels and/or blood glucose levels is significant from pre-to post-treatment. Additionally, independent sample t-tests can determine if there is a significant difference between the treatment and control groups.

Chapter 6: Results

6.1 Participant Recruitment

Attendance to pre-diabetes management classes were thought to be the ideal venue for recruitment of potential participants. According to the diabetes care program of Nova Scotia, close to 5,000 newly diagnosed cases of diabetes, pre-diabetes and gestational diabetes are referred to them each year (8). From this data, it has been estimated that over 800 new cases of pre-diabetes is diagnosed every year, which provides a large population to sample from (8). Through the recruitment period, the research coordinator attended about 15 separate pre-diabetes management classes. Attendance at these sessions could range from 10 to 20 people, therefore it is estimated that the research coordinator presented the study material to about 150 to 300 people throughout the recruitment period. From that pool of people, only 23 potential participants expressed interest in taking part in this study. Sources of recruitment for these individuals were diverse: 12 were recruited at pre-diabetes information sessions that were held at local diabetes management center, 1 from study posters that were displayed in HRM hospitals and 3 from the Dalplex of Dalhousie University, 2 by word of mouth, while 2 contacted the research coordinator after reading the newspaper article and 3 after watching the television program.

6.2 Inclusion into the Study

All 23 potential participants contacted the research coordinator with their interest in participating in the study. Either by phone or one-on-one conversations, 20 potential

participants successfully underwent the telephone screening questionnaire, with 3 expressing disinterest when contacted. The remaining 20 participants agreed to meet the coordinator at the Dalplex to read and sign the consent form and receive the OGTT blood requisition form. Two participants did not require the OGTT blood test due to a previous OGTT, which was completed within 3 months prior to agreeing to be a part of the study. Two participants withdrew from the study before taking the OGTT citing scheduling issues as the reason for the inability to complete the blood work. A further 9 participants were excluded from the study because their OGTT revealed that they were euglycemic and therefore did not meet the criteria to continue to the next stages of the study. One participant, after completion of the OGTT was excluded because he or she was found to have DM2. This participant was contacted by an endocrinologist on the committee to inform him or her of the results and deliver advice as to the further steps that should be taken. One participant was excluded from the study due to the inability by the study coordinator to get in contact with that participant over a 3 month time period. Figure 8 displays a diagram illustrating recruitment of participants for this study.

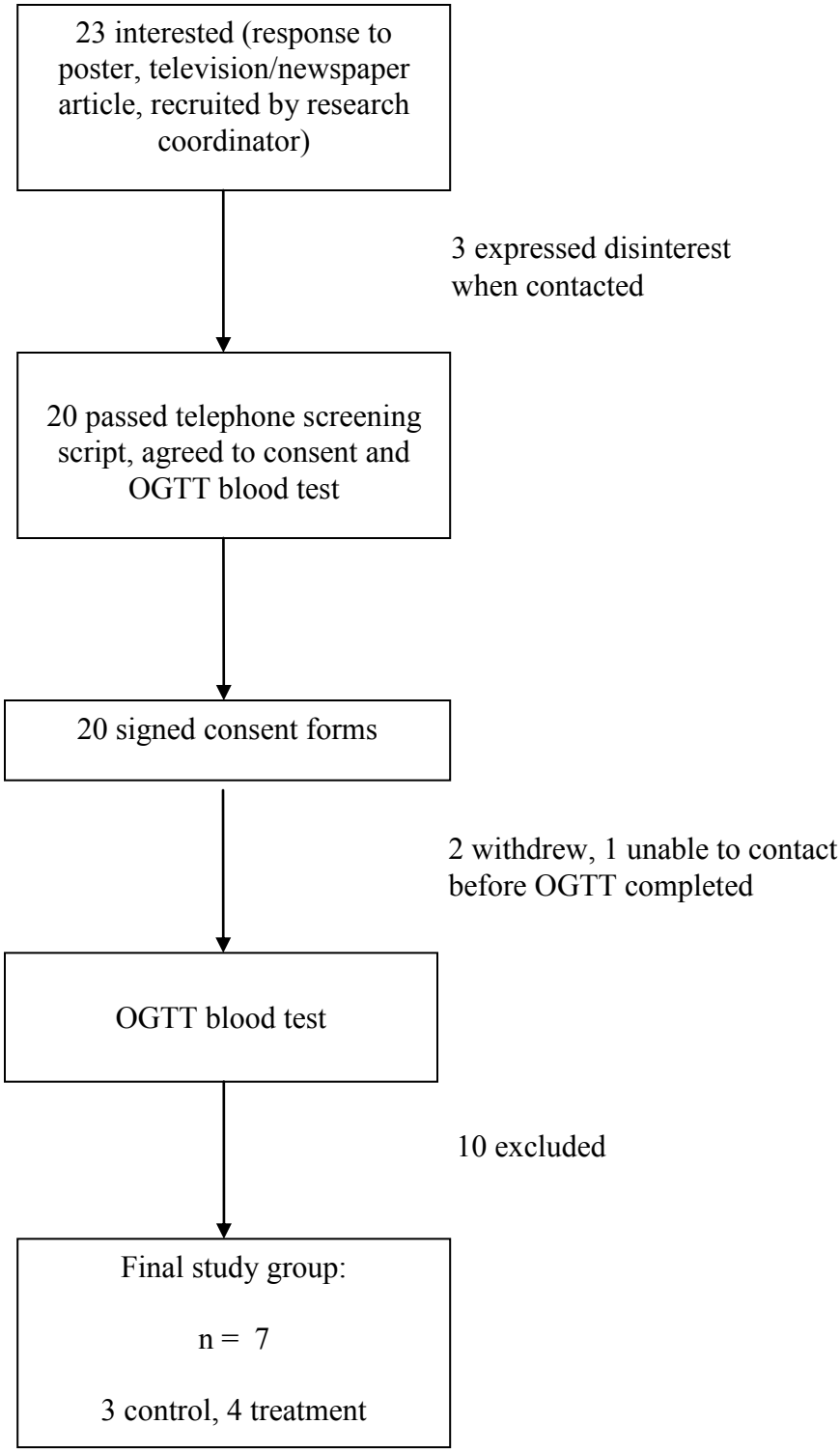

Figure 8. Recruitment of individuals interested in participating in the study

6.3 Participant Demographics and Anthropometric Data

Table 2 displays and summarizes the anthropometric measurements of the 4 treatment and 3 control study participants. Categorization of the participants by BMI was based on Health Canada's levels of BMI and risk (147). Three participants (2 treatment, 1 control) were of normal weight. Three participants (all treatment) belonged in the category Obese Class I, while 1 participant of the control group belonged in the category of Obese Class II.

Participant data was compared to the normative data for BMI for Nova Scotians, which estimates that 60 % of Nova Scotian adults are overweight or obese. In this study, 4 out of 7 participants met the criteria for this category, which indicates that the participants in this study were representative of Nova Scotian residents over the age of 18 (147). The waist circumference (WC) of the participants was compared to the Canadian cutoffs for elevated health risk, which are 102 cm in males and 88 cm in females. Four of the 7 participants had a WC that indicated an elevated health risk.

Table 2. Descriptive values for demographic and anthropometric data.

	Age (yr)	Sex	Height (m)	Weight (kg)	BMI (kg/m^2)	WC (cm)
Treatment						
T1	62	Female	1.65	94.0	34.5	105.0
T2	62	Female	1.50	69.0	30.7	101.6
T3	68	Female	1.64	59.0	21.9	83.4
T4	56	Female	1.52	73.0	31.6	96.5
Control						
C1	67	Female	1.64	63.0	23.4	76.2
C2	53	Female	1.65	58.0	21.3	71.1
C3	45	Male	1.72	111.1	37.6	106.3

yr- years of age
m- meters
kg- kilograms
kg/m^2- kilograms per meters squared
cm- centimeters

6.4 Health, Exercise and Lifestyle Questionnaire Data

Three of the 4 participants in the treatment group described themselves as "about as healthy as others their age", while the fourth participant considered her health to be "less healthy than others". In the control group, 1 participant considered herself to be healthier than those of her age, 1 about as healthy, and the third less healthy then those in her age group. All participants in both groups were currently non-smokers, with 2 participants in the treatment group and 1 participant in the control group having quit smoking 3, 7 and 20 years ago respectively. Advil and Tylenol, or their generic equivalents were used by 3 participants in the therapy group and 1 in the control group;

these were the only non-prescription drugs taken by participants in this study. All prescription drugs that were used by participants did not meet exclusion criteria for this study. Specifically, no participants reported taking any prescription drugs that are known to impact either vitamin D status or glucose control. Only 1 participant indicated that she had an intestinal diseases or skin condition, which was psoriasis, a skin condition that is not exacerbated by vitamin D phototherapy.

All participants had been previously diagnosed with pre-diabetes before being accepted into the study. Diagnosis of pre-diabetes for all participants was completed by their family physicians between 4 months to 10 years previously. No participants reported any active blood glucose monitoring or management.

The treatment group walked an average of 30 minutes a day, while the control group walked 15-20 minutes. One participant in the treatment group and 2 participants in the control group participated in physical activity other than walking. The activities included participating in aerobics classes, gardening and outdoor activities like sailing and softball.

6.5 Vitamin D and Sun Exposure Questionnaire Data

All participants had resided in Halifax for the past 12 months. Two participants (1 treatment, 1 control) had gone on vacation to tropical climates in the past year, and were exposed to sunshine while traveling. Four participants indicated that they were rarely outside from 10 am to 3 pm, while 3 participants (1 control, 2 treatment) indicated that they are in the sun sometimes between 10 am and 3 pm. During times in the sun, 3 treatment participants indicated they usually wear sunscreen, with the other 1 only

sometimes wore sunscreen, whereas in the control group, 2 participants sometimes wore sunscreen while 1 never used sunscreen when outside.

6.6 Phototherapy Sessions

The 4 participants randomized to the phototherapy group agreed to arrive at the Dalplex 3 times a week for a 3-month period for phototherapy. The date, session number, and time in the lamp were recorded each time they arrived for phototherapy. Figure 9 displays the session, and time spent in the lamp over the 3-month study period. When asked to report on their skin type and the effect that sun exposure has on their skin, all participants reported that they "sometimes burn, always tan", indicating that phototherapy should begin at 1:30 seconds per anterior and posterior exposure.

On average, participants missed a total of 4.5 of the 36 phototherapy session. No more than 2 sessions of phototherapy were missed in succession. The average time spent in the phototherapy lamp was 3:32 minutes, with a maximum exposure time of 5:06 minutes by 1 participant. None of the missed sessions were a result of the participants stating that their skin looked or felt to have significant erythema after any of the phototherapy sessions.

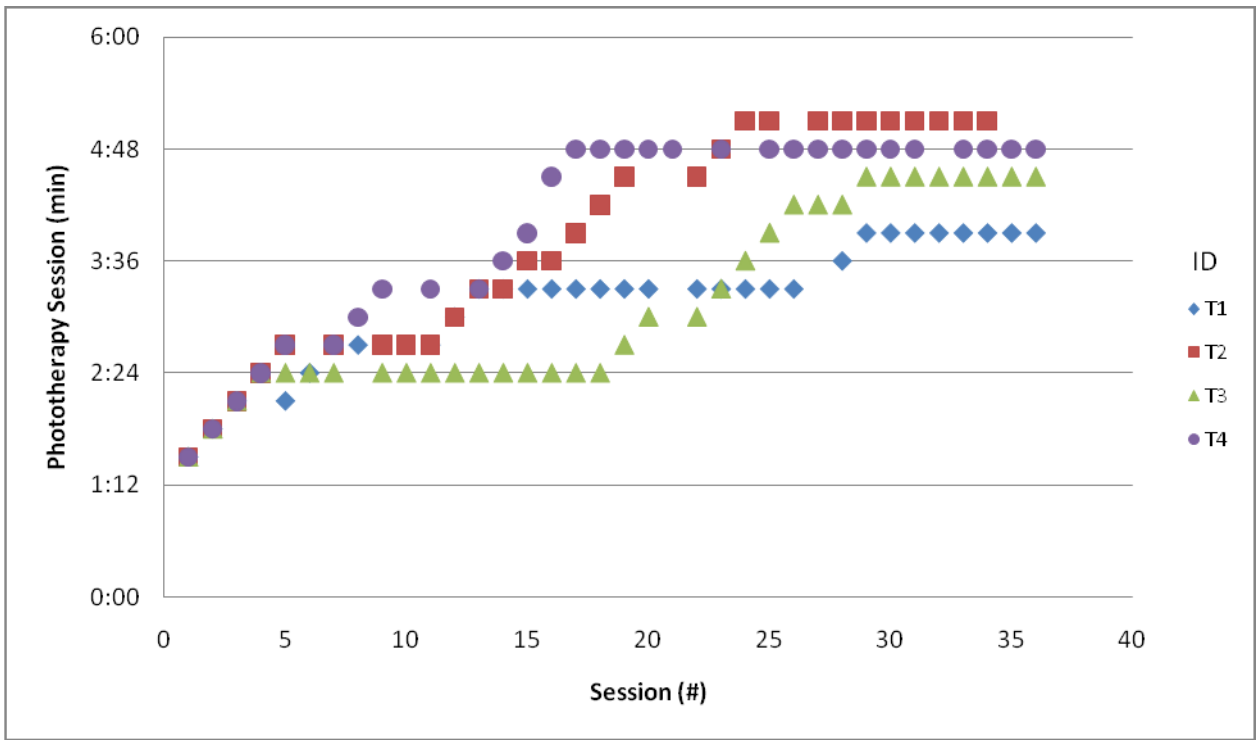

Figure 9. Vitamin D sessions for four participants in the therapy group

6.7 Serum Vitamin D Levels

All participants were required to have their serum 25(OH)D measured pre- and post-intervention. Vitamin D measures were based on the lab's (Hospital-In-Commons) definition of vitamin D sufficiency, which closely resembled the limits found in the literature (99, 169, 170). The nmol/L ranges for severe vitamin D deficiency to vitamin D toxicity are found in Table 3.

Table 3. Values in nmol/L for serum vitamin D deficiency to vitamin D toxicity (167)

Vitamin D Level (nmol/L)	Definition
<30	Severely deficient
30-49	Deficient
50-74	Insufficient
74-250	Sufficient
>250	Toxic

The post-intervention serum 25(OH)D value in each participant in the treatment group increased with the phototherapy treatment; these changes ranged from 0.6 % to 110.6 %. One participant jumped from deficient to sufficient in 25(OH)D status. Serum 25(OH)D levels in 2 of the 3 control participants decreased, one by 35.2 % and the other by 48.7 %. One of the control participants dropped in vitamin D status from sufficient to deficient. However, the other control participant increased her 25(OH)D by 27.8 % (Table 4, Figure 10).

Table 4. Vitamin D values and their respective category for study participants pre- and post-intervention, including percent change

	Pre-Study Vitamin D (nmol/L)	Pre-Study Category	Post-Study Vitamin D (nmol/L)	Post-Study Category	Percent Change (%)
Treatment					
T1	47	Deficient	99	Sufficient	110.6
T2	59	Insufficient	71	Insufficient	20.3
T3	159	Sufficient	160	Sufficient	0.6
T4	75	Sufficient	83	Sufficient	10.7
Control					
C1	158	Sufficient	202	Sufficient	27.8
C2	162	Sufficient	105	Sufficient	-35.2
C3	78	Sufficient	40	Deficient	-48.7

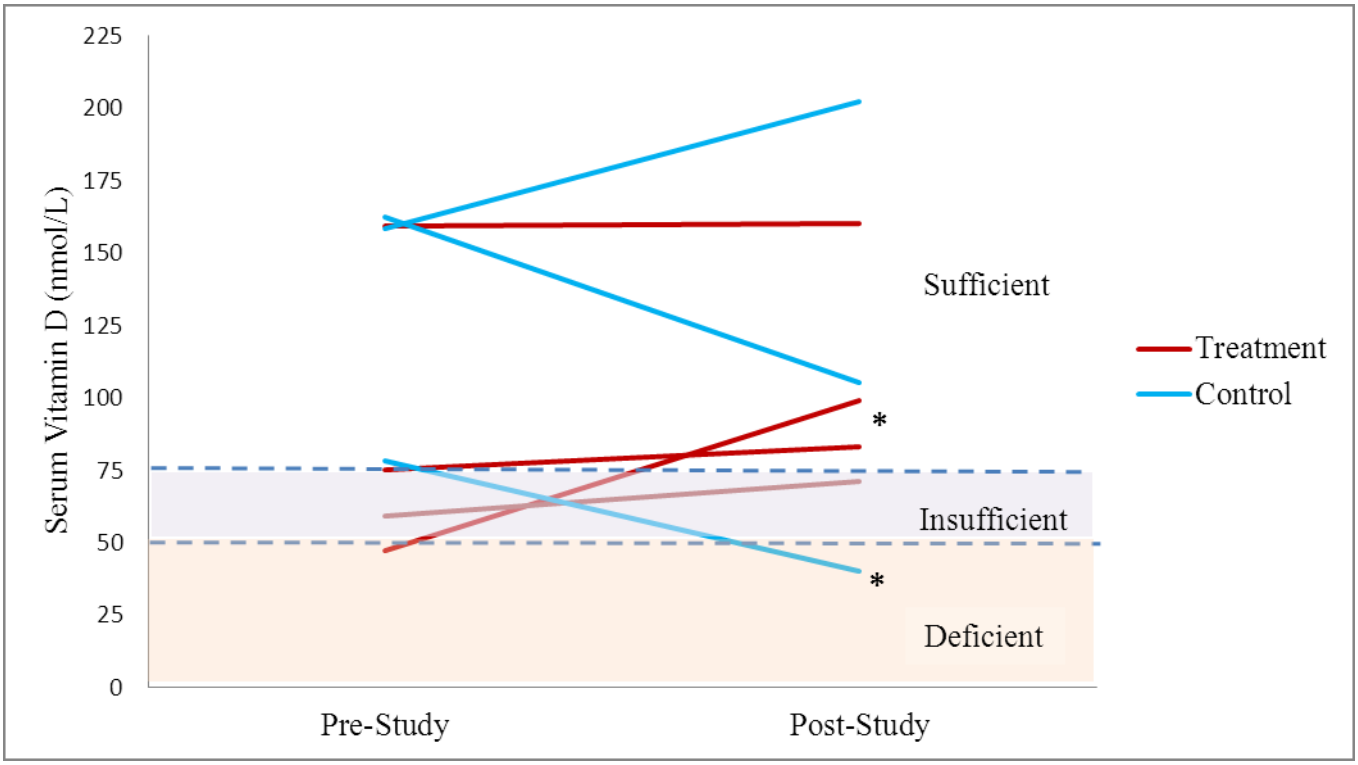

Figure 10. Participant pre- and post-intervention vitamin D values.

* indicates change in serum level from pre- to post-intervention

6.8 Oral Glucose Tolerance Test

OGTTs were administered to each participant before inclusion into the study as well as post-intervention in order to determine if there was a change in glucose clearance due to the effects of vitamin D therapy. Five of the 7 participants completed both sets of OGTT; one therapy participant refused to have the second OGTT administered, while another did not complete the test within a time period where the results would be considered usable, however a fasting blood glucose level of 7.2 mmol/L could indicate DM2 (Table 5). Pre-diabetes categories were based on the Canadian Diabetes Association's 2008 Clinical Practices Guide (2), as shown in Table 1.

After completion of the 3-month vitamin D treatment, 2 out of the 4 participants who had completed a post-intervention OGTT showed improvements in their blood glucose. Their OGTT results indicated that one of these participants had reduced her fasting blood glucose level, while the other participant demonstrated reduced blood glucose 2 hours after glucose ingestion. This changed their categorization from combined IFG plus IGT to isolated IGT and isolated IFG, respectively. The control group saw the opposite occur. Two of the 3 participants experienced an adverse change in categorization, with 1 developing combined IFG plus IGT, while the other control participant changed to DM2 from isolated IFG. The glucose metabolism of the third control participant did not change.

Table 5. OGTT results and category of pre-diabetes pre- and post-intervention

	Pre-Intervention			Post-Intervention		
	FBG (mmol/L)	**2hr Post OGTT (mmol/L)**	**Pre-diabetic category**	**FBG (mmol/L)**	**2hr Post OGTT (mmol/L)**	**Pre-diabetes category**
Treatment						
T1	6.3	4.2	Isolated IFG	N/A*	N/A*	N/A*
T2	6.9	9.5	IFG & IGT	7.2	N/A*	DM2
T3	6.7	8.6	IFG & IGT	5.6	8.4	Isolated IGT
T4	6.9	9.9	IFG & IGT	6.8	6.3	Isolated IFG
Control						
C1	5.5	9.5	Isolated IGT	6.2	9.6	IFG & IGT
C2	6.9	7.2	Isolated IFG	7.5	8.1	DM2
C3	6.4	6.0	Isolated IFG	6.2	6.2	Isolated IFG

* T1 refused to consent to a second OGTT; T2 did not complete the test within an acceptable time for analysis

6.9 Hemoglobin A1c Test

Hemoglobin A1c testing was completed to determine if there was a change in average circulating blood glucose levels between pre- and post-intervention. According to Diabetes Canada Clinical Guidelines, an HbA1c of above 7.0 % is at high risk for both microvascular and macrovascular complications (2). An HbA1c of 6.0 % is considered good for those exhibiting signs of pre-diabetes or DM2.

Before phototherapy, only 1 of the treatment participants exhibited an HbA1c result indicative of high risk. However, 2 of the treatment group participants and 1 of the control group exhibited elevated HbA1c values above the recommended 6.0 %. The final treatment participant and the other 2 control participants had HbA1c values within the optimal range. After the 3-months of treatment a change in HbA1c values was noted. Within the treatment group, 1 participant showed a positive change, while 2 others showed a negative change in HbA1c. The participant who showed the largest decrease had a change in her risk range from elevated to optimal as a result. The fourth treatment participant showed no change. A control participant also changed categories, moving from the optimal range to an elevated risk range. Two control participants, though showing a decrease did not change HbA1c categories from pre- to post-intervention (Table 6, Figure 11).

Table 6. HbA1c values of participants pre- and post-treatment and the subsequent risk category associated with values

	Pre-study HbA1c (%)	Reference Range	Post-study HbA1c (%)	Reference Range	Percent Change (%)
Treatment					
T1	7.0	High Risk	7.3	High Risk	4.3
T2	6.5	Elevated Risk	6.5	Elevated Risk	0.0
T3	5.7	Optimal	5.6	Optimal	-1.8
T4	6.1	Elevated Risk	5.8	Optimal	-4.9
Control					
C1	5.7	Optimal	6.0	Elevated Risk	5.3
C2	6.4	Elevated Risk	6.3	Elevated Risk	-1.6
C3	5.3	Optimal	5.2	Optimal	-1.9

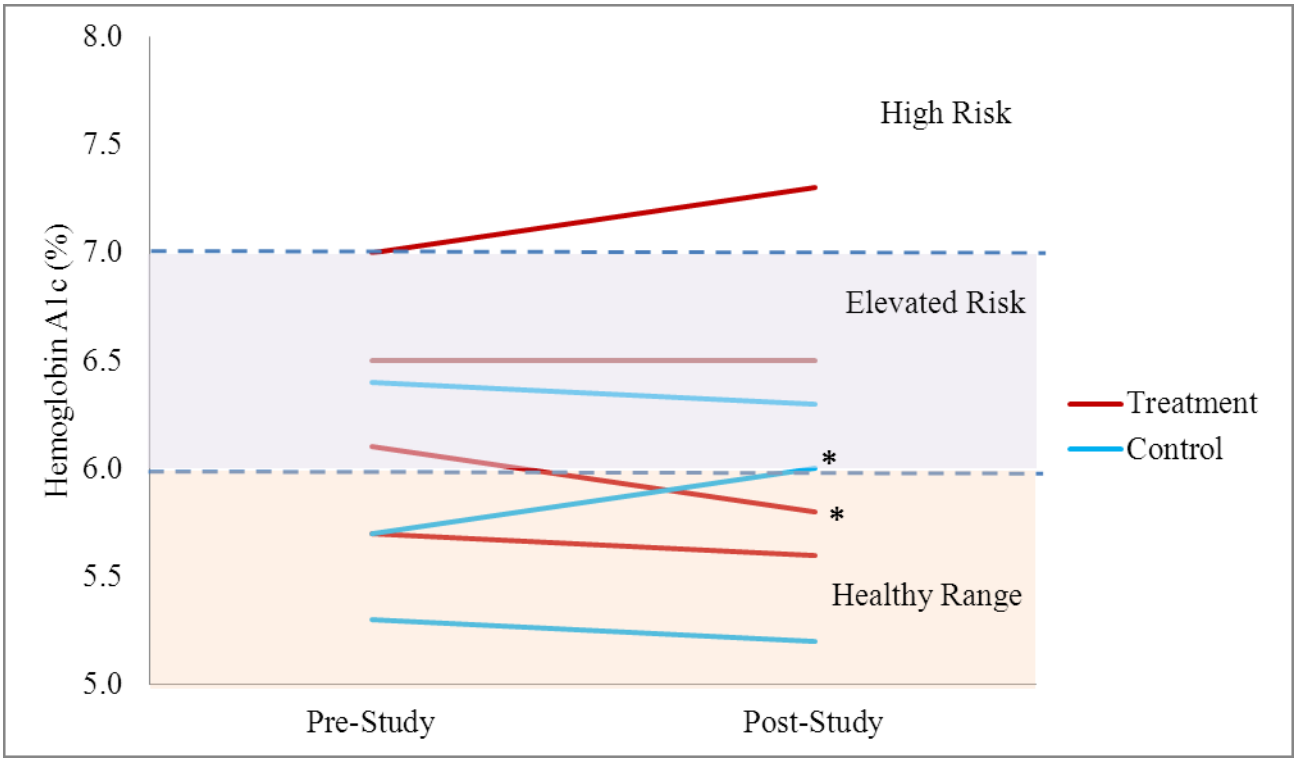

Figure 11. Participant pre- and post-intervention Hemoglobin A1c (HbA1c) levels and the associated risk range.

* indicates change in risk range from pre- to post-intervention

6.10 Liver and Kidney Function Tests

A variety other blood tests were performed that are typically completed in individuals with diabetes in order to determine if elevated blood glucose levels are affecting other systems such as liver and kidney function. These blood tests were assessed pre- and post-intervention in order to determine if vitamin D caused a change in their values.

In order to determine if there was any effect of vitamin D on kidney function, creatinine was sampled. Reference range for optimal creatinine levels differ for each

laboratory assessing the blood sample. While Capital Health considers 37 to 96 µmol/L to be optimal, Hospital In-Common considers 52 to 112 µmol/L for males and 42 to 102 µmol/L for females to be optimal (167). In a review of the literature, Erlandsen et al. (171) determined that an optimal reference range for creatinine in females is 57 to 95 µmol/L and 69 to 111 µmol/L in males. Because kidney function was tested using Capital Health Blood Services, the reference range provided by them were used for analysis of any change in values pre- to post-intervention. Both the phototherapy and control groups had mixed changes in creatinine pre- to post-intervention (Table 7, Figure 12). However, all 7 participants' pre-intervention and post-intervention creatinine values fell within the normal range.

Table 7. Results of creatinine concentration pre- and post-intervention

	Pre- study Creatinine (µmol/L)	**Post-study Creatinine (µmol/L)**	**Percent Change (%)**
Treatment			
T1	54	58	7.4
T2	77	69	-10.4
T3	62	69	11.3
T4	61	60	-1.6
Control			
C1	73	69	-5.5
C2	44	52	18.2
C3	80	68	-3.2

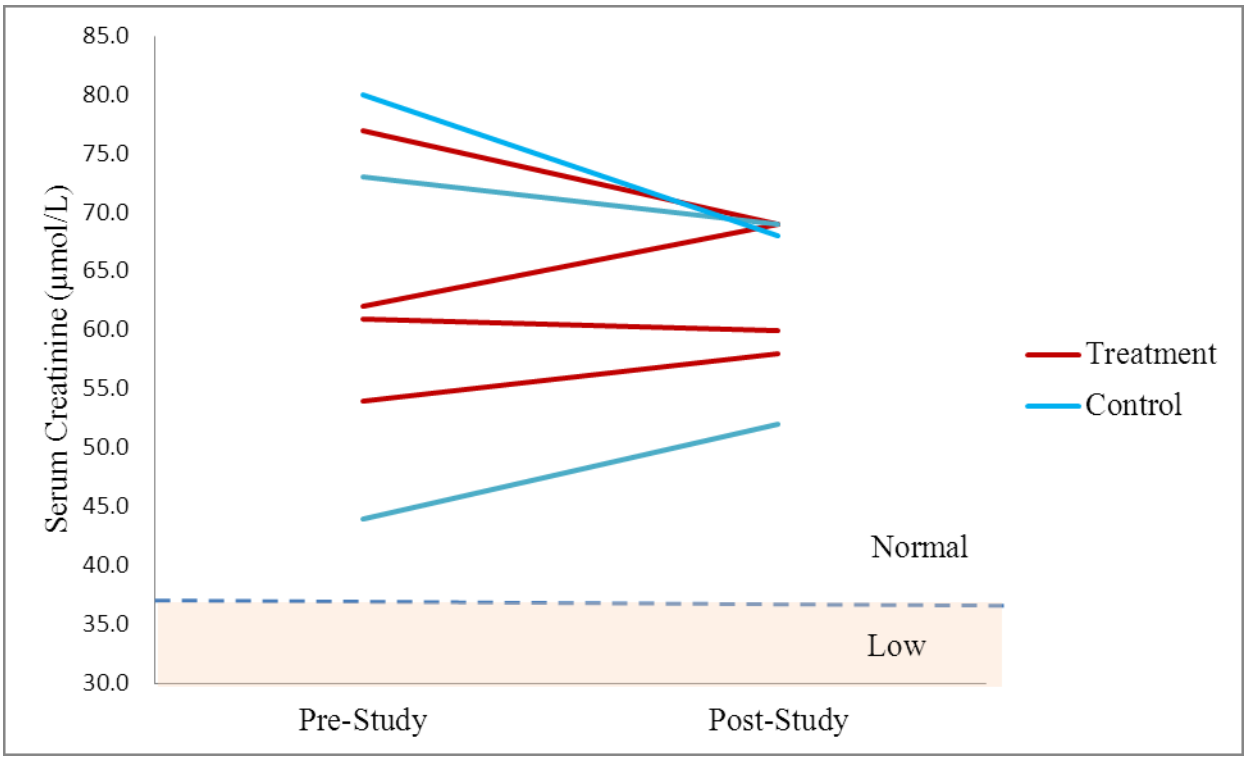

Figure 12. Serum creatinine level pre- to post-intervention

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are blood tests that assess liver function and may be elevated in people with poor glucose control. Reference values used by Capital Health Blood Services state that normal levels for ALT are 17 to 63 U/L while normal AST values are 15 to 41 U/L. A review by Ceriotti et al. (172) determined that the reference ranges for ALT and AST were common among most laboratories, and therefore the use of Capital Health's range is applicable for analysis of ALT and AST values in this study.

Based on Capital Health reference values, only 1 control participant had a change in reference range. His ALT concentration went from 19 U/L to 72 U/L, which changed from optimal to high. This shift was also noted in his AST values, which went from 36 U/L to 48 U/L, a change in category from normal to elevated. One treatment participant

exhibited low ALT values, but did not change ranges from pre- to post-intervention. One treatment participant showed a negative percent change pre- to post-intervention, but still remained within the normal reference range (Tables 8 and 9; Figures 13 and 14).

Table 8. Participant ALT values, reference range and percent change from pre- to post-intervention

	ALT (U/L)	**Reference Range**	**ALT (U/L)**	**Reference Range**	**Percent Change (%)**
Treatment					
T1	17	Normal	17	Normal	0.0
T2	18	Normal	18	Normal	0.0
T3	11	Low	13	Low	18.2
T4	23	Normal	20	Normal	-13.0
Control					
C1	24	Normal	27	Normal	12.5
C2	19	Normal	23	Normal	21.1
C3	39	Normal	72	High	84.6

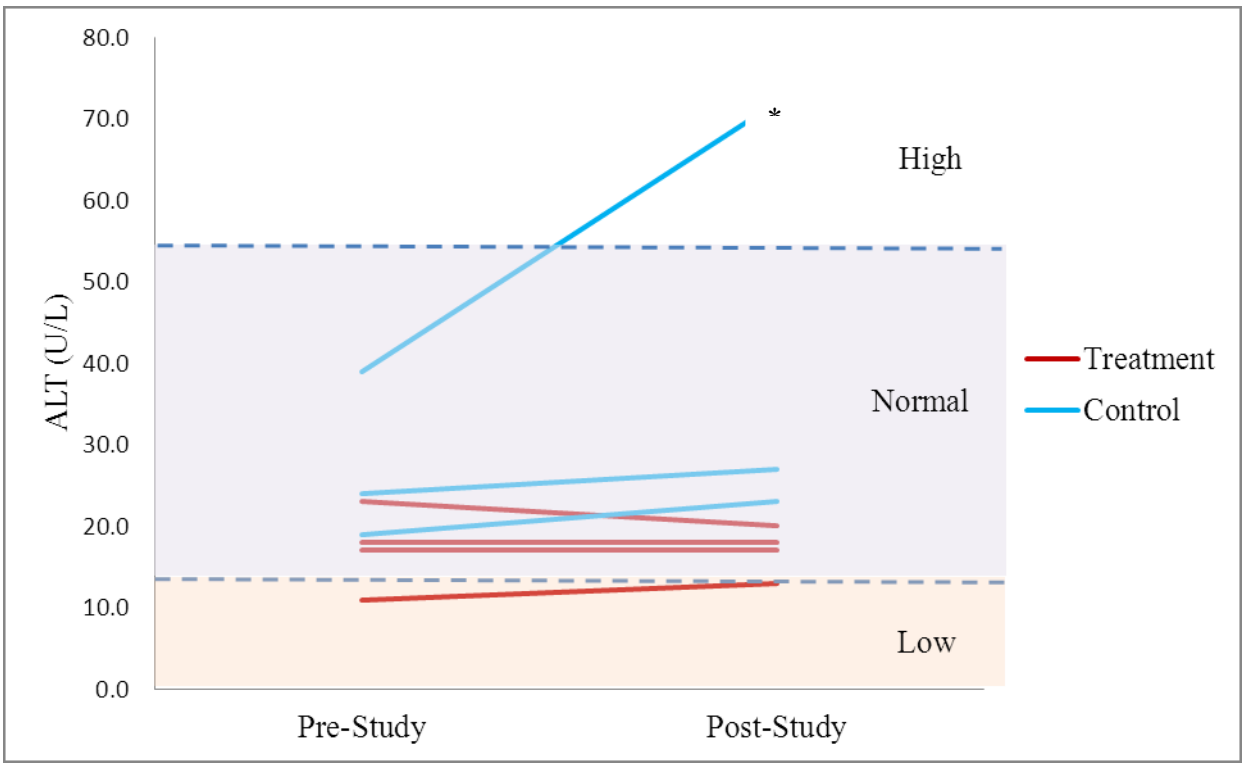

Figure 13. Serum ALT pre- and post-intervention.

* indicates change in status from pre- to post-intervention.

Table 9. Participant AST values, reference range and percent change from pre- to post-intervention.

	AST (U/L)	Reference Range	AST (U/L)	Reference Range	Percent Change (%)
Treatment					
T1	19	Normal	19	Normal	0.0
T2	20	Normal	20	Normal	0.0
T3	22	Normal	27	Normal	22.7
T4	30	Normal	23	Normal	-23.3
Control					
C1	31	Normal	28	Normal	-9.7
C2	19	Normal	20	Normal	5.3
C3	36	Normal	48	High	33.3

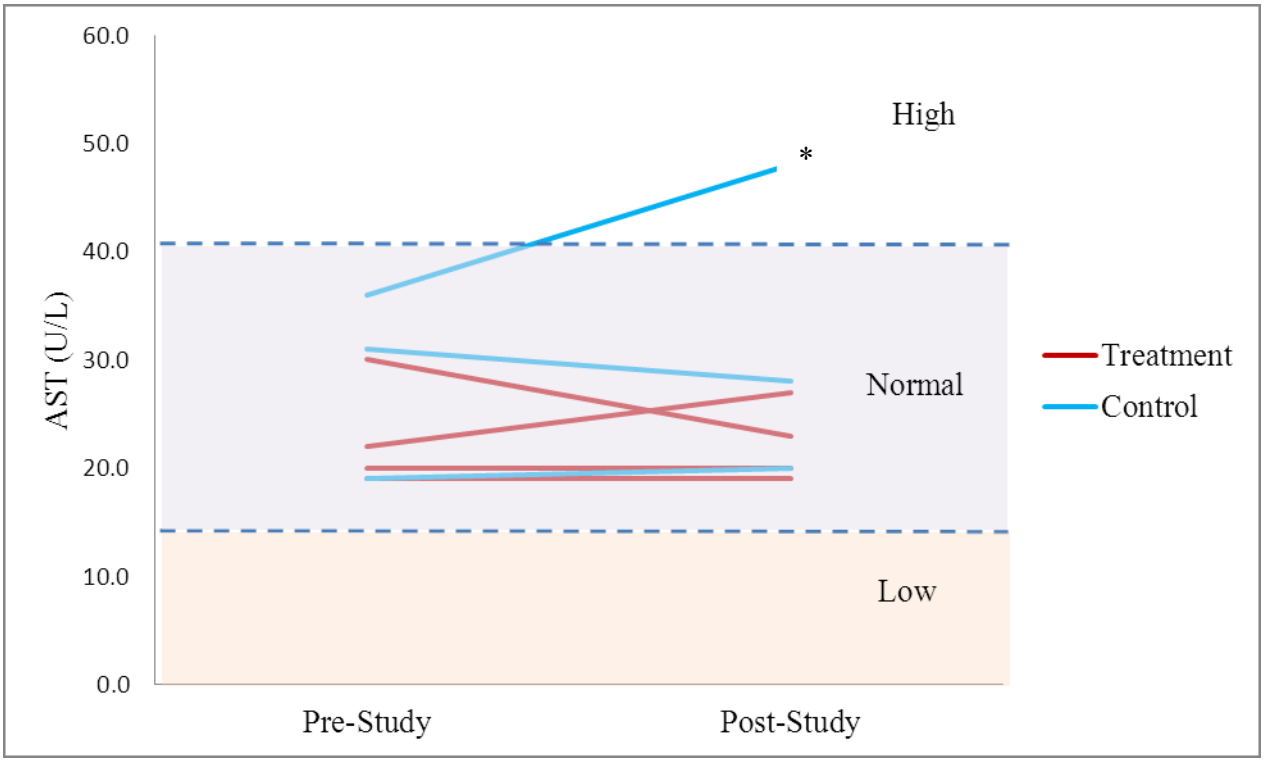

Figure 14. Serum AST pre- and post-intervention.

* indicates a change in status from pre- to post-intervention.

Analysis of pre- to post-treatment ALT and AST showed some change in serum levels, only 2 of which resulted in a change in risk level. ALT values remained unchanged in 2 of the treatment participants, while 4 participants (1 treatment, 3 controls) showed positive percent change, one of whom displayed a change in risk value from normal to high. The final treatment participant had a negative percent change. AST values showed similar changes. Two treatment participants had no change, while 3 participants (1 treatment, 2 controls) had a positive percent change, with 1 participant changing risk value from normal to high. The remaining 2 participants (1 treatment, 1 control) had a negative percent change.

6.11 Nutritional Intake

Dietary information acquired from 24-hour dietary recalls completed during inclusion to the study was entered into EatRight computer software, which was used to determine the nutrient content of the participants' diets. Multi-vitamin, vitamin D and calcium supplements were included in the dietary recall. Selected micronutrients that might affect blood glucose management were included. Terry et al. (173) reported that the average adult Canadian woman ingested 190 g of carbohydrate per day. Study participants carbohydrate consumption ranged from 200 to 450 g per day (Figure 15). All but 1 treatment participant had ingested more carbohydrates than the average Canadian female. When averaged, the study participants ingested 295 g of carbohydrates, 55 % more carbohydrates than the average adult Canadian woman.

Garriguet (174) reported that total fat consumption in adult Canadian women is 62.4 g per day. In the study population, total fat ingestion was above the Canadian average only in 2 treatment participants who ingested 79 and 136 g of fat per day. The remaining treatment participants ingested below the average adult Canadian woman (Figure 16). When averaged, the study participants ingested 53.4 g of fat, 14 % less than the average adult Canadian woman.

Health Canada recommended that people ingest greater than 400 IU to 600 IU of vitamin D per day (134), with ingestion of 1,000 IU having the greatest health benefits (175). None of the participants in this study ingested enough vitamin D via natural and fortified foods or supplements to reach the recommended value of 400 IU to 600 IU (Figure 17). The average Canadian woman 40 to 50 years of age has a serum vitamin D level of 69.4 nmol/L (103). In comparison, the average vitamin D level for participants in

this study was 86.2 nmol/L, 24 % more vitamin D than the average Canadian, though still significantly lower than what is recommended.

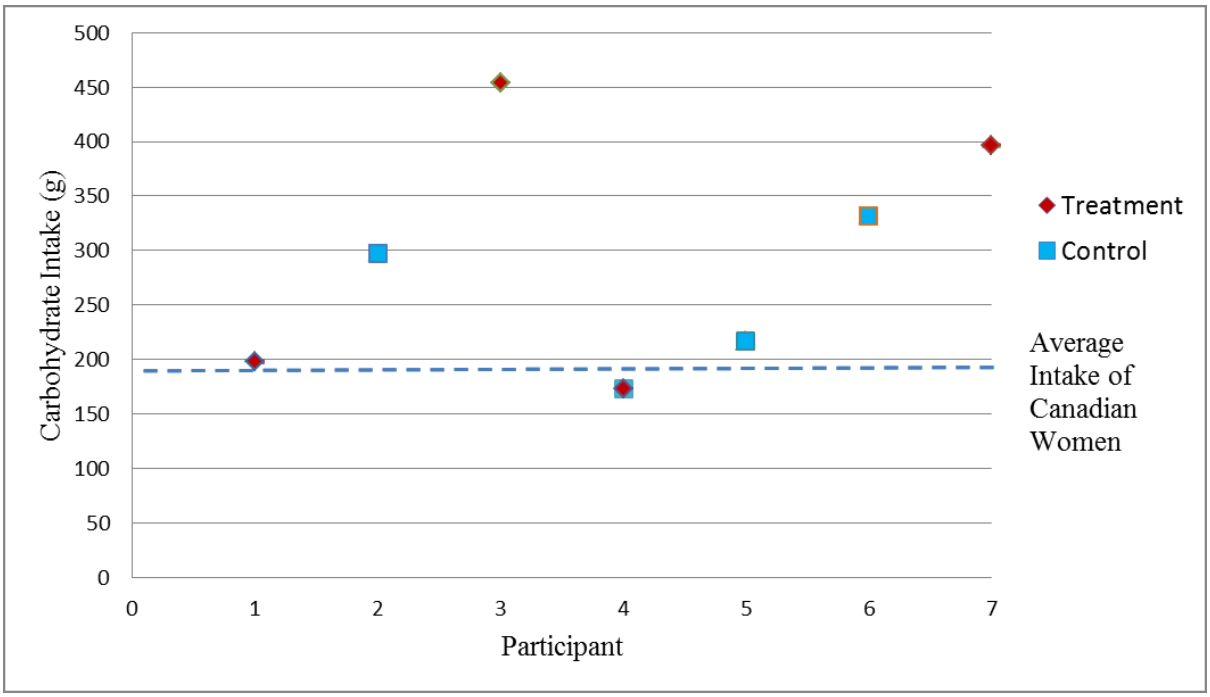

Figure 15. Consumption of carbohydrates by participant compared to Canadian Females

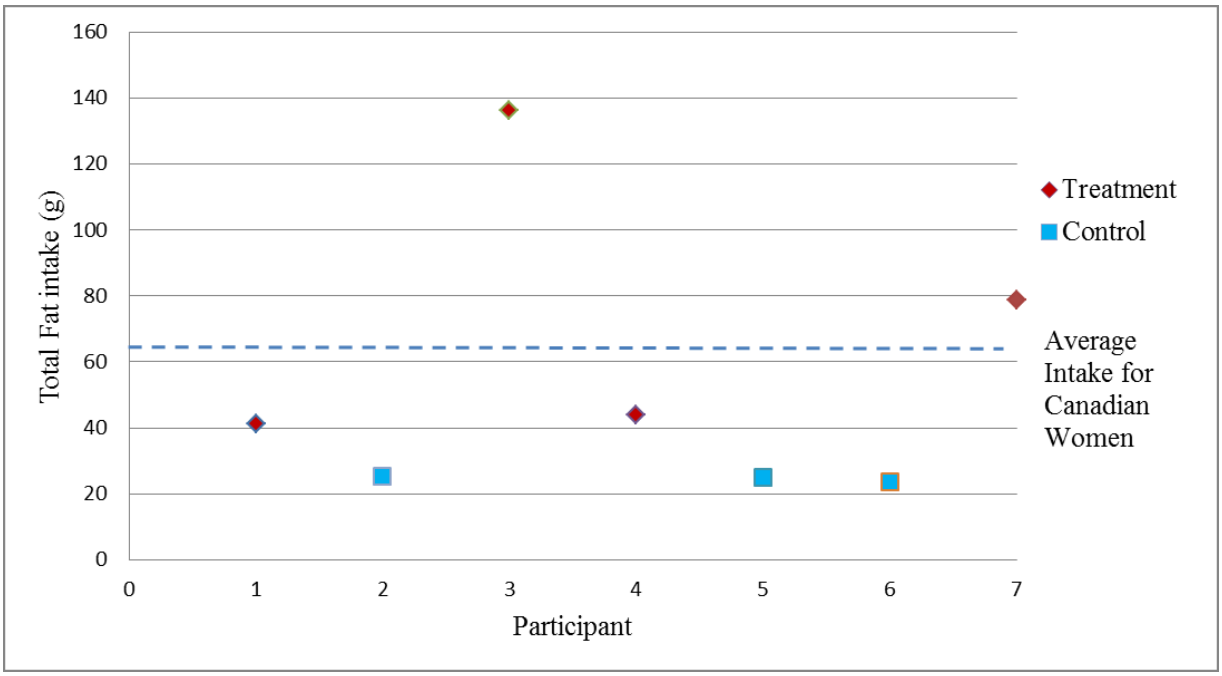

Figure 16. Consumption of fats by participant compared to the Canadian Females

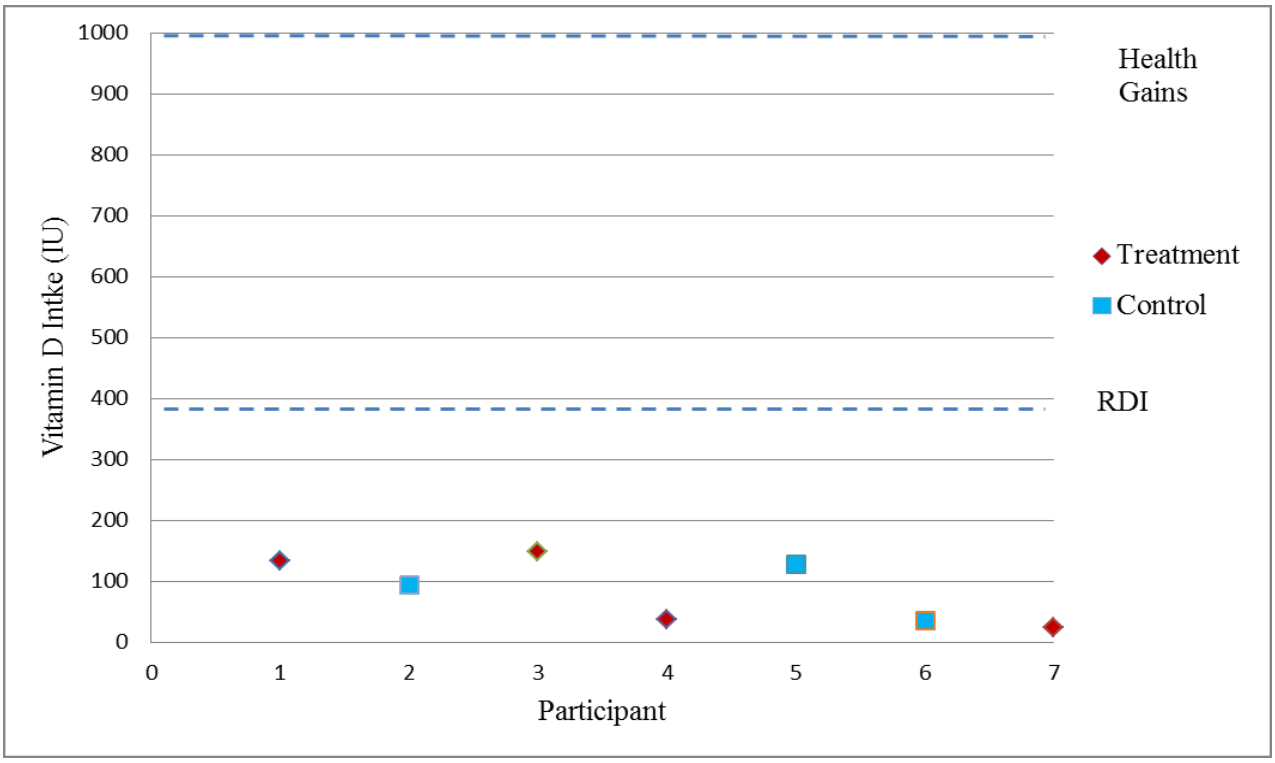

Figure 17. Consumption of vitamin D by participant compared to the Recommended Daily Intake (RDI)

Chapter 7: Discussion

This study was conceived with the main goal of assessing the effect of vitamin D phototherapy on blood glucose metabolism in adults with pre-diabetes. Changes in blood glucose levels, both in the fasted state and after a glucose challenge, glucose status over a 3-month period, and other indicators of disease progression were tested. A secondary goal of the study was to determine the effect of phototherapy on vitamin D status. Four participants completed the phototherapy treatment while 3 others acted as controls. Within these two groups, participants displayed 3 different forms of pre-diabetes, namely iIFG, iIGT, or combined IFG & IGT. From this limited data, several inferences could be made with respect to single participant changes in serum vitamin D levels, OGTT and HbA1c values after being treated with vitamin D phototherapy. The results suggested that the vitamin D phototherapy lamp could be useful in increasing serum vitamin D levels, and may have a beneficial effect on blood glucose levels.

7.1 Serum Vitamin D

Serum 25(OH)D levels between 75 and 225 nmol/L are indicative of vitamin D sufficiency (96, 99, 167, 169, 178). Levels between 50 and 75 nmol/L are defined as insufficient, while levels less than 49 nmol/L are considered to be deficient (99, 167, 169, 178). Vitamin D levels in individuals who reside in areas above 42 degrees latitude typically fall within the insufficient to deficient range for most of the year (105, 109-111), due in part to changing zenith angles of the Earth (108,110). The vitamin D status for participants in this study prior to treatment were similar to the average vitamin D levels found in populations that live above 42 degrees latitude (105, 109-111).

Travel to tropical locations is common for some Nova Scotians during the winter months and such exposure to UVB radiation would confound the ability to interpret serum vitamin D levels attained from phototherapy use. However, none of the participants in this study travelled to a southern location within the 6 months prior to, or during, the study.

Ideally, the treatment period for this study would have occurred during the winter months to avoid the confounding factor of accumulation of vitamin D from UVB radiation. However, 3 out of the 4 treatment participants began their vitamin D phototherapy during the spring, which meant that their treatment ended in the early summer months. Although the time period in which phototherapy treatment had occurred may have contributed to some unexpected vitamin D levels in some participants, most said they were rarely in the sun during peak sun hours, and the others were in the sun only occasionally. Therefore, it was expected that the relative lack of exposure to the sun during peak hours would not augment any additional vitamin D conversion outside of the phototherapy lamp. However, according to Environment Canada, the months of June through August ranged from 15 degrees Celsius in June to 19 Celsius in August (176). Furthermore, Halifax experienced a heat wave during July with temperatures feeling like 35 degrees Celsius due to the humidity (177). Although most participants indicated on their initial questionnaire that they rarely spent any time outdoors it is possible that, due to the weather, they may have spent more time outdoors during that time than they had indicated. This could have caused an unintended increase in vitamin D levels from a source other than the vitamin D lamp. This unintended increase may have been the cause

of the positive percent change found in the single control participant whose vitamin D levels increased after the 3 month study period.

Sunscreen is meant to reflect harmful UVA and UVB rays from being absorbed by the skin. Using a sunscreen with a sun protection factor (SPF) of greater than 8 can reduce vitamin D production by up to 95 % (92). Considering that vitamin D is converted cutaneously due to UVB radiation, sunscreen protection would cause a reduction in the total accrual of vitamin D from the sun even during prolonged exposure (178). In a study investigating the use of sunscreen and vitamin D levels in renal transplant patients, subjects who used sun protection had lower serum vitamin D levels versus their counterparts who did not use sun protection (178). In our study all participants, except for 1 control participant, indicated that they wore sunscreen on a regular basis when going outdoors. It could, therefore, be hypothesized that minimal vitamin D would have been absorbed from the sun when these participants went outside. Only in the case of the one control participant, who indicated that she used sunscreen "sometimes", might there have been unintended accrual of vitamin D from the sun.

Vitamin D can be attained from ingestion of natural and fortified foods as well as supplements. The 24-hour dietary recall and additional questions that detailed their consumption of vitamin D fortified foods were used to assess their intake of vitamin D. Current Health Canada recommendations indicate that the daily intake needed for adults is 400 to 600 IU of vitamin D per day (134). However, current literature has stressed the need to increase that value due to new information indicating that 400 to 600 IU is not enough to cause a positive change in vitamin D levels. Grant et al. (175) reported that health benefits occur with an average of 1,000 IU of vitamin D per day; while others

reported that as much as 5,000 IU is safe to ingest (135). However, a study that investigated vitamin D intake in Canadian children and adults concluded that Canadian children and adults consume less than the current recommendations despite the fortification of foods with vitamin D (179). Similarly, all study participants ingested less than the recommended RDI for their age group, even though 6 of the 7 participants noted that they ingested some form of multivitamin daily. Therefore, it is unlikely if that the participants would have achieved a significant change in their vitamin D status due to their food and supplement ingestion.

A vitamin D lamp could be employed to increase serum vitamin D levels. Devgun et al. (180) investigated vitamin D synthesis in the human body achieved from exposure to 4 commercially available sunlamps. All commercially available sunlamps tested in their study successfully increased serum concentrations of vitamin D, while control groups showed a decrease in serum vitamin D levels (180). These conclusions were further corroborated by Tangpricha et al. (181) who investigated how tanning is associated with optimal vitamin D status. They discovered that those subjects who used tanning beds regularly had a 90 % greater serum vitamin D levels versus controls (115 nmol/L and 60 nmol/L respectively) (181). Chandra et al. (182) investigated vitamin D lamps in adults with cystic fibrosis and short bowel syndrome, and determined that a vitamin D lamp successfully increased or maintained vitamin D levels in these groups over the winter months. They reported a significant mean rise in serum vitamin D levels from 50 nmol/L to 65 nmol/L (182). Our study data shows similar changes in vitamin D levels as a result of vitamin D phototherapy lamp use. Participants who underwent

vitamin D therapy all showed some increase in serum vitamin D as a result of phototherapy treatment while 2 of the 3 control participants had a marked decrease.

The amount of time exposed to phototherapy could determine the amount of vitamin D accrued. The studies that investigated vitamin D accrual from commercially available tanning beds involved individuals who attended whole body tanning bed sessions, which ranged from 3 to 15 minutes at a time (180, 182). The phototherapy lamp in this study was not a full body lamp, so it required the participants to expose both sides of their bodies separately, but for equal amounts of time. In total, participants spent 3 to 10 minutes at a time in front of the lamp, with both sides receiving equal time of exposure. These times are consistent with those of the tanning bed studies.

The increases in serum vitamin D levels due to the phototherapy lamp were similar to the increases in serum vitamin D reported from using vitamin D supplements (183-185). In supplement studies, an oral vitamin D supplement that was ingested daily by individuals with vitamin D deficiency or insufficiency improved their vitamin D status to within the sufficient range. Moreover, vitamin D supplements were beneficial in maintaining adequate serum vitamin D levels during times when sun exposure was less than adequate (183-185). Therefore, our study shows that a vitamin D lamp can increase vitamin D levels similarly to those reported from ingestion of vitamin D supplements.

7.2 Blood Glucose Levels

One of the goals of this study was to determine if there would be a benefit to circulating blood glucose levels with an intervention of vitamin D phototherapy. When creating this study, it was of interest to determine if vitamin D would affect two major

diabetes blood tests: the OGTT and the HbA1c test. While the OGTT examines glucose clearance after consuming a bolus of glucose solution, the HbA1c test is designed to assess blood glucose levels in the bloodstream over a 3 month period.

Several studies have reported that a deficiency in vitamin D can result in glucose tolerance impairment (151, 156, 186) and decreased insulin sensitivity (151, 156, 157, 186). However, in studies where vitamin D therapy led to vitamin D repletion, improvement in glucose tolerance and insulin sensitivity were noted (151, 156, 157, 186). In a population of London-based Bangladeshi people, reduced serum vitamin D levels were associated with greater incidences of DM2 (158). After acute vitamin D replenishment, increased insulin secretion was noted in the short-term, and improved overall glucose tolerance was noted after long-term replenishment (158). Chiu et al. (187) found a positive correlation between serum vitamin D concentration and insulin sensitivity as well as alteration in beta-cell function. In a large prospective study, Pittas et al. (188) demonstrated that men and women who ingested 1,000 mg/day of calcium and 800 IU/day vitamin D had a 33 % lower risk of DM2. Similar to those studies, this study showed improvement in glucose tolerance in 2 of the 4 participants in the phototherapy group, concomitant with increases in vitamin D levels. Both participants had reductions in both fasting and 2-hour post glucose load as displayed in Table 5. Those with no change, or reduction in vitamin D levels showed greater glucose tolerance impairment.

The HbA1c test was employed in order to determine if vitamin D therapy would cause a change in circulating blood glucose levels. A study investigating vitamin D status and glucose homeostasis found that there was a small decrease in HbA1c values as serum vitamin D levels was increased (189). Similarly, Mitri et al. (190) reported that a short-

term supplementation of vitamin D had a marginal effect on attenuating the rise in HbA1c levels in adults with high risk for diabetes. In the current study, a similar but slight improvement in overall glucose levels was noted in the HbA1c levels of 2 of the phototherapy participants after replenishment of vitamin D, while 1 participant maintained the same level. This shows that changes in circulating blood glucose levels, as exhibited by changes in HbA1c levels, may be improved with increased serum vitamin D levels.

Due to the relatively small percent change found in both the OGTT and HbA1c tests pre- to post-treatment, it is possible that natural variability within blood glucose levels were responsible for changes found in the study and not from vitamin D phototherapy. In a study investigating the short-term variability of glycemic measures with respect to diabetes classification, Selvin et al. (191) determined that there was differing within-person variability in both OGTT and HbA1c blood tests. For the OGTT test, within-person coefficient of variation was highest, with a variation of 16.6 % between tests (191). Variation within HbA1c tests was smaller, with a variation of 3.2 % between tests (191). It is therefore possible that normal fluctuations in blood glucose levels were responsible for the changes in OGTT and HbA1c results found in the study results and not due to phototherapy. Multiple testing would be required to determine if the participant's natural fluctuations in blood glucose levels were responsible for percent changes found.

A major risk factor for DM2 is obesity, which is characterized by overconsumption of carbohydrates and fats combined with lack of physical activity. Left

untreated, excessive consumption of high caloric foods can worsen blood glucose levels and counteract any positive effects from vitamin D.

Carbohydrates are catabolized in order to utilize their constituent glucose molecules for energy. When too much carbohydrate is ingested, the unused glucose can be stored in muscle tissue and in the liver as well as some being converted to fat. Consumption of high glycemic index foods such as white bread and rice have been connected to elevated HbA1c levels (192) and may also be responsible for chronically elevated blood glucose levels. Garg et al. (77) found that a diet high in glucose and other simple carbohydrates caused a reduction in glycemic control as well as worsening of hyperinsulinaemia. The study participants, when averaged, ingested 295 g of carbohydrates, 55 % more carbohydrates than the average adult Canadian woman. This overconsumption of carbohydrates is typical in people who exhibit signs and symptoms of pre-diabetes and DM2.

Several studies have investigated the effect of high fat diets on obesity. Marshall et al. (75) investigated how dietary fats intake could predict conversion of pre-diabetes into DM2 in subjects with IGT. They reported that subjects who ingested 43.4 % of their caloric intake in dietary fats subsequently became DM2 versus those who ingested 40.6 % who maintained their IGT status and 38.9 % who reverted to normal glucose tolerance (75). Meyer et al. (193) corroborated Marshall's results in a study investigating the association between dietary fat intake and incidence of DM2 in a large population of older adults who initially did not have diabetes. They noted that ingestion of animal fat and cholesterol were positively associated with DM2 (193). Fat intake was expected to be elevated in the study participants due to poor eating habits that are usually associated

with individuals who have pre-diabetes or DM2 (5). However, total fat intake for all but 2 participants in the study was found to be below that of the average Canadian female (174) (Figure 16). When averaged, the study participants ingested 53.4 g of fat, 14 % less fat than the average adult Canadian woman. Therefore, it could be surmised that fat intake contributed less to weight gain and pre-diabetic status than did ingestion of carbohydrates.

Dietary intake is accepted as a primary determinant to a participant's pre-diabetic status and is one of the main areas targeted to combat the condition (2). It is unknown if increased vitamin D status and reductions in carbohydrate and fat intake could exert additional benefits to blood glucose levels. Further investigation of diet in conjunction with vitamin D therapy should be assessed.

7.3 Liver and Kidney Function

In diabetes, high blood glucose levels can cause secondary complications to other vital organs. Creatinine, ALT, and AST are blood tests that are completed in order to examine functionality of the kidneys and the liver. For this study, creatinine, ALT, and AST were tested to see how pre-diabetes might have affected the participant's liver and kidney function (Table 7, 8, 9; Figure 12, 13, 14).

Creatinine is a product of creatine phosphate breakdown, and elevation of creatinine in the bloodstream could be indicative of kidney dysfunction. The relationship between creatinine levels and vitamin D were investigated in a study by Wolf et al. (194), who examined vitamin D levels and mortality rates in hemodialysis patients. Vitamin D values were measured in 825 patients, and found that 78 % were considered deficient

while 18 % were severely deficient. Wolf et al.'s study found that serum creatinine levels were weakly correlated to vitamin D level (194). Semba et al (195) also reported a marginal association between vitamin D deficiency and elevated creatinine levels in a study investigating vitamin D deficiency in older women with and without a disability. Others investigated if vitamin D might affect how the kidneys filter waste products, or the glomerular filtration rate (GFR) of the kidneys. It was found that vitamin D supplementation did not change the progression of GFR or affect renal function (196). Our study showed similar results, with vitamin D levels causing only small fluctuations in creatinine levels in all participants regardless of treatment group.

The liver plays an essential role in the synthesis of vitamin D, and liver markers in turn may be affected by circulating vitamin D levels. Miroliaee et al. (197) looked for an association between vitamin D levels with chronic liver disease. They noted that inadequate serum vitamin D levels paralleled the severity of liver dysfunction. Vitamin D insufficiency was higher in participants with cirrhotic livers, and the degree of vitamin D insufficiency has a correlation to the severity and progression of liver disease (197). Other studies have equated lower serum vitamin D levels with predictors of liver diseases such as coagulopathy and hypoalbuminemia (198, 199). Unlike these studies, the participants in this study were otherwise healthy and did not exhibit liver dysfunction. In both ALT and AST tests, 1 control participant changed risk range from optimal to high, while the remaining participants had little change in their ALT and AST levels as a result of the study.

For the purposes of this study, assessment of renal and liver function was completed for evaluating any possible complications these participants may have had due

to their diagnosis of pre-diabetes. It was not expected that vitamin D was going to have an effect on these systems. Furthermore, secondary complications such as kidney and liver dysfunction do not manifest themselves until later on in the diseases progression due to prolonged period of poor diabetes management. With this in mind, it is possible that elevated creatinine, ALT and AST levels due to renal or liver dysfunction would have not manifested itself in these participants due to their relatively recent diagnosis of pre-diabetes.

7.4 Osteocalcin and Insulin

Emerging research has focused on a proposed link between osteocalcin and the production of insulin. Osteocalcin is a bone derived protein that is secreted by osteoblasts, which are cells responsible for bone formation (200). When osteocalcin is released it causes an increase in osteoblast activity, thereby increasing bone production (201). However, osteocalcin also has also been noted to act as a hormone and may promote insulin production and concentration within the blood (200).

Lee et al. (201) introduced the theory of the osteocalcin/insulin relationship in 2007. This relationship between osteocalcin and insulin is based on feedback loops. Circulating insulin attaches to insulin receptors located on the osteoblasts of the bone. This connection causes an increase in osteoblastic bone formation (201). The increase in osteoblastic activity stimulates its antagonist, osteoclast, to begin breaking down old bone, causing a higher amount of bone turnover (201). With the increase in osteoclastic activity, Tcirg1, an acidifier of the extracellular matrix begins to change the plasma pH to be more acidic. The acidity transforms inactive osteocalcin, Gla-OCN, into Glu-OCN, the

active form of osteocalcin by an uncarboxylation (201). The active form of osteocalcin travels as a hormone through the blood stream to the pancreas where it interacts with the beta-cells (201). This causes a stimulation of beta-cell production of insulin. A visual representation can be seen in Figure 19.

Gla-OCN- Inactive osteocalcin
Glu-OCN- Active osteocalcin
Opg- Inhibitor of bone resorption
Tcirg1- Acidifier of extracellular matrix

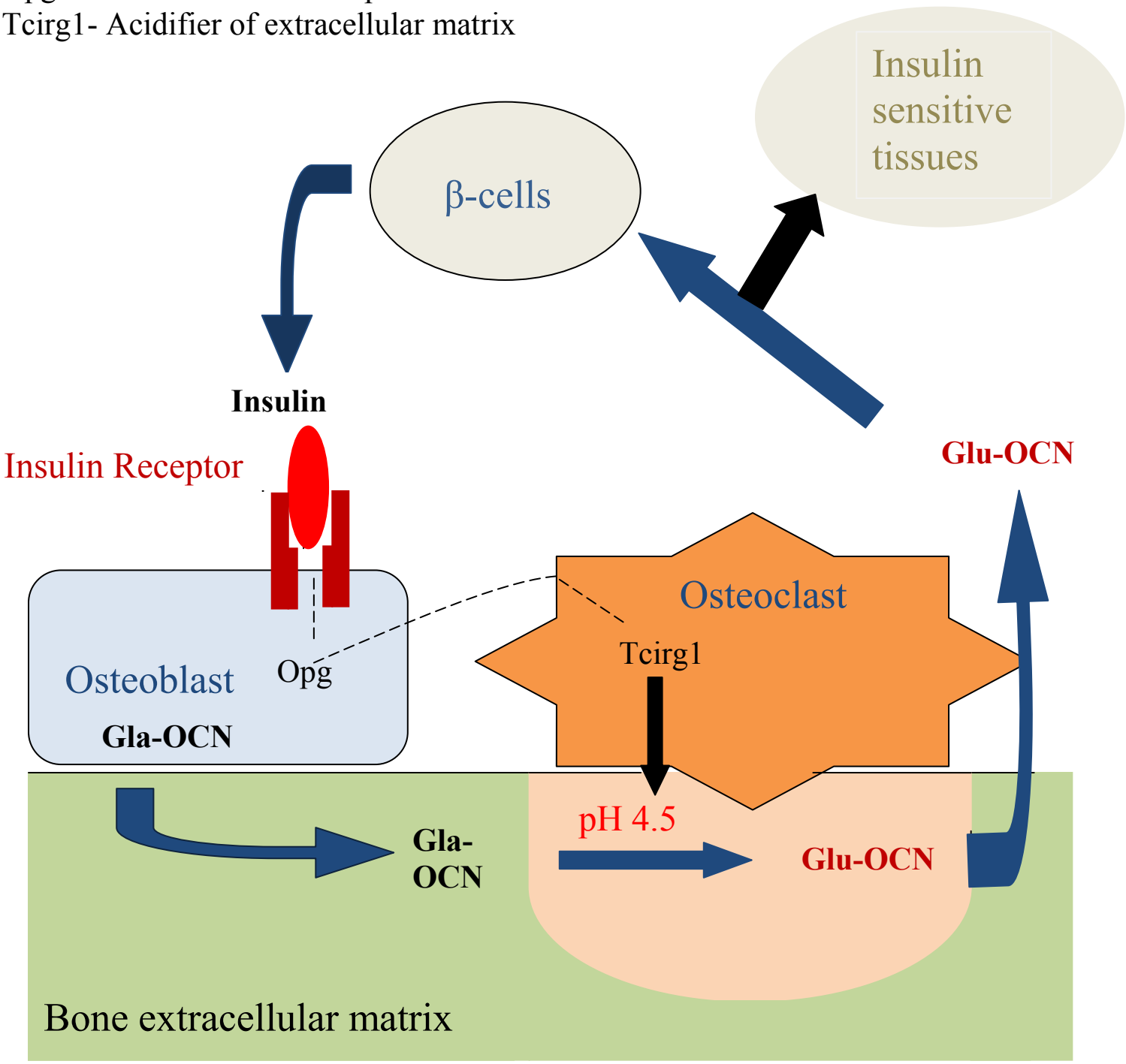

Figure 18. Visual representation of the osteocalcin-insulin connection, adapted from cell.com (202).

With this new relationship, researchers have begun investigating the possible connections between osteocalcin and insulin production and DM2. Kanazawa et al. (203) reported an association between plasma glucose levels and active osteocalcin in patients with DM2. Participants with DM2 were tested for serum osteocalcin and fasting plasma glucose and HbA1c (203). Serum osteocalcin was negatively correlated with fasting plasma glucose levels and HbA1c results. Zhou et al. (200) reported similar results when they investigated the effect of serum osteocalcin levels with glucose metabolism. Zhou showed that serum osteocalcin levels in patients with diabetes were significantly lower than those with no diabetes (200).

Osteocalcin was further positively associated with beta-cell function (200). Im et al. (204) similarly observed a reduced serum osteocalcin in those with DM2. However, Im noticed that those with the highest quartile of osteocalcin had significantly decreased fasting glucose and HbA1c values, with osteocalcin inversely proportional to glucose, insulin, HbA1c and insulin resistance in those of the lowest quartile (204). In both the Zhou and Kanazawa studies, there was also a connection between osteocalcin and lipid metabolism. In both instances, it was found that percent fat as well as HDLs were inversely associated with serum osteocalcin levels (200, 203).

Vitamin D is essential in bone health due to its effects on calcium and phosphorous absorption in the small intestine (100). During vitamin D deficiency, there is enhanced resorption of calcium and phosphorus in bone. This deficiency increases bone loss. Considering that osteocalcin is a marker of bone formation, there is a potential connection between vitamin D and the osteoclast/insulin matrix. Liant et al (205) investigated changes in osteocalcin levels in a vitamin D deficient chicken bone. They

concluded that in vitamin D deficient chicken bones there was a decrease in osteocalcin levels by 50 % (205).

It would seem that there is an intimate connection between vitamin D levels and circulating insulin levels and that osteocalcin is involved. Current literature is sparse with respect to how vitamin D may be a factor in osteocalcin concentration and insulin production. It would be of interest to investigate the relationship between serum vitamin D, osteocalcin and fluctuations in insulin concentrations. Based on current literature, elevated vitamin D levels may prompt elevated osteocalcin levels due to the interaction of vitamin D with osteoblasts. The increase in osteocalcin correlates with a rise in insulin production and such a change in serum insulin levels may be sufficient to improve glucose control in people with pre-diabetes and DM2. Further research in this area is required.

Sclerostin, a product of osteocyte activity, limits bone formation. Sclerostin acts through the inhibition of osteoblast activity and is prominently found in unloaded bone (206). Recently, elevated sclerostin levels have been noted in patients with DM2. In a study by Garcia-Martin et al. (206), serum sclerostin was found to be significantly higher in patients with DM2 compared to a group of non-diabetics. The elevated sclerostin levels were correlated with the duration of DM2 as well as their elevated HbA1c results and overall bone status (206). Sufficiency in vitamin D has been shown to have a positive effect on bone status and higher bone turnover. With increased serum vitamin D levels it may be possible to reduce the concentration of sclerostin within bone. Furthermore, increased bone loading would aid in reducing sclerostin, which is hypothesized to help in reducing HbA1c levels and increasing bone turnover (206).

7.5 Study Limitations and Future Recommendations

Several limitations were noted in the completion of this study. Participant recruitment was difficult and the total sample size was well below the required number for statistical testing. Further participant recruitment should be completed to obtain a larger sample size. Greater attendance to diabetic management clinics is advised, as face-to-face interactions proved more beneficial in accumulating adequate sample numbers then posters and leaflets. An alternate experimental design may prove efficacious with regards to study numbers and the subsequent results attained. Using a 2 x 2 split plot study design requires a large numbers of participants who ideally would be similarly matched (sex, age, weight) in order to conclude that the therapy used was beneficial in changing serum vitamin D and blood glucose levels. With the difficulty faced in attaining the 7 participants for this pilot study, it may be beneficial to modify the experimental design to one that is a within subject design. In this case, the treatment participant would act as his or her own control. Though this style of experimental design would require a longer study period in order to completed both the 3 month treatment period and the 3 month control period, this type would eliminate the need to match pair the participants, and would lessen the amount of participants needed to be recruited. It would also provide results that are more homogeneous. However, this design would need to be performed in consecutive years due to seasonal changes in vitamin D synthesis, which adds potential confounding variables.

Data collection occurred predominantly during the spring and summer months. Because vitamin D levels are elevated during those months due to exposure to the sun,

future study periods should be limited to months where vitamin D would be its lowest, specifically between October and April.

During the study period, those participants allocated to the therapy group may have modified their behavior with respect to their dietary, sun, vitamin D or lifestyle habits due to the fact that they knew they were a part of a clinical study, also known as the Hawthorne effect. This could have caused unintended changes in the data attained for serum vitamin D and blood glucose levels. In order to minimize the Hawthorne effect from occurring, the study may wish to create a second lamp, which would not provide any vitamin D. When a participant arrives for their phototherapy session, they would be unaware of which lamp they were under. This may minimize the tendency for those receiving the actual treatment from modifying their behavior.

At the end of the treatment or control periods, the health, exercise and dietary questionnaire, along with the vitamin D and sun exposure questionnaire were not again completed. However, it would be of interest to have the participants redo these questionnaires post-intervention to determine if there was any change in habits from the beginning to the end of the study, especially with changes in vitamin D intake or time spent outdoors. This would help to determine if the phototherapy was the sole and primary method of accruing vitamin D during the study period. Furthermore, anthropometric measures done pre-treatment should also be completed at the end of the study period. Changes in those measures, specifically those of weight and waist circumference could affect the results of both serum vitamin D and blood glucose levels. Considering a reduction in overall weight and waist circumference could alter blood glucose and serum vitamin D levels, it would be important to reassess those measures

post-treatment and determine if any change in those factors were a result of weight loss/gain or due to the therapy provided.

The study was only completed over a 3 month span. It is difficult to assume that only 3 months of phototherapy could cause a change on blood glucose levels in this type of population. Further research should try a longer treatment period, with treatments being completed over several months or over a calendar year.

Overall, the apparent success of this pilot study is evidence of an effectiveness of the vitamin D phototherapy lamp in changing serum vitamin D and blood glucose levels. Furthermore, the use of the phototherapy lamp to accrue vitamin D was proven to result in no adverse effects to any participant who used it. It could be concluded that use of a phototherapy lamp is both effective and safe for vitamin D accrual in this population and should remain as the primary form of vitamin D accrual as the study continues on with more participants.

7.6 Conclusions

In conclusion, improvement in blood glucose levels as a result of 3 months of vitamin D therapy occurred in some of the treatment participants. Serum vitamin D levels increased in the treatment group after the treatment period, while controls saw a reduction in their vitamin D levels. Nutritional intake of vitamin D was found to fall below the RDI of vitamin D and therefore would not have had a major effect on vitamin D levels. However, the completion of primary treatment during the summer for most participants, in combination with a warmer than usual summer, may have caused an increase in vitamin D levels due to greater exposure to the sun. Commercially available vitamin D

therapy lamps could be utilized to maintain or increase serum vitamin D levels during periods where sun exposure is difficult. The increase of circulating vitamin D year round could be beneficial to health and could be protective to many diseases including diabetes. With a lack of dietary ingestion of vitamin D, the use of vitamin D supplementation via oral ingestion or vitamin D lamp should be emphasized.

Blood glucose values, as determined by both the OGTT and HbA1c tests showed a positive change likely due to increase in serum vitamin D levels. It is widely accepted that pre-diabetes can usually be managed with diet and exercise alone. With the inclusion of vitamin D as part of a preventative therapy, it is possible that pre-diabetes could be further diminished or the progression to DM2 be delayed.

This study provides a novel method of combating pre-diabetes that has previously been scarcely investigated. It showed the ability of phototherapy to elevate vitamin D levels and that it may have a beneficial effect on blood glucose levels in people who have elevated blood glucose levels with no adverse effects. Further research into the effects of vitamin D in pre-diabetes may show that phototherapy could be important in reducing the rate of disease progression from pre-diabetes to DM2, which has become an epidemic in our society. It is imperative that this study continue until a sample size is reached that is large enough to confirm the trends that have been found in this initial investigation.

Appendix A: *Recruitment Poster*

DALHOUSIE UNIVERSITY
Inspiring Minds

DO YOU HAVE PRE-DIABETES

Are you 20-70 years old?

A new study will examine how vitamin D from phototherapy may affect blood glucose levels in people with pre-diabetes.

You are invited to participate in this study!

Version 2.0 Jan 27, 2010

For more information please contact:
Noam Ami, Research Coordinator (902) 494-3572 or noam.ami@dal.ca

Vit D & Blood Glucose Study
Noam (902) 494-3572
noam.ami@dal.ca

Appendix B: *Recruitment Leaflet*

Does vitamin D lamp exposure affect blood glucose control in pre-diabetics?

Capital Health

WHO IS DOING THIS STUDY?
This is a joint study among researchers at Dalhousie University and Capital Health. The primary investigators are Dr. Jo Welch, Dalhousie University, Dr. Dale Clayton and Dr. Stephanie Kaiser of the Endocrinology & Metabolism Division of the Faculty of Medicine and Capital Health.

WHAT IS THE STUDY TESTING?
Individuals with pre-diabetes experience difficulty with blood sugar control. Small studies have suggested that Vitamin D may improve blood sugar control in people with pre-diabetes. Phototherapy can increase Vitamin D levels. The aim of this study is to determine if phototherapy can improve blood sugar control in individuals with pre-diabetes.

WHAT IS BEING COMPARED?
Measures of blood sugar control will be compared between subjects who will participate in the phototherapy group or the control group.

WHAT WILL SUBJECTS IN THIS STUDY DO?

Both Groups
All subjects will complete exercise and diet questionnaires. They will also have blood tests and an oral glucose tolerance test (OGTT) before and after a three month period.

Phototherapy Group
This group will be treated with phototherapy 3 times a week for 3 months.

Control Group
This group will be asked to continue their usual daily routine for 3 months.

If you are interested,

Please contact the Research Coordinator, Noam Ami:
(902) 494-3572 or noam.ami@dal.ca

DALHOUSIE UNIVERSITY
Inspiring Minds

Appendix C: *Preliminary Telephone Script*

Does vitamin D lamp exposure affect glycemic control in pre-diabetics?

Preliminary Screening Telephone Script

Background information:

Name: _____

Age: _____

M / F: _____

Diagnosis: _____

Date of diagnosis: _____

To be read to potential study participant over the phone by a research team member:

For this study, we will be looking at the relationship between vitamin D levels and glycemic control in people with pre-diabetes who live in Nova Scotia. Participation in this study is voluntary and you may withdraw at anytime without consequence.

I need a little information from you, which will determine if you might be eligible for this study. These questions will take about 5 minutes. Is this a good time to ask you these questions?"

You were diagnosed with pre-diabetes? _____

Have you been tested for diabetes mellitus since then? Yes No

If yes: 1(B) When were you tested? _____
1(C) What did the testing show? _____

1. Do you take Vitamin D supplements?

Yes No

If Yes: 2(B) Approximately how much? _____

2. Do you use tanning beds?

Yes No

3. Do you plan to travel to a sunny place this fall, between September and January?

Yes No

4. Some medications and supplements can cause increased sensitivity of the skin to sunshine or phototherapy. We will need to check if what you are taking might increase your photosensitivity. What medications and supplements are you currently taking, and do you expect to be still taking then in September?

Capital Health

Appendix D: *Consent Form*

Consent Form

Category B

CONSENT TO TAKE PART IN A RESEARCH STUDY
Participant Information

STUDY TITLE: **Does vitamin D lamp exposure affect glycemic control in pre-diabetics?**

PRIMARY OR QUALIFIED INVESTIGATOR:

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PART A

RESEARCH STUDIES – GENERAL INFORMATION

1. INTRODUCTION

You have been invited to take part in a research study. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains this study.

Please read this carefully. Take as much time as you like. If you like, take it home to think about it for a while. Mark anything you don't understand, or want explained better. After you have read it, please ask questions about anything that is not clear.

The researchers will:

- discuss the study with you,
- answer your questions,
- keep confidential any information which could identify you personally, and
- be available during the study to deal with problems and answer questions.

We do not know if taking part in this study will help you. You may feel better. On the other hand, it might not help you at all. It might even make you feel worse. We cannot always predict these things. We will always give you the best possible care no matter what happens.

If you decide not to take part or if you leave the study early, your usual health care will not be affected.

PART B
EXPLAINING THIS STUDY

2. WHY IS THIS STUDY BEING DONE?

This study will investigate if raising the levels of vitamin D in people with pre-diabetes improves their ability to regulate blood glucose levels. Unfortunately, pre-diabetes often progresses into Type 2 diabetes mellitus. In this study, we are testing if increasing

vitamin D levels in the body might delay the progression to Type 2 diabetes by improving glucose control. In this study we will use a vitamin D lamp to increase vitamin D levels. Therefore, it is the purpose of this study to test if **vitamin D lamp exposure** can aide in better maintenance of healthy blood glucose levels.

Vitamin D_3 is a naturally occurring vitamin or hormone that is created by your body in response to UVB rays from the sun. The most effective way to increase the amount of vitamin D_3 that is circulating in your body is by exposure of your skin to ultraviolet B (UVB) radiation in sunshine. But getting enough sunshine, or the right wavelength from sunshine, can be tough in Nova Scotia. Nova Scotia lies at a latitude where, from fall to early spring, there is a lack of adequate UVB radiation. Also, during cool and cold months, our clothing blocks radiation from interacting with the skin, as does our fog, rain, and sunscreen during summer days. Canadians have been advised to ingest vitamin D to keep their vitamin D levels normal but most Canadians don't consume enough foods that contain vitamin D.

Tanning beds have been shown to improve vitamin D production but they provide too much radiation, which is not healthy for skin. A manufacturer has created a vitamin D lamp that uses special fluorescent light bulbs to radiate a specific wavelength of ultraviolet light (UVB). That wavelength stimulates vitamin D production. The vitamin D lamps use only a very narrow band of radiation and for only a few seconds per session. Therefore, this treatment is unlikely to damage your skin. Narrowband phototherapy is already approved for some medical conditions such as psoriasis. We would like to find out if this type of exposure will cause a positive or negative effect on your blood glucose levels.

The vitamin D lamp that will be used for this study has been approved by Health Canada, which means that its radiation level is within the safe limits of exposure. However, how effective this lamp is in raising vitamin D levels is not yet known. We also do not know if raising vitamin D levels improves blood glucose control in pre-diabetics. If you decide to be a part of this study, please keep in mind that there is very little information regarding the effectiveness of this type of exposure on vitamin D levels. Also, we are not yet sure if vitamin D lamps are effective for blood glucose control or in forestalling Type 2 diabetes.

3. WHY AM I BEING ASKED TO JOIN THE STUDY?

You have been invited to enroll in this research study because you were identified by the Research Group of the Division of Endocrinology at Queen Elizabeth II Hospital as a pre-diabetic who is 20 to 70 years old and you previously indicated on a "Diabetes Research Permission Form" that you would like to be contacted by the research team if a new study became available.

4. HOW LONG WILL I BE IN THE STUDY?

This study will take about four months. After one session of initial blood tests, you will be placed in either the phototherapy group or in the non-phototherapy group. If you are placed in the phototherapy group, then you will attend three phototherapy sessions per week, for three months. Each of these sessions will take approximately 5-10 minutes of your time, and will occur in a special room in the Dalplex building at Dalhousie University. Final tests will be done immediately after your three months of phototherapy are finished. If you are placed in the non-phototherapy group, then you will have final blood tests about 13 weeks after your initial tests. Before you begin the study we will ask you to complete questionnaires that will take approximately 20 minutes each time to complete.

5. HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

This study is taking place only in Nova Scotia. Approximately 40 people will take part in this study, half of whom will be placed in the phototherapy group.

6. HOW IS THE STUDY BEING DONE?

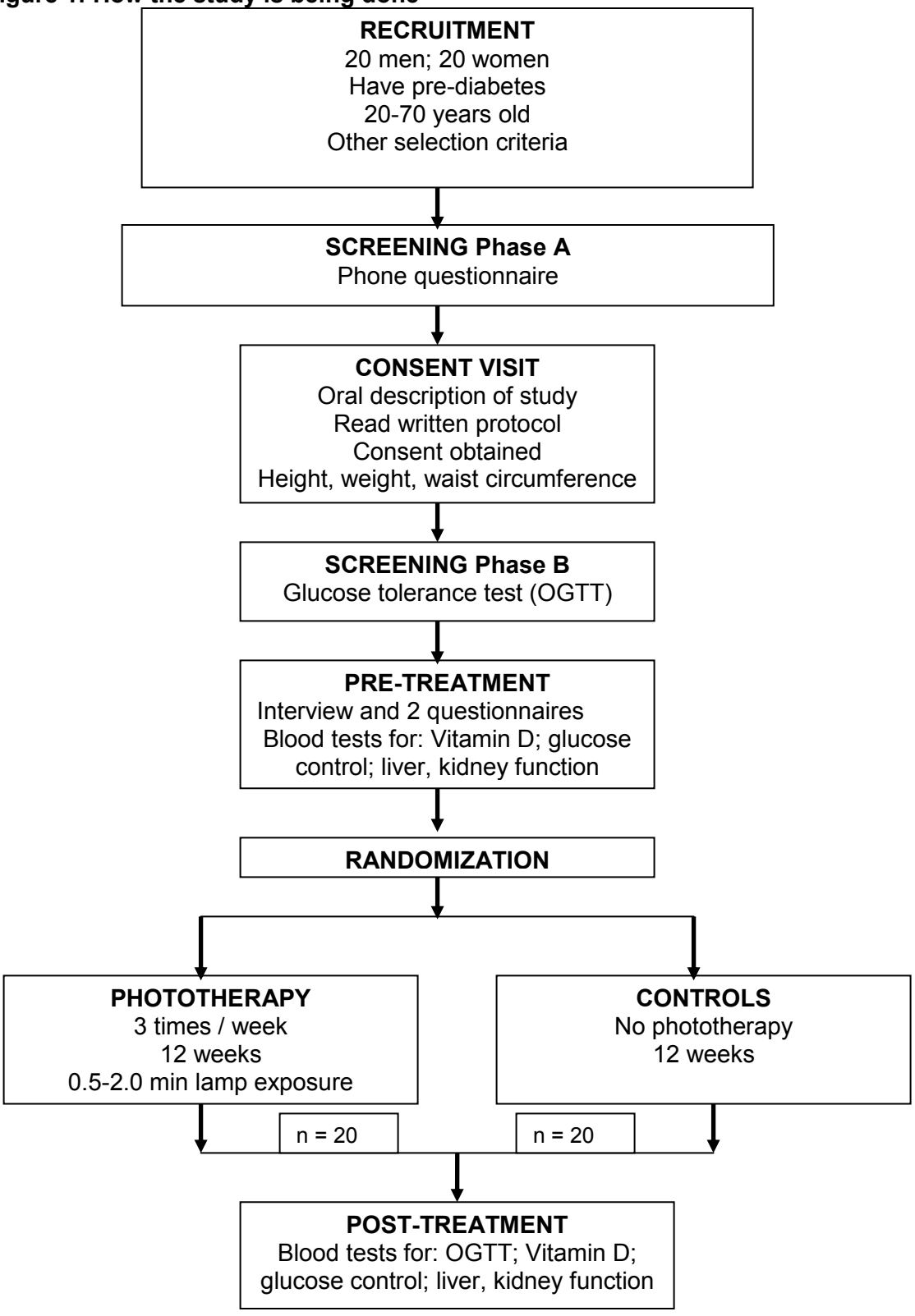
Only people with a previous diagnosis of pre-diabetes will be recruited for this study. To make sure volunteers still have pre-diabetes, they will be tested again. Those who still have pre-diabetes will then have additional blood tests so that we can fully understand how the glucose and insulin is behaving in each individual. Subjects will also complete two questionnaires that will help us understand what medical, dietary, and exercise factors might be contributing to their pre-diabetes.

Subjects will be assigned to either a phototherapy group or a control group for 12 weeks. People in the phototherapy group will be exposed to a vitamin D lamp for between one (1) and four (4) minutes, three (3) times a week, while those in the control group will not

be exposed. After 12 weeks, people in both the phototherapy and the control groups will again have the blood tests done to see if phototherapy results in different glycemic control and/or changes in vitamin D levels compared to those who were not exposed to the phototherapy. If so, we will be able to suggest that exposure to the lamp caused greater vitamin D synthesis and that this greater vitamin D level improved control of their glucose and insulin levels.

Please see the flowchart below for a brief reference guide to how this study is being done.

Figure 1: How the study is being done



7. WHAT WILL HAPPEN IF I TAKE PART IN THIS STUDY?

SCREENING

If you would like to participate in this study, a research investigator will ask you several questions over the telephone to determine if you might be eligible to participate in this study. This will take about five (5) minutes. If the results of these questions indicate that you might be eligible, then you will be invited to meet with a study researcher, at which time the study will be explained to you in person and you will be asked to sign this consent form. Subsequently, if you have not had an oral glucose tolerance test within the past 3 months, you will go to a blood draw lab and participate in a 2-hour oral glucose tolerance test to see if you are still pre-diabetic and therefore eligible to be in the study. This test requires about 10 mL (2 tsp) of blood. This process is called "screening". It is possible that this screening process will show that you cannot be in the study.

STUDY

If the screening process indicates that you are eligible for this study, then you may become a subject in the study. If so, additional blood tests will be done. The tests that will be done on your blood are for vitamin D, glucose control, and liver and kidney function. A phlebotomist at a designated blood draw clinic in the HRM will collect about 20 mL (4 tsp) of blood from you. You will also be asked to complete the following questionnaires:

- Dietary questionnaire, which will be administered by a researcher. It takes approximately ten (10) minutes to complete.
- A health and physical activity questionnaire, which takes approximately ten (10) minutes to complete.

During the study, subjects should avoid travel to areas within +30 and -30 degrees latitude. Similarly, the use of tanning beds during the study should be avoided.

PHOTOTHERAPY

You will be randomly placed into either the phototherapy or the control group, which does not receive phototherapy. If you are placed in the phototherapy group, you will be asked to attend phototherapy sessions three (3) times per week for 12 weeks. The lamp used for the phototherapy is six (6) feet tall, consists of several long lamp bulbs, and is

placed in a private booth. You will remove all clothing except underwear, put on protective glasses that will be provided, face the lamp and press the "on" button. After a pre-set time between 30 and 120 seconds, the lamp will turn off. You will then press the "on" button again and face away from the lamp. Subjects who have a fair complexion will be exposed for fewer seconds than those with a darker complexion. Apart from travel to the sessions at Dalhousie University, each session will take about 5 minutes. If you are placed in the control group, you will not take part in any activities related to this study for a 12 week period.

AT THE END OF THE STUDY, AFTER PHOTOTHERAPY

After the 12 week period has elapsed and, if you are in the phototherapy group, your 12 weeks of lamp use has been completed, all subjects in both groups will be reassessed by blood tests. You will have another 2-hour glucose tolerance test, which will require about 8 mL (2 tsp) of blood from you. In addition, your blood will also be used to determine your vitamin D level, glucose control, and liver and kidney functions. This will require about 20 mL (4 tsp) of blood.

Of course, at any time you may ask not to have further tests done.

It is important that you tell a study researcher about any drugs or medications you are taking or wish to take. You must also tell the Principal Investigator about anything unusual that is happening with your health. This includes any medical problems that seem to be getting worse. If you have to see a doctor or have to go to a hospital, you must let the doctors know that you are in a research study and, if you are in the phototherapy group, that it involves phototherapy. You should also tell your own doctor as quickly as possible, for your safety.

8. ARE THERE RISKS TO THE STUDY?

There are risks with this, or any study. To give you the most complete information available, we have listed many *possible* risks, which may appear alarming. We do not want to alarm you but we do want to make sure that if you decide to try this study, you have had a chance to think about the risks carefully. Please also be aware that there may be risks in participating in this study that we do not know about yet.

BLOOD SAMPLE

You may experience some temporary discomfort when blood samples are taken. There is a small risk of bruising, infection or swelling at the site where the needle is inserted, and some people may feel faint or dizzy.

QUESTIONNAIRES

You may find the interviews and questionnaires to be tiresome or you may not like all the questions that you will be asked. You do not have to answer any question that you find distressing.

UV LAMP

If you are assigned to the phototherapy group, you might notice a change in skin tone due to the exposure of your skin to UVB light. Excessive exposure to this lamp would result in a burn, especially for a light skinned person. To ensure that you do not burn, the timer for your first session will be set for a length of time that is shorter than the recommended length. This will be increased a little each session until the recommended exposure time is reached. However, although the lamp will be pre-set to turn off before it can cause a burn, it is possible that a slight redness will occur, especially due to the first session. Any slight redness of the skin due to the lamp exposure will disappear within a few days. However, if any reddening occurs, please tell the researcher about this right away. After each session the researcher will check for redness and before each session, he or she will inquire if redness occurred after the previous session.

DRUG INTERACTION

Sun exposure is not recommended when certain photosensitizing medications are taken or applied. Therefore, if you are using any of these medications, then you cannot be accepted into this study. If you are assigned to the phototherapy group and after the beginning of the study begin taking or using one of these medications, then we will need to stop your phototherapy. It is therefore important that you tell the researcher if you begin any new medication or have any new ailments. Also, if you are placed in the phototherapy group and feel that you are having any reaction to the lamp exposure, tell one of the study personnel immediately. The researchers may need to interrupt or discontinue your phototherapy.

9. WHAT HAPPENS AT THE END OF THE STUDY?

At the end of the study the information collected throughout the testing session will be stored and locked in a confidential location to ensure no one except the investigators can access it. You will be given a copy of the final publication when the study is finished if you wish to receive it.

10. WHAT ARE MY RESPONSIBILITIES?

As a study participant you will be expected to:

- Follow the directions given by the Principal Investigator
- Report all medication being taken or that you plan on taking
- Report any change in your health to the Principal Investigator
- Report any problems that you experience if you think it might be related to participating in the study

11. CAN I BE TAKEN OUT OF THE STUDY WITHOUT MY CONSENT?

Yes. You may be taken out of the study at any time, if:

- There is new information that shows that being in this study is not in your best interests.
- The Capital Health Research Ethics Board or the Principal Investigator decides to stop the study.
- In the opinion of the Investigators you are experiencing side effects that are harmful to your health or well-being
- You do not follow the directions of the Principle Investigator

If you might need to withdraw from the study, you will be told what the reasons are.

12. WHAT ABOUT NEW INFORMATION?

It is possible (but unlikely) that new information may become available while you are in the study that might affect your health, welfare, or willingness to stay in the study. If this happens, you will be informed in a timely manner and will be asked whether you wish to continue to take part in the study or not.

13. WILL IT COST ME ANYTHING?

COMPENSATION

You will not be paid to be in this study.

RESEARCH RELATED INJURY

If you become ill or injured as a direct result of participating in this study, necessary medical treatment will be available at no additional cost to you. Your signature on this form only indicates that you have understood to your satisfaction the information regarding your participation in the study and agree to participate as a subject. In no way does this waive your legal rights nor release the Principal Investigator, the research staff, or involved institutions from their legal and professional responsibilities.

14. WHAT ABOUT MY RIGHT TO PRIVACY?

Protecting your privacy is an important part of this study. A copy of this consent will be put in your health record.

When you sign this consent form, you give us permission to:

- Collect information from you
- Collect information from your health record
- Share information amongst the research personnel connected with this study
- Share information with the people responsible for protecting your safety while participating in this research

However, complete privacy cannot be guaranteed. For example, the investigator may be required by law to allow access to research records. A copy of this consent form will be put in your health records. Your family doctor may be told that you are taking part in this study.

ACCESS OF OTHERS TO YOUR RECORDS

The members of the research team will see health and study records that identify you by name. Other people, during visits to this health care facility, may need to look at the health and study records that identify you by name. These people might include:

- The CDHA Research Ethics Board and people working for, or with, the Research Ethics Board
- Health Canada personnel

USE OF YOUR STUDY INFORMATION

The research team will collect and use only the information they need to complete the study. This information will only be used for purposes of this study.

This information will include your:

- Date of birth
- Gender
- Medical conditions

- Medications
- The results of tests and procedures you had before and during the study
- Information from the study interviews and questionnaires

Your name and contact information will be kept secure by the research team in the Bone Lab of Dalhousie University. It will not be shared with others without your permission. Information collected for this study will be kept as long as required by law. This could be 7 years or more. Your name will not appear in any report or article published as a result of this study.

After your part in the study ends, we may continue to review your health records for safety and data accuracy until the study is finished.

If you decide to withdraw from the study, the information collected up to that time will continue to be used by the research team. It may not be removed.

Information collected and used by the research team will be stored at the Bone Lab of Dalhousie University. The Principle Investigator is the person responsible for keeping it secure.

The Research Ethics Board and people working for or with Research Ethics Boards may also contact you personally for quality assurance purposes.

YOUR ACCESS TO RECORDS

You may ask the study coordinator or Principal Investigator to see the information that has been collected about you.

15. WHAT IF I WANT TO QUIT THE STUDY?

If you choose to participate and later decide to change your mind, you can say no and stop your participation in the research at any time. If you wish to withdraw your consent, please inform the Principal Investigator. Your health records may be examined in connection with this study or further analyses related to it. All of your data up to the date you withdraw your consent will remain in the study records, to be included in study related analyses. A decision to stop participating in the study will not affect your health care.

16. DECLARATION OF FINANCIAL INTEREST

This study will be funded by small grants. The amount of these grants is sufficient to cover the costs of conducting the study. The Principal Investigator has no financial interests in conducting this research study. Solarc Systems Inc. (Barrie, Ontario) has kindly provided the phototherapy lamp for this study.

17. WHAT ABOUT QUESTIONS OR PROBLEMS?

For further information about the study, please contact Dr. Jo Welch. Dr. Welch is in charge of this study at Dalhousie University. (She is the Primary Investigator.) Dr. Welch can be reached at (902) 494-2475. If you can't reach the Primary Investigator, please refer to the attached Research Team Contact Page for a full list of the people for further information about the study.

If you experience any symptoms, possible side effects or other medical problems, please contact either Dr. Stephanie Kaiser or Dr. Dale Clayton. Their contact information is found on the cover page.

If you cannot reach the above researchers, contact the Dr. Jo Welch, the Principal Investigator or the Research Coordinator, Mr. Noam Ami.

Principle Investigator: Dr. Jo Welch
Phone: (902) 494-2475

Associate Investigator: Dr. Stephanie Kaiser
Phone: (902) 473-3712

Associate Investigator: Dr. Dale Clayton
Phone: (902) 473-3712

Research Coordinator is Mr. Noam Ami
Phone: (902) 494-6786

18. WHAT ARE MY RIGHTS?

After you have signed this consent form you will be given a copy.

If you have any questions about your rights as a research participant, contact the **Patient Representative** at **(902) 473-2133.**

In the next part you will be asked if you agree (consent) to join this study. If the answer is "yes", you will need to sign the form.

PART C.

19. CONSENT FORM AND SIGNATURES

I have reviewed all of the information in this consent form related to the study called:
Does vitamin D lamp exposure affect glycemic control in pre-diabetics?

I have been given the opportunity to discuss this study. All of my questions have been answered to my satisfaction.
I agree to allow the people described in this consent form to have access to my health records.
This signature on this consent form means that I agree to take part in this trial. I understand that I am free to withdraw at any time.

_____	_____	____ / _____ / ____
Signature of Participant	Name (Printed)	Year Month Day*
_____	_____	____ / _____ / ____
Witness to Participant's Signature	Name (Printed)	Year Month Day*
_____	_____	____ / _____ / ____
Signature of Investigator	Name (Printed)	Year Month Day*
_____	_____	____ / _____ / ____
Signature of Person Conducting Consent Discussion	Name (Printed)	Year Month Day*

Note: Please fill in the dates personally

I WILL BE GIVEN A SIGNED COPY OF THIS CONSENT FORM.

Thank you for your time and patience!

Appendix E: *Informed consent comprehension questionnaire*

Informed Consent

PATIENT ID #_____ INITIALS:_____
STUDY SHORT NAME:_____
PROTOCOL NUMBER:_____

Persons obtaining informed consent in conjunction with the Division of Endocrinology will ask subjects the following three questions and record their responses and subsequent discussion below:

1. **What is the purpose of the research?**

_____
_____
_____
_____
_____
_____
_____
_____

2. **What are the risks?**

_____
_____
_____
_____
_____
_____
_____
_____

3. **What are the benefits?**

_____
_____
_____
_____
_____
_____
_____
_____

Further questions/comments

_____
_____
_____
_____
_____
_____
_____
_____

_____ _____

Signature of person obtaining informed consent **Date**

This document will be attached to the written informed consent discussion and filed as a source note.

*** This form is based in part on the Division of Endocrinology Clinical Trials Source Documentation document.**

Appendix F: *Health, exercise, and dietary information questionnaire*

Name: _____

Subject no.: _____

Height: _____

Weight: _____

Waist circumference: _____

Vitamin D supplementation and its effects on blood glucose levels in pre-diabetics

Health, Exercise, and Dietary Information Questionnaire

Health section

1. How would you describe your general overall health?
   - ☐ I am healthier than others my age.
   - ☐ I am about as healthy as others my age.
   - ☐ I am less healthy than others my age.
   - ☐ My overall health varies considerably.

2. Do you smoke cigarettes?
   - ☐ No, never.
   - ☐ I quit a while ago. If so, how long ago? _____
   - ☐ Yes, I smoke or have smoked within the past year
   - ☐ No, but my partner smokes.

3. Please list any **non**-prescription drugs you take regularly (e.g. Aspirin, Tylenol etc.).
   _____
   _____
   _____
   _____

4. Please list all prescription drugs you are currently taking.
   _____
   _____
   _____

5. Have you been diagnosed with Crohn's disease, ulcerative colitis or celiac disease?
   - ☐ Yes
   - ☐ No

6. Do you often have diarrhea?
   - ☐ Yes
   - ☐ No

7. Have you been diagnosed with any skin condition (Eczema, Psoriasis, etc.)?
   - ☐ Yes
   - ☐ No

8. If yes, please describe the type of condition, the severity, how long you have had it, and any medications prescribed for it.

Skin Condition	Severity 1=mild; 5=severe	When it began; when it ended	Medications

9. When were you first diagnosed with pre-diabetes? _____

10. Who made the diagnosis? _____

11. How many times a day to you monitor your blood glucose levels?
   - ☐ 0 times a day
   - ☐ 1-3 times a day
   - ☐ 3+ times a day

12. Do you require the use of daily insulin?
   - ☐ Yes
   - ☐ No

13. If yes, please describe the type of insulin (e.g. Fast acting, etc.), and dosage.

_____

Exercise section

14. How much do you walk in a normal day?
    - None.
    - 1-10 minutes
    - 11-30 minutes
    - 31-45 minutes
    - more than 45 minutes

15. Do you do exercise other than walking?
    - Yes
    - No

16. If yes, please describe the nature of the activity, when you first started the activity, when you stopped the activity and approximately how many hours/week you participated.

Sport/Activity	Start Date	End Date	Hours/Week

Nutrition section

17. How much milk do you consume daily?
    - None
    - 1 cup
    - 2 cups
    - 3+ cups
    - Other (describe
      _____)

18. Do you take cod-liver oil or omega-3fatty acids (fish oils)?
    - Yes
    - No

19. How much yogurt or calcium fortified juice do you consume daily?
    - None
    - 1 cup
    - 2 cups
    - 3+ cups
    - Other (describe)
      _____)

20. How much margarine do you consume daily?
    - None
    - 1 tbsp
    - ½ cup
    - 1+ cups
    - Other (describe
      _____)

21. If the amount of milk, yogurt, calcium fortified juice, or margarine consumption has changed over the years, please describe

    _____

22. Do you add salt to your food?
    - Never
    - Sometimes
    - Often

23. Do you eat packaged food?
    - Never
    - Sometimes
    - Often

Appendix G: *24-hour dietary recall*

24-Hour Dietary Recall Exercise (Interview by study coordinator)

PRE BREAKFAST SNACKS (Estimate Quantity)	**WHERE EATEN**
FOODS**:**	
BEVERAGES:	
BREAKFAST (Estimate Quantity)**:**	**WHERE EATEN**
FOODS:	
BEVERAGES:	
MORNING SNACKS (Estimate Quantity)**:**	**WHERE EATEN**
FOODS:	
BEVERAGES:	
LUNCH (Estimate Quantity)**:**	**WHERE EATEN**
FOODS:	
BEVERAGES:	
AFTERNOON SNACKS (Estimate Quantity)**:**	**WHERE EATEN**
FOODS:	
BEVERAGES:	
SUPPER (Estimate Quantity)**:**	**WHERE EATEN**

FOODS:	
BEVERAGES:	
AFTER DINNER SNACKS (Estimate Quantity):	WHERE EATEN
FOODS:	
BEVERAGES:	
LATE NIGHT SNACKS (Estimate Quantity):	WHERE EATEN
FOODS:	
BEVERAGES:	

Name: _____Today's date: _____

Appendix H: *Vitamin D and sun exposure questionnaire*

Vitamin D and Sun Exposure Questionnaire

Please complete the following questionnaire to the best of your ability. If you have any questions please do not hesitate to ask for clarification. Fill in the blanks as necessary or check the appropriate boxes.

1. Name _____
2. Your date of birth _____
3. Have you lived in Nova Scotia for the past 12 months?
   - ☐ Yes
   - ☐ No
4. Have you travelled to a sunny location within the last year?
   - ☐ Yes
   - ☐ No
5. If yes to #4:
   A) Where did you go? _____
   B) How long were you there? _____
   C) Were you exposed to the sun when you were there?
      - ☐ Yes
      - ☐ No
6. During the daytime this summer were you exposed to the sun?
   - ☐ No
   - ☐ Only in the early mornings or evenings
   - ☐ Rarely between 10 am and 3 pm
   - ☐ Sometimes between 10 am and 3 pm
   - ☐ Often between 10 am and 3 pm
   - ☐ I live in the sun in the summer (e.g. lifeguard

7. Do you wear sunscreen when you are in the sun?
   - ☐ I'm never in the sun
   - ☐ No, I do not wear sunscreen
   - ☐ Not often
   - ☐ Sometimes
   - ☐ Usually
   - ☐ Always

8. Do you tan when exposed to sunshine?
   - ☐ No, I only burn
   - ☐ I burn a little, then it turns into a light tan
   - ☐ I occasionally burn a little, but tan quite easily
   - ☐ I rarely burn but tan easily

7. Do you take any multi-vitamins supplements?
   - ☐ Yes
   - ☐ No

8. If yes, please state the brand name: _____Dose? _____

9. Do you take any vitamin D or fish oil supplements?
   - ☐ Yes
   - ☐ No

10. If yes, please state the brand name: _____Dose? _____

11. Do you take any calcium supplements?
   - ☐ Yes
   - ☐ No

12. If yes, please state the brand name: _____Dose? _____

Signature _____ Today's Date _____

Appendix I: *Initial Vitamin D Form*

Initial Visit Vitamin D Lamp Exposure Sheet

Subject Name: _____ID#:_____

Initial Visit

Date of initial visit:

Clothing worn:

In your opinion what would you consider your skin type to be?

I (1) Always burns, never tans
II (2) Always burns, sometimes tans
III (3) Sometimes burns, always tans
IV (4) Never burns, always tans
V (5) "Brown" or moderately pigmented. Includes: Asiatic, Mexican, American Indian, others
VI (6) "Black"

Initial treatment time:

Maximum treatment time allowed per week:

Notes:

Appendix J: *Weekly Vitamin D log*

Weekly Vitamin D Exposure Log

Subject Name: _____ ID#:_____

Week #:

Session 1

Current date:

After your previous treatment session (12-24 hours after), how did your skin look/feel?

A. No or minimally noticeable effect
B. Light pink. Very moderate burn. "Sub-erythema"
C. Significant erythema (burns) red
D. Significant erythema (burns) edema/blisters.

Exposure time based on answer:

Notes:

Session 2

Date of last treatment:
Current date:

After your previous treatment session (12-24 hours after), how did your skin look/feel?

A. No or minimally noticeable effect
B. Light pink. Very moderate burn. "Sub-erythema"
C. Significant erythema (burns) red
D. Significant erythema (burns) edema/blisters.

Exposure time based on answer:

Notes:

Session 3

Date of last treatment:
Current date:

After your previous treatment session (12-24 hours after), how did your skin look/feel?

A. No or minimally noticeable effect
B. Light pink. Very moderate burn. “Sub-erythema”
C. Significant erythema (burns) red
D. Significant erythema (burns) edema/blisters.

Exposure time based on answer:

Total exposure for the week:

Notes:

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