The Nutritional Intake of Persons With Type 2 Diabetes Mellitus Who Have Peripheral Neuropathy, Compared To Those Who Do Not Have Peripheral Neuropathy

by

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A Thesis submitted to the Faculty of Graduate Studies of

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FACULTY OF GRADUATE STUDIES
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Abstract

Objectives: The incidence of type 2 diabetes mellitus (DM2) is on the rise worldwide. The primary objective was to determine the prevalence of nutrient inadequacy and excessiveness in persons with DM2 with and without diabetic peripheral neuropathy (DPN).

Study Design: A validated semi-quantitative food frequency questionnaire was used to determine the prevalence of inadequacy of nutrients with an estimated average requirement; the mean intake of nutrients with an adequate intake; and the proportion of persons not meeting the recommendations for the acceptable macronutrient distribution range (AMDR).

Results: Differences were observed in the prevalence of inadequacy of vitamin A and the proportion of persons not meeting the AMDR for total fat, linoleic acid and carbohydrate.

Conclusion: The aforementioned nutrients may have a significant role in the progression/development of DPN and should be studied in further detail. We recommend a balanced diet and use of a multi-vitamin for persons with DM2.

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List of Abbreviations

ADA: American Dietetic Association

AI: Adequate Intake

AMDR: Acceptable Macronutrient Distribution Range

BMI: Body Mass Index

CDA: Canadian Diabetes Association

DCCT: Diabetes Control and Complications Trial

DM: Diabetes Mellitus

DM1: Type 1 Diabetes Mellitus

DM2: Type 2 Diabetes Mellitus

DN: Diabetic Neuropathy

DPN: Diabetic Peripheral Neuropathy

DRI: Dietary Reference Intakes

EAR: Estimated Average Requirement

FBS: Fasting Blood Sugar

FFQ: Food Frequency Questionnaire

$HbA1_c$: Glycosylated Hemoglobin

HDL: High Density Lipoprotein

Kcal: Kilocalories

LDL: Low Density Lipoprotein

MNCV: Motor Nerve Conduction Velocity

NCI: National Cancer Institute

NCV: Nerve Conduction Velocity

NHANES: Nurses Health and Nutrition Examination Survey

PAL: Physical Activity Level

PUFA: Polyunsaturated Fatty Acid

RDA: Recommended Dietary Allowance

SD: Standard Deviation

2HABF: Two Hours After Breakfast

UKPDS: United Kingdom Prospective Diabetes Study

UL: Tolerable Upper Intake Level

WHO: World Health Organization

1.0 Introduction

1.1 Diabetes Mellitus

Diabetes mellitus (DM) is a condition in which: 1) the pancreas in unable to produce insulin in sufficient amounts, or 2) the body is unable to adequately utilize the amount of insulin that the pancreas produces.[1] Both scenarios lead to hyperglycemia. DM has various forms including: 1) type 1 diabetes mellitus (DM1); 2) type 2 diabetes mellitus (DM2); and 3) gestational diabetes mellitus.

1.1.1 Type 2 Diabetes Mellitus

DM2 occurs in two circumstances: 1) when the pancreas is unable to produce a sufficient amount of insulin, and 2) when the body is unable to utilize the amount of insulin that the pancreas created.[2] Insulin is "a hormone produced by the β cells of the pancreas that regulates blood glucose levels".[3] Insulin maintains levels of glucose in the blood by "transporting glucose into cells where it is then used as a source of energy for the body"[3] However, in the presence of DM2, rather than providing energy for the body, glucose remains in the bloodstream[2] which can lead to severe damage of the nerves. DM2 is the most common form of DM and is presumed to affect 90% of persons with DM.[4] DM2 is usually observed in adults, but is now also being observed in children and youth.[4] Management of DM2 is generally through proper nutrition and exercise. However use of oral hypoglycemic agents and insulin injections is common. A diagnosis of DM can be made when fasting blood glucose levels are ≥ 7 mmol/L or through a 2 hour oral glucose tolerance test with levels > 11 mmol/L.[4]

1.2 Incidence of Diabetes Mellitus in Canada

DM is a leading cause of morbidity and mortality amongst members of all societies.[5,6] Manitoba has been noted to have one of the highest rates of DM in Canada.[7] As of July 1, 2006, the total population of Manitoba was 1, 177, 165.[8] It is known that more than 67, 000 people residing in Manitoba have DM.[9] However, it is speculated that an additional 19, 000 persons have DM but are unaware.[9] Combined, this equals more than 7% of the population of Manitoba.[9] It is estimated that on average, there are 16 newly reported cases of DM in Manitoba every day, whereas the average across the remainder of Canada is 11%.[9]

1.3 Complications of Diabetes Mellitus

The complications associated with DM can be both severe and debilitating. Complications are due to both macro and micro-vascular injury and can include but are not limited to: 1) cardiovascular, 2) ophthalmologic, 3) renal and 4) neurologic. The focus of this study is on diabetic neuropathy (DN).

1.3.1 Diabetic Neuropathy

DN is a broad term which encompasses a number of nerve disorders affecting persons with DM.[10] DN can be described as "destruction of the nerves".[10] DN can be classified as 1) peripheral, 2) autonomic, and 3) proximal or focal.[10] DN can develop at any time during DM, but the longer an individual has DM, the greater the risk and magnitude of the nerve injury.[10] It has been reported that DN develops most frequently in persons who have had DM for approximately 25 years[11]; however, DN can also occur in persons who

have had DM for less than 25 years which can lead to a diagnosis of DM in persons not previously diagnosed.[12,13] It is believed that approximately 50% of Canadians with DM will be affected by some form of DN, but the actual numbers may be significantly higher as not all individuals experience symptoms.[10,14]

1.3.2 Diabetic Peripheral Neuropathy

Diabetic Peripheral Neuropathy (DPN) is the most common form of DN. Peripheral neuropathy is a condition which affects the nerves of the extremities, specifically the legs, feet, toes, arms, hands and fingers.[10] DPN most commonly affects the legs and feet.[10] The exact mechanisms leading to DPN are still not fully established, however, prolonged hyperglycemia appears to be one of the primary mechanisms behind the development of this condition.[11,12,13] Symptoms of DPN may include; numbness, tingling sensation and, pain in the legs, feet, toes, hands, fingers and arms.[10] Symptoms can go undetected and further injury may result. If injuries go undetected, infection may develop and spread to the underlying bone. Amputation of the affected area may be necessary to control the infection.[10,14] It is estimated that the risk of amputation in persons with DM is 20 times greater than persons without DM.[15] It is also estimated that 50% of all lower extremity amputations that occur in Canada are due to complications of DM.[16]

1.3.3 Risk Factors for Diabetic Peripheral Neuropathy

Known risk factors for DPN include, hyperglycemia, duration of DM, height, hypoinsulinemia, and abnormal lipid levels in the blood.[10] Dysglycemia is a defining

characteristic of DM, with hyperglycemia being a risk factor for many end organ complications. The benefits of aggressive glycemic control have been clearly demonstrated to minimize and prevent the complications of DM.[17,18] Blood glucose levels in persons with DM2 are significantly higher than in persons without DM2, and it is these elevated levels which can lead to end organ damage.[17] Glycosylated hemoglobin ($HbA1_c$) can provide an indication of glycemic control over the preceding 120 days. The Canadian Diabetes Association (CDA) states that adequately controlled blood glucose levels will yield a $HbA1_c$ level of $\leq 7.0\%$.[19] Approximately half of all persons with DM2 in Manitoba do not have adequately controlled blood glucose levels which leaves them at high risk for complications of diabetes.[19,21] The Diabetes Control and Complications Trial (DCCT) involved 1,441 persons with DM1, and concluded that managing blood glucose levels as close to normal as possible may diminish the progression of the complications of DM.[18] Results from the United Kingdom Prospective Diabetes Study (UKPDS) further stress that optimal glycemic control is imperative in minimizing the risk of complications of DM2 including neuropathy.[17]

Hyperglycemia leads to the end organ complications associated with diabetes and is also thought to affect the transmitting ability of nerves and decrease the antioxidative abilities of persons with DM.[14,20,21] Hyperglycemia can also decrease the antioxidative abilities of antioxidants including vitamins A, C, and E.[23] It is thought that oxidative stress may be a causative factor in the development of the complications of DM. Research on experimental DM has shown that oxidative stress can cause dysfunction in the sciatic nerve. It was also found in experimental DM that decreased levels of vitamin E were

present in the nerves as compared to controls without DM.[22] When the animals were administered doses of antioxidants, nerve function improved.[22] Plasma levels of biomarkers of oxidative stress have been evaluated in persons with DM in relation to the presence/absence of polyneuropathy.[21] Results showed that the persons with polyneuropathy had increased markers of oxidative stress and decreased levels of antioxidants.[21] This study concluded that oxidative stress is higher in persons with DM prior to the onset of polyneuropathy, and is considerably higher in those who develop polyneuropathy.[21,22,23]

1.3.4 Diagnosing Diabetic Peripheral Neuropathy

The technique most frequently used to screen for DPN in lower extremities is the Semmes-Weinstein ten gram monofilament. The inability to appreciate the monofilament when applied to selected areas of the foot (the pulp space of the first toe and the first and fifth metatarsal heads) on one or more repetition will help determine whether there is a loss of protective sensation which places the person at high risk of foot injury.[24]

1.4 Nutrition

There are specific daily recommendations for both micro- and macronutrients. Data on these recommendations is shown in the section that follows.

1.4.1 Dietary Reference Intakes

The dietary reference intakes (DRIs) are a group of dietary reference values comprised of four reference values used in both Canada and the United States. The basis for the establishment of these reference values were scientifically proven relationships between the nutrients themselves and signs of adequate intake and the prevention of chronic disease in a population deemed as healthy.[25] The purpose of the DRIs is to "evaluate and prepare nutrition plans for persons of good health".[25] The four reference values that make up the DRIs are the: 1) Estimated Average Requirement (EAR), 2) Recommended Dietary Allowance (RDA), 3) Adequate Intake (AI), and 4) Tolerable Upper Intake Level (UL). These four reference values were established for most micronutrients, and replace the previous Recommended Nutrient Intake values in Canada and the Recommended Dietary Allowance values in the United States.[25]

1.4.2 Estimated Average Requirement

The EAR is defined as "the typical level of daily nutrient intake that is anticipated to meet the needs of 50% of the persons in a particular life stage and gender group that are deemed as being in good health".[25]

1.4.3 Adequate Intake

Most nutrients have a set of established DRI values.[25] However, there are situations when an EAR and RDA cannot be established for the nutrient, in these cases, an AI was then established.[25] The AI is defined as "the typical level of daily intake of a particular nutrient established from observed or experimentally determined approximations or

estimates of nutrient intake by a group (or groups) of people deemed as being in good health, that are presumed to be adequate; they are used when an RDA could not be established".[25]

1.4.4 Tolerable Upper Intake Level

The UL is defined as "the highest average level of daily nutrient intake that is unlikely to cause a threat of negative health outcomes to nearly every person in the general population. As daily intake exceeds the UL, the potential threat of negative health effects is markedly higher".[25]

1.4.5 Recommended Dietary Allowance

The RDA is defined as "the typical level of daily nutrient intake that has been deemed satisfactory to exceed the nutrient needs of 97 – 98% of persons in a particular life stage and gender group deemed as being in good health".[25] The DRI values for the nutrients discussed in this paper as well as food group data can be found in Tables 5 - 8.

1.4.6 Acceptable Macronutrient Distribution Ranges

The macronutrients, protein, fat and carbohydrate, have the ability to replace each other to some degree for the purpose of fulfilling the body's energy requirements.[25] It is for this reason that when the intake of one macronutrient is increased, the intake of the other two must decrease to prevent over consumption of energy.[25] The Acceptable Macronutrient Distribution Range (AMDR) is defined as "a range of intake for the macronutrients that is associated with minimal risk of chronic disease (including DM)

while supplying sufficient intakes of indispensable nutrients".[25] The AMDRs are presented in terms of the percent of total energy consumed. The AMDRs are expressed in terms of a range which differs from the DRI values for the micronutrients. This range should help individuals to obtain sufficient amounts of nutrients required in the diet as well.[25] When a person consumes amounts that are either less than or greater than the AMDR, the risk of nutrient inadequacy and the development of chronic disease is then increased.[25]

An AMDR has not been established for dietary fiber because it provides little energy to the overall diet and there does not appear to be any negative consequences associated with its intake.[25] An AI has been established for dietary fiber however. An AMDR has also not been established for monounsaturated fatty acids because they are not considered indispensable to the human diet and there was insufficient evidence to support its intake (either high or low) in relation to chronic disease.[25] It has been shown that both over- and under consumption of macronutrients coupled with physical inactivity lead to the increased risk for chronic disease such as DM2, obesity and coronary heart disease.[25]

1.5 Evaluating Nutrient Intakes of Groups

The primary objective when evaluating the nutrient intake of a group is to establish the frequency of insufficient or extreme intake of nutrients.[25] In order to establish the frequency of insufficient intake, the EAR can be used, if it has been established. The frequency of insufficient intake can then be established by using one of two methods; 1) the probability approach or 2) the EAR cut-point method which is a quick method originating from the probability approach.[25] The probability approach is "a statistical

process that requires the determination of the probability of inadequacy of the typical nutrient intake level for each person in the group to determine an approximation of the group's prevalence of inadequacy".[25] The EAR cut-point method is "a statistical method which is derived from the probability approach and when specific conditions are fulfilled, the proportion of the group with intakes less than the EAR will be similar to the proportion of the group that does not meet their requirement".[25]

For nutrients for which an EAR has not been established, the AI values can be used to determine the level of inadequate intake,[25] however, the assumptions must be taken with caution. Because an EAR could not be established, this means that the distribution of requirements is unknown and thus, the proportion of a group with intakes that are less than the recommended values cannot be determined.[25] However, it can be presumed that when the mean or median intake of an entire group are either at, or exceeds the AI that the prevalence of inadequate intake of the group is minimal.[25] However, if the mean or median intakes of the group are below the AI, it cannot be presumed that the group does not have adequate intakes as the distribution of requirements is not known.[25] One can however determine the proportion of a group with insufficient intake but once again, it cannot be presumed that this proportion of the group has insufficient intake well below their requirements.[25]

The UL is used to determine the proportion of persons within a group that are exceeding the requirements of a specific nutrient and consequently placing persons at risk for any possible negative health effects of the nutrient.[25] ULs for nutrients were established based upon the various sources of each nutrient.[25] Some nutrients have ULs that were

based upon typical intake of all sources (for example, food, water and supplements), while others are based solely upon fortified foods and supplements. For this reason, the typical intake distribution must be taken into account in order to determine what proportion of a group (if any) exceeds the UL.[25]

For nutrients with AMDRs, determining the proportion of the group that are within, below or exceeding the AMDRs will provide data on the "level of adherence within the group".[25]

The purpose of this study is to examine the nutritional adequacy of persons with DM2 with DPN (cases) and without DPN (controls) to determine if differences exist between the two groups. A semi-quantitative food frequency questionnaire (FFQ) will be used to assess nutrient intakes.

2.0 Review of Literature

2.1 Nutrition and Diabetic Peripheral Neuropathy

Previous research has been conducted on the topic of nutrition and DPN, however, the majority did not utilize the DRI values, and typically does not focus on nutritional intake as a whole, but rather focuses on intake of single nutrients. The following nutrients have been determined to have some influence upon DPN, but research is limited.

2.1.1 Polyunsaturated Fatty Acids

Studies have shown that there are two metabolic irregularities that may contribute to DN. The first is altered lipid metabolism which occurs due to altered or inhibited activity of the enzymes Δ 5 and 6 desaturases. These enzymes are responsible for the conversion of linoleic and α-linolenic acids from food sources to polyunsaturated fatty acids (PUFAs) such as γ- linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids.[26] The second is reduced Na^+, K^+ATPase activity which may be attributed to a decreased incorporation of n-6 fatty acids in the membrane phospholipids.[26]

It has been shown in experimental models of diabetes that PUFAs such as γ-linolenic acid[27,28] and fish oils[28,29] can prevent the reduction normally seen in nerve conduction velocity (NCV) in persons with DM, which is an indicator of DPN.[30] It has been proposed that these PUFAs bypass the inhibited Δ6 desaturase which occurs in DM.[31]

A study to assess the relationship of PUFA intake to peripheral neuropathy among adults with diabetes was undertaken using the Nurses Health and Nutrition Examination Survey (NHANES) 1999 – 2004[32]. Twenty-four hour recalls and the confirmation of the presence or absence of DPN of 1 602 participants with DM2 and ≥ 40 years of age was utilized as part of this study. Total PUFA intake as well as the intake of seven specific PUFAs including C18:2, C18:3, C18:4, C20:4, C20:5, C22:5 and C22:6 were examined.[32] Results showed that persons with DPN consumed less total PUFA and linolenic acid than persons without DPN.[32] This study concluded that the intake of linolenic acid is associated with a decreased risk of DPN.[32] These investigators suggest that a probable

mechanism behind this decreased risk of DPN is that PUFAs reduce the threat of hypertension, and adequate vascular health is important for reduced risk of DPN.[32]

It is known that persons with DM generally have abnormal lipid profiles [characterized by reduced high density lipoprotein (HDL) cholesterol levels, elevated low density lipoprotein (LDL) cholesterol levels and high triglycerides]. Since it has been shown that intake of n-3 PUFAs may help to improve triglyceride levels and increase HDL cholesterol levels, it is possible that intake of n-3 PUFAs could lead to a decreased risk of DPN as abnormal lipid profiles have been implicated to be a risk factor for the onset of DPN.[33]

2.1.2 Vitamin B6

Studies have shown that persons with DM generally have lower levels of vitamin B6 in the body.[34-44] It has also been shown that when persons with DM2 are supplemented with vitamin B6, $HbA1_c$ levels were improved.[43,45] It has also been shown that when persons with DM received nutritional supplementation with vitamin B6, the rate of neuropathy decreased drastically.[42,44,45]

2.1.3 Vitamin D

Low serum vitamin D concentrations are commonly observed in patients with DM2.[46] Vitamin D has also been shown to improve glycemic control.[47] The extent of the effects of vitamin D repletion on the severe pain caused by neuropathy in persons with DM2 has been examined.[48] The presence of DPN was determined by monofilament and radioimmunoassay was used to determine serum vitamin D concentrations.[48] Results

showed that all participants had insufficient vitamin D stores, but when vitamin D stores were improved, the degree of pain from neuropathy diminished.[48] This study concludes that a decrease in serum vitamin D concentrations may contribute to the onset of DPN in persons with DM2, and that an increase in serum vitamin D concentrations through supplementation may decrease the pain associated with DPN.[48]

2.1.4 Vitamin E

Research on the topic of vitamin E and DPN is sparse, but appears to suggest that supplementation with vitamin E may improve NCV of persons with DM2 and DPN.[49-51] The dietary intake of persons with DM (the majority being DM2) has been evaluated. Half of the participants had foot ulcers, and the other half did not. Results showed that nutritional intake was comparable between the two groups in terms of energy, carbohydrates, lipids, proteins, fiber, vitamins A, B, C and folic acid.[49] However, intake differed between the two groups in terms of alcohol, and vitamin E. This study notes that vitamin E deficiency has been implicated as a cause for DPN.[49] This study concludes that additional research is required on supplemental vitamin E at large doses as it may aid in the improvement of NCV in motor nerves of persons with DM2.[49]

Another study examined the effects of vitamin E supplementation on NCV in patients with DPN. Twenty one participants were randomly assigned to receive either a 900 mg dose of vitamin E, or a placebo for a six month period.[51] NCV, fasting plasma glucose, HbA_1c, and postprandial plasma glucose levels were all examined at both the commencement and conclusion of the study. Results showed that the participants

receiving vitamin E supplementation did not have any change in terms of markers of glycemic status, however, NCV improved.[51] This study concludes that NCV may improve from vitamin E supplementation, but further research is required.[51]

While both of the aforementioned studies conclude that vitamin E supplementation can improve NCV, both studies also concur that additional research is required to verify these results.

2.1.5 Thiamin

Research on thiamin and DPN is extremely limited. However, a study examined the response to clinical doses of thiamin on persons with DPN.[53] Two hundred participants were enrolled in the study with one hundred participants receiving a combination of 25 mg/day of thiamin and 50 mg/day of pyridoxine and the other one hundred receiving only one 1 mg of each thiamin and pyridoxine. Results showed that the degree of severity of the signs of DPN decreased in approximately 48.9% of patients receiving the higher dose of thiamin and pyridoxine.[53] Results also showed that there was an inverse relationship between plasma thiamin levels, and the level of severity of the symptoms of DPN.[53] This study concludes that in the location in which this study took place, thiamin deficiency is in fact related to the onset of DPN, and that persons with DM should consume a balanced diet to ensure adequate intake of nutrients.[53]

2.1.6 Zinc

Research on zinc and DM2 suggests that zinc plays a major role in DM2. It has been shown that zinc is involved in both the production and the action of insulin, and that hyperglycemia can cause a depletion of zinc stores in the body.[54] It has also been demonstrated that oxidative stress contributes to the development of DPN.[54] Zinc functions as an antioxidant, therefore, depleted zinc stores may contribute to the development of DPN. Zinc cannot be produced by the human body, therefore it must be provided through the diet.

The effects of zinc supplementation on DPN have been investigated.[55-57] In this study,[57] participants were divided into three groups a) persons without DM or neuropathy, b) those with DM and neuropathy who received a placebo for a six week period and c) those with DM and neuropathy who received zinc supplementation in the form of 660 mg zinc sulphate for six weeks.[57] Parameters measured included serum zinc level, fasting blood sugar (FBS), blood sugar 2 hours after breakfast (2HABF) and motor nerve conduction velocity (MNCV).[57] Results show that initial serum zinc levels in both groups of participants with DM were lower than those of participants without DM.[57] After six weeks, significant improvements were seen in FBS, 2HABF, and MNCV in the participants with DM and neuropathy receiving zinc supplementation.[57] Changes in the participants with DM and neuropathy not receiving supplementation were not significant.[57] This study concludes that persons with DM and neuropathy may benefit from zinc supplementation, through improvement of glycemic control, thereby assisting in the alleviation of the symptoms of neuropathy.[57]

In another study considering the effects of zinc supplementation upon DPN, participants received either a placebo or 660 mg of zinc sulphate for a period of six weeks.[58] Parameters examined included serum zinc, fasting blood glucose, postprandial blood glucose and NCV.[58] Results showed that at baseline, serum levels of zinc were substantially lower in both groups with DM as compared to controls without DM.[58] It was also shown that significant modifications were seen in fasting blood glucose, postprandial blood glucose and MNCV in the participants with DM receiving zinc supplementation, but was not seen in participants receiving the placebo.[58] This study also concludes that zinc supplementation improves both glycemic control as well as the severity of symptoms of DPN.[58]

The effect of zinc supplementation upon oxidative stress in persons with DM2 has been considered.[59] In this study, participants received either 30 mg/day of zinc gluconate or a placebo for a period of six months. Results showed that zinc supplementation caused a decrease in lipid peroxidation.[59] This study concludes that consuming zinc at increased levels may be a key component in the prevention of the complications such as DPN, which are associated with DM.[59]

2.1.7 Magnesium

In persons with DM2, hypomagnesaemia (which can occur due to deficiency in the diet, or to increased excretion in the urine) has been linked to the development of neuropathy in persons with DM2.[60] The proposed mechanism behind this relationship is that hypomagnesaemia can lead to hyperglycemia through the development of insulin

resistance.[61] Based on the present state of knowledge, it can be maintained that hypomagnesemia increases the risk for the development of complications of DM such as DPN.[61,62] It has been confirmed that there is a causal relationship between insulin resistance and DPN.[63] Hypomagnesemia may contribute to the development of insulin resistance which leads to the development of hyperglycemia. Therefore, it may be possible that decreased magnesium intake may be associated with the development of DPN.

The association between serum magnesium levels and the development of foot ulcers in persons with DM2 has been investigated.[60] Serum magnesium levels were measured, and compared to controls with DM2 without foot ulcers. Results showed that 93.9% of persons with DM2 and foot ulcer(s) had hypomagnesemia compared to only 73.1% of controls".[60] This study concludes that there is a strong positive association between hypomagnesemia and DPN.[60]

2.1.8 Dietary Patterns

The associations between dietary patterns and the risk of insulin resistance in males and females have been considered.[64] In this study nutritional intake was assessed using a FFQ. This study classified participants into three groups based upon their nutritional intake. The groups were a) traditional (increased consumption of high fat dairy products, refined grains and low consumption of poultry and fish), b) prudent (high consumption of fruits, vegetables, poultry and whole grains), and c) alcohol/convenience (high intake of alcohol and meat and low intake of fruits and vegetables and whole grains). Results

showed that persons in the traditional and alcohol/convenience groups had lower intakes of "healthy", nutrient dense foods including fruits and vegetables.[64] Results also showed that the prudent diet group had the lowest risk of developing insulin resistance.[64]

The association between dietary patterns and risk of DM2 has been investigated in a cohort of women.[65] This study collected nutrition data with a FFQ including kilocalories (Kcal), and grams of cholesterol, and saturated fat consumed per day. This study also considered servings of fruits and vegetables per day. This study found that a diet considered to be "prudent" can be characterized by increased intakes of fruits, vegetables, and whole grains for example[65], and that a more Westernized diet included higher intakes of red meat and refined grains for example.[65] This study concluded that the Westernized diet may lead to an increased risk for DM2.[65]

Research has also examined the association between major dietary patterns and risk for DM2. Participants included males, age 40 – 75 years of age.[66] Dietary data was obtained using a 131-item semi-quantitative FFQ. Foods were classified into groups based upon similar nutrient profile. Results were expressed as servings/day, including fruits and vegetables, and whole grains. A more Westernized type of diet was associated with higher intakes of fat, and lower intakes of fibre.[66] Results showed that the individuals consuming the Westernized diet were at a considerably increased risk for DM2 compared to the prudent diet.[66]

The association between food groups and indices of glycemic control has been considered.[67] Diet was evaluated using a 156-item semi-quantitative FFQ reflecting the previous year. Results showed that there were differences in intake between the males and females. Specifically, males consumed a greater amount of red meat and meat products, and less fruits fruit and dairy products than females.[67] This study concluded that a higher consumption of red meat and its products may increase the risk for hyperinsulinemia as well as insulin resistance in persons without diabetes.[67]

2.2 Summary of Nutrition and Diabetic Peripheral Neuropathy

Research on nutrition and DPN is limited, and appears to focus on the intake of single nutrients rather than total nutritional intake. It has been shown that the aforementioned nutrients have an effect upon the development or progression of DPN, but additional research is required to determine if other nutrients may play a role in the development or progression of this condition, as well as to verify the research that has already been conducted. Some studies have suggested that nutritional supplementation may be necessary to achieve optimal levels to assist in the decreased risk of developing DPN. Conversely, some studies have suggested that optimal levels of intake for nutrients can be achieved through consumption of a nutritionally adequate diet. Consuming a nutritionally adequate diet is in line with the recommendations of the CDA; however, despite numerous research studies cited herein suggesting that nutritional supplementation may decrease risk factors for DM and DPN, the CDA does not recommend the use of nutritional supplements and suggests that people meet their nutritional requirements through nutritionally adequate diets.[4]

2.3 Methodologies to Collect Nutritional Intake

Two common methods used to collect nutritional intake are FFQs, and 24-hour recalls which are summarized below.

2.3.1 Food Frequency Questionnaires

A FFQ is a tool used for the purpose of reviewing an individual's nutritional histories with the focus on frequency of consumption of various food items over a specified period of time, either per day, week, month or year.[68] FFQs allow the investigator to obtain information regarding each participant's typical nutritional intake[69] as they are reflective of a previously specified extended period of time. This information can then be extracted from FFQs as the questionnaire probes the participant to answer questions about the frequency of specific foods, or foods that have previously been amalgamated to form groups of foods that are common in terms of nutritive value,[69] over a specified, prolonged period of time. FFQs are typically designed in a user friendly format with the ideation that participants will be able to complete it independently.[70]

2.3.2 24-Hour Recalls

A 24-hour recall can be described as "a complete list of every food item and beverage the individual ingested over the past 24-hours".[25] However, this form of nutritional assessment requires the individual to be able to accurately recall all foods and beverages consumed over the past 24-hours and this can prove to be burdensome for certain individuals, and can lead to under and over reporting which is commonly seen when this method of nutritional assessment is used.[68] Twenty four-hour recalls can be completed

by the individual themselves, or by the investigator conducting an interview with the individual. Typically, more than a single 24-hour recall should be collected from individuals to account for variability between days.[25]

3.0 Research Design

Given the preceding summary about DM, DPN, and nutrition, it is clear that much remains to be known about the interaction between nutritional choices and peripheral neuropathy in persons with diabetes. Therefore, the following study has been undertaken.

3.1 Objectives

The objectives of this study are to:

1) Examine the nutritional intake of persons with DM2 with and without foot complications to determine: the prevalence of inadequacy of nutrients with an EAR; the mean intake of nutrients with an AI; and the proportion of persons not meeting or exceeding the AMDR.
2) Characterize the sample of patients in terms of their social, demographic/physical and medical parameters.

3.2 Hypothesis

We hypothesize that:

Persons with DM2 and DPN will not meet the recommended DRI values for n-3 PUFAs, dietary fat, carbohydrates, vitamin B6, vitamin D, vitamin E, thiamin, zinc and magnesium compared to persons with DM2 without DPN.

3.3 Rationale

DM2 is rapidly becoming an epidemic amongst all populations in Canada. This can be due in part to an increase in diets which are high in fat, specifically, saturated and trans fats, high in added sugars, and low in fiber, combined with increasingly sedentary lifestyles. With the prevalence of DM2 increasing at such alarming rates, we recognize that the prevalence of DPN will also increase alarmingly. Currently, there is no cure for DPN, and the complications that can occur as a result of DPN are deleterious which reveals the severity of this condition. Presently we know that adequate control of blood glucose levels and maintenance of lipid profiles and vascular health are imperative in the prevention of DPN.[75] However, other nutrients may have a significant impact upon the development or progression of DPN. To our knowledge, data regarding the nutritional intake of persons residing in Manitoba, Canada with DM2 and DPN is unknown. This study will help to expand our understanding of, and define the nutritional intake of this patient population. Ultimately, this information may be able to influence the nutritional behaviors of persons with DM2, and may optimistically play a role in the prevention, development or progression of DPN.

3.4 Participants

Participants for this study were persons with DM2 with DPN and persons with DM2 without DPN. They were recruited from the Diabetic Foot and Complicated Wound Clinic located at the Health Sciences Centre, Winnipeg, Manitoba, as well as Endocrinology Clinics located at the Health Sciences Centre and the Winnipeg Clinic, also both located in Winnipeg. Participants were selected based upon criteria that were predetermined. The study protocol received approval from The University of Manitoba Health Research Ethics Board.

3.5 Data Collection

Data were obtained through the following means: questionnaires for assessing nutritional intake, demographics, laboratory parameters, medication use, medical history and physical activity. A Semmes-Weinstein 10-gram Monofilament was used to perform the lower extremity examination. A semi-quantitative FFQ was used to obtain nutritional data.

4.0 Research Methods

4.1 Participants

4.1.1 Sample Size

Based upon crude observations of the Diabetes Education Centre, Health Sciences Centre, Winnipeg, Manitoba, it is presumed that approximately 20% of persons presenting with DPN will have an adequate diet compared to approximately 60% of those without DPN. With a sample size of 60 participants per group, we will have at least 80%

power to detect an odds ratio of 2.5 with a level of significance of $p < 0.05$. The sample size calculation was undertaken by Ms. M. Cheang, Biostatistical Consulting Unit, University of Manitoba, Winnipeg, Manitoba.

4.1.2 Study Population

Sixty participants (30 cases and 30 controls) between the ages of 40 and 75 years were recruited for this study. The original goal for recruitment was a total of 120 participants (60 cases and 60 controls). However, due to the constraints of recruiting study participants, this original number proved implausible for the given time frame and thus the goal was reduced to half of the original total (30 cases and 30 controls) but our power was unchanged. When analysis of the FFQs was completed, seven questionnaires from the control group and six questionnaires from the case group were either incomplete, or had to be eliminated from analysis due to implausible results. The resulting total sample size was then 47. The final number of FFQs was determined through a plausibility analysis which was undertaken by M Jabbour, Dt.P. from the Assistante de recherché Institut universitaire de gériatrie de Montréal located in Montreal, Québec, Canada.

4.1.3 Inclusion Criteria

4.1.3.1 Cases

Any persons with the following criteria were invited to participate in this study:

- Caucasian adults.
- 40 - 75 years of age.
- DM2.

- Confirmed presence of DPN.
- Resident of Manitoba.
- Able to communicate verbally and non-verbally in the English language.

4.1.3.2 Controls

- Caucasian adults.
- 40 - 75 years of age.
- DM2.
- Confirmed absence of DPN.
- Resident of Manitoba.
- Able to communicate verbally and non-verbally in the English language.

4.1.4 Exclusion Criteria

Any persons with the following criteria were not invited to participate in this study:

- Of descent other than Caucasian.
- < 40 or > 75 years of age.
- DM1.
- End stage renal disease on hemodialysis or peritoneal dialysis.
- Any known cancers (except non-melanoma skin cancer).[66]
- Known vitamin B12 deficiency.
- A current unresolved neurological disorder other than DPN.
- Current chronic alcoholism (> 2 drinks/day everyday for males; > 1 drink per day everyday for females).[4]

- Pregnant/lactating.
- Unable to communicate both verbally and non-verbally in the English language.

4.1.5 Recruitment Strategy

The principal investigator and the research assistant recruited cases from the Diabetic Foot and Complicated Wound Clinic located at the Health Sciences Centre, and controls from Endocrinology Clinics located at either the Winnipeg Clinic, or the Health Sciences Centre. All clinics were located in Winnipeg, Manitoba

Advertisements (approved by the University of Manitoba Health Research Ethics Board) were placed in the waiting rooms of the clinics, and were also published in the Health Sciences Centre weekly General Notices. Clinic staff inquired of the clinic attendees whether they had numbness and loss of feeling in their feet and whether they would be interested in discussing the study protocol with the investigators. The study protocols were then explained to the potential study participant by the research assistant in a private room. The research assistant then obtained written informed consent from the individual who then participated in the study protocol.

To ensure the anonymity/confidentiality of all participants, each participant was assigned a participant number. Cases were represented as 'case # _' and controls were represented as 'control # _'. Participants who completed the requirements of the study in full received a $20 gift card for a local Supermarket.

4.2 Ethics Committee Approval

Ethics approval for this study was obtained from the University of Manitoba Health Research Ethics Board.

4.3 Materials

The FFQ was obtained from Chercheure, Centre de recherche, Institut universitaire de gériatrie de Montréal Montreal, Québec, Canada. This 16 page, 73-item semi-quantitative FFQ was adapted from the Block National Cancer Institute Health Habits and History Questionnaire.

4.4 Data Collection and Analysis

4.4.1 Demographics and Physical Characteristics

This data was collected for the purposes of defining the patient population and ensuring that persons with any of the exclusion criteria were not included in the study. The demographic questionnaire obtained data such as: age, gender, type of employment, income level, financial situation, and highest level of education completed. The height, weight, and body mass index (BMI) value were also obtained.

The demographic data was obtained through a brief, ten minute interview which took place at the commencement of the study between the participant and the principal investigator (C. Ross) or the participant and the research assistant (T. Bzura).

The height and weight of the participants were obtained using standard methods, a stadiometer and scale, respectively. Mean values were obtained and comparisons were made between the cases and the controls by chi-square analysis as well as t-tests to determine if differences were present between the two groups and allowed us to illustrate the characteristics of our patient population. Values were represented as mean height and weight ± standard deviation (SD), mean BMI value ± SD and mean duration (years) of DM ± SD. The BMI values were calculated based upon the formula:

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

4.4.2 Laboratory Parameters

Laboratory parameters included serum lipid values (LDL cholesterol, HDL cholesterol, total cholesterol) and HbA_1c, a marker of glycemic control, were collected for the purpose of determining the level of lipidemic and glycemic control by the participants. The mean values were determined for the serum lipid values, and were then compared to the CDAs guidelines which include maintaining LDL cholesterol levels of ≤ 2.0 mmol/L, and maintaining total cholesterol to HDL cholesterol ratio of < 4.0.[75] Mean HbA_1c levels were determined for each group and were also then compared to the CDAs guidelines. The laboratory parameters were obtained through the participants' family physicians. Fax requests were sent out to the respective physician's offices requesting the information. Patients consented to us obtaining this information when the informed consent was initially obtained.

4.4.3 Medication Use

Information regarding participant medication use was obtained to determine the level of control our participants had on their serum lipid and blood glucose levels. The frequency of participants in each group using and not using insulin, oral hypoglycemic agents, and antihyperlipidemic agents was determined. This information was obtained during the initial face to face interview with the participant. Participants simply answered 'yes' or 'no' as to whether or not they were currently using the specified medications (insulin, oral hypoglycemic agents and oral antihyperlipidemic agents). This information was collected to better understand the control (or lack thereof) that participants had on their blood glucose and lipid levels, as at elevated levels, both are risk factors for DPN.

4.4.4 Medical History

Co-morbidities that may develop as a result of DM2 include ophthalmologic cardiovascular and renal complications. Therefore, data obtained regarding additional co-morbidities of participants was obtained through the initial face to face interview and included history of myocardial infarction and current ophthalmologic (retinopathy) difficulties. The status of dentition (natural teeth, edentulous, dentures) was also considered. Participants were required to answer 'yes' or 'no' for all co-morbidities. This information was obtained through the initial face to face interview with the participant as well. Information was self reported. This information was obtained to determine the frequency of previous complications of DM in each group, and to further classify the participants. Values were represented as duration (years) of DPN (if applicable), the frequency of participants with a history of myocardial infarction,

ophthalmologic complications, and denture use, previous nutrition education, duration of nutrition education, vitamin/mineral supplement use and frequency of vitamin/mineral supplement use. Information regarding nutrition education was collected to help us obtain an understanding of the level of nutrition knowledge the two groups had.

4.4.5 Physical Activity

This portion of the questionnaire has also been validated on persons between the ages of 18 and 82 with various educational backgrounds[70] and was developed by a Québec based government program for the purpose of promoting physical activity called Kino-Québec.[76] The purpose of this aspect of the questionnaire is to classify individuals into physical activity levels (PALs), specifically, light, moderate, and heavy intensities.[76] These PALs are based on the World Health Organization's (WHO) recommendations for physical activity.[76] Missing information was obtained by the investigator/research assistant. Data on the frequency and duration of physical activity was obtained from the participants in order to be able to assess the energy requirements for the participants, as well as to obtain a snapshot of the physical abilities of the two groups. Participants were asked about the frequency of low, moderate and high intensity physical activity that they participate in. Low intensity included activities such as walking at a normal pace and golf. Moderate intensity activities included swimming and badminton. High intensity activities included running and hockey. The data on physical activity was analyzed by the principal investigator. The percent of participants falling into each PAL was determined.

4.4.6 Status of Lower Extremities

The examination acknowledged the signs of DPN including loss of sensation and a brief examination with a Semmes-Weinstein 10 gram monofilament.[24] The Semmes-Weinstein monofilament is composed of calibrated nylon monofilaments that has the ability to generate reproducible pressure and is commonly used to diagnose DPN.[24] The most widely used monofilament is the 5.07 which requires a force of 10 grams to bend.[24] It is thought that this same amount of force will be required to cause damage to the respective tissue(s).[24] For this component of the study, participants were required to remove footwear and stockings. While in a sitting position and with their eyes covered (to ensure they could not witness what parts of the foot the monofilament was applied to), the monofilament was applied to three areas of the foot (specifically the pulp space of the first toe, as well as the first and fifth metatarsal heads), with three repetitions on each area.[24] When insensitivity to the monofilament was detected at one or more sites on more than one repetition, this constituted the presence of DPN.[24]

4.4.7 Nutrition

Following collection of the additional parameters and completion of the physical examination, the participants were instructed on how to appropriately and accurately complete the sixteen page, 73-item semi-quantitative FFQ. To ensure the participant understood the instructions as clearly as possible, the principal investigator or the research assistant read the instructions for the FFQ (located on the first three pages of the FFQ) aloud to the participant, while the participant followed along on the hard copy. The principal investigator or research assistant then briefly reviewed the remainder of the

FFQ with the participant in order to provide them with a feel for the length and requirements of the FFQ.

The FFQ contained questions regarding the frequency of consumption as well as the portion size of 73-items that were amalgamated into groups based upon similar nutrient/meal composition. For example, groups included, 'breads and cereals and related foods' and 'meat and poultry'.

Each question consisted of two components; 1) frequency of consumption, and 2) portion size of foods consumed. Participants were instructed to start with the initial question, and to first answer the frequency component of that question. Participants were instructed to answer each question based upon their typical frequency of intake over the preceding twelve months. Frequencies ranged from 'never or rarely' to '2 times or + per day'. For example, 'commercial sliced whole wheat breads', if the participant typically consumed this item one time per day, they would then select 'once per day' and so forth. Following completion of the frequency component, participants were instructed to continue directly horizontally to the portion size component of the question.

Portion sizes for the food items varied accordingly with each food group. However, every question was identical such that the participant was responsible for determining whether the typical portion size they consumed at a single sitting was 'smaller than', 'similar to', or 'larger than' the sample portion size(s) provided. Food items which had easily identifiable portion sizes had a single portion size denoted. For example,

‘commercial sliced whole wheat breads’, the sample portion size is easily identifiable and in this case was ‘1 to 2 slices’. For foods in which portion sizes were not easily identifiable, sample portion sizes were denoted in two forms, both of which represented identical sizes. For example, ‘high fiber breakfast cereals’, the sample portion size was denoted as both ‘125 to 500 mL’; and ‘½ to 2 cups’. This was to ensure the FFQ was as user friendly as possible. Participants were instructed to answer each question based upon their typical portion size at a single sitting over the preceding twelve months. If their typical daily intake was ‘smaller than’ the smallest sample portion size, they would fill in the ‘smaller’ response. If their typical daily intake fell anywhere ‘within’ the sample portion sizes provided, they would fill in the ‘similar’ response. If their typical portion size was ‘larger than’ the larger portion, they would fill in the ‘larger’ response. To illustrate this process, use the ‘commercial sliced whole wheat bread’ example. If the participant typically consumed ‘less than’ ‘1 slice’ of bread at one sitting, then they would fill in the ‘smaller’ response. If the participant typically consumed ‘between’ ‘1 and 2 slices’ of bread at one sitting, they would fill in the ‘similar’ response. And if the participant typically consumed ‘more than’ ‘2 slices’ of bread at one sitting, they would fill in the ‘larger’ response.

Sample portion sizes were depicted as black and white photographs located on the opposing pages for select food items. Food items depicted as photographs would demonstrate both the ‘smaller’ and the ‘larger’ portion size and corresponded to the written portion sizes. In the exact method used when the participant opted to use the written portion sizes, participants had to select if their typical portion size at one sitting

was ‘smaller than’ the smaller photograph, ‘within’ the two photographs, or ‘larger than’ the larger photograph, and answer accordingly. Participants were encouraged to refer to the photographs when available to assist in the clarification of portion sizes and to ease participant burden in determining typical portion size. The photographs were adapted from the American Dietetic Associations (ADA) “Portion Photos of Popular Foods”[77], and the SU.VI.MAX food portion photo manual.[78]

Once the participants had completed both the frequency and portion size components of the first question, they were instructed to complete the remainder of the FFQ in the same manner.

When the explanations of the FFQ were complete, the principal investigator or the research assistant then completed the first two questions from the FFQ with each participant so they would be familiar with, and feel comfortable in completing the FFQ independently. Additional questions were completed with the participant if the investigator felt they would benefit from further explanation. Participants were instructed to complete each response with pencil so that the investigator would be able to interpret their response and so any potential errors in the completion process could be easily be rectified by the participant. The principal investigator/research assistant instructed each participant to answer each question as honestly as possible, and to reflect carefully about their typical intake. Participants had the option of completing the FFQ in a private room in their respective clinic setting, or if they were unable to stay for the predetermined amount of time, taking the FFQ home to complete. This was determined following

completion of both the informed consent and the lower extremity examination. If the participant chose to remain in their respective clinic setting, to prevent bias, the principal investigator or research assistant left the participant to complete the FFQ in the private room. The principal investigator/research assistant was available outside the room to answer questions at any time. After approximately five to ten minutes, the principal investigator/research assistant inquired of the participant if they had any questions regarding the FFQ. The principal investigator/research assistant would then inquire of the participant one additional time to ensure the participant was comfortable with the completion of the FFQ. When the FFQ was complete, participants were instructed to bring it out of the room to the principal investigator/research assistant.

If the participant opted to complete the FFQ at home, they were still instructed on the accurate completion of the FFQ in the same manner as the participants that opted to remain in their clinic setting. Participants were then given a self addressed, stamped envelope along with their FFQ and requested to return the FFQ within ten days.[70] The participant was given the contact information for the principal investigator and instructed to contact the principal investigator if they had any questions or concerns. If the participant did not return the FFQ within the allotted ten day time period, the research assistant contacted the participant by telephone to remind them that the FFQ was to be returned as promptly as possible. Regardless of the setting in which the FFQ was completed, each FFQ should have taken approximately 30 to 45 minutes to complete (depending upon the pace of the participant). FFQs were checked and any information that was either missing, or required clarification from the participant was attempted to be

clarified by either the principal investigator or the research assistant in person or by telephone.

Because the data obtained from the FFQ encompassed each participant's intake over the previous twelve months, the nutrition information obtained was reflective of weekdays, weekends, special events, holidays and all four seasons.[70] This compilation of information allowed the principal investigator to obtain information about each participant's typical nutritional intake.[70]

Prior to commencement of the study, both the principal investigator and the research assistant were extensively trained by the researchers who created the FFQ in how to accurately explain the instructions for the FFQ to the participant. They were also trained on how to properly conduct the lower extremity examination by a physician.

The nutrition data was entered by the principal investigator into an Access file. The data entry was completed in the same manner in which the participant completed the FFQ. Starting with the first food, the principal investigator entered the frequency component of the question and then the portion size component of that same question using the numeric key pad on the keyboard. Each numeral represented a different frequency. For example, '1' represented 'never or rarely', '2' represented '1 to 7 times per month' and so forth. For portion size data entry, '-', '/' and '+' were used to represent 'smaller', 'similar' and 'larger' portion sizes respectively. For the questions included at the end of the FFQ such as vitamin/mineral supplement use, the mouse was used to check off the appropriate

response. This process was done for every question in every FFQ. Once every question from the FFQ was complete, all of the data was entered into the Access file a second time by the investigator for verification purposes. The same process was used during the second entry. After both sets of entries were completed, the data was verified. During this process, the computer program verified every question to ensure there were no discrepancies between the two entries. If there were discrepancies, the principal investigator then verified with the hard copy of the FFQ to determine which entry required correction. The correction was then made in the verification section of the program. The two entries were then verified a second time to ensure all discrepancies were corrected. The data from the Access program was then automatically exported into an Excel document which listed both the micro- and macronutrients in their respective units. The Excel files were then examined along with the hard copies of the FFQs by a research dietitian who determined the plausibility of the nutrient intakes using DietSys software (National Cancer Institute [NCI] Dietary Analysis system, version 4.01).[70] From this plausibility report, we then knew which FFQs to eliminate from analysis. FFQs that we eliminated from analysis included a.) those that had < 10% of the questionnaire completed; and b.) those that had a total energy intake of ≤ 800 kcal or ≥ 4000 kcal.[70] Individual questions that were omitted from analysis included a.) those with blank frequencies (as they were considered to be incomplete); and b.) those with more than one portion size or frequency denoted.[22] For responses that did not include a portion size, the smallest portion size was assumed in order to avoid over estimation.[81] Nutrients were expressed in terms of the units represented by the DRI values and were based upon data from the Canadian Nutrient File and the United States Department of Agriculture

food composition database.[70] The majority of the nutrients in the nutrient database were identical except for sodium and potassium which were converted from 'milligrams per day' to 'grams per day' in order to correspond with the units used by the DRI values.

The mean intake of all nutrients was then determined for both groups of participants. The group means were then compared to the DRI values and the prevalence of inadequacy for nutrients with an EAR value was calculated. The prevalence of inadequacy for each nutrient with an EAR value was determined by calculating the number of participants in each group *at* and *below* the EAR value and dividing this number by the total number of participants in that group. This method is known as the EAR cut-point method.[25] The mean intake of each age and gender group was then determined and chi square analysis was then used to determine if there were differences in terms of group inadequacy between the cases and controls.

For micronutrients without an EAR value and dietary fiber, group intakes were compared to the respective AI values. However, AI values were established for these nutrients on the premise that an EAR value could not be established and it is not possible to determine the prevalence of inadequacy of these nutrients.[25] The only assumptions that can be made are that if the mean intake of the nutrient is at or above the AI value, it can be assumed that the prevalence of nutrient inadequacy in that group is low.[25] If the mean group intake is below the AI, it is not possible to make assumptions about the prevalence of inadequacy.[25]

4.5 Rationale for Food Frequency Questionnaire

The FFQ was selected for this study for the following reasons:

- The questionnaire can generally be completed quickly and independently by the participants as they are designed to be user friendly,[69]
- Bias from the researchers can be reduced as they were not present for the completion of the FFQ.
- This FFQ was relatively simple for the participants to complete.
- This FFQ has been validated on individuals of both genders between the ages of 18 and 82 years and on persons of various educational backgrounds.[70]
- The FFQ has been used in previous research on the topic of nutrition, DM2, and its complications.[79]

It is important to note, however, that this FFQ has not been validated in our particular study population, but it has been validated previously in other similar populations of persons with DM2.[70]

4.5.1 Advantages and Limitations of the Food Frequency Questionnaire

There are several advantages to the FFQ used for this study, which are summarized as follows:

- The FFQ is very user friendly,
- Photographs of sample portion sizes were included in order to assist participants in completing the FFQ as accurately as possible,

- This FFQ measures amount of fat in food preparation which allowed the principal investigator to obtain as much detailed information as possible about the amount of fat included in the diet,
- The FFQ took all four seasons and holidays into account which allowed for collection of nutrition data throughout the entire twelve months and included foods that the participant might not consume on a daily basis, but may consume more frequently at certain times of the year.

There were numerous methods to increase the accuracy of the participant's responses and reduce reporting errors, including[70]:

- A complete set of instructions that enabled the participants to complete the FFQ independently,
- Detailed explanations of the food items,
- When required, portion sizes were presented in two forms,
- Photographs of sample portion sizes were available for select food items.

There were, however, potential limitations to the use of this FFQ, which are summarized as follows:

- FFQs rely on memory, and may be more complex for certain individuals,
- Users must be able to read and write in English which potentially lead to the exclusion of participants that may have been valuable to this study.

4.5.2 Modifications to the Food Frequency Questionnaire

Some minor modifications were made to the FFQ which included, the addition of the University of Manitoba emblem to the cover page, and the addition of simple sugars, selenium and trans fatty acids to the nutrient analysis database to ensure we were capturing data on as many relevant nutrients as possible.

5.0 Data Management

The data collected for the purposes of this study were stored on a computerized database. When the computer was not stored in the Medical Records Office of the Health Sciences Centre, it was maintained in a locked study office, MS 673 or MS7 of the Health Sciences Centre, Winnipeg, Manitoba. Data will be kept until the final report (thesis) is completed and the manuscript has been published in a peer reviewed journal. It is anticipated that this process will likely take two years from the end of the time of data collection. After the manuscript is published, and the time required by the respective journal to keep documentation from the study has passed, all paper documents will be shredded and disposed of in confidential waste, and the computer database will be purged. Only persons who are immediately involved in the study will have access to any of the information obtained.

6.0 Statistical Analysis

Statistical analysis was undertaken by Ms. M. Cheang, Biostatistical Consulting Unit, University of Manitoba. Chi-square analysis was used to determine the nutrient intake of the two groups that deviated from the expected (DRI) values. However, due to the small number of participants in each life stage and gender group, the nutrients with an AI value were only analyzed by stating whether or not each group met the recommendation or not. It was not possible to detect statistical significance for these nutrients. Chi-square analysis was also used to determine differences between the two groups in terms of: previous nutrition education; duration of nutrition education; vitamin/mineral supplement use; medication use; history of myocardial infarction, ophthalmologic complications and denture use; presence or absence of DPN; education level; type of employment; household income; financial situation; gender; place of residence; physical activity; and nutritional intake.

T-tests were used to determine if there were differences between the two groups in terms of diabetes duration, age, height, weight and BMI values. For each test, $p < 0.05$ was considered significant.

7.0 Results

7.1 Demographics and Physical Characteristics

Both chi square analysis and t-tests were used to compare the two groups in terms of demographics and physical characteristics ($p < 0.05$). A total of 60 participants were recruited for this study. Table 1 summarizes the demographics and physical

characteristics for the study subjects. Differences were not detected in terms of height, p=0.08; weight, p=0.09; or BMI, p=0.45 A statistically significant difference between the two groups in terms of gender, education, place of residence, annual household income and perception of financial situation was also not observed.

7.2 Laboratory Parameters

Due to suboptimal response from the participant's physicians, there was an insufficient amount of data to report on the laboratory parameters.

7.3 Medication Use

Chi square analysis was also used to determine differences between the two groups in terms of medication use. Results showed that 57% (17) of cases reported they were currently taking insulin compared to 47% (14) of controls, p=0.44. Results also showed that 76% of cases (22) reported they were currently taking oral hypoglycemic agents compared to 90% (27) of controls, p=0.15. It was also determined that 66% of cases (19) reported they were currently using antihyperlipidemic agents compared to 73% (22) of controls, p=0.52. Table 2 summarizes the results for medication use.

7.4 Medical History

Chi square analysis was used to determine differences between the two groups in terms of medical history. Table 3 summarizes the medical history of the study participants. With regards to the duration of DM, the cases had a longer mean duration of diabetes (17.5±9.1

years), compared to the subjects with DM2 without neuropathy (11.0 ± 7.6 years), p=0.003. The mean duration of DPN was 7.0 ± 7.8 years.

Differences were not observed between persons with and without DPN in terms of previous myocardial infarction, and ophthalmologic complications, p=0.10. Denture use between the two groups was comparable, p=0.07. Previous nutrition education, p=0.51; duration of previous nutrition education, p=0.59; and vitamin/mineral supplement use, p=0.50 also did not show any significance.

7.5 Physical Activity

Chi square analysis was also used to detect differences between the two groups in terms of physical activity. Table 4 summarizes the participants' reported levels of physical activity. Differences were not detected between the two groups in terms of participation in low intensity physical activity which included activities such as walking at a regular speed, p=0.16; or high intensity physical activity including activities such as running, p=0.08. However, it is important to note that a difference existed between the study participants in terms of moderate intensity physical activity including walking at a quick speed, with the persons with DPN participating in this intensity of physical activity less often, p=0.03.

7.6 Nutritional Intake

7.6.1 Micronutrients

7.6.1.1 Nutrients with Estimated Average Requirements

Table 5 summarizes the results for nutrients with an EAR. For each nutrient, group means and SD for both the cases and controls were first calculated based upon gender and age group. This division took place because a number of nutrients have different daily requirements based upon gender and/or age group. The prevalence of inadequacy was then determined for the cases as a whole and for the controls as a whole and prevalence of inadequacy was compared using chi square analysis. The level of significance was defined as $p < 0.05$.

Overall, the prevalence of inadequacy was relatively low in both groups. There were no differences between the two groups in terms of prevalence of inadequacy for the following nutrients: vitamin C, vitamin E, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, copper, magnesium, phosphorus, selenium or zinc.

Both groups were found to have a moderate prevalence inadequacy for the following nutrients: 1) vitamin C, cases 33% and controls 38%, p=0.92; 2) thiamin, cases 33% and controls 38%, p=0.92; 3) folate, cases 46% and controls 48%, p=0.17; and 4) magnesium cases 67% and controls 48%, p=0.41.

Both groups showed a high prevalence of inadequacy for vitamin E, cases 88% and controls 91%, p=0.67.

Differences between the two groups was detected in the prevalence of inadequacy of vitamin A with a moderate prevalence of inadequacy found in the cases, 38% compared to a low prevalence of inadequacy seen in the controls, 9%, p=0.02.

The micronutrient with the highest prevalence of inadequacy was the same in both groups and was vitamin E, (cases, 88% and controls, 91%). Cases had a higher prevalence of inadequacy for vitamin A (38%), riboflavin (4%), vitamin B12 (13%), magnesium (67%), selenium (4%) and zinc (17%) compared to controls. The investigator used < 30% as the cut-off for low prevalence of inadequacy; between 31% and 70% for the cut-off for moderate prevalence of inadequacy; and > 70% as the cut-off for high prevalence of inadequacy However, statistical differences between the two groups were only found for vitamin A.

7.6.1.2 Nutrients with an Adequate Intake

Complete results for nutrients with an AI can be found in Table 6. The mean intakes for dietary fiber were well below the AI for both groups for both men and women, specifically all cases (n=24) and 17 controls, except for female controls in the 51 – 70 + age category. The mean intakes of calcium were well below the mean for both male cases and controls in the 51 – 70 and 70 + age categories. Mean intakes of calcium were also well below the AI for female cases in the 51 – 70 age category and for female controls in the 70 + age category. Both groups were well below the AI for potassium. The mean intake for males in the 40 – 70 + age category in both groups were well below the AI for vitamin K. Males in both groups in the 51 – 70 and 70 + age categories had

mean intakes of vitamin D well below the AI. Female cases in the 51 – 70 age category also had a mean intake well below the AI. The female control in the 70 + age category had a mean intake well below the AI as well. Both cases and controls had mean intakes of water well below the AI.

7.6.2 Macronutrients

The recommendations for macronutrients are expressed as percentages of total energy intake. The DRI value for macronutrients are expressed in terms of ranges and therefore have both a lower and a higher value. To assess each group's intake of macronutrients, first each group's mean intake was established. The percent of each group that was below and above the recommendations for the macronutrients was then calculated. Chi square analysis was used to determine if there were differences between the two groups, $p < 0.05$.

Complete results for the AMDRs can be found in Table 7. Significant differences were observed between the two groups in terms of total fat intake, which had the greatest proportion of persons being above the AMDR for both groups; however, cases had a greater proportion lying above the AMDR, 54% compared to the controls, 17%, p=0.009. Significant differences were also detected between the two groups in terms of linoleic acid with the proportion of cases lying below the AMDR being 4% compared to 39% of controls, p=0.005. Significant differences were found between the two groups in terms of total carbohydrate intake. However, the cases had a greater proportion of persons lying *below* the AMDR 67% compared to the controls 33%, p=0.01.

8.0 Discussion

The primary objective of our study was to compare the nutritional adequacy of persons with DM2, with and without DPN by the following means:

a) determining the prevalence of inadequacy for nutrients with an EAR value,
b) determining the proportion of persons laying outside the recommended AMDR value for macronutrients, and
c) examining the mean intake of nutrients with an AI value and comparing the mean intake of each group to the AI.

While a number of nutrients were found to have a moderate to high prevalence of inadequacy in persons with DM2 with and without DPN, our results showed fewer differences between the two groups than we had previously anticipated.

8.1 Demographics and Physical Characteristics

The majority of participants in both groups were male. The mean age of the cases was slightly older than that of the controls although this did not reach significance. Differences in height and weight between the two groups did not reach significance, $p=0.08$, $p=0.09$ respectively. However differences did approach significance with the average height of the cases being taller and the average weight of the cases being heavier. Differences were also not detected between the two groups in terms of BMI with the mean BMI of both groups falling in the grade II obesity category ($BMI \geq 30$)[68], $p=0.20$. The majority of both groups reported residing in an urban setting; however, differences were not found between the two groups, and theoretically, all participants would have equal access to locations from which food can be obtained. Both groups also reported

completing comparable levels of education, secondary school or grade 12. This means that both groups were theoretically equal in terms of education levels and results were not skewed with one group having a greater number of persons with higher education levels than the other. A greater percentage of controls rated their financial situation as 'sufficient to meet their basic needs', although the cases reported having an average annual household income higher than that of controls.

8.2 Laboratory Parameters

As previously mentioned, we were unable to collect sufficient data to report on the laboratory parameters for this study.

8.3 Medication Use

There were no differences found between the two groups in terms of medication use. The majority of participants in both groups were taking oral hypoglycemic agents as well as antihyperlipidemic agents.

8.4 Medical History

In terms of duration of DM, the participants with DPN had a longer mean duration of DM2 compared to those without DPN, $p=0.003$ which was to be expected since duration of DM is a risk factor for DPN.[10] No differences were detected between the two groups in terms of previous myocardial infarction, $p=0.45$, or ophthalmologic complications, $p=0.1$. Differences approached significance with denture use, $p=0.07$. This displays the similarities of our two groups.

In terms of previous nutrition education and duration of nutrition education, there were no differences detected between the two groups. The majority of both groups reported that they had in fact received nutrition education, and the majority of both groups also reported that they had received it on more than one occasion. This was an important finding because this suggests that both groups have received instruction on nutrition and diabetes management, and that one group was not disadvantaged by not having received guidance about DM2 management compared to the other group of participants. We also did not find differences between the two groups in terms of vitamin/mineral supplement use, however, a greater percentage of controls reported taking a vitamin/mineral supplement on a daily basis compared to the cases.

8.5 Physical Activity

Differences between the two groups were not observed in terms of low intensity physical activity. The greatest percentage of cases reported participating in activities of low intensity such as walking at a normal speed 'never or occasionally' while the greatest percentage of controls reported participating in low intensity activities '4 times or more per week'. We did however find a difference between the two groups in terms of activities of moderate intensity such as walking at a quick speed. The vast majority of cases and controls reported that they 'never or occasionally' participated in activities of this intensity. However, a greater proportion of controls reported participating in activities of this intensity 'one or more times per week' compared to cases. Not surprisingly the two groups approached significance in terms of high level of physical activity. The vast majority of cases and controls both reported participating in activities

of high intensity such as running 'never or occasionally', however, a greater percentage of the controls reporting participating in high intensity activities 1 - 3 times per week. Overall, the controls participated in more physical activity (albeit very little) as compared to the cases which was to be expected. This finding was not unanticipated as persons with DPN have been advised to minimize trauma to their feet, and to avoid ambulation, and therefore do not participate in the same level of physical activities as persons without DPN.

8.6 Nutritional Intake

The method of dietary recall used for this study was a semi-quantitative FFQ. This method was appropriate for our sample population because it is especially user friendly due to the presence of photographs to assist in estimation of portion size. Additionally, the FFQ required a relatively low level of respondent burden. These factors were of vital importance and assisted our patient population in completing the FFQ as accurately as possible and also helped us to achieve our revised recruitment goal. When participants were informed of the relatively minimal time commitment involved in completing the FFQ, this frequently became a deciding factor for them, and they were then willing to participate in our study. Minimal respondent burden also helped us ensure an adequate rate of return for the participants who opted to complete the FFQ at home. The nutrient intake of the participants was then compared to the DRI values and nutritional adequacy was determined.

This study illustrated that a large proportion of cases and controls had mean intakes of several nutrients well below the recommended DRI values, and found significant differences for vitamin A, total fat, linoleic acid and carbohydrate. Both groups could potentially be at risk for inadequate intakes of vitamin A, vitamin C, vitamin E, thiamin, folate, magnesium, zinc, total fat, α-linolenic acid, carbohydrate, dietary fiber, calcium, potassium, vitamin K, vitamin D and water due to levels of consumption lower than their respective DRI value.

More specifically, cases are potentially at risk for inadequate intakes of vitamin A, vitamin C, vitamin E, thiamin, folate, magnesium, dietary fiber, calcium, potassium, vitamin K, vitamin D, water, α-linolenic acid and carbohydrate. Similarly, controls are also potentially at risk for inadequate intakes of vitamin C, vitamin E, thiamin, folate, magnesium dietary fiber, calcium, potassium, vitamin K, vitamin D, water, α-linolenic acid and carbohydrate. However, controls are also potentially at risk for inadequate intake of linoleic acid. Our results only detected significant differences between persons with and without DPN in terms of vitamin A, total fat, linoleic acid and carbohydrate.

8.6.1 Nutrients with an Adequate Intake

In terms of cases, the mean intake of dietary fiber for all life stage groups were below the AI with notably low intakes in males in the 40 – 50 life stage group and as previously mentioned, dietary fiber assists in the maintenance of adequate blood glucose levels. Therefore, decreased dietary fiber intake may be a contributing factor in the development and or progression of DPN. Males in the 51 – 70 + life stage group, as well as females in

the 51 – 70 life stage group had mean intakes of calcium below the AI with notably low intake of the single male in the 70 + life stage group. All life stage and gender groups had mean intakes below the AI for potassium with particularly notably low mean intake in females. Males in the 40 – 70 life stage group had mean intakes of vitamin K below the AI. Males in the 51 – 70 + life stage group had mean intakes of vitamin D below the AI with particular low intake in the single male in the 70 + life stage group. As well, all life stage groups had mean intakes of water below the AI with notably low intake in males.

In terms of controls, the mean intake of dietary fiber for all life stage and gender groups except females in the 51 - 70 + life stage group were below the AI with particularly low intake seen in males in the 40 – 50 life stage group. Males 51 – 70+ and the single female in the 70 + life stage groups all had mean intakes of calcium below the AI with notably low intake found in the single female in the 70 + life stage group. All life stage and gender groups had mean intakes below the AI for potassium. All life stage groups for the males had mean intakes well below the AI for vitamin K. Males in the 51 – 70 + and the single female in the 70 + life stage groups had mean intakes below the AI for vitamin D with considerably low intakes for the female in the 70 + life stage group. All life stage and gender groups had mean intakes of water below the AI with notably low intakes in males.

As previously discussed, there is a relative paucity of research on vitamin D and DPN and the research that is available is not conclusive. It has however been suggested that

decreased vitamin D intake may potentially lead to the development of DPN. Our results showed that a greater number of cases had mean vitamin D intakes that did not meet the AI value compared to controls. This coincides with the notion that vitamin D intake and DPN are perhaps related.[48] If any association exists between calcium, potassium and vitamin K and DPN, this still needs to be investigated.

A mean group intake below a nutrient's respective AI value, however, does not mean that the group is not meeting their nutrient requirement.[25] A mean intake below the AI simply implies that the persons with intakes below the AI are at an increased risk of not meeting their daily nutrient requirements. Increased intakes of these nutrients may be warranted in the specified groups to prevent nutrient inadequacy.

8.6.2 Nutrients with as Estimated Average Requirement

8.6.2.1 Vitamin A

The prevalence of inadequacy determines the proportion of each group that is lying below the EAR value and means that this proportion of the group may be at risk of not meeting their daily requirements for that particular nutrient. To our knowledge, previous research has not been conducted on the topic of vitamin A and DPN. Our results indicated that a moderate proportion of cases (38%) may be at risk of consuming inadequate amounts of vitamin A. This is compared to a lower proportion of controls (9%). It is important to note that other investigators have stated that diabetic neuropathy is a condition which occurs in various stages, and that neuronal damage can occur from and be accelerated by oxidative stress.[83] It is possible that vitamin A, specifically beta

carotene, assists in the reduction of oxidative damage caused by reactive oxygen species. Controls had a lower prevalence of inadequacy compared to cases and may exemplify the possible antioxidative abilities of vitamin A in relation to the prevention of DPN. It is our belief that this is the first study to examine vitamin A intake in persons with DM2 with and without DPN however, the relationship between vitamin A and DPN has yet to be defined.

8.7 Nutrients with an Acceptable Macronutrient Distribution Range

Our results show that there were differences between cases and controls in terms of certain aspects of their macronutrient distribution. When a proportion of a group does not meet the AMDR for a macronutrient, they are potentially at risk of not meeting their daily requirement for that macronutrient and are potentially at risk for any negative effects associated with under consumption of that particular macronutrient.[25] Conversely, when the proportion of a group exceeds the AMDR, this proportion is potentially at risk for any negative effects associated with over consumption of that particular macronutrient.[25] Previous research has shown the immeasurable importance of optimal nutrition in the adequate control of DM2 to prevent the onset of debilitating complications including DPN. Specifically, optimal nutrition can assist in the achievement of adequate blood glucose levels, lipid profiles and in the reduction of oxidative stress. Achievement of optimal nutritional intake requires balance between intake of macronutrients.[25] For example, when the intake of one macronutrient is increased, the intake of the others should ideally be decreased to compensate and

similarly, when the intake of one macronutrient is decreased, the intake of the others should be increased to compensate accordingly.[25]

Our results showed that cases had a significant proportion of persons with mean intakes of α-linolenic acid and carbohydrate below the recommended AMDR. A large proportion of cases exceeded the AMDR for total fat.

Similarly, there were a significant number of controls whose intakes did not meet the AMDR for carbohydrate, α-linolenic acid and linoleic acid. Because our results were similar in both cases and controls, it is possible that the association between the PUFAs and DPN is not as profound as previously thought. It is our suggestion that additional research with more than one method of nutritional data collection be completed to obtain more sensitive results.

Our results revealed that cases (54%) had a greater proportion of persons who exceeded the AMDR for total fat compared to a much smaller proportion of controls (17%), $p=0.009$. Our results also detected a difference in mean carbohydrate intake. Specifically, cases had a greater proportion of persons whose intake of carbohydrate was below the AMDR (67%) compared to controls (30%), $p=0.01$. Of note, however, neither of the two groups had mean intakes of carbohydrate which exceeded the AMDR. Our results also showed that there was a greater proportion of controls (39%) lying below the AMDR for linoleic acid compared to cases (4%), $p=0.005$.

8.7.1 Dietary Fat

Our results revealed that a significant proportion of cases (54%) exceeded the AMDR for total fat. This is in stark contrast to the smaller proportion of controls (17%) exceeding the AMDR. The remaining proportion of participants in both groups had mean intakes of total fat within the AMDR. Neither of the two groups reported having mean intakes of total fat below the AMDR.

Our findings concur with those of other investigators who have shown that mean total fat intake of persons with DM2 was also within recommendations.[84] Overall, their results showed a lower percentage of participants lying within the recommendations for total fat (62%) than controls in our study, but a higher percentage than cases in our study. It has, however, been postulated that differences in mean intakes could be attributed to multiple factors including variations in geographic location, availability of foods and participant preferences.[85]

It has been suggested that persons with DM who are either overweight or obese have a greater risk of developing neuropathy.[33] It is known that high fat diets contribute to obesity.[5] The proportion of persons in our study with DPN who exceeded the AMDR for fat was greater than those without DPN. This finding is in keeping with published reports and may support the suggestion that total fat intake may be associated with DPN.[26-28] Given that the mean BMI values for both cases and controls were within the obese range, we would have expected a greater percentage of participants to exceed the AMDR for at least one of the macronutrients. Again, under reporting may have been a factor.

It has also been suggested that an inverse relationship exists between BMI and insulin sensitivity which may lead to insulin resistance in persons without DM.[86] In persons without DM, it has also been shown that obesity may lead to the decreased sensory action potential of nerves throughout the body which may well lead to the development of neuropathy.[87] Once again this exemplifies the observation that high fat intake may be a contributing variable in the development of DPN. Given these revelations, obesity and its contributing factors may play a significant role in the development of DPN.

Our results are also consistent with those of other investigators who have shown that persons with DM2 consumed an average of 34 – 40% of total calories from fat.[89] Our results revealed that cases consumed a mean intake of total fat of roughly 38% of total Kcal, and controls roughly 33% of total Kcal. We know that saturated and trans fatty acids contribute to elevated levels of LDL cholesterol in the blood which is a known contributing factor for DPN.[25] Sources of saturated fatty acids include high fat meats. When the servings of meat and alternatives per day is considered in our participants, our results showed that both cases and controls consumed daily servings of meat and alternatives above the daily recommendations, however, cases consumed an overall greater number of servings per day 6.0 for males and 5.0 for females, compared to controls, 4.6 for males and 5.7 for females. This could contribute to daily fat intake and helps to solidify our findings that cases had a greater proportion of persons lying above the AMDR for total fat. Diets which are high in total fat may have a potential link to the development or progression of DPN, however, this has yet to be confirmed. The exact

relationship between total fat and DPN remains unclear; however we have postulated a few possible mechanisms.

8.7.2 Linoleic Acid

Our results show that a significantly greater proportion of controls had a mean intake below the recommended AMDR for linoleic acid compared to cases. Our results also show that a greater proportion of cases fall within the recommended AMDR for linoleic acid compared to controls. These results are not unanticipated as cases also had a greater proportion of persons who exceeded the AMDR for total fat compared to controls. Given that cases had higher mean intakes of linoleic acid than controls, perhaps more research is required to determine or redefine the relationship between linoleic acid and DPN.

8.7.3 Carbohydrate

In our study both cases and controls were either below or within the AMDR for total carbohydrate with none of the study participants exceeding the AMDR. A greater proportion of cases (67%), did, however, have carbohydrate consumption below the AMDR compared to controls (30%). It was unanticipated that such a large proportion of the cases would be below the AMDR as suboptimal glycemic control and hyperglycemia are known risk factors for DPN. In terms of added sugars, however, 100% of persons with and without DPN were within the AMDR. We anticipated that a greater proportion of cases would exceed the AMDR for carbohydrate. One reason for our presumption is that increased intake of refined carbohydrate can not only contribute to hyperglycemia, but when consumed in excess amounts, can contribute to an increased BMI, both of

which are risk factors for DPN. Our result could be attributed to underreporting and our small sample size. It has also been suggested that persons with obese BMI levels tend to under report carbohydrate intake[88] and the mean BMI of both persons with and without DPN in our study fell within the obese range. It is possible that we may have seen higher intakes of carbohydrate had our sample size been larger.

Our observation that cases had a greater proportion of persons who did not achieve their AMDR for carbohydrate intake is consistent with the observations of other investigators who have shown that persons with DM2 have lower intakes of carbohydrate than recommended.[90] The recommendations used for their comparison were the guidelines established by the ADA which are very similar to the current DRI values. The ADA guidelines suggest that persons with DM2 should consume 55 – 60% of their daily energy requirements from carbohydrates. Their study showed that only 26% met the guidelines for carbohydrate intake, however, this study only considered the proportion of persons who met the guidelines, and did not take into account the proportion of persons who were either above or below.[92] The purpose of determining the proportion of individuals who lay outside the AMDRs is to determine the risk of negative outcomes that coincide with consuming macronutrients in either inadequate or excessive amounts.

Our findings also concur with other investigators who used 3-day food records to assess nutritional intake and who demonstrated that persons with DM2 consume carbohydrate within recommendations at 49% of total energy.[84] The recommendations they used for comparison were from the Diabetes and Nutrition Study Group of the European

Association for the Study of Diabetes which are similar to the AMDR values. Our results revealed that 67% of the cases consumed less than the AMDR for carbohydrate. Given that a larger proportion of cases reported having intakes of carbohydrate below the AMDR compared to controls, we feel our results may be implausible as it has been shown in numerous studies[14,17,18,20,21] that optimal glycemic control is imperative in the prevention of complications of DM including DPN. Thus, with such a large proportion of cases having their carbohydrate intake below the AMDR, under reporting could be a possibility in this group. Additionally, other metabolic factors may play a major role in the development of DPN.

It was not unanticipated that the vast majority of persons in both groups also did not meet the AI for dietary fiber.[25] Our results showed that all cases had mean intakes of dietary fiber well below the AI. This potentially suggests that these persons are consuming increased amounts of refined carbohydrate which increase blood glucose levels rapidly as opposed to complex carbohydrates which do not raise blood glucose levels as rapidly. Similarly, all persons with the exception of the female controls in the 51 – 70 + life stage group had reported fiber intake well below the AI. When some high sources of dietary fiber are considered, for example, grain products and fruits and vegetables, a greater proportion of cases (75%) reported consuming a lesser number of servings per day of grain products compared to controls (0). When fruits and vegetables are considered, it was noted that a greater proportion of cases (96%) reported consuming a lesser number of servings per day compared to controls (61%). Thus our results showing that cases had a

greater proportion of persons not meeting the AMDR for carbohydrate may in fact be true.

The fact that we did not find numerous differences between cases and controls in terms of prevalence of inadequacy of nutrients with an EAR, proportions of persons with mean intakes of macronutrients above or below the suggested levels the AMDRs and mean intakes of nutrients below the AI values was not unanticipated. With our relatively small sample size (47 FFQs), the power to detect significant differences was decreased. If a larger sample size had been utilized in this study, we may have had the power to detect differences between the two groups.

8.8 Additional Nutrients

Our study found a high prevalence of inadequacy of vitamin E and magnesium in both cases and controls; a moderate prevalence of inadequacy of vitamin B6 and thiamin in both cases and controls; a moderate prevalence of inadequacy of zinc in cases; a moderate proportion of controls with intakes of linoleic acid below the AMDR; and a moderate proportion of both cases and controls below the AMDR for α-linolenic acid. Our results support previous findings which suggest that persons with DM2 may require supplementation of several nutrients including vitamins B6, E, thiamin, magnesium, zinc and may also require higher intakes of PUFAs[32,33,42,44-46,50,51,53,57-62] in order to assist in the prevention of DPN. Our study was unable to detect significant differences between persons with DM2 with and without DPN in terms of the prevalence of inadequacy for vitamin B6, vitamin E, thiamin, magnesium and zinc. We also failed to detect

differences between persons with DM2 with and without DPN in terms of mean vitamin D intake or in terms of the proportion of cases and controls with intakes that were not within the recommended AMDR range for α-linolenic acid.

8.8.1 α-Linolenic Acid

Previous research has shown that PUFAs such as α-linolenic acid may contribute to a reduced risk of DPN.[30,32] However, our research has revealed that the proportion of controls not meeting the AMDR (39%) for α-linolenic acid was higher than the proportion of cases (21%). It has been postulated that PUFA inadequacy may contribute to the development of DPN through inhibited fatty acid metabolism due to impaired function of the Δ 5,6 desaturase enzymes.[27] It is important to note that despite a lack of observed differences between the two groups, controls had a greater proportion of persons not meeting the AMDR for PUFAs compared to cases. Based upon the possible relationship between PUFAs and DPN, we had anticipated that the proportion of cases who had intake below the AMDR for PUFAs to be greater than the proportion of controls. Our results contradict this assumption and also prior studies which have suggested that there is in fact an inverse relationship between α-linolenic acid and the risk of DPN.[32] Our results showed that cases had a greater proportion of persons with intakes of these nutrients meeting the recommended AMDR compared to controls. With the higher proportion of cases exceeding the AMDR for total fat, this may, once again explain the higher intake of α-linolenic acid. Not dissimilar to other reports, our study only utilized one method of dietary recall which does not allow for day to day variability to be considered.[32] This inability to consider day to day variation may have impacted our

ability to detect differences between persons with and without DPN in terms of α-linolenic acid. Rich sources of α-linolenic acid include canola, flaxseed and soybean oils. It is possible that a greater proportion of controls are choosing to use other types of oil for food preparation.

8.8.2 Vitamin B6

Research on experimental models has shown that vitamin B6 deficiency may contribute to the risk of DPN through decreased density of nerve fibers and increased axon to myelin ratios.[63] Both of these alterations are indicators of peripheral neuropathy.[63]

The prevalence of inadequacy of vitamin B6 for both cases and controls was low and differences between the two groups did not reach significance. Our results indicate that the prevalence of inadequacy was low and surprisingly, similar in both cases and controls (17%). It is known that persons with DM2 generally have low levels of vitamin B6 in the body.[34-44] However our results did not show any difference in the prevalence of inadequacy for vitamin B6 which could suggest that low intakes of vitamin B6 may only play a small role in the development and progression of DPN in persons with DM2. It was not unexpected that our study did not detect differences in the prevalence of inadequacy of vitamin B6. Major sources of vitamin B6 include meat and alternatives of which all participants in both groups exceeded the daily recommended servings. Whole grains are another major source of vitamin B6. The majority of the controls met or exceeded the recommended servings of grain products per day. Cases who did not meet the recommended servings per day were very close to meeting the recommendations.

8.8.3 Vitamin D

Vitamin D supplementation has been shown to reduce the pain associated with DPN and it has been proposed that decreased levels of vitamin D in the body may lead to the development of DPN.[48] Our research revealed that a higher proportion of cases had mean vitamin D intakes below the AI when compared to controls. An exception was found in the female controls in the 51 – 70 life stage group. The cases in this life stage group all had mean intakes below the AI compared to the controls in the same life stage group all of whom had mean intakes above the AI. This however does not mean that either of the two groups have intakes that should be considered inadequate as the AI cannot be used to assess inadequate intake in the same manner that the EAR can. Previous research shows that supplementation with vitamin D may decrease the risk for DPN,[48] but perhaps the level of vitamin D that must be consumed to achieve the protective effects may only be achievable through supplemental levels rather than through diet alone. A major food source of vitamin D includes fortified milk of which a greater number of cases reported having intakes well below the recommended servings per day compared to controls.

8.8.4 Vitamin E

Vitamin E has been implicated to improve NCV.[50,51] Our results showed a high prevalence of inadequacy for both groups, (88% for cases and 91% for controls), p=0.67. This result was not anticipated as oxidative stress has been implicated as a potential risk factor for DPN and it is known that vitamin E is a potent antioxidant.[25] Fruits and vegetables are sources rich in vitamin E and when this food group is considered, a large percentage of both cases and controls consumed less than the daily recommended

servings of fruits and vegetables. This could be a potential explanation for the high prevalence of inadequacy of vitamin E. Deficiency of vitamin E is extremely uncommon because it is so widely available in foods. However, a deficiency of vitamin E can cause peripheral neuropathy in the general population.[25] With such a high prevalence of inadequacy seen in both cases and controls, controls may potentially be at risk for peripheral neuropathy, however, this seems unlikely.

8.8.5 Thiamin

Limited research suggests that thiamin intake at clinical doses may help to reduce the risk of DPN.[53] Our study failed to detect a difference in the prevalence of inadequacy between cases and controls in terms of thiamin intake. We found that both groups showed a moderate prevalence of inadequacy (33% of cases, and 35% of controls). The prevalence of inadequacy of thiamin intake was not surprising when sources of thiamin are considered. Major sources include carbohydrate rich foods including whole-grains and foods that have been either enriched or fortified with thiamin. A larger proportion of cases reported consuming levels of carbohydrate below the recommended AMDR as compared to controls which could potentially help to explain the moderate level of inadequacy seen for thiamin. Conversely, controls reported consuming levels of carbohydrate within the recommended AMDR. However, it is possible that the moderate prevalence of inadequacy of thiamin seen in those persons may be explained by a higher consumption of refined grain products such as white breads compared to cases. While it has been suggested that thiamin deficiency is commonly seen in persons with DM2, this may not be related solely to dietary intake. There is limited research on the dietary intake

of thiamin and persons with DM2, but the available data suggests that the intake of thiamin in persons with DM2 is sufficient.[91,92] Our study contradicts these findings and shows that persons with DM2 with and without DPN show a moderate prevalence of inadequacy. Our results may vary from those of the previously mentioned study due to the extremely small sample size, six subjects, of that particular study.

8.8.6 Magnesium

Our results did not demonstrate a difference in the prevalence of inadequacy for the two groups with regards to magnesium. Our results revealed that both cases and controls had a moderate prevalence of inadequacy. Cases did, however, have a higher prevalence of inadequacy (67%) compared to controls (48%). Given the newly determined relationship between magnesium and hyperglycemia,[61] the prevalence of inadequacy seen in cases was not surprising. It has been shown that magnesium supplementation can help to reduce plasma glucose levels and the moderate prevalence of inadequacy seen in both cases and controls may illustrate the importance of magnesium in the maintenance of blood glucose levels. Our results may suggest that persons with DM2 with and without DPN could potentially be at risk for inadequate intake of magnesium and consequently persons without DPN may potentially be at risk for the development of DPN. It is known that persons with DM in general are at risk for hypomagnesemia[25] and it could be suggested that persons in both groups of this study should increase their intake of magnesium. Rich sources of magnesium include bright green leafy vegetables, whole grains as well as nuts.[25] A greater number of cases did not meet the recommended servings of fruits and vegetables per day as compared to controls. A greater number of

cases also did not meet the daily recommended servings for grain products and it is possible that these two factors may have played a role in the level of prevalence of inadequacy. We would have expected to find a difference in the prevalence of inadequacy for magnesium with cases having a higher prevalence of inadequacy compared to controls.

8.8.7 Zinc

Previous research suggests that zinc may help to improve glycemic control.[57,58] If this is correct, zinc may play a role in reducing the risk of DPN. Our results did not find a difference in the prevalence of inadequacy between cases and controls and showed a low prevalence of inadequacy in both groups with cases showing a slightly higher prevalence (17%) compared to controls (9%). Our results are in contrast with previous reports which have shown that persons with DM2 without complications have lower intakes of zinc than recommendations.[90] The majority of controls in our study had mean intakes of zinc above the EAR. The low prevalence of inadequacy seen in both of the study groups is not surprising as zinc deficiency is uncommon.[25] Zinc has been suggested to promote positive immune function as well as to assist in wound healing, thus some research suggests that persons with DM2 increase their daily intake of zinc.[90] However, a flaw of the previous report was in the comparison of the groups mean intake to the RDA which is an incorrect assessment of nutritional intake as it over estimates the requirements of the majority of the group.[90] This could explain why our results differed from this prior study. Food sources rich in zinc include red meat and whole grains.[25] Since a greater proportion of cases reported having carbohydrate intake below the AMDR value, it is possible that

they are not consuming enough sources of zinc to fulfill the DRI requirements. It is also possible that with a larger sample size, we may have discovered additional differences between persons with DPN versus those without in terms of their nutritional adequacy.

8.9 Methods to Increase Validity

To increase the validity of our results we could have used our FFQ in conjunction with another form of dietary recall such as three day food records, or multiple 24-hour recalls.[25] This would have, however, increased our respondent burden which could lead to decreased respondent retention. We could have also attempted to obtain a larger sample size, however it became evident during the study period that the time required for subject recruitment would have to be extended significantly, which was not realistic for this protocol. Finally, we could have narrowed our life stage groups down in order to obtain a more representative sample which would have provided us with saturated information for that particular life stage group, however, this would have required a much larger sample size, and longer period for subject recruitment, which was not practical for this preliminary study to investigate the differences in nutritional intake between the persons with DM with and without DPN.

9.0 Conclusion

To our knowledge, this study is currently the first to examine the nutrient adequacy in persons with DM2 with and without DPN using the newly revised DRI values. Our study has shown that while the prevalence of nutrient inadequacy was moderate to high for a number of nutrients for persons with DM2 with and without DPN, there were only differences in the prevalence of inadequacy between the two groups in terms of vitamin A as well as the proportion of the groups lying outside the AMDRs for total fat, linoleic acid and carbohydrate.

Our results indicate that our hypothesis that 'persons with DM2 and DPN will not meet the recommended DRI values for n-3 PUFAs, dietary fat, carbohydrates, vitamin B6, vitamin D, vitamin E, thiamin, zinc and magnesium compared to persons with DM2 without DPN' was partially true. When the mean dietary intakes of the two groups were compared, we determined that there were in fact differences between the two groups in terms of prevalence of nutrient inadequacy and proportion of persons whose nutritional intake lay outside the recommended AMDRs, albeit for less nutrients than we had originally anticipated.

We have demonstrated a difference in the prevalence of inadequacy for vitamin A intake between persons with DM2 with and without DPN which has not been previously discovered in this patient population. We have suggested the possible relevance of this difference between the two populations; however, additional larger studies that have greater power to determine differences in nutritional intake are required to explore this

difference further, and to investigate if there is a possible association between vitamin A intake and DPN.

We have also found that cases had a greater proportion of persons not achieving the AMDR for carbohydrate. It is suspected that under reporting may be responsible for this difference as the importance of glycemic control has been demonstrated in the prevention of complications of DM2 through improved blood glucose levels as well as possible improved serum lipid levels. We conclude that there are in fact differences between persons with DM2 with and without DPN in the prevalence of nutrient inadequacy and also in the proportion of persons whose nutritional intakes lay outside of the AMDRs. In addition we believe that these differences may play a significant role in the development or progression of DPN. We recommend consumption of a balanced diet to help ensure optimal intake of nutrients. We also recommend that persons with DM2 take a multi-vitamin supplement to assist in the prevention of the development or progression of DPN.

10.0 Study Limitations

The major limitation of this study is the small sample size. A greater number of differences may have been observed between the groups in terms of the: prevalence of inadequacy, proportion of participants whose nutritional intakes lay outside the AMDRs and the mean intake of nutrients with an AI if more participants had been enrolled in our study, however there were constraints on recruitment based upon the time allotted for the study. Another limitation could be related to recall of food intake, specifically, it is possible that participants inaccurately recalled their nutritional intake which may have

lead to either an over- or under estimation of their typical daily nutritional intake and thus their nutritional adequacy. Persons not communicating in the English language and of ethnicities other than Caucasian were excluded from this study which may have lead to an underestimation of the target population and selection bias. Use of a single method of dietary recall, specifically the FFQ, did not take within person variability into consideration. Thus, our results may be inaccurate and may have over estimated the proportion of participants in each group not meeting the DRI requirements. Participants were not matched for variables such as age, HbA_1c levels among other variables which may be of interest for future study.

11.0 Relevance to Practice

Both DM2 and DPN are serious conditions. Symptoms of DM2 and DPN can be minimized and/or alleviated, but to date there is no cure for either condition. DPN may be preventable and it is our hope that by examining the nutritional adequacy of persons with DM2 with and without DPN, our research along with future research may provide insight towards the prevention and development or progression of DPN. This study has confirmed previous observations in terms of dietary fat consumption and also revealed that cases are potentially at risk of consuming inadequate amounts of vitamin A, vitamin C, vitamin E, thiamin, folate, magnesium, dietary fiber, calcium potassium, vitamin K, vitamin D, water, α-linolenic acid and carbohydrate. We have also demonstrated that controls are potentially at risk for inadequate intake of vitamin C, vitamin E, thiamin, folate, magnesium, dietary fiber, calcium, potassium, vitamin K, vitamin D, water, linoleic acid, α-linolenic acid and carbohydrate. To our knowledge, this is the first study

that examines the nutritional adequacy of persons with DM2 with and without DPN with the newly revised DRI values. It is therefore that we can infer the importance of nutritional counseling for persons with DM2 with and without DPN. Future considerations in the field of DM2 and DPN should include additional research into the possible associations between vitamin A and DPN as well as further research into the possible association between PUFAs and DPN. Future research should also consider using multiple methods of dietary recall to obtain nutrition information to verify the results found in this study. We also suggest that research be undertaken with a larger sample of participants, one that can examine the nutrient intake of persons with DM2 with and without DPN and have an adequate sample size in all life stage and gender groups. By including a larger sample size, future research will be able to examine and compare the nutritional intake of persons with DM2 and DPN and determine nutrient inadequacies, proportion of persons lying outside the AMDRs as well as compare the mean intake of nutrients with AIs in each life stage group. It is our hope that this research coupled with future research will benefit persons with DM2 with and without DPN.

Appendix A

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: A Comparison of the Nutritional Intake of Persons with Type 2 Diabetes Mellitus Who Have Peripheral Neuropathy, Versus Those Who Do Not Have Peripheral Neuropathy

Principal Investigator: John M. Embil, Infection Prevention and Control Unit, Health Sciences Centre, Winnipeg, Manitoba, Canada, 787-4654

Co-Investigator: Gregoire Nyomba, Endocrinology Clinics, Health Sciences Centre, Winnipeg, Manitoba, 787-2870

You are being asked to participate in a research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this study and you may discuss it with your friends, family or your doctor before you make your decision. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand.

Purpose of Study

This research study is being conducted to study the food choices in persons with type 2 diabetes who do not have lower limb problems and in those persons with type 2 diabetes who have lower limb problems. We are particularly interested in learning about the food choices that are made in persons with type 2 diabetes. The study consists of a diet history questionnaire as well as an examination of the lower limbs. There are no medications to take, and there will be no long-term follow up.

A total of 120 participants will participate in this study.

Study procedures

If you take part in this study, you will have the following procedures coordinated with your outpatient clinic visit:

1. You will be asked to complete a food frequency questionnaire which should take approximately 30 minutes to complete. In addition, you will also be asked to complete a general questionnaire which should take approximately 15 minutes to complete. If there are any questions which you feel uncomfortable answering, you do not need to answer them. If you are unable to complete either questionnaire on the clinic visit, you will be asked to take it home and complete and return it either by prepaid postage envelope or by facsimile transmission to 787-2989.

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2. An examination of your lower limbs to determine whether sensation is preserved or absent.

3. The researchers will also review your medical record to learn more about your medical history and the hemoglobin A1C and cholesterol profile. If these are not available in the Health Sciences Centre record, we would like to obtain copies of the most recent results from whomever is managing your diabetes.

Participation in the study will be only on the day you complete the questionnaire and your lower limbs are examined. Long-term follow up will not be undertaken**.**

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff first.
If any serious abnormality is detected at the time of the physical examination or studies of your blood supply in the upper limb, your doctors will be notified immediately.

The results of this study will likely be available in 2009 and can be communicated to you should you desire.

Risks and Discomforts

It is not expected that you will experience any discomfort participating in this study. You may experience slight pressure in your wrist and feet as the researchers check the pulses in your feet. Pressure will also be experienced in your finger tips when the blood pressure is checked in the vascular laboratory.

Your condition may not improve, or may worsen while participating in this study.

Benefits

There may or may not be direct benefit to you from participating in this study. We hope the information learned from this study will benefit other people with type 2 diabetes and lower extremity complications.

Costs

All the procedures, which will be performed as part of this study, are provided at no cost to you. The Study will be done on the same day as your clinic appointment, so no extra visits will have to be made.

Payment for participation

You will receive no payment or reimbursement for any expenses related to taking part in this study**.**

Alternatives

You do not have to participate in this study to receive treatment for your condition. Please talk to your regular doctor (or therapist) about all your treatment options.

Confidentiality

Information gathered in this research study may be published or presented in public forums; however your name and other identifying information will not be used or revealed. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. All study related documents will bear only your assigned study number and/or initials.

Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba.

Organizations that may inspect and/or copy your research/medical records for quality assurance and data analysis include the Health Research Board at the University of Manitoba.

The University of Manitoba Health Research Ethics Board may review records related to the study for quality assurance purposes.

All records will be kept in a locked secure area and only those persons identified will have access to these records. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave the Health Sciences Centre.

Voluntary Participation/Withdrawal from the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision not to participate or to withdraw from the study will not affect your care at this centre. If the study staff feel that it is in your best interest to withdraw you from the study, they will remove you without your consent.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

Medical Care for Injury Related to the Study

In the case of injury or illness resulting from this study, necessary medical treatment will be available at no additional cost to you.

You are not waiving any of your legal rights by signing this consent form nor releasing the investigator(s) or the sponsor(s) from their legal and professional responsibilities.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact the study doctor and the study staff: Dr. John Embil (787-4654) or Dr. Gregoire Nyomba (787-2870). For questions about your rights as a research participant, you may contact The University of Manitoba, Bannatyne Campus Research Ethics Board Office at (204) 789-3389

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Drs. Embil and/or Nyomba and/or their study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statements or implied statements. Any relationship (such as employer, supervisor or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of any of my records that relate to this study by The University of Manitoba Research Ethics Board, for quality assurance purposes.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

I agree to be contacted for future follow-up in relation to this study,
Yes _ No _

Participant signature_____Date _____
(day/month/year)

Participant printed name: _____

I, the undersigned, attest that the information in the Participant Information and Consent Form was accurately explained to and apparently understood by the participant or the participant's legally acceptable representative and that consent to

participate in this study was freely given by the participant or the participant's legally acceptable representative.

Witness signature_____ Date _____

_

(day/month/year)

Witness printed name: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____Date _____

(day/month/year)

Signature: _____

Role in the study: _____

Relationship (if any) to study team members:_____

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Table 1
Demographic and Physical Characteristic Data for Cases and Controls

Participant Demographics and Physical Characteristics	Cases (n=30)	Controls (n=30)	Significance (p<0.05)
Gender (Mean)			
Males	**22 (73.3%)**	**20 (66.7%)**	**0.57**
Females	**8 (26.7%)**	**10 (33.3%)**	
Height (cm)			
Mean ± SD	**176.0 ± 10.8**	**171.0 ± 8.2**	**0.08**
Weight (kg)			
Mean ± SD	**102.0 ± 24.6**	**91.0 ± 18.2**	**0.09**
Body Mass Index (BMI)[a]			
Mean ± SD	**33.0±6.5**	**30.9±5.6**	**0.2**
Age (years)			
Mean ± SD	**60.1±7.5**	**57.8±8.2**	**0.26**
Place of Residence (n)			
Urban	**24 (80.0%)**	**28 (93.3%)**	
Rural	**5 (16.7%)**	**2 (6.7%)**	**0.27**
Reserve	**1 (3.3%)**	**0 (0%)**	
Highest Level of Education Completed (n)			
Between Grades 8 - 11	**8 (26.7%)**	**2 (7.1%)**	
Grade 12	**10 (33.3%)**	**10 (35.7%)**	
Some years at Trade School or Commercial Studies	**3 (10.0%)**	**5 (17.9%)**	
Trade School or Commercial Studies Completed	**3 (10.0%)**	**3 (10.7%)**	**0.43**
Some Years at University	**4 (13.3%)**	**3 (10.7%)**	
University Undergraduate Degree	**1 (3.3%)**	**4 (14.3%)**	
University Post-Graduate Degree	**1 (3.3%)**	**1 (3.6%)**	
Missing Data	**0**	**2**	
Type of Employment (n)			
Unemployed/Retired	**14 (46.7%)**	**15 (50.0%)**	
Sedentary Worker	**5 (16.7%)**	**8 (26.7%)**	**0.64**
Clerical/Administrative	**7 (23.3%)**	**4 (13.3%)**	
Indoor Labourer	**4 (13.3%)**	**3 (10.0%)**	
Annual Household Income (n)			
< $ 20 000	**3 (12.0%)**	**4 (16.0%)**	
$ 20 000 - $ 49 000	**8 (32.0%)**	**12 (48.0%)**	
$ 50 000 - $ 100 000	**11 (44.0%)**	**6 (24.0%)**	**0.49**
> $ 100 000	**3 (12.0%)**	**3 (12.0%)**	
Missing Data	**5**	**5**	
Perceived Financial Situation (n)			
Financially Well-Off	**2 (7.1%)**	**5 (18.5%)**	
Sufficient Income	**19 (67.9%)**	**21 (77.8%)**	
Poor	**5 (17.9%)**	**1 (3.7%)**	**0.11**
Very Poor	**2 (7.1%)**	**0 (0%)**	
Missing Data	**2**	**3**	

[a] Body Mass Index, calculated as weight (kg) divided by height (m^2)

Table 2
History of Medication Use for Cases and Controls

Medication Use	Cases (n=30)	Controls (n=30)	Significance (p<0.05)
Insulin (n)			
Yes	**17 (56.7%)**	**14 (46.7%)**	**0.44**
No	**13 (43.3%)**	**16 (53.3%)**	
Oral Hypoglycemic Agents (n)			
Yes	**22 (75.9%)**	**27 (90.0%)**	
No	**7 (24.1%)**	**3 (10.0%)**	**0.15**
Missing Data	**1**	**0**	
Antihyperlipidemic Agents (n)			
Yes	**19 (65.5%)**	**22 (73.3%)**	
No	**10 (34.5%)**	**8 (26.7%)**	**0.52**
Missing Data	**1**	**0**	

Table 3
Medical History of Cases and Controls

Medical History	Cases (n=30)	Controls (n=30)	Significance (p<0.05)
Duration of Diabetes (years)			
Mean ± SD	**17.5±9.1**	**11.0±7.6**	**0.003**
Duration of Neuropathy (years)			
Mean ± SD	**7.0 ± 7.8**	**0**	
Previous Myocardial Infarction (n)			
Yes	**5 (16.7%)**	**3 (10.0%)**	**0.45**
No	**25 (83.3%)**	**27 (90.0%)**	
Previous Ophthalmologic Complications (n)			
Yes	**13 (43.3%)**	**7 (23.3%)**	**0.1**
No	**17 (56.7%)**	**23 (76.7%)**	
Dentures (n)			
Yes	**16 (53.3%)**	**9 (30.0%)**	**0.07**
No	**14 (46.7%)**	**21 (70.0%)**	
Previous Nutrition Education (n)			
Yes	**25 (83.3%)**	**25 (89.3%)**	
No	**5 (16.7%)**	**3 (10.7%)**	**0.51**
Missing Data	**0**	**2**	
Duration of Nutrition Education (n)			
One Time Only	**11 (44.0%)**	**12 (46.1%)**	
More than One Time	**14 (56.0%)**	**13 (50.0%)**	**0.59**
Missing Data	**5**	**5**	
Vitamin/Mineral Supplement Use (n)			
No	**14 (46.7%)**	**10 (33.3%)**	
Yes, Not Daily	**7 (23.3%)**	**7 (23.3%)**	**0.5**
Yes, Daily	**9 (30.0%)**	**13 (43.3%)**	

Table 4
Physical Activity Information for Cases and Controls

Physical Activity Level[a]	Cases (n=30)	Controls (n=30)	Significance (p<0.05)
Low (n)			
Never or Occasionally	**14 (46.7%)**	**7 (23.3%)**	
One to three times per week	**7 (23.3%)**	**11 (36.7%)**	**0.16**
Four times + per week	**9 (30.0%)**	**12 (40.0%)**	
Moderate (n)			
Never or Occasionally	**27 (90.0%)**	**18 (60.0%)**	
One to three times per week	**2 (6.7%)**	**8 (26.7%)**	**0.03**
Four times + per week	**1 (3.3%)**	**4 (13.3%)**	
High (n)			
Never or Occasionally	**29 (96.7%)**	**26 (86.7%)**	
One to three times per week	**0 (0%)**	**4 (13.3%)**	**0.08**
Four times + per week	**1 (3.3%)**	**0 (0%)**	

[a] Adapted from Shatenstein B, Nadon S, Godin C, Ferland G. Development and validation of a food frequency questionnaire. Can J Diet Pract Res 2005;66:67-75.[70]

Table 5
Prevalence of inadequacy for cases and controls based upon twelve months of nutrition assessment from a food frequency questionnaire

Nutrient[a]	Life Stage Group[a]	EAR[a,b]	Mean Case Intake ± SD (n=24)	Prevalence of Inadequacy of Cases (%)[c]	Mean Control Intake ± SD (n=23)	Prevalence of Inadequacy of Controls (%)[c]	Significance (p<0.05)
Vitamin A (µg/day)[d]	Males (40 - > 70)	625	926.5 ± 443.2 (18)	37.5	1469.3 ± 884.0 (14)	8.7	0.02
	Females (40 - > 70)	500	983.3 ± 1093.0 (6)		1344.4 ± 1183.0 (9)		
Vitamin C (mg/day)	Males (40 - > 70)	75	92.4 ± 44.6 (18)	33.3	89.6 ± 60.9 (14)	34.8	0.92
	Females (40 - > 70)	60	102.0 ± 44.6 (6)		132.3 ± 84.5 (9)		
Vitamin E (mg /day)[e]	Males (40 - > 70)	12	7.7 ± 5.8 (18)	87.5	4.8 ± 1.9 (14)	91.3	0.67
	Females (40 - > 70)		7.5 ± 4.0 (6)		7.6 ± 4.3 (9)		
Thiamin (mg/day)	Males (40 - > 70)	1	1.7 ± 0.7 (18)	33.3	1.4 ± 0.5 (14)	34.8	0.92
	Females (40 - > 70)	0.9	1.4 ± 0.5 (6)		1.5 ± 0.4 (9)		
Riboflavin (mg/day)	Males (40 - > 70)	1.1	2.5 ± 0.9 (18)	4.2	2.6 ± 0.8 (14)	0	0.32
	Females (40 - > 70)	0.9	2.2 ± 0.8 (6)		2.4 ± 1.1 (9)		
Niacin (NE)[f]	Males (40 - > 70)	12	45.2 ± 11.7 (18)	0	41.2 ± 11.5 (14)	0	1
	Females (40 - > 70)	11	38.5 ± 15.5 (6)		46.2 ± 19.8 (9)		
Vitamin B6 (mg/day)	Males (40 – 50)	1.1	2.5 (1)	16.7	2.0±0.9 (3)	17.4	0.99
	Males (51 - > 70)	1.4	2.0±0.6 (17)		1.8±0.5 (11)		
	Females (40 – 50)	1.1	0 (0)		1.7±0.2 (3)		
	Females (51 - > 70)	1.3	1.7±0.7 (6)		2.4±1.1 (6)		

Table 5 Continued

Prevalence of inadequacy for cases and controls based upon twelve months of nutrition assessment from a food frequency questionnaire

Nutrient[a]	Life Stage Group[a]	EAR[a,b]	Mean Case Intake ± SD (n=24)	Prevalence of Inadequacy of Cases (%)[c]	Mean Control Intake ± SD (n=23)	Prevalence of Inadequacy of Controls (%)[c]	Significance (p<0.05)
Folate (µg/day)	Males (40 - > 70) Females (40 - > 70)	320	379.4 ± 139.9 (18) 370.0 ± 157.8 (6)	45.8	369.3 ±152.1 (14) 380.0 ± 183.6 (9)	47.8	0.17
Vitamin B12 (µg/day)	Males (40 - > 70) Females (40 - > 70)	2	5.8 ± 2.7 (18) 5.2 ± 5.0 (6)	12.5	8.4 ± 3.8 (14) 8.2 ± 6.2 (9)	4.4	0.32
Copper (µg/day)	Males (40 - > 70) Females (40 - > 70)	700	1600 ± 600 (18) 1500 ± 700 (6)	0	1800 ± 600 (14) 1800 ± 900 (9)	0	1
Magnesium (mg/day)	Males (40 - > 70) Females (40 - > 70)	350 265	327.2 ± 98.3 (18) 260.3 ± 84.6 (6)	66.7	330.7 ± 86.0 (14) 351.7 ± 138.4 (9)	47.8	0.41
Phosphorus (mg/day)	Males (40 - > 70) Females (40 - > 70)	580	1608.2 ± 551.9 (18) 1182.2 ± 472.7 (6)	0	1579.7 ± 364.4 (14) 1782.7 ± 905.6 (9)	0	1
Selenium (µg/day)	Males (40 - > 70) Females (40 - > 70)	45	127.4 ± 33.8 (18) 103.7 ± 51.7 (6)	4.2	129.6 ± 41.7 (14) 148.6 ± 65.2 (9)	0	0.32
Zinc (mg/day)	Males (40 - > 70) Females (40 - > 70)	9.4 6.8	14.4 ± 4.3 (18) 11.3 ± 5.8 (6)	16.7	13.8 ± 3.6 (14) 12.9 ± 4.8 (9)	8.7	0.55

[a] Adapted from Institute of Medicine. Dietary reference intakes: the essential guide to nutrient requirements. Washington (DC): The National Academies Press; 2006.[25]

[b] EAR: Estimated Average Requirement.

[c] Percent of each group below the EAR. This was determined by calculating the total number of participants below the EAR in each group and dividing by the total number of participants in that group.[25]

[d] Adjustments have not been made to the new conversion factors for carotene to retinol.[25]

[e] The intake of α-Tocopherol was calculated s total vitamin E x 0.8.[25]

[f] NE: Niacin Equivalents

Table 6
Adequate intakes of nutrients based upon twelve months of nutrition assessment from a food frequency questionnaire

Nutrient[a]	Life Stage Group[a]	AI[a,b]	Mean Case Intake ± SD (n=24)	Mean Control Intake ± SD (n=23)
Dietary Fiber (g/day)	Males (40 - 50)	38	27 (1)	18.7±9.1 (3)
	Males (51 - > 70)	30	21.2±9.9 (17)	20.9±9.4 (11)
	Females (40 - 50)	25	0 (0)	16.0±4.6 (3)
	Females (51 - > 70)	21	17.3±5.9 (6)	26.3±9.8 (6)
Calcium (mg/day)	Males (40 - 50)	1000	1600 (1)	1333.3±850.5 (3)
	Males (51 - 70)	1200	893.8±423.4 (16)	930.0±323.4 (10)
	Males (> 70)	1200	600 (1)	900 (1)
	Females (40 - 50)	1000	0 (0)	1000±346.4 (3)
	Females (51 - 70)	1200	733.3±294.4 (6)	1260.0±743.6 (5)
	Females (> 70)	1200	0 (0)	500 (1)
Potassium (g/day)	Males (40 - > 70)	4.7	3.6±1.2 (18)	3.3±0.9 (14)
	Females (40 - > 70)		2.9±1.1 (6)	3.6±1.6 (9)
Sodium (g/day)	Males (40 - 50)	1.5	5.8 (1)	4.0±0.2 (3)
	Males (51 - 70)	1.3	3.5±1.3 (16)	3.2±1.1 (10)
	Males (> 70)	1.2	3.0 (1)	2.8 (1)
	Females (40 - 50)	1.5	0 (0)	3.2±1.0 (3)
	Females (51 - 70)	1.3	3.4±1.8 (6)	4.0±2.1 (5)
	Females (> 70)	1.2	0 (0)	3.1 (1)
Pantothenic Acid (mg/day)	Males (40 - > 70)	5	7.1±2.6 (18)	6.9±2.0 (14)
	Females (40 - > 70)		5.8±2.3 (6)	6.8±3.2 (9)
Vitamin K (µg/day)	Males (40 - > 70)	120	116.7±53.8 (18)	111.4±85.4 (14)
	Females (40 - > 70)	90	130.0±44.7 (6)	158.9±111.4 (9)
Vitamin D (µg/day)	Males (40 - 50)	5	11 (1)	9.7±5.5 (3)
	Males (51 - 70)	10	7.4±4.1 (16)	6.9±2.8 (10)
	Males (> 70)	15	5 (1)	7 (1)
	Females (40 - 50)	5	0 (0)	6.0±1.7 (3)
	Females (51 - 70)	10	4.7±2.4 (6)	13.8±12.0 (5)
	Females (> 70)	15	0 (0)	2 (1)
Manganese (mg/day)	Males (40 - > 70)	2.3	3.7±1.3 (18)	4.2±2.0 (14)
	Females (40 - > 70)	1.8	3.1±1.0 (6)	4.4±1.6 (9)
Water (L/day)	Males (40 - > 70)	3.7	2.3±1.1 (18)	2.2±0.7 (14)
	Females (40 - > 70)	2.7	2.0±0.9 (6)	1.9±0.7 (9)

[a] Adapted from Institute of Medicine. Dietary reference intakes: the essential guide to nutrient requirements. Washington (DC): The National Academies Press; 2006.[25]
[b] AI: Adequate Intake

Table 7
Acceptable Macronutrient Distribution Ranges for Cases and Controls

Macronutrient[a]	Life Stage Group[a]	Mean Case Intake (% total energy) [n=24]	Recommendation (% total energy)[a]	Proportion of Cases Below AMDR[b] (%)	Proportion of Cases Above AMDR[b] (%)	Mean Control Intake (% total energy) [n=23]	Proportion of Controls Below AMDR[b] (%)	Proportion of Controls Above AMDR[b] (%)	Significance (p<0.05)
Protein	Males (40 - > 70) Females (40 - > 70)	21.1 ± 4.0 [18] 19.0 ± 2.4 [6]	10 - 35	0	0	20.6 ± 3.3 [14] 20.4 ± 4.0 [9]	0	0	
Total Fat	Males (40 - > 70) Females (40 - > 70)	38.2±5.0 [18] 37.7±6.7 [6]	20 - 35	0	54.2	33.2±4.9 [14] 33.2±2.6 [9]	0	17.4	0.009
n-6 Polyunsaturated Fatty Acids (Linoleic Acid)[c]	Males (40 - > 70) Females (40 - > 70)	6.9±2.3 [18] 6.5±2.3 [6]	5 - 10	4.2	12.5	5.1±1.5 [14] 5.8±1.3 [9]	39.1	0	0.005
n-3 Polyunsaturated Fatty Acids (α-Linolenic Acid)[c]	Males (40 - > 70) Females (40 - > 70)	0.8±0.2 [18] 0.9±0.3 [6]	0.6 - 1.2	20.8	4.2	0.6±0.3 [14] 0.6±0.2 [9]	39.1	4.4	0.38
Carbohydrate	Males (40 - > 70) Females (40 - > 70)	41.7 ± 5.8 [18] 43.7 ± 7.5 [6]	45 - 65	66.7	0	44.9±6.4 [14] 46.7 ± 3.9 [9]	30.4	0	0.01
Sugar	Males (40 - > 70) Females (40 - > 70)	15.4±4.2 [18] 15.3±4.8 [6]	≤ 25	0	0	17.1±3.8 [14] 17.3±4.5 [9]	0	0	

[a] Adapted from Institute of Medicine. Dietary reference intakes: the essential guide to nutrient requirements. Washington (DC): The National Academies Press; 2006. [25]

[b] AMDR: Acceptable Macronutrient Distribution Range

[c] Roughly 10 % of energy can come from long chain n-3 or n-6 polyunsaturated fatty acids

Table 8
Food Group Data for Cases and Controls

Food Group[a]	Life Stage Group[a]	Recommended Servings[a]	Mean Case Servings ±SD (n=24)	Mean Control Servings ±SD (n=23)
Grain Products	Males 40 - 50	8	6 (1)	8.7±3.2 (3)
	Females 40 - 50	6 - 7	0 (0)	7.3±3.2 (3)
	Males > 51	7	6.1±2.5 (17)	7.0±4.0 (11)
	Females > 51	6	6.7±5.3 (6)	7.5±3.1 (6)
Fruits and Vegetables	Males 40 - 50	8 - 10	8 (1)	8.7±3.2 (3)
	Females 40 - 50	7 - 8	0 (0)	5.0±1.0 (3)
	Males > 51	7	5.8±2.1 (17)	5.4±3.6 (11)
	Females > 51		5.7±2.6 (6)	8.5±5.2 (6)
Milk and Alternatives	Males 40 - 50	2	4 (1)	3.3±3.2 (3)
	Females 40 - 50		0 (0)	2.7±0.6 (3)
	Males > 51	3	2.1±1.4 (17)	2.0±1.6 (11)
	Females > 51		1.5±0.8 (6)	3.0±1.8 (6)
Meat and Alternatives	Males 40 - > 51	3	6.0±1.8 (18)	4.6±1.7 (14)
	Females 40 - > 51	2	5.0±2.3 (6)	5.7±3.1 (9)

[a] Adapted from Health Canada 2007. Eating well with canada's food guide. A resource for educators and communicators. http://www.healthcanada.gc.ca/foodguide.[93]