

**SOCIAL AND HEALTH IMPLICATIONS OF
PLANT REMEDIES OF THE JAMES BAY CREE FOR
SYMPTOMS OF TYPE 2 DIABETES MELLITUS**

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ABSTRACT

In order to develop a culturally appropriate strategy to contend with the rising type 2 diabetes mellitus (DM2) epidemic among Canadian Aboriginals, quantitative and qualitative analyses were performed. The study assessed current consumption trends of traditional foods (TF) and medicines (TM) and *in vitro* antioxidant and anti-atherogenic potential of eight medicinal plants used for treatment of symptoms of DM2. Interviews with 173 adults of Mistissini revealed a decreased consumption of TFs and TMs in younger generations in comparison to elders ($p<0.05$). Four plants demonstrated free radical scavenging activity akin to standard controls ($p<0.05$). All plants effectively protected against the cytotoxic effects of oxidized low-density lipoproteins (ox-LDL) in relation to controls ($p<0.05$). All plants significantly inhibited lipid peroxidation compared to ox-LDL ($p<0.05$). Further animal and clinical trials are needed, but these plants, may in the least, contribute positively to an intervention strategy incorporating traditional plants in treatment of symptoms of DM2.

RÉSUMÉ

À fin de développer une stratégie qui contraind à l'augmentation marqué du diabète du type 2 (DM2), l'épidémie affectant les peuples Indigènes du Canada, certaines analyses qualitatives et quantitatives ont été réalisées. L'étude à démontré les habitudes de consommation des aliments traditionnels et médicinales ainsi que le potentiel antioxydants et cardioprotecteurs *in vitro* de huit plantes médicinales utiliser pour le traitement des symptômes du DM2. Des entrevues avec 173 adultes venant de Mistissini a révélé une diminution de la consommation d'aliments et de médecines traditionnelles pour la génération plus jeune contrairement aux aînés ($p<0.05$). Quatre plantes ont révélé une activité comparable aux contrôles pour réduire les radicaux oxydants ($p<0.05$). Toutes les plantes ont démontré une protection contre les effets cytotoxiques du lipoprotéine à basse densité oxyder (ox-LDL) par rapport aux contrôles ($p<0.05$). Toutes les plantes ont empêché significativement la peroxydation des lipides comparés à ox-LDL ($p<0.05$). Des études cliniques et *in vivo* seront nécessaires, mais ces plantes, néanmoins, contribuent positivement à une stratégie interventionnel pour DM2.

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STATEMENT OF ORIGINALITY

Sonia Grandi prepared the thesis under the supervision of Dr. Timothy Johns (thesis supervisor). Experiments were performed by Sonia Grandi in the laboratories of Dr. Timothy Johns at McGill University and Dr. Martin Sirois at the Montreal Heart Institute. The preparation of plant extracts, the DPPH and the TBARS assays were performed in the laboratory of Dr. Timothy Johns at McGill University. The cellular manipulations and the LDH assay were performed in the laboratory of Dr. Martin Sirois at the Montreal Heart Institute. Patrick Owen, a PhD candidate under the supervision of Dr. Timothy Johns, assisted in the formulation of protocols and laboratory experiments. Paul-Eduard Neagoe, a PhD candidate under the supervision of Dr. Martin Sirois, assisted in cellular manipulations. The Canadian Institutes of Health Research (CIHR) provided funding for fieldwork and laboratory analysis.

TABLE OF CONTENTS

ABSTRACT	i
RÉSUMÉ.....	ii
ACKNOWLEDGEMENTS.....	iii
STATEMENT OF ORIGINALITY.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES AND FIGURES.....	ix
LIST OF ABBREVIATIONS.....	x
1. INTRODUCTION.....	1
2. LITERATURE REVIEW.....	5
2.1 Diabetes Mellitus	5
2.1.1. Diabetes and Indigenous People.....	6
2.1.2. Symptoms, causes, and therapies of DM2.....	7
2.1.3. Susceptibility of diabetic patients to atherosclerosis and CVD.....	8
2.1.4. Western and non-western treatments for DM2.....	9
2.2 Oxidative Stress and Antioxidant Defences.....	10
2.2.1 Oxidative stress and free radical generation.....	10
2.2.2 Antioxidant defences.....	12
2.2.3 Sources of antioxidants.....	14
2.2.4 Role of antioxidants in atherosclerosis.....	15
2.3 LDL peroxidation.....	17
2.3.1 Cytotoxic effects of oxidized low-density lipoprotein	17
2.3.2 Role of ox-LDL in diabetic patients.....	18
2.4 Traditional Lifestyle of the Cree.....	18
2.4.1 Modernization and its effects on Cree health.....	19
2.4.2 Sociocultural aspects surrounding the use of traditional remedies among the Cree.....	20
2.5 Global View on Traditional Medicines.....	21

5.3.2	Traditional food consumption.....	35
5.3.3	Plant species in use for treatment of symptoms of DM2.....	40
5.3.4	Frequency of consumption of traditional medicines and trends associated with use of traditional medicines.....	43
5.3.5	Traditional knowledge and adherence to a traditional lifestyle.....	45
5.4	DISCUSSION.....	46
6.0	BRIDGING STATEMENT FOR CHAPTER SIX.....	49
6.1	CHAPTER 6: Antioxidant Activity and Cardioprotective Effects of Traditional Plants for Symptoms of Diabetes Mellitus.....	50
6.1	INTRODUCTION.....	50
6.2	MATERIALS AND METHODS.....	52
6.2.1	Plant selection and acquisition.....	52
6.2.2	Preparation of plant extracts.....	52
6.2.3	Free radical scavenging activity (DPPH).....	52
6.2.4	LDL preparation and oxidation.....	53
6.2.5	Conjugated dienes formation	53
6.2.6	Cell preparation	53
6.2.7	Trypan blue viability assay.....	54
6.2.8	Incubation with extracts and addition of ox- LDL.....	54
6.2.9	Ox-LDL cytotoxicity via Lactate Dehydrogenase (LDH) release.....	55
6.2.10	Inhibition of lipid peroxidation (TBARS).....	55
6.2.11	Statistical analysis.....	56
6.3	RESULTS.....	56
6.3.1	Free radical scavenging ability of plant extracts.....	56
6.3.2	Characterization of LDL oxidation (CD).....	57

6.3.3	Cytoprotective effects.....	58
6.3.4	Inhibition of lipid peroxidation.....	59
6.3.5	Correlation between TBARS and LDH.....	61
6.4	DISCUSSION.....	62
7.	CONCLUSIONS.....	66
7.1	LIMITATIONS.....	66
7.1.1	Nutritional Assessment	66
7.1.2	Bioactivity of plant extracts.....	66
7.2	GENERAL CONCLUSIONS AND FUTURE DIRECTIONS.....	67
7.2.1	General Conclusions.....	67
7.2.2	Future Research.....	69
9.	LITERATURE CITED.....	71
10.	APPENDICES.....	99
	APPENDIX 1: Food Frequency Questionnaire.....	99
	APPENDIX 2: Socio-behavioural Questionnaire.....	107
	APPENDIX 3: Letter of Consent.....	113
	APPENDIX 4: Ethics Approval Form.....	117
	APPENDIX 5a: Plant Percent Yields (Post Lyophilization).....	118
	APPENDIX 5b: Plant Percent Yields (Post Extraction).....	118

LIST OF TABLES

CHAPTER FIVE

Table 5.3.1.1: Population Demographics.....	36
Table 5.3.3.1: Frequency of Use of Traditional Medicines by Species and Part.....	42
Table 5.3.4.1: Frequency of Use and Reasons for Non-Use of Traditional Medicines by Age Group.....	44

CHAPTER SIX

Table 6.3.4.1: Correlation coefficients and p-values for TBARS and LDH results.....	62
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LIST OF FIGURES

LITERATURE REVIEW

Figure 1: The early events in the pathogenesis of atherosclerosis incited by Hyperglycemia.....	11
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CHAPTER FIVE

Figure 5.2.1.1: Geographical Representation of the Nine Cree Communities of the James Bay Region.....	33
Figure 5.3.2.1a: Frequency of Consumption of Large and Small Game Meats.....	37
Figure 5.3.2.1b: Frequency of Consumption of Fish Species.....	37
Figure 5.3.2.1c: Frequency of Consumption of Wild Berries.....	38
Figure 5.3.2.1d: Frequency of Consumption of Traditional and Non-Traditional Bannock.....	38
Figure 5.3.3.1: Frequency of Consumption of Plants for Symptoms of DM2	41

CHAPTER SIX

Figure 6.3.1.1: 1, 1 diphenyl-2-picryl-hydrazyl (DPPH) scavenging ability of crude ethanol plant extracts and standards.....	57
Figure 6.3.2.1: Comparative lag times of ox-LDL and n-LDL.....	58
Figure 6.3.3.1: Cytoprotective effects of plant extracts against ox-LDL using the LDH assay.....	59
Figure 6.3.4.1: Inhibition of lipid peroxidation by plant extracts found within the supernatant.....	60
Figure 6.3.4.2: Inhibition of lipid peroxidation by plant extracts found within the cellular lysate.....	61

LIST OF ABBREVIATIONS

BAEC:	Bovine aortic endothelial cells
DM2:	Type 2 Diabetes Mellitus
DPPH:	1,1 diphenyl-2-picryl-hydroazyl, used as radical donor in the free radical scavenging assay
FFQ:	Food frequency questionnaire
LDH:	Lactate dehydrogenase
LDL:	Low-density lipoprotein
n-LDL:	Native low-density lipoprotein
ox-LDL:	Oxidized low-density lipoprotein
ROS:	Reactive oxygen species
SBQ:	Socio-behavioural questionnaire
TBARS:	Thiobarbituric acid-reacting species; measure of level of lipid peroxidation
TF:	Traditional food
TK:	Traditional knowledge
TM:	Traditional medicine

1.0 INTRODUCTION

Plants form the basis upon which much of societies' material wealth originates. They were and continue to be the pivotal resource from which all living organisms thrive. From the beginning of time plants have offered humans the resources for food, shelter and transportation (Balick and Cox, 1997). Indigenous People who still adhere to a traditional lifestyle, characterized by reliance on the land for sustenance, represent the prehistoric relationship that existed between plants and humans (Kuhnlein, 2000).

Canadian Indigenous People have relied on the land for food, shelter, and remedial treatment for over 2000 years (Martijn, 1969; Berkes, 1978; Arnason, 1981; Johnson, 1995; Kuhnlein, 2000; Duhaime, 2001). They possessed extensive knowledge of the species found both in their immediate and surrounding areas. Oral transmission of traditional knowledge ensured the sacredness and conservation of plant species (Berkes, 1999). The traditional knowledge of the Indigenous People is based on a harmony with their surroundings in accordance with the laws of nature.

Loss of traditional knowledge accompanied the increased exposure to Western influences. The traditional hunting/gathering lifestyle of Indigenous People was gradually infiltrated by Western culture. Acculturation resulted in a decrease in the consumption of traditional foods, the performance of physically exerting tasks, the attachment to the land, and the engagement in traditional rituals. Alongside the shift to a more Western lifestyle came an increase in the incidence of chronic diseases, such as diabetes mellitus, obesity, hypertension, and cardiovascular disease. Many researchers have attributed this increase to the replacement of the traditional diet with market foods alongside a decrease in the level of daily physical activity (Delorimier, 1999; Young, 2000; Dewailly, 2002). Of major concern is the decreasing consumption of traditional foods that are typically high in omega-3 fatty acids and low in saturated fats, with the increased

reliance on market-based foods. As a result of lifestyle modifications and genetic predispositions, Indigenous populations of Canada are found to be 3 to 5 times more susceptible to diabetes mellitus in comparison to the remainder of the Canadian population (Brassard, 1995; Young, 2000).

Diabetes mellitus is a disease of the endocrine system specifically linked to the pancreas. Diabetes mellitus is categorized by a state of hyperglycemia and subsequent increases in oxidative stress. The oxidative state of DM2 is a result of overproduction of free radicals and subsequent depletion of endogenous antioxidants (Guerci, 2001). Oxidative stress generates lipid peroxides, hydroperoxides and glycoxidated by-products, which compromise endothelial integrity (Esterbauer, 1992; Eckel, 2002). This state of oxidative stress can lead to further complications, such as, atherosclerosis and cardiovascular disease, two major complications of type 2 diabetes mellitus (DM2) patients. Canadian Indigenous populations are one such population affected by complications of DM2, with ~13% of diabetic patients having experienced a cardiovascular event (Statistics Canada, 2004).

Traditional plant remedies, for use in treatment of DM2, have been previously highlighted by Marles and Farnworth (1995). They summarized over 1200 species known for use in treatment of DM2 worldwide. Although many of the plants found within the list are known to possess blood glucose lowering potential, their antioxidant properties offers potential mechanisms to counter the onset and propagation of lipid peroxidation and oxidative stress alike (Halliwell, 1989; Li, 1993; Antolovich, 2002).

Numerous plants of the Boreal forest, of Northern Quebec and Ontario, have been observed to possess high levels of phenolic compounds (McCune, 2003), which are known to be rich in antioxidant potential (Cotelle, 1996; Johns, 1996; Benito, 2002; Tsai, 2003). Various well-known antioxidants, such as vitamin C, α -tocopherol, and phenols have been found to significantly lower the rate of free

radical production (Halliwell, 1989; Niki, 1995; Li, 1993; Antolovich, 2002). However, epidemiological and animal studies have to date shown inconsistent results with respect to antioxidant effects on vascular integrity. Several studies have revealed an inverse relationship between vitamin E levels and cardiovascular events, while other studies have highlighted the cytotoxic effects of ascorbic acid induced by modified lipid by-products (Stampfer, 1993; Morel, 1994; Ashidate, 2003). Recent studies performed with various polyphenols have demonstrated their cardioprotective effects by their ability to improve prostaglandin production and maintain vascular integrity (Shutenko, 1999; Benito, 2002; Anter, 2004; Steffen, 2005). These recent findings provide the basis for the potential of antioxidants, found within the plants of the Boreal Forest, in reducing the deleterious effects of oxidative stress induced by DM2 and the occurrence of atherosclerotic events common to DM2 patients.

No study to date has used the knowledge obtained directly from Indigenous people for guided laboratory analysis of medicinal efficacy of plant remedies to counter the symptoms of DM2. Therefore, the objectives of the study are two-fold; the first is a quantitative assessment of the frequency of consumption of traditional foods and medicines within the Cree community of Mistissini. A socio-behavioural questionnaire (SBQ) administered in conjunction with the food frequency questionnaire (FFQ) is used to assess the perceptions underlying the use of traditional medicines, the acceptability of administration of traditional medicines and the notions surrounding the health of the Cree people. The second objective is to assess the antioxidant and anti-atherogenic potential of the plants most frequently used for treatment of symptoms of DM2 outlined within the FFQ. Antioxidant potential will be determined using a free radical generating assay, which is then confirmed by the level of protection conferred against the deleterious effects of oxidized low-density lipoproteins (ox-LDL) by-products. Anti-atherogenic potential is determined by the ability of the plants to confer protection within a vascular system.

Indigenous populations are known to possess diverse notions of health and wellness in comparison to Western societies (Boston, 1997; Robinson, 1995). As such, the findings aim to build the basis for a more integrative approach to diabetes treatment within the James Bay Cree communities.

2.0 LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus is a disease of global proportions that is estimated to affect approximately 150 million people worldwide (WHO, 2002). Moreover, this number is estimated to double by the year 2025 (WHO, 2002). Canada is appreciably affected by this epidemic with approximately two million Canadians currently suffering from diabetes (CDA, 2003).

Diabetes mellitus is a disease of the endocrine system, which results in insulin deficiency. Diabetes is characterized by hyperglycemia and if not properly controlled can lead to more long-term complications such as cardiovascular disease (Harris, 2002), micro- and macrovascular complications, blindness, peripheral vascular disease (Fabsitz, 1999), renal disease (Young, 2000) and bacterial infections. Two main categories exist, classified as type 1 or insulin-dependent diabetes mellitus (IDDM) or type 2 diabetes mellitus (DM2). Approximately 90% of diabetic cases are type 2, while 7-9% are type 1. A third category known as gestational diabetes is found to represent 1-3% of the cases.

Type 1 diabetes mellitus is characterized by an earlier onset and involves the autoimmune destruction of β -cells in the pancreas resulting in cessation of insulin production. IDDM is a more severe form of diabetes, which requires insulin therapy as a means of sustaining life.

DM2 characteristically emerges in adulthood and results in insulin resistance within the body's tissues, which alters proper insulin secretion from the pancreas. Insulin resistance in turn leads to decreased uptake of glucose in the cells, leading to hyperglycemia. DM2 occurs gradually over the course of months or years and is not easily detected if mild symptoms occur.

Diabetes results from two primary defects, insulin resistance due to decreased tissue sensitivity and impaired β -cell function, which delays or causes inadequate insulin production (Shils, 1999). Insulin, a key hormone within the body, ensures the proper delivery of glucose, the body's key energy source, across the intracellular membrane for utilization by the body. Without insulin, or in the presence of resistance to insulin by the body's tissues, glucose accumulates in the blood, leading to hyperglycemia.

2.1.1 DIABETES AND INDIGENOUS PEOPLE

Canadian Indigenous populations have been greatly affected by the rising incidence of diabetes. In 2001, the Cree Board of Health and Social Services of James Bay (CBHSSJB) reported a prevalence rate of 11.7% among the Eeyouch Istchee (Crees of the James Bay Region) (Statistics Canada, 2004). Factors such as an increase in market food consumption, a more sedentary lifestyle (Boston, 1997), a decrease in traditional food use (Kuhnlein, 1996) and the "thrifty" genotype (Young, 2000) have been proposed as key contributors to the rise in diabetic cases.

Initiatives have been taken by the Canadian government to help diminish the burden of diabetes within the Indigenous communities of Canada. Health Canada announced in 1999 the allocation of funds to the Aboriginal Diabetes Initiative (ADI) as a means of dealing with the diabetes epidemic. At a provincial level, the Quebec government in collaboration with the First Nations of Quebec and Labrador Health and Social Services Commission (FNQLHSSC) offered support for activities and programs such as native healing and healthy living workshops, community gardens, and cooking courses. Despite these initiatives the incidence rates of diabetes continue to rise (Statistics Canada, 2004).

2.1.2 SYMPTOMS, CAUSES AND THERAPIES OF DM2

DM2 is characterized diagnostically by polydipsia, polyuria, rapid weight loss, excessive thirst, fatigue, elevated blood glucose levels (over 11.1 mmol/L) and fasting plasma glucose levels over 7.0 mmol/L (CDA, 2005). The underlying determinants of diabetes are still under investigation. However, studies have shown factors such as excess weight, genetics and a sedentary lifestyle as probable contributors to the epidemic proportions of diabetes. Most DM2 patients display few, if any, symptoms early on in the disease, leading to late-stage diagnosis. DM2 is typically treated with exercise, nutritional therapy, and oral medications and less often, insulin.

The Canadian Diabetes Association (CDA) recommendations include a regimen of physical activity, dietary modification, decreased alcohol consumption, and proper medical monitoring of physiological levels of glucose and insulin. DM2 patients are recommended to participate in at least 150 minutes of moderate-intensity exercise at least three times per week (Boulé, 2001; CDA, 2005). Exercise in concert with resistance training has been found to improve glycemic control and lipid profiles, as well as, decrease insulin resistance and weight gain (Boulé, 2001; Dunstan, 2002). Large-scale cohorts have also confirmed the reduced risk of morbidity associated with regular physical activity (Hu, 2001).

Nutritional therapy is a central part of successful intervention strategies for diabetic patients. The CDA recommends a diet rich in low-glycemic index foods, a reduced intake of saturated and trans fats equal to <10% of energy, with an emphasis on consumption of polyunsaturated fatty acids (CDA, 2005). Integration of low-glycemic foods in the diet is beneficial in reducing blood glucose responses in diabetic patients, while a diet low in carbohydrate-rich foods has been found to lower postprandial glycemic levels, triglyceride levels and increase insulin sensitivity (Parillo, 1992; Garg, 1994). Protein intake is recommended to be a source of 15-20% of energy, while fats should be restricted to <30% of energy. The type of fats ingested should favour a low saturated and

high polyunsaturated ratio with emphasis on consumption of omega-3 fatty acids. Past studies show a diet high in mono- and polyunsaturated fatty acids is beneficial in maintaining healthy lipid profiles and glucose metabolism (Howard, 2002; Grundy, 2002; Jenkins 2002).

2.1.3 SUSCEPTIBILITY OF DIABETIC PATIENTS TO ATHEROSCLEROSIS AND CVD

Cardiovascular disease (CVD) has become a major affliction of developed societies and is beginning to affect developing countries. Many developing countries experiencing nutritional transition have seen an alarming rise in the rates of chronic diseases such as obesity, hypertension, diabetes, and cardiovascular disease (Hamilton, 1988; Bogin, 1997). The rate of DM2 worldwide has reached pandemic levels, with its complications giving rise to disquieting rates of mortality (CDC, 2003).

Diabetic patients are two-to-four times more likely to experience cardiovascular events versus their non-diabetic counterparts (Haffner, 1998). In 2003, 5.2 million people worldwide were afflicted by cardiovascular conditions associated with diabetes. (CDC, 2003) As such, cardiovascular events are currently the major affliction of diabetic patients worldwide. Eeyou Istchee communities of Canada are among these afflicted communities, with 13% of diabetic patients suffering from cardiovascular complications (Statistics Canada, 2004).

These rates can be largely attributed to various metabolic conditions such as hypertension, hypercholesterolemia, obesity, and insulin resistance (Libby, 2002; Mokdad, 2001; Doualhy, 2005). Hyperglycemia increases the levels of lipid peroxidation and glycoxidation, which in turn propagates a decrease in arterial wall function (Baynes, 1991). This relationship between DM2 and cardiovascular events is a result of endothelial dysfunction, which has been linked to increased levels of oxidative stress (Beckman, 2002).

2.1.4 WESTERN AND NON-WESTERN TREATMENTS FOR DM2

Western treatments currently promoted for diabetic patients focus on optimizing insulin action within the body. As such, their actions target pivotal organs implicated in proper insulin function. Various pharmacological agents are administered to diabetic patients pending the state of diabetes and the duration of the disease (CDA, 2005). Medications vary from their action to their site of target; glucophages and biguanides (i.e. metformin) target the liver to reduce the production and output of sugar; thiozolidinediones (i.e. Actos®, Avandia®) act on the muscle cells to stimulate uptake of glucose; meglitinides and sulfonylureas (i.e. Diabeta, Diamicon, Amaryl®, Gluconorm® and Starlix®) act on the pancreas in promotion of insulin secretion; and α -glucosidase inhibitors (i.e. Prandase®), act on the small intestines by delaying digestion and absorption of glucose from the gut (CDA, 2005; ADA, 2005). Due to the multifaceted nature of DM2, these drugs may also be given in combination with medications, which act to lower the other risk factors of diabetic patients (i.e. hypertension).

The limited efficacy and dangerous side effects of hypoglycemic treatments has lead to the investigation of traditional remedies. Many hypoglycemic agents are found in effective quantities in medicinal plants ingested for centuries by Indigenous communities worldwide. The most widely used traditional plants used for the treatment of diabetes include *Momordica charantia*, *Catharanthus roseus*, *Anacardium occidentale* and *Trigonella foenum-graecum* (Abdel-Barry, 1997; Vats, 2002; McCune, 2003).

Many of the plants known to possess hypoglycemic activity contain key components, which target specific organs and tissues and act through various mechanisms. *Trigonella foenum-graecum* (fenugreek) has been known for many years to exert anti-diabetic activity through its ability to decrease postprandial glucose levels (Madar, 1998).

The antioxidant potential of plants has been shown to be a major determinant of their anti-diabetic properties. The antioxidant potential of a plant has been shown to inversely correlate to the degree of hyperglycemia-induced oxidative stress (Antolovich, 2002). Antioxidants exert their action on lipid membranes by inhibiting the degree of lipid peroxidation resulting from oxidized lipid by-products, primarily ox-LDL (Halliwell, 1989; Li, 1993).

A diet rich in fish & marine mammals has also been found to correlate to lower prevalence rates of diabetes and glucose intolerance (Dyerberg, 1986; Harris, 1989; Von Shacky, 1992; Kuhnlein, 1994). This is exemplified by the lower rates of DM2 within Canadian Inuit communities in comparison to the remainder of Indigenous populations.

2.2 OXIDATIVE STRESS AND ANTIOXIDANT DEFENCES

2.2.1 OXIDATIVE STRESS AND FREE RADICAL GENERATION

Oxidative stress is characterized by an imbalance in the pro-oxidant / antioxidant levels within the cells. This state of oxidative stress is a result of increased levels of intracellular reactive oxygen species (ROS) or pro-oxidants. Normal oxygen metabolism within the body produces metabolites such as the super oxide anion (O_2^-), the hydroxyl radical (OH^\cdot) and the peroxy radical (ROO^\cdot). An increase in oxygen radicals occurs either as a primary defence mechanism or through the hypoxanthine-xanthine oxidase reaction (Junod, 1989). These radicals are deleterious to erythrocyte membranes due to their unpaired electron, which dictates their reactivity.

Free radical oxidation proceeds by a chain reaction initiated by a sole radical. The deleterious level of these radicals is due to their ability to propagate numerous chain reactions (Nidi, 1991). It is these non-enzymatic, free radical-mediated mechanisms, which are notably observed in many pathologies of chronic disease (Nidi, 1991).

Free radical generation is a recurrent condition within diabetes resulting from hyperglycemia or high blood glucose levels. Hyperglycemia increases the level of oxidative stress via glucose auto-oxidation, decreases in antioxidant defences, and glycosylation of functional proteins (Figure 1) (Baynes, 1991; Guerci, 2001; Chew, 2004). The increase in advanced glycated end products (AGE) concomitantly decreases the availability of nitric oxide (NO), leading to subsequent increases in the susceptibility of LDL to oxidation (Chew, 2004; Duvall, 2005; Steffen, 2005). The disturbance in the antioxidant/ pro-oxidant equilibrium as a result of DM2 has direct implications on vascular integrity and endothelial and metabolic function (Guerci, 2001; Benito, 2002; Anter 2004; Chew, 2004; Steffen, 2005).

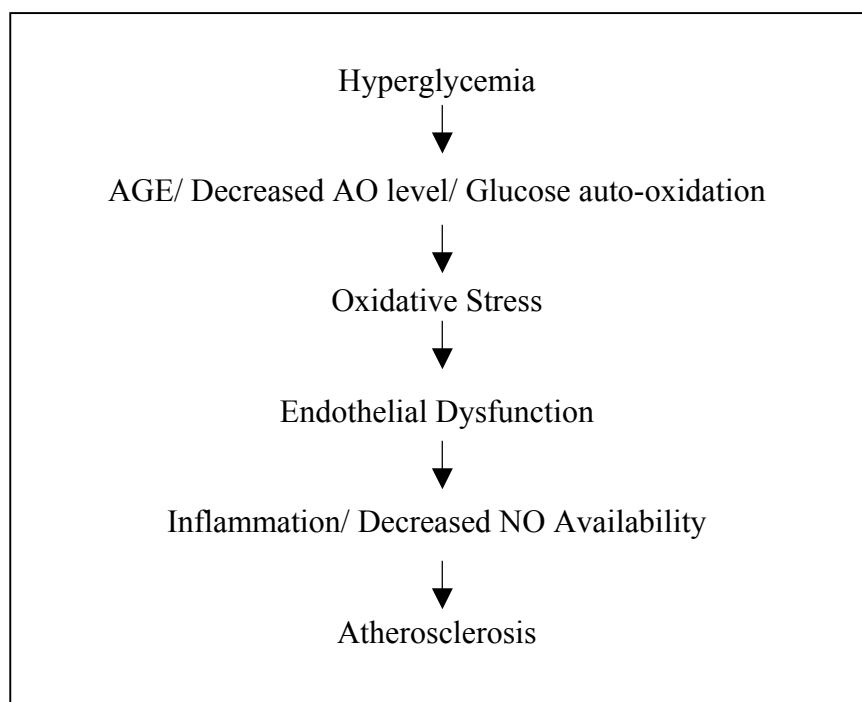


Figure 1: The early events in the pathogenesis of atherosclerosis incited by hyperglycemia. Modified from Chew et Watts, 2004 and Guerci et al., 2001. AGE: Advanced glycated end-products; AO: Antioxidant; NO: Nitric Oxide.

2.2.2 ANTIOXIDANT DEFENCES

An antioxidant can be characterized by its ability to significantly delay or inhibit the oxidation process of a substance (Halliwell, 1989). Enzymatic and non-enzymatic antioxidant defences work jointly to ensure the proper recycling and shunting of ROS (Diplock, 1998).

Researchers have for years been attempting to discern defence mechanisms to counter the damaging effects of oxidative stress and help decrease the rates of chronic disease. Early hypotheses have suggested an increase in the levels of antioxidants, in view of the fact that low levels of antioxidants in relation to pro-oxidants characterize oxidative stress (Halliwell, 1989). Studies in support of this hypothesis have showed an inverse correlation between oxidative stress and antioxidant levels, primarily those of α -tocopherol and carotenoids (Antolovich, 2002). Subsequent studies have shown a sequential loss of endogenous antioxidants as a result of lipid peroxidation (Brandi, 1992).

Antioxidants act as a primary defence against ROS at the site of chain initiation within the free-radical mediated chain reaction (Niki, 1995). Their position, therefore, makes them important as scavengers of chain-carrying radicals (Niki, 1995). Endogenous and exogenous antioxidants, such as α -tocopherol, ascorbic acid (Vitamin C), carotenoids and flavonoids play an essential role in scavenging of free radicals. Two major endogenous antioxidants, superoxide dismutase (SOD) and glutathione reductase play a major role in free radical defence. They maintain the levels of antioxidants in the system and help protect against oxidation of low-density lipoproteins (LDL) (Halliwell, 1989; Li, 1993).

Ascorbic acid (Vitamin C) is a water-soluble analog able to scavenge superoxide, hydrogen peroxide and hydroxyl radicals. Its major function is the transport of radicals across the membrane for deterioration and elimination from the body (Niki, 1987). Ascorbic acid's major role is the recovery of Vitamin E via an electron-mediated transfer of the Vitamin E radical (Jailal, 1991; Sies, 1992;

Simon, 1992). However, in the presence of free metal ions, ascorbic acid can also play a role as a pro-oxidant increasing the incidence of lipid peroxidation (Cao, 1997).

Alpha-tocopherol (Vitamin E) is a lipophilic antioxidant found in cellular membranes. Its strategic positioning within the membrane accounts for its major role in inhibition of lipid peroxidation (Niki, 1995; Diplock, 1998). It is highly reactive with free radicals, primarily that of nitric oxide (Ahmad, 1999). As such it is implicated in the maintenance of microvascular circulation.

Carotenoids are lipophilic compounds present in lipoproteins such as high-density lipoprotein (HDL) and LDL (Esterbauer, 1991). Their role as antioxidants lies in their ability to quench free radicals, primarily the superoxide and peroxy ions (Paloza, 1992). The major role of carotenoids within lipid peroxidation is defence against LDL oxidation.

Non-nutritive antioxidants such as phenolics, flavonoids, and terpenes are water-soluble antioxidants able to break free radical chain reactions and chelate metal ions (Halliwell, 1996; Rice-Evans, 1996; Cao, 1997). Their free radical scavenging ability makes them ideal candidates as co-antioxidants to major antioxidants such as α -tocopherol and ascorbic acid (Kandaswami, 1993; Thomas, 1996). Flavonoids have been found to be highly effective free radical scavengers due to their ideal positioning at the aqueous surface of phospholipids (Brandi, 1992). Studies have also shown the ability of flavonoids to conserve endogenous α -tocopherol in LDL (de Whalley, 1990). This latter property allows for an effective defence system to aid in reducing levels of oxidative stress.

Industry has also been successful in producing synthetic compounds with well-established antioxidant potential. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were originally used as food additives and are of note due to their ability to inhibit LDL oxidation (Shahidi, 1992). Their action can be attributed to their ability to react with lipid peroxy, oxygen, and nitric

oxide radicals (Ahmad, 1999). BHT and BHA's aromatic ring structure contributes to their antioxidant ability in terminating radical chain reactions (Ahmad, 1999).

Past research has focused on the supplementation of individual antioxidants as possible mechanisms against oxidative stress and disease. Studies both *in vitro* and *in vivo* with human samples have shown a positive correlation between oxidative stress and disease incidence (Esterbauer, 1991; Vaya, 1997; Kuyvenhoven, 1999; Beckman, 2002; Chew, 2004). However, few single supplementation programs have shown promising effects. Clinical trials involving vitamin E supplementation are among the few that exhibit positive effects however; the effects are more pronounced with combination therapy of vitamin supplementation (Gey, 1991; Singh, 1995a; Singh, 1996). As a result, public health initiatives have focused on more whole food research to help in promotion of overall health.

2.2.3 SOURCES OF ANTIOXIDANTS

Natural antioxidants are primarily found in plant sources. Ascorbic acid and α -tocopherol are found in large quantities within fruits and vegetables. They are of interest due to their nutritive value alongside their antioxidant potential. They have been shown to be key preventative agents in many illnesses of both developed and developing countries (Block, 1992; Hertog, 1993; Halliwell, 1994; Fuhrman, 1995; Ide, 1997; Laplaud, 1997; Wilson, 1998).

Phenolic compounds are highly prevalent in plant species and are a major contributor to the flavour and colour of many fruits and vegetables. They are readily available in citrus fruits, chocolate, grapes, olives, strawberries, tomatoes, tea, and wine (Stagg, 1975; Hertog, 1993; Frankel, 1995). They have received much attention related to the benefits of a diet rich in fruits and vegetables in prevention of chronic diseases and cancers (Block, 1994; Halliwell, 1994; Rice-Evans, 1996; Enstrom, 1992; Hu, 2000).

Flavonoids comprise a subgroup of compounds, within the phenol and polyphenol category, and are found in numerous foods, spices, cosmetics, and pharmaceuticals (McCune, 2003). Notable contents of polyphenolic antioxidants have been found in vegetables, fruits, and beverages, such as berries, wine, and tea (Ishikawa, 1997). Many spices have also been studied and found to have appreciable amounts of antioxidants; such items include rosemary, oregano, sage, thyme, and pepper (Huang, 1994; Plumb, 1995; McCune, 2003).

The French and Mediterranean diets have stimulated the investigation of highly active plant constituents as possible protective agents against chronic disease (Renaud, 1992; Frankel, 1993; Groenbaek, 1995; Miyagi, 1997). The paradox of the Maasai culture is also of interest due to the low rates of cardiovascular disease in light of a diet rich in saturated fats and cholesterol (Johns, 1996). Further investigation into the Maasai diet unveiled a cardioprotective effect of the bark constituents found to have high phenolic content. China offers another example of low rates of cardiovascular events as result of high tea consumption (Stensvold, 1992; Green, 1992; Imai, 1995). The cardioprotective effect of tea has been also attributed to its high phenolic content.

2.2.4 ROLE OF ANTIOXIDANTS IN ATHEROSCLEROSIS

LDL normally occurs with antioxidants, of which, α -tocopherol, γ -tocopherol, β -carotene, lycopene, and ubiquinol are included (Esterbauer, 1990). The strategic positioning of these antioxidants, under normal physiological conditions, provides considerable protection against oxidative modification of lipids. It is only upon exhaustion of these reserves that LDL is susceptible to oxidation and thus takes a pro-oxidant role. Lipid hydroperoxides cause a loss of endothelial cell integrity precipitating an increased permeability of macromolecules across the endothelium (Peng, 1998; Ashdate, 2003; Mehta, 2006). Research has shown ox-LDL's ability to induce endothelial damage, increase recruitment of macrophages to the arterial wall, compromise vascular tone, and induce

inflammatory parameters (Esterbauer, 1990; Ceriello, 2003; Chew 2004). Vascular cells are further exposed to oxidation during inflammation, endotoxic shock, and hypertension. Atherosclerosis is thus characterized by a chronic inflammation of the arterial wall caused by endothelial dysfunction (Mano, 1995; Guerci, 2001; Ceriello, 2003; Mehta, 2006).

Antioxidants act by inhibiting key oxidative chain reactions responsible for the lipid peroxidation implicated in endothelial dysfunction. Ascorbic acid is involved in this process through its ability to shunt free radicals and thus avert damage to biological membranes. The latent action of ascorbic acid was validated in animal studies of which deficiency resulted in myocardial injury (Steinberg, 1989; Stadtman, 1991; Jailal, 1991).

Vitamin E has distinct effects on cardiovascular parameters, which categorizes it as the most effective antioxidant with respect to lipid peroxidation (Jailal, 1992; Dutta, 2003; Munteanu, 2004). Epidemiological and animal studies have shown an inverse relationship of serum levels of vitamin E and rates of cardiovascular events (Gey, 1991; Riemersma, 1991; Stampfer, 1993; Morel, 1994). This action however, is dependent on the ubiquitous supply of reducing antioxidants primarily that of ascorbic acid and selenium (Jailal, 1991; Sies, 1992; Simon, 1992).

Metal-chelating polyphenols have also been found to be effective in preventing ROS production and providing protection against oxidation of LDL (Laughton, 1991). Polyphenols may also be implicated in cardioprotection due to their effect on prostaglandin production (Fitzpatrick, 1998; Huang, 1999; Shutenko, 1999; Benito, 2002). Flavonoids, more generally, have been shown to induce vasorelaxation in rat aorta through increased nitric oxide (NO) production (Benito, 2002).

2.3 LDL PEROXIDATION

Lipid peroxidation, a result of prolonged oxidative stress, is the process in which unsaturated lipids in LDL react with oxygen species to yield hydro peroxides.

The process is initiated by an oxygen free radical, interacting with lipids on the membrane surface and proceeds by a chain reaction. LDL particles are made of complex molecules, such as, cholesterol esters and triacylglycerols of n-3 and n-6 polyunsaturates. Oxygen radicals, metal ions, and endothelial cell radicals originating from the arterial wall readily oxidize polyunsaturated lipids.

Hydroperoxides formed by lipid oxidation decompose into aldehydes and interact with apolipoprotein B (apo B) (Zhang, 1994). The modified lipoproteins are no longer recognized by the lipoprotein receptors and subsequently taken up by the “scavenger” receptor system (Vaya, 1997). Due to lack of regulation, there is often an accumulation of ox-LDL, better identified as foam cells (Vaya, 1997).

The oxidization of LDL by metal ions is the most extensively studied mechanism in *in vitro* studies and is readily characterized by binding of Cu^{2+} to apoB and reduction of LDL by Cu^{2+} (Antolovich, 2002). Metal ions catalyze the initiation and decomposition of hydroperoxides to produce high levels of volatile products. The metal ions function by similar mechanisms as oxygen free radicals to produce parallel degrees of damage to arterial wall function. Oxidative damage to various tissues throughout the body has been credited as the cause of chronic diseases, such as, atherosclerosis and diabetes (Vaya, 1997).

2.3.1 CYTOTOXIC EFFECTS OF OXIDIZED LOW-DENSITY LIPOPROTEINS

LDL is responsible for lipid transport and cholesterol synthesis (Myers, 1996). Oxidation of LDL is characteristically found in areas of low antioxidant concentrations (Bankson, 1993). Free radical generation is initiated by the

oxidation of polyunsaturated fatty acids (PUFA), which in turn causes a decrease in the levels of endogenous antioxidants. As LDL is oxidized, it becomes a potent chemoattractant to circulating monocytes, in turn inhibiting the mobility of macrophages found within the surrounding tissues.

Under normal physiological conditions, the endothelium's function is to vasodilate, inhibit platelet formation and suppress inflammation via production of prostacyclin and NO (Chew, 2003). The cytotoxicity of ox-LDL to endothelial cells can be attributed to its ability to modify prostaglandin synthesis, modulate expression of cytokines and affect coagulation (Bankson, 1993; Vergnani, 2000). Once modified, macrophages are able to bind ox-LDL with a greater affinity. The increased binding of ox-LDL leads to the eventual formation of foam cells, characterized as fatty streaks (Steinbrecher, 1990; Ross, 1993; Virella, 1995). Mineralization of fatty streaks exemplifies the early stages of atherosclerosis.

2.3.2 ROLE OF OX-LDL IN DIABETIC PATIENTS

Several chronic conditions, such as, DM2, hypertension, and atherosclerosis are implicated in the alterations seen within the endothelium (Luscher, 1993; Luscher, 1994). Damaging factors resulting from DM2, such as, high glucose levels, lipid hydroperoxides, increased free fatty acids, and oxidation end-products may be the catalyst for endothelial dysfunction seen within macrovascular complications (Eckel, 2002).

2.4 TRADITIONAL LIFESTYLE OF THE CREE

The Cree have inhabited the James Bay Region for over 5000 years (Adelson, 1992). Traditionally, they were a nomadic, hunter/gatherer society relying solely on the land for sustenance (Kuhnlein, 2000). The Crees traveled in small to large groups across numerous hunting grounds within the northern James Bay region. Since the arrival of the Europeans in the 18th century, the trading posts were a meeting ground in the summers to sell their furs, socialize, and gather supplies for their return to the land (Robinson, 1988). The Cree culture is characterized

by a harmony with the animals and the plants as a means of survival and respect for their creator (Berkes, 1999). The use of traditional medicines was an integral part of the Cree culture passed through generations by oral transmission (Ohmagari, 1997).

2.4.1 MODERNIZATION AND ITS EFFECTS ON CREE HEALTH

The Cree population was introduced to Western influences with the arrival of the first settlers in the early 1700's (Berkes, 1978). The infiltration of missionaries and Hudson Bay posts into the James Bay Region introduced the Cree to amenities never experienced in the past. With the trading of furs came the reliance on non-native products seen by the Cree as markers of craftsmanship and prosperity. The installation of the Hydro Quebec projects, beginning in 1975, forced the nomadic Cree hunters to settle in more permanent communities (Adelson 1992; Hydro-Québec, 1993a-b). With the shift to more communal settlements came a decreased reliance on the land and subsequently decreased levels of physical activity and consumption of traditional foods.

The development of communal infrastructures further exacerbated the loss of cultural identity with the infiltration of larger numbers of non-native people into the communities (Adelson, 1992; Robinson, 1988). The development of communities produced local employment opportunities entailing less time for hunting, gathering, and traditional activities. The Cree communities were further influenced by the government assimilation initiative in 1955 (Hydro-Québec, 1993a-b). The removal of the younger generations from their homes and their culture caused a dramatic loss in cultural transmission. Children were no longer exposed to their ancestral activities, which defined them as a people.

As time progressed, communities gained greater access to southern goods, which brought an increased consumption of western foods. The communities moved to a monetary based society with acquisition of funds from western industry (Robinson, 1995). Communities began seeing an emergence of Western foods as a normal part of daily life. In 1991, Santé Québec documented an increased

consumption of market foods among young Crees, versus their older counterparts, consisting of wheat flour, refined sugar and saturated fats (Daveluy and Bertrand, 1998). The decreased consumption of traditional foods among the younger Cree generation was highlighted by only a 15% consumption rate of wild game meats (Young, 2000; Hydro-Québec, 1993b). Bériault (1992) observed similar trends with increased preference for store-bought and canned meats among the younger Cree populations.

2.4.2 SOCIOCULTURAL ASPECTS SURROUNDING THE USE OF TRADITIONAL REMEDIES AMONG THE CREE

Within Indigenous cultures of the past, the distinction between food and medicine was typically not clearly defined (Johns, 1990). The Maasai of East Africa provide an example of this pattern with foods and medicines equally represented in their traditional cuisine. The anomaly of the low incidence of cardiovascular disease despite a high saturated fat and cholesterol diet was discovered to be a result of the cardioprotective effects of bark constituents used as an additive in Maasai cuisine (Johns, 1996). Similar properties of ingested plants may have also been important for Canadian Indigenous people, as the incidences of chronic diseases, such as, diabetes and cardiovascular disease have only been a concern of the last 50 years. Previous research highlights the plethora of knowledge of Canadian Indigenous groups in regard to flora pharmacopoeia (Leighton, 1985; Johnson, 1995; Kuhnlein, 1996; Marles, 2000).

The Anglican missionaries and Western paradigms influenced the Cree in ways that diminished their beliefs in their spiritual connection with the land (Zieba, 1990). Alongside this spiritual alteration came the loss of traditional knowledge and traditional activities. As a result, traditional use of plants took on a new form with clear distinctions formed between foods and medicines.

2.5 GLOBAL VIEW ON TRADITIONAL MEDICINES

Traditional medicines provide remedies to over 80% of Indigenous People in developing countries worldwide (Bennerman, 1983). Many well-known drugs in use today in Western medicine have their roots in the folk culture of Indigenous groups. A few examples of notable worth are vincristine, vinblastine, and aspirin, which are in current use for cancer treatment and anti-inflammatory responses. The WHO acknowledged the importance of traditional remedies in the healthcare system and the need for standardization, in 1995, with the initiation of its plant monographs. The monographs are an attempt to outline the safety, quality, and efficacy of plant materials currently in use in both developed and developing countries (WHO Report, 1998).

The sale of natural remedies within developed countries, primarily Europe and the United States, has increased tremendously over the last ten years becoming a billion-dollar market (Eisenberg, 1998). The minimal attention to traditional medicinal research in the past is influenced by Western colonialism throughout the world (Balick and Cox, 1997). With the settlement of Europeans within various Indigenous communities came dominance of European influences, including medicine. New medicines were often viewed by Europeans to have liberated Indigenous people from past maladies. As a result of this intrusion into Aboriginal culture and society and the eventual infiltration into Indigenous lands, traditional remedies took on a more primitive and unrecognized position. In Canada, the arrival of missionaries' to the northern Quebec regions further aggravated the situation with the eventual tabooing of natural remedies in practice by the Cree (Hydro Quebec, 1993a).

3.0 HYPOTHESES AND OBJECTIVES

3.1 HYPOTHESES

- 1) H_0 : The frequency of use and attitudes toward the use of medicinal plants and traditional foods will not differ among the various age groups and genders.

H_a : The frequency of use and attitudes toward the use of medicinal plants and traditional foods will differ among the various age groups and genders.

- 2) H_0 : Plant extracts highlighted for use for symptoms of DM2 will not show significantly greater antioxidant activity than standard controls.

H_a : Plant extracts highlighted for use for symptoms of DM2 will show significantly greater antioxidant activity than standard controls.

- 3) H_0 : Plant extracts shown to have antioxidant potential will not significantly inhibit the effects of oxidized LDL on cardiovascular parameters.

H_a : Plant extracts shown to have antioxidant potential will significantly inhibit the effects of oxidized LDL on cardiovascular parameters.

3.2 SPECIFIC OBJECTIVES

- 1) To assess the quantitative and qualitative aspects of anti-diabetic plants used by the Cree
 - a. To determine the frequency of consumption of traditional medicines and foods
 - b. To determine the patterns of consumption in the varying age groups
 - c. To determine the views surrounding the use of anti-diabetic plants

- 2) To assess the *in vitro* antioxidant potentials of the plant extracts from *Sarracenia purpurea*, *Larix laricina*, *Rhododendron groenlandicum*, *Abies balsamifera*, *Alnus incana* spp. *rugosa*, *Picea mariana*, *Pinus banksiana*, *Sorbus decora* by their ability to,
 - a. Scavenge free radicals
 - b. Provide cytoprotection
 - c. Inhibit lipid peroxidation

4.0 METHODOLOGY

4.1 QUESTIONNAIRE DEVELOPMENT

The choice of research tools in a culturally diverse setting is pivotal to successful data collection and participant compliance. As Teufel (1997) states, “cultural competency” is the key to successful research and culturally appropriate data collection. Due to the changing lifestyle habits of North American Indigenous People and the increasing incidence of chronic diseases, many researchers have looked to the dietary habits of people for reasonable solutions. Epidemiological and census studies have shown a change in dietary habits among Indigenous People with a strong correlation to increased incidence of chronic diseases (Robinson, 1995; Young, 2000; Harris, 2002).

Studies have highlighted the reduced consumption of traditional foods in the last century; however the consumption of traditional medicines has not been assessed within the Indigenous communities of Canada, particularly among the Cree communities of the James Bay Region. In addition to considerations of frequency consumption are the questions concerning the use of traditional medicines and the reasons for the decrease in the use of traditional medicines across the years.

4.1.1 FOOD FREQUENCY QUESTIONNAIRE

A food frequency questionnaire (FFQ) is the most commonly used tool for assessment of dietary intake. A common format consists of a list of food items grouped into their respective food groups (Teufel, 1997). The food items included are based on the individual researchers’ objectives.

Respondents are asked their frequency of consumption of each food item on a time-based scale outlined by the researcher. FFQs can be quantitative or semi-quantitative, such that, respondents are asked to quantify a usual amount of the respective food item consumed at a seating. FFQs are a commonly used tool in

large-scale studies due to their ability to assess usual intake over a longer period of time relative to a 24-hour recall. FFQs are also easy to administer and thus do not require lengthy periods of time. They are able to provide a thorough overview of consumption trends and can be easily correlated to lifestyle behaviours. (Togo, 2003) The FFQ therefore, becomes a valid research tool in assessment of dietary and health trends.

The FFQ used in this study was a non-quantitative FFQ designed to assess both medicinal plant and traditional food consumption trends. The FFQ was developed using a seasonal hunting calendar constructed by local hunters. The respective food and plant items were grouped according to species or genus. The list of plant items was based on a previously developed list obtained from interviews with key elders within the community in 2003 (Leduc, 2005). The list was derived from responses given by elders and/or healers on an open-ended questionnaire developed to highlight plants used for symptoms of DM2 (Leduc, 2005). Expert medical practitioners in the domain of DM2 treatment derived the list of symptoms. The questionnaires were developed in close conjunction with the Cree Board of Health and Social Services of the James Bay Region (CBHSSJB) to help preserve cultural integrity.

Food and plant items were assessed on a daily, weekly or yearly consumption depending on the respective respondent's habits. Trends were then broken down into seasonal consumption based on community suggestions and seasonal availability of game animals and plants.

4.1.2 SOCIO-BEHAVIOURAL QUESTIONNAIRE

The socio-behavioural questionnaire was developed to assess social and behavioural trends in relation to use of traditional medicines. The questionnaires consist of structured questions aimed to outline several key ideas surrounding the use of traditional medicines. The first theme highlights adherence to a traditional lifestyle. Traditional lifestyle is defined as a reliance on ancestral traditions for

sustenance. The second theme aims to highlight the concerns surrounding the consumption of traditional foods. The third theme highlights the type and level of transmission of traditional knowledge. The emphasis in the third theme was placed on traditional knowledge in relation to traditional medicines. However, all responses were encouraged in open-ended questions. The final objective aimed to assess the community's perceptions of health and well-being.

4.2 IN VITRO CELL CULTURE

Cell culture is the process in which a specific cell line is isolated from the *in vivo* state. It is capable of living independently from its homeostatic environment and to mimic *in vivo* conditions. Cell culture is advantageous in that it allows for a controlled environment with a degree of consistency and reproducibility (Butler, 1996). Cell cultures are readily used systems due to their ability to investigate a diversity of objectives. Cell cultures allow for a basic investigation of biochemical and physiological systems within specific cellular structures (Butler, 1996). The disadvantages of working with cell culture is the ability of the cell cultures to adapt to various manipulative stimuli (i.e. medium additives), change in cell characteristics due to the lack of *in vivo* surrounding conditions and the survival of the fittest condition, which is not representative of *in vivo* conditions (Butler, 1996). All these combined may lead to positive, however misleading conclusions in regard to the relationships seen in culture.

4.2.1 BOVINE AORTIC ENDOTHELIAL CELLS

Bovine aortic endothelial cells (BAEC) are a primary cell culture isolated from the aorta of livestock. The aorta is surrounded by epithelial tissue and as such produces a monolayer of cells with a characteristic cobblestone form (Butler, 1996). Endothelial cells rapidly adapt with the addition of culture medium supplied with the necessary nutrients and serum. BAEC grow ideally in Dulbecco Modified Eagle Medium (DMEM) with addition of essential amino acids, fetal bovine serum and antibiotics.

4.2.2 ANTIOXIDANT AND CARDIOPROTECTIVE ASSAYS

4.2.2.1 In *vitro* Antioxidant Assays

Numerous assays have been utilized over the years for validation of antioxidant activity of plant materials. Assays are chosen based on plant constituents and researcher's objectives. Numerous assays for analysis of antioxidant activity have been proposed and proven to have high efficacy in detection of antioxidant activity. The most notable assays are the 1,1-diphenyl-2-picryl-hydrazyl (DPPH), thiobarbituric acid-reacting substances (TBARS), xanthine oxidase, oxygen radical absorbance capacity (ORAC) and the DCF/AAPH assays. For the purposes of this study we found two of these assays to be adequate and effective assays to meet the study objectives.

4.2.2.2 1, 1 diphenyl-2-picryl-hydrazyl (DPPH) Assay

The DPPH assay is one of the earliest synthetic methods for analysis of antioxidant potential. It is a simple and easy method to detect anti-radical efficiency of potential anti-diabetic plants. The DPPH assay unveils preliminary information on the reactivity of compounds with a stable free radical. Due to DPPH's odd electron, a strong absorption band is seen in visible spectroscopy at 517nm (Cotelle, 1996). The band formed is a result of the odd electron of DPPH pairing with a free radical scavenger. The tapering and eventual consistency of absorbance is inversely related to the uptake of electrons. The variance in decay slopes and absorbance levels is dependent on the nature and concentration of antioxidants within the extracts (Ratty, 1988). The activity of the extracts is expressed as the inhibitory concentration at 50% (IC₅₀), which relates to the concentration needed to decrease the absorbance by 50% in comparison to ascorbic acid (Cotelle, 1996).

The DPPH assay is limited due to the reactivity of DPPH radicals with various other radicals and the relatively irregular shape of the response curve relative to altering ratios of antioxidants to DPPH radicals (Ratty, 1988). Overall, the DPPH

bleaching test has proven to be a valid tool in determination of anti-radical efficiency of antioxidant constituents.

4.2.2.3 Thiobarbituric acid-reacting substances (TBARS) Assay

The TBARS (thiobarbituric acid-reacting substances) assay is a convenient method of evaluating free radical-mediated oxidation of polyphenolic components found within plant extracts. The TBARS assay measures the level of malonaldehydes (MDA) produced as a result of lipid peroxidation.

The primary aldehyde produced from lipid peroxidation is malonaldehyde (MDA), a hydrophilic aldehyde found within the aqueous phase of the membrane. MDA reacts readily with thiobarbituric acid, resulting in a red species, which can be detected spectrophotometrically at an absorbance of 532-535 nm (Zhang, 1994). The amount of detectable thiobarbituric acid reacted MDA produced over time, correlates to decreased LDL peroxidation and thus increased antioxidant activity. Therefore, low levels of MDA correlate to increased levels of antioxidants within the plant extracts (Zhang, 1994). The concentration of TBARS is calculated using a standard curve of MDA and results are expressed as nmol of MDA equivalents/g LDL protein (Owen, 2000). The plant extracts are compared to BHT, a phenolic preservative known to possess effective antioxidant activity.

The TBARS assay is limited due to the lack of specificity of the results, as well as, the synergistic effect of alpha-tocopherol and other key antioxidants on lipid peroxidation (Antolovich, 2002). The former limitation can be alleviated through the use of HPLC techniques to further separate the characteristic components of the fractions. Overall, the TBARS assay offers valid insight into the relative ability of plant extracts to effectively decrease lipid peroxidation within the cellular membrane.

4.2.2.4 Lactate Dehydrogenase (LDH) Assay

Assays for cellular toxicity allow researchers to decipher non-toxic doses of plant extracts of interest. Methods for determination of cellular toxicity commonly involve the use of dyes and radioisotopes as a result of cellular release (Korzeniewski, 1983). The ^{51}Cr -release assay was a well-established technique for cytotoxic analysis, but limited in its reliability (Korzeniewski, 1983). Korzeniewski and Callewaert found that lactate acid dehydrogenase (LDH) was a more efficient method due to its quantifiable and sensitive nature. The LDH assay is a measure of membrane integrity as it relates to LDH release into the cellular medium. LDH is an intracellular enzyme that leaks from the cells upon cell membrane damage (Li, 1993). The assay involves the reduction of NAD (NADH) via the LDH. The resulting NADH interacts with a tetrazolium dye to produce a coloured substance visible by spectroscopy (Legrand, 1992). Lower LDH concentrations relate to increased protection against cellular stresses. The LDH method is commonly used because of its accuracy, easy manipulation and reliable results.

5.0 CHAPTER FIVE: PRACTICES AND ATTITUDES SURROUNDING THE USE OF TRADITIONAL FOODS AND MEDICINES AMONG THE JAMES BAY CREE

5.1 INTRODUCTION

Diabetes mellitus is estimated to affect approximately 150 million people worldwide (WHO, 2002). Canadian Indigenous populations have epidemic levels of type 2 diabetes mellitus (DM2) with prevalence rates three to five times higher than the general Canadian population (Boston, 1997; Gray-Donald, 2000; Young, 2000). Factors such as an increase in market food consumption (Boston, 1997; Dewailly, 2002), a sedentary lifestyle (Antolovich, 2002), a decrease in traditional food use (Kuhnlein, 1996; Delorimier, 1999), and the “thrifty” genotype (Young, 2000) have been proposed as key contributors to the rise in DM2.

Multifaceted intervention strategies embodied in federal and provincial public health directives have proven ineffective in combating the rising rates of DM2. This has been attributed to a lack of sensitivity toward local traditions and social changes that have occurred as a result of Westernization (O’Neil, 1993; Boston, 1997). As such, integrative strategies more aligned with Cree traditions could prove to be effective in alleviating some of the burden of DM2 within these communities.

Traditional knowledge (TK) is a broad term used to define the knowledge, of a distinctive group of people, handed down from generation to generation (Ohmagari, 1997). It encompasses the skills and aptitudes of Indigenous People acquired across the ages (WIPO Report, 2000). Spradley takes a psychosocial approach in defining TK as, “the acquired knowledge people use to interpret and generate behaviour” (Balick and Cox, 1997, p.41). Maintenance of a traditional lifestyle therefore, relies on the oral transmission of TK over generations. Ethnobotanists and anthropologists are equally concerned as the transmission and

extent of knowledge is being compromised with increased Western exposure (Balick and Cox, 1997).

The uses of traditional foods (TF) and medicines (TM) are encompassed within the global definition of TK and as such are likewise subject to erosion and eventual extinction. TMs are most at risk due to the global decrease in plant biodiversity as a result of adoption of monetary-based economies and environmental pressures (Balick and Cox, 1997). Prohibitions against TMs as forms of witchcraft, by missionaries and Europeans more generally, has further exacerbated reduction in the exposure of younger generations to TM (Hydro-Quebec, 1993 a-b). Nonetheless, TMs still remain a primary source of therapy for ~80% of Indigenous populations worldwide (Bennerman, 1983). In addition, many drugs used globally in Western medicine are a direct result of plant-derived constituents (Arnason, 1981; Marles, 1995). The integration of Western and traditional medicines has been successfully exemplified within the medical systems of Mexico, Nigeria, Thailand, China, and Yukon (Balick and Cox, 1997). Thus TMs offer a direct link to possible intervention strategies for DM2 treatment more inline with Cree Indigenous culture.

Numerous studies have highlighted the benefits of TF consumption, with its increased omega-3 fatty acids, favourable PUFA content, and less market food substitutes often cited (Robinson, 1995; Belinsky, 1996; Kuhnlein, 1996; Rosol, 2005). An increase in TF consumption is also correlated to an increase in physical activity, as a result of the inherent exertion of hunting and gathering, the required travel to the bush, and the carrying of items to and from hunting sites. The indirect increase in the level of physical activity as a result of TF consumption proves to be beneficial based on previous studies highlighting the positive effects of physical activity on glucose homeostasis and weight control (Boulé, 2001; Dunstan, 2002). Elders have also been shown to attribute feelings of pride and comfort to acquisition and consumption of TFs (Robinson, 1995; Bobbish-Rondeau, 1996). This association has been recently acknowledged by

Canadian health professionals with the creation of culturally appropriate food guides for Indigenous communities.

As such, ethnobotanical and nutritional studies offer the opportunity for preservation of cultural behaviours, primarily those associated with traditional foods and medicines. They offer the communities a means of revival of ancestral customs amid the influence of a rapidly growing, urbanized setting.

This study was carried out to assess the prevalence of use and attitudes surrounding the use of traditional foods and traditional medicines. The overall objective is to provide an integrative approach to diabetes intervention more inline with Cree traditions.

5.2 MATERIALS AND METHODS

5.2.1 Study Site

The study was carried out in the community of Mistissini, one of ten Cree communities, located between the 49th and 55th parallel, within the James Bay region. It is one of four inland Cree communities within the Boreal forest region of Quebec (Figure 5.2.1.1). Mistissini has 2597 inhabitants with approximately 58.7% of the population over the age of twenty (Statistics Canada, 1995). The community of Mistissini was first established in 1679 with the development of the Hudson Bay Company posts. Mistissini has its well-established health and social infrastructures including a community clinic, primary and secondary schools, and community administrative offices.

5.2.2 Study Design

The study followed a cross-sectional design with participants interviewed in-person about their frequency of consumption of traditional foods (TF) and medicines (TM) over the last 12 months using a food frequency questionnaire (FFQ) (Appendix 1). A semi-structured socio-behavioural questionnaire (SBQ)

(Appendix 2) was used to assess participants' beliefs and perceptions toward use of medicinal plants and foods and concepts of health and DM2. Interviews performed in Cree were recorded and translation was validated by a secondary Cree source.



Figure 5.2.1.1: Geographical representation of the nine Cree communities of the James Bay Region. Source: <http://www.mcc.gouv.ca/region/10/dir10/cartecri.htm>

5.2.3 Population Sampling and Recruitment

Participants were recruited through door-to-door invitations, health care workers, and radio announcements. Participants received a consent form (Appendix 3), which was explained to them by an interviewer or interpreter in their language of choice (English or Cree). Confidentiality was explained to participants and consent forms were signed as confirmation of agreement to participate in the study.

Inclusion Criteria: Community members who identified as Cree, aged twenty years and older, and residents of the community of Mistissini.

Exclusion Criteria: Community members under the age of twenty years

The study sample was divided into two age groups, 20-59 and >59 years of age, as a result of previous study findings demonstrating decreased consumption of

traditional foods and medicines in younger generations in comparison to elders (Ohmagari, 1997; Daveluy, 1999; Young, 2000; Dewailly, 2002).

5.2.4 Ethics

Ethics approval was obtained from McGill University prior to commencement of the study (Appendix 4). Study approval was also received from the Cree Board of Health and Social Services of James Bay (CBHSSJB) in conjunction with the Council of the Cree Nation of Mistissini.

5.2.5 Frequency Determination and Statistical Analysis

Answers from both questionnaires were coded and entered into Microsoft Excel (n=173). A frequency score was determined based on the frequency in which the participant consumed the individual food item with respect to time (i.e. day, week, month, year and/or season). Each frequency was then converted to a score to reflect a yearly consumption. Participants were placed into their appropriate age grouping with their respective frequency score and an average was determined for each age group, with respect to total number of participants per group. The use of medicinal plants was recorded as a yes or no answer for use in the past year. The proportion of participants having used a respective plant mentioned in the questionnaire was used for tabular representations. Further analysis of use of TMs was done using a list of 15 symptoms of DM2 formulated by a team of expert physicians in DM2 treatment. Responses for use of TMs were then categorized into the 15 symptoms and used for graphical and tabular representations reported. This list was previously used in ethnobotanical studies performed by Leduc et al. (2005) to outline key plants in use for symptoms of DM2. The plants highlighted in the above-mentioned work formed the basis for the plant listing found within the FFQ. Responses to the SBQ were coded and proportions of responses were reported.

The Excel data sheets were exported into the SAS Statistical Analysis v9.1 program. Statistical analysis was performed to yield means, medians, standard

deviations and frequencies. Chi-squared and logistic regression analyses were performed to test the association between variables and the effects on consumption of TMs. A Student's t-test was used to test the differences of consumption of TMs between age and gender groupings. All tests had a significance set at $p < 0.05$.

5.3 RESULTS

5.3.1 Population Demographics

Interviews with a sub-set of the participants ($n=137$) using the SBQ gave an overview of population demographics (Table 5.3.1.1). The mean age of the study population was 60 ± 18 years and the median age was 57. The participant ages ranged from 23-108 years. Females represented a larger proportion within both age groups but were comparable in numbers between the two groups.

The occurrence of DM2 was self-reported and found to be higher among elders with almost double the occurrence in comparison to the younger group (31.40% vs. 17.24%). The majority of the study sample was originally from Mistissini (92.7%) and had little or no previous formal education (58.39%).

5.3.2 Traditional Food Consumption

Traditional food (TF) in our study is defined as the hunting and gathering of wild game and/or fish for personal consumption. Consumption of traditional food (TF), of any sort over the last year, was confirmed by 97.08% of participants. The large majority of participants confirmed consumption of large and small game meats, as well as, birds and ducks (Figure 5.3.2.1a). Elders were more likely to have eaten mammalian species such as lynx, muskrat, otter, and porcupine over the last year (Figure 5.3.2.1a). Fish was less frequently consumed by the younger generation, primarily species such as sucker, pike, sturgeon, and whitefish (Figure 5.3.2.1b).

Total Population (N=173)	Age Group 20-59 (n=87)		Age Group ≥ 59 (n=86)	
	Male	Female	Male	Female
Gender Distribution	31	56	39	47
Mean Age	42 ± 7.8 years		73 ± 6.7 years	
Occurrence of DM2 (n=173)	17.24% (n=15)		31.40% (n=27)	
Originally from Mistissini (n=137) **	92.7% (n=127)			
Average number of people living in the home (n=137) **	1-4 people: 54.75% (n=75) 5-10 people: 44.53% (n=61) >10 people: 0.73% (n=1)			
Level of education Completed (n=137) **	No formal education: 58.39% (n=80) Primary school: 5.11% (n=7) Secondary school: 8.76% (n=12) Post-secondary: 25.55% (n=35) Continuing Education: 2.19% (n=3)			
Employment of family members (n=137) **	Full-time: 1-3 people- 94.89% (n=130) 4-7 people- 5.11% (n=7) Part-time: 1-3 people- 97.81% (n=134) 4-7 people- 2.19% (n=3)			

Table 5.3.1.1: Population Demographics. **Number of participants does not add to 173; 36 participants did not complete the SBQ.

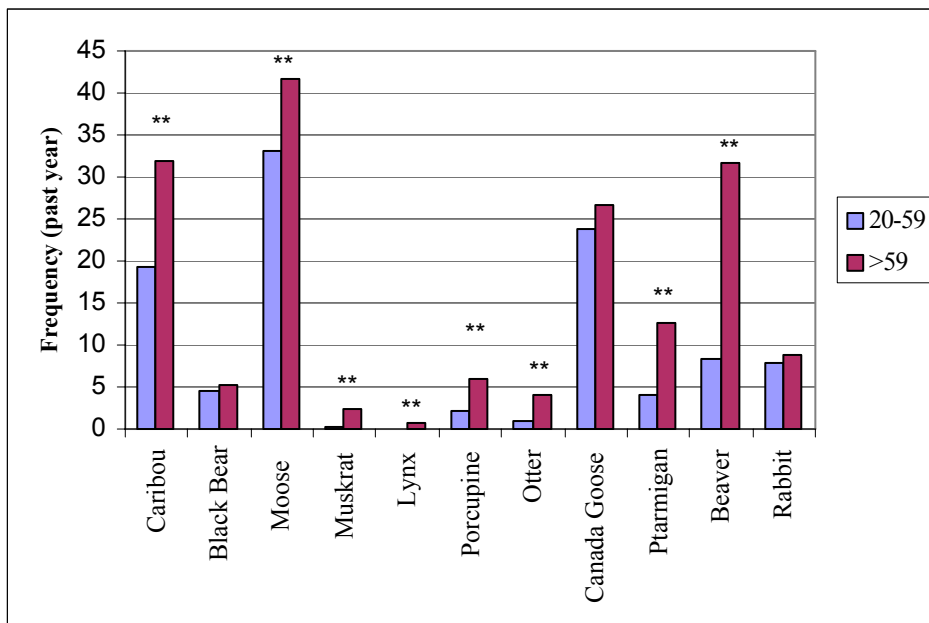


Figure 5.3.2.1a: Frequency of Consumption of Large and Small Game Meats

** Denotes a statistically significant difference between the two age groups.

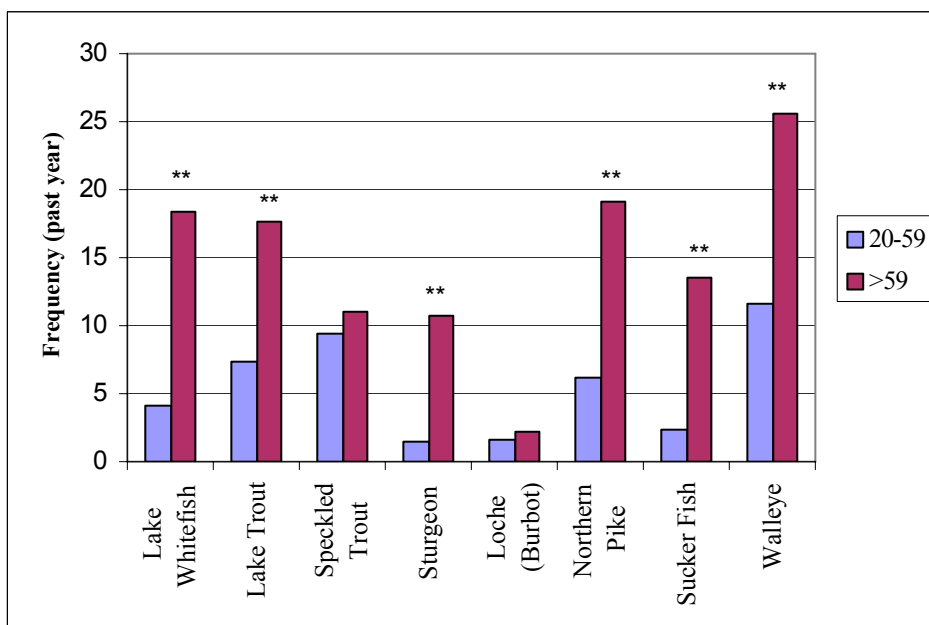


Figure 5.3.2.1b: Frequency of Consumption of Fish Species

** Denotes a statistically significant difference between the two age groups.

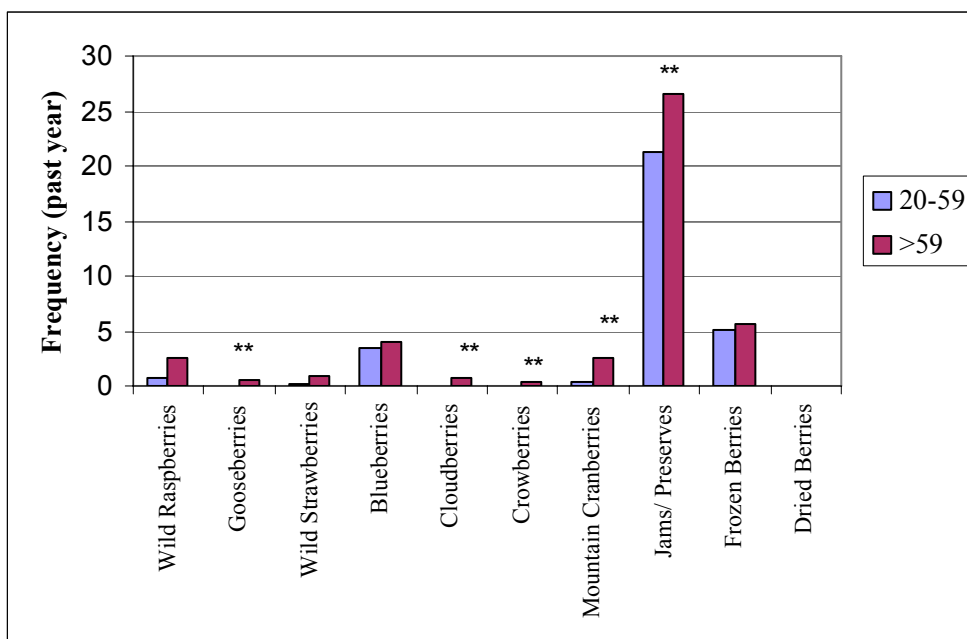


Figure 5.3.2.1c: Frequency of Consumption of Wild Berries

** Denotes a statistically significant difference between the two age groups.

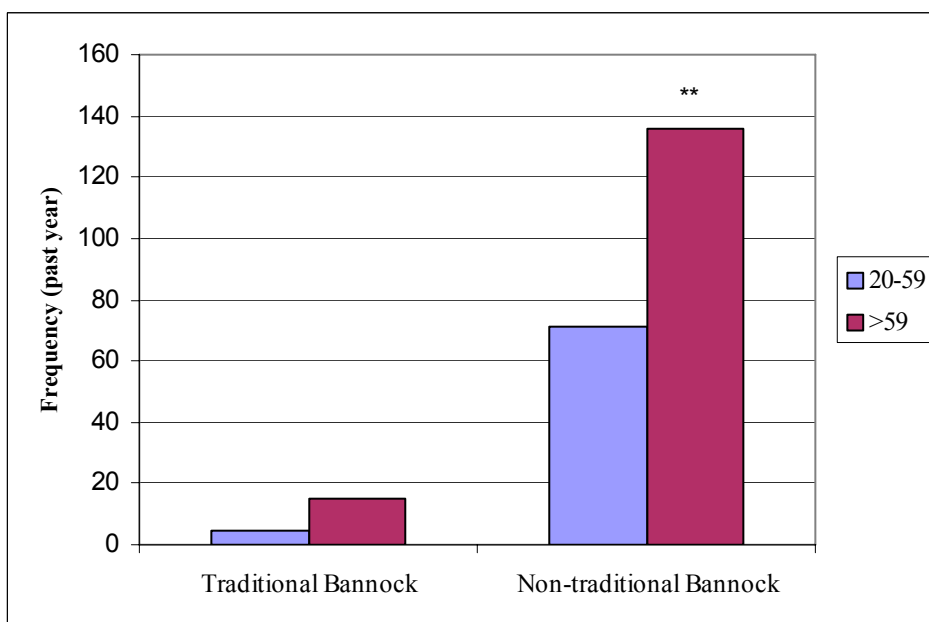


Figure 5.3.2.1d: Frequency of Consumption of Traditional and Non-Traditional

Bannock. ** Denotes a statistically significant difference between the two age groups.

Berry consumption followed similar trends to those of fish consumption with the less frequent species of berries, such as, gooseberries, cloudberries, crowberries, and mountain cranberries being consumed in greater frequency by elders (Figure 5.3.2.1c). All participants reported consuming traditional bannock (cooked on an open-fire) less frequently with consumption most often at feasts and gatherings. Non-traditional bannock (cooked on a stove-top or baked in the oven) remains a staple for elders with a significantly higher consumption ($p < 0.05$) in comparison to the younger generation (Figure 5.3.2.1d).

The majority of participants reported storing their traditional foods in chest freezers (96.35%), while smoking and drying of meats has become a less frequent method of preservation (35.77%). Smoking and drying of meats is reserved primarily for first trappings, feasts, and while in the bush. The primary source of acquisition of traditional foods is through immediate or extended family members (86.13%). The local grocery store and the Cree Trappers Association (CTA) were less frequently cited as sources of traditional food (34.31% and 36.5%, respectively) due to lack of availability and reliability.

Few participants responded having family members who did not consume traditional foods (3.65%). Among the non-consumers, aversion due to smell of traditional foods was the major contributing factor (96.35%). A large number of participants (54.74%) reported a change in traditional food consumption from earlier years as a result of increased access to market foods (primarily fast foods), less time spent in the bush, and lack of hunting skills for acquisition of food sources.

Past mercury programs and animal advisories cautioning the consumption of game animals and fish, due to contaminant exposure has caused a concern for decreased consumption of traditional foods (CCSSBJ et al., 1988; Delormier, 1993). However, when prompted on the impact of previous programs and

advisories on TF consumption, ~80% of participants reported no affect of past advisories on current consumption habits.

5.3.3 Plant species in use for treatment of symptoms of DM2

Interviews with the aid of a FFQ revealed a list of 11 plants, with their characteristic parts, as most frequently used for treatment of symptoms of DM2 (Figure 5.3.3.1 and Table 5.3.3.1). Analysis using a list of 15 symptoms, determined by physicians to be most relevant to DM2, was used as the basis for frequency counts. Top ranked plants based on frequency of use were used for further laboratory analysis of antioxidant activity and cardioprotective effects (see chapter 1).

Rhododendrum groenlandicum (Labrador Tea) was cited in use for the greatest number of symptoms; however it was not the most frequently consumed by the study participants. Labrador Tea was used traditionally as a heal-all medicine as a result of its increased availability throughout the region. *Larix laricina* (Tamarack) a common tree of the Boreal forest was cited for 6 of the 15 symptoms of DM2 and was consumed in high proportion among the participants. *Sarracenia purpurea* (Purple Pitcher Plant) is used for only one symptom of DM2, primarily rashes and sores, but is consumed in high frequency among the participants. This may be attributed to the elevated occurrence of foot sores among diabetic and non-diabetic patients alike.

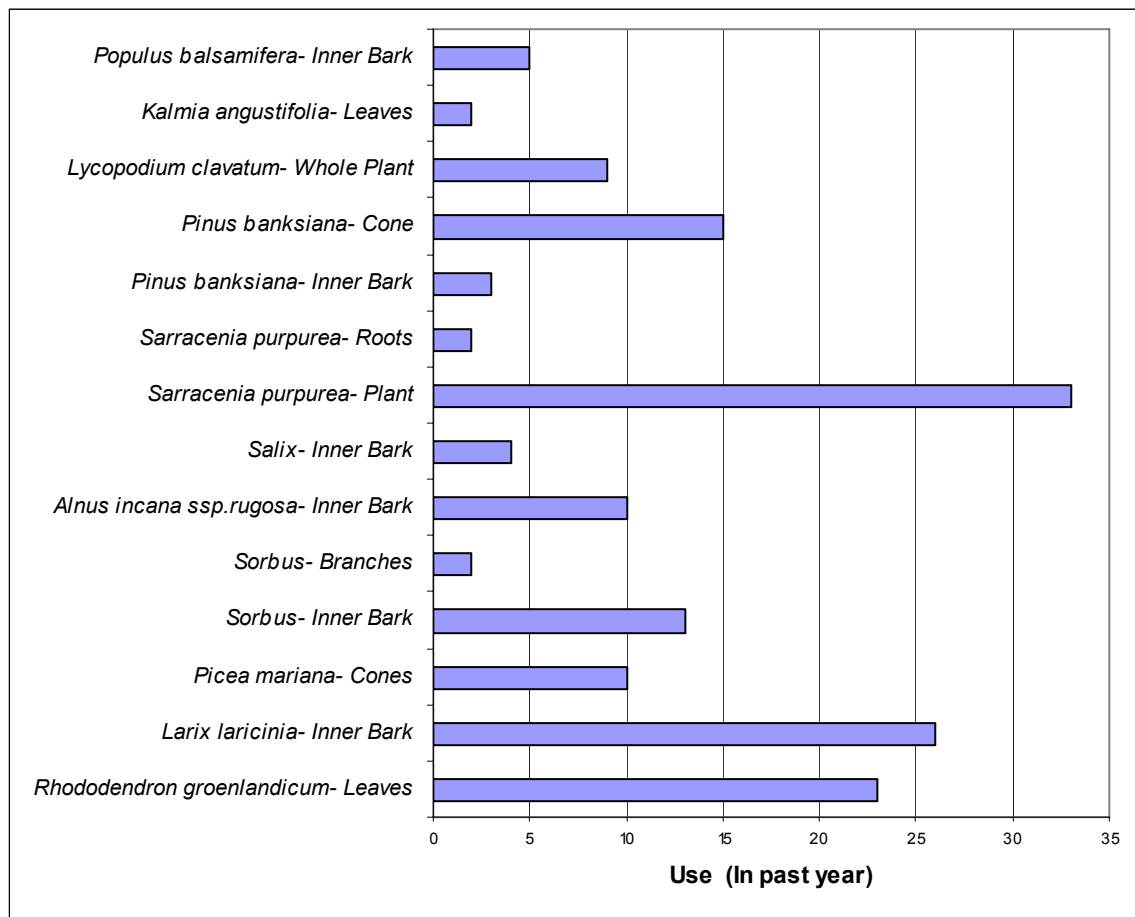


Figure 5.3.3.1: Use of Plants for Symptoms of DM2. Use refers to the number of participants that responded to having used the respective plants at least once in the past year. Categories of symptoms used for analysis were determined by physicians to be the most recurrent symptoms of DM2 patients. Symptoms included: increased thirst, increased urination, increased appetite, loss of strength, skin infection, blurry vision, foot sores, diarrhea, arthritis, chest/ heart pain, inflammation, sore/ swollen limbs, headaches, backaches and kidney problems and abscesses/ boils.

Latin Name	Common Name	Cree Name	Part(s) Used	Use (In Past Year) (N= 173)	# Of Symptoms ^a (Out of 15)
<i>Rhododendron groenlandicum</i>	Labrador Tea	<i>Kachebuk</i>	Leaves	23 (13.3%)	10
<i>Larix laricina</i>	Tamarack	<i>Waachinaakan</i>	Inner Bark	26 (15.0%)	6
<i>Lycopodium clavatum</i>	Stag's Horn	<i>Pasnacowon</i>	Whole Plant	9 (5.2%)	4
<i>Sorbus decora</i>	Mountain Ash	<i>Muskuanna Natuk</i>	Inner Bark	13 (7.5%)	4
<i>Populus balsamifera</i>	Poplar	<i>Miitus</i>	Inner Bark	5 (2.9%)	3
<i>Alnus incana ssp. rugosa</i>	Speckled Alder	<i>Muikutusphi</i>	Inner Bark	10 (5.8%)	2
<i>Salix spp.</i>	Willow	<i>Utusphi</i>	Inner Bark	4 (2.3%)	2
<i>Juniperus communis</i>	Juniper		Roots	2 (1.2%)	1
<i>Kalmia angustifolia</i>	Sheep Laurel		Leaves	2 (1.2%)	1
<i>Nuphar lutea</i>	Yellow Pond Lily		Entire Plant	2 (1.2%)	1
<i>Picea mariana</i>	Black Spruce	<i>Inaatuk</i>	Cones	10 (5.8%)	1
<i>Pinus banksiana</i>	Jack Pine	<i>Ushichishk</i>	Cones	15 (8.7 %)	1
<i>Pinus banksiana</i>	Jack Pine	<i>Ushichishk</i>	Inner Bark	3 (1.7%)	1
<i>Populus tremuloides</i>	Trembling Aspen		Inner Bark	2 (1.2%)	1
<i>Sarracenia purpurea</i>	Purple Pitcher Plant	<i>Ayigadash</i>	Roots	2 (1.2%)	1
<i>Sarracenia purpurea</i>	Purple Pitcher Plant	<i>Ayigadash</i>	Whole Plant	33 (19.1%)	1
<i>Sorbus decora</i>	Mountain Ash	<i>Muskuanna Natuk</i>	Branches	2 (1.2%)	1

Table 5.3.3.1: Use of Traditional Medicines by Species and Part for Symptoms of DM2.

Medicinal use is representative of usage at least once in the past year. ^a Symptoms include: increased thirst, increased urination, increased appetite, loss of strength, skin infection, blurry vision, foot sores, diarrhea, arthritis, chest/ heart pain, inflammation, sore/ swollen limbs, headaches, backaches and kidney problems and abscesses/ boils.

5.3.4 Frequency of Consumption of Traditional Medicines and Trends Associated with Use of Traditional Medicines

Analysis of use of TMs was categorized by age, gender, and reason for non-use (Table 5.3.4.1). Chi-squared analysis of general use of traditional medicines and age demonstrated a significant association (Mantel-Haenszel Chi-Square=12.7125, $p<0.0004$). A Student's t-test found the frequency of use of traditional medicines to be significantly different between the two age groups ($p=0.0003$). Logistic regression analysis, grouped by age, showed elders to be 2.38 times more likely to consume traditional medicines than their younger counterparts. Logistic regression grouped by gender showed a trend towards higher frequency of consumption of traditional medicines by males; however, this value was not found to be statistically significant ($p=0.1917$). The medical status of a person, in relation to DM2, also showed no significant effect on use of traditional medicines ($p=0.6727$). Further analysis of frequency with various demographic characteristics (education, time spent in bush) produced non-significant effects.

Reasons for non-use of TMs varied between the two age groups with the younger generation offering reasons such as lack of traditional knowledge and use of modern medicines as the major contributors. Among elders, spirituality and use of modern medicines were the most frequently cited reasons for non-use of TMs (Table 5.3.4.1).

Consumers of traditional medicines were found to ingest traditional medicines primarily upon need and not as a regular part of their diet. An exception is Labrador Tea, which is frequently consumed as an herbal tea. This tea is not as frequently used as in previous years but is still quite customary among elders. Respondents reported a trend toward decreased consumption of traditional medicines (21.9%) from previous years in accordance with traditional food.

Consumers of TMs reported receiving remedies from local healers within the elder population known for their expertise with the specific ailment. Knowledge of the use of plants was most often transmitted from local elders or relatives who had experience with the medicine or who themselves prepared medicines at one time.

Among participants interviewed, 92.7% supported increased use of TMs if made more available in the community. Many participants were in favour of integration of TMs within the clinic (77.37%); however, a strong consensus highlighted a preference for administration of TMs in their original form (or closest form possible) by healers and/or elders. Overall, 86.13% of participants believe a program integrating TMs into the healthcare system would be beneficial for Cree communities.

Total Population (N=173)	Age Group 20-59 (n=87)	Age Group ≥ 60 (n=86)
Occurrence of DM2	17.24% (n=15)	31.40% (n=27)
Use of Traditional Medicines	33.33% (n=29)	60.47% (n=52) **
Non- Use of Traditional Medicines	66.67% (n=58)	39.53% (n=34) **
Reasons for Non-Use		
Spirituality	0	32.4% (n=11)
Less Time in Bush	0	14.7% (n=5)
Use of Modern Meds	54.4% (n=31)	41.2% (n=14)
Decreased Mobility	0	11.8% (n=4)
No TK about TM	47.4% (n=27)	0

Table 5.3.4.1: Use and Reasons for Non-Use of Traditional Medicines by Age Group.

** Denotes a statistically significant difference between the age groups ($p < 0.05$). Percentages represent the frequency of which study participants reported the specific occurrence or behaviour within the respective age groups.

When prompted about the overall health of the Cree population, 60.58% of participants believed the health of the Cree population is below average. A healthy lifestyle or good health is a wide-ranging term, which takes on variant meanings in diverse cultural settings. Therefore, participants were questioned on their perceptions on good health and a healthy lifestyle. Participant responses were quite in concert with Western concepts of good health with 48.18% defining physical activity and good eating habits as essential elements in maintaining good health. Less frequent responses, primarily by elders, were shown to involve the adherence to a traditional lifestyle implicit with the consumption of traditional foods and medicines.

5.3.5 Traditional Knowledge and Adherence to a Traditional Lifestyle

Traditional knowledge and its dissemination across generations has been a concern since the infiltration of western influences into the Cree culture. Participants were prompted on their understanding of the meaning of TK and responses included daily life tasks (6.57%), bush/survival skills (17.52%), traditional way of life (14.6%), and traditional medicines (16.06%). Numerous participants (62.77%) agreed to the use of traditional knowledge in their daily lives.

As a result of past studies showing a decreased transmission of TK, participants were questioned on their opinion toward transmission of TK in the home and in the community. Participant responses' confirmed earlier findings of a decreased transmission of traditional knowledge among the younger generations. Responses were equally distributed with 50.36% believing there was pressure from elders to learn TK, while 42.34% did not believe there was pressure. Despite the decreased transmission of traditional knowledge to the younger generations, 88.32% of participants confirmed participation in traditional activities for pleasure.

When prompted on number of days spent in the bush most individuals reported spending 2-4 weeks a year in the bush (56.94%). The remainder of the participants, primarily elders, showed an increased number (>6 months) of days spent in the bush (23.36%). The majority of the participants (76.64%) were found to have spent a large part of the past year in Mistissini (>10 months) with few participants on income security (14.6%).

5.4 DISCUSSION

Numerous studies have effectively outlined the epidemic state of DM2 within Indigenous populations (Boston, 1997; WHO, 1999; Young, 2000; Gray-Donald, 2000). Many studies have also attempted to comprehend the reasons behind this epidemic state, with nutrition transition, sedentary lifestyle, and ineffectiveness of past government initiatives as plausible rationales (Robinson, 1995; Boston, 1997; Delorimier, 1999; Dewailly, 2002).

Our results confirm past findings of decreased cultural transmission across generations, with the younger generations showing a decreased consumption and use of traditional foods and medicines. The decreased use of traditional foods within the younger generation suggested to have been aggravated by modernization, residential school, and lack of transmission of traditional knowledge follows the same trends seen within previous studies (Ohmagari, 1997; Young, 2000; Dewailly, 2002). Significant differences observed in game meats and fish can be attributed to a decreased occurrence of many of these sources as a result of dam and forestry projects (Niezen, 1993). Animal species, such as, lynx, muskrat, otter, and squirrel are among the few that have become locally extinct as a result of environmental stresses. The erection of Hydro-Quebec dams and the forestry industry have caused the migration of species to the northeastern regions of Quebec, thus limiting the availability of food sources (Elton, 1942; Berkes, 1979).

The decreased consumption of fish species within the younger generations is not surprising as this trend has existed since the 1950's (Rogers, 1963). Fish consumption, in the past, was regarded as a complementary source of energy during periods of famine (Feit, 1973). These trends have continued through the years leaving fish consumption relatively low in comparison to game meats. Mercury advisories were found to have precipitated a more pronounced decrease in consumption of fish, although our results show little, if any, such effect. Recent community-based initiatives have been implemented to help increase the awareness of the benefits associated with consumption of fish in order to restore fish consumption (Mistissini Fish Project, Summer 2005).

Plant species outlined by the participants in this study were in accordance with previous ethnobotanical works of Leduc et al. (2005). Numerous species outlined by participants in use for treatment of symptoms of DM2 are among those highlighted in previous literature for medicinal use (Arnason, 1981; Marles, 1995; Kuhnlein, 1995; McCune, 2003).

Participants expressed a willingness to use TMs due to previous experiences and recount healings within the community. Due to the fairly recent emergence of DM2 in Indigenous communities, programs targeting symptoms of DM2 versus the disease itself are suggested. The frequencies of use of TM reported by the participants could be a result of the commonality of symptoms of DM2 and other medical conditions. The increased use of plant remedies within the *Pinaeae* (Pine) family could be a result of their common occurrence in the Boreal forest and their known antioxidant activities attributable to their high phenol and tannin constituents (Arnason, 1981; Marles, 1995; Cotellet, 1996; Rice-Evans, 1996; McCune, 2003).

Our results may show a slight bias due to the use of a non-random sample; however this still allows for qualitative and descriptive insights of possible trends and behaviours within the population. This does however hinder our ability to

draw precise conclusions about the general population. The findings do provide a basis for future community-based intervention strategies and clinical trials to further investigate the plausibility of integration of TMs and traditional practices into the Western healthcare system.

In the past, the transmission of traditional knowledge was the basis for survival in the bush. The decreased transmission over the years could be attributed to the differences in generational perceptions toward the use of traditional medicines, foods, and adherence to a traditional lifestyle (Bobbish-Rondeau, 1996). Lack of educational institutions, above the secondary level, within Indigenous communities exposes advanced students to more Southern influences which can further precipitate loss of culture, of which traditional foods, medicines and knowledge are included (Ohmagari, 1997). The decrease may also be a direct result of the distinctions Westernization has placed on TMs and TFs. This is highly in contrast to past traditions, in which the distinction of TMs and TFs was not clearly delineated (Johns, 1990).

Traditionally, Indigenous groups perceived health as “a balance between self and others, rooted in spirituality and the laws of Nature” (Royal Commission of Aboriginal Peoples, 1996). The current Cree endeavour is to maintain a balance between the evolution of their people with respect to their relationships with each other and their land. As a result, a balanced intervention program integrating community and regional public health objectives proves to be a challenge for local health professionals.

This study outlines a substantiated need for alternative measures for treatment of symptoms of DM2. Responses from community members outline the readiness of the community to return to past traditions in order to combat the epidemic rates of chronic disease among their people.

6.0 BRIDGING STATEMENT FOR CHAPTER SIX

The previous chapter highlighted the plants and parts that are most frequently used for treatment of symptoms of DM2. This following chapter aims to scientifically validate the knowledge obtained from participants in the nutritional assessment. The plants most frequently cited were assessed for their antioxidant ability and protective effects using an *in vitro* cellular model. Antioxidant potential has been previously implicated in prevention of oxidative damage resulting from DM2 (Halliwell, 1989; Vaya, 1997; Antolovich, 2002; Tsai, 2003). Oxidized LDL was used as the pro-oxidant source due to its pre-established implication in oxidative damage resulting from DM2 (Steinberg, 1989; Esterbauer, 1990; Esterbauer, 1992; Steinberg, 1997). The use of the BAEC system is a result of the increased occurrence of cardiovascular events among diabetic patients.

6.1 CHAPTER SIX: ANTIOXIDANT ACTIVITY AND CARDIOPROTECTIVE EFFECTS OF TRADITIONAL PLANTS FOR SYMPTOMS OF DIABETES MELLITUS

6.1 INTRODUCTION

Cardiovascular complications are the major cause of mortality and morbidity for 135 million individuals worldwide afflicted by DM2 (Alberti, 1998; Nathan, 1999; Expert Committee on DM2, 1999). As such, diabetic patients are two-to-four times more likely to experience cardiovascular events versus their non-diabetic counterparts (Haffner, 1998). In 2003, 5.2 million people worldwide were afflicted by cardiovascular conditions associated with diabetes (CDC, 2003). Eeyou Istchee (Crees of the James Bay Region) communities of Canada are one such community, with 13% of diabetic patients suffering from cardiovascular events (Statistics Canada, 2004).

These rates are largely attributed to various metabolic conditions associated with DM2 such as hypertension, hypercholesterolemia, obesity, and insulin resistance (Libby, 2002; Mokdad, 2001; Doualhy, 2005). Hyperglycemia has been shown to increase the level of oxidative stress through glycosylation of functional proteins, glucose auto-oxidation, and decreased function of the glutathione-redox cycle (Chew, 2004). Damaging factors resulting from DM2 may be the catalyst for endothelial dysfunction associated with micro- and macrovascular complications (Eckel, 2002).

Endothelial dysfunction is implicated in the development of atherosclerosis and microvascular complications associated with DM2 (Tooke, 1995; Mano, 1996; Beckman, 2002) and as such is a major contributor to morbidity in diabetic patients (Dogra, 2001; Van de Ree, 2001; Weis, 2001). Endothelial dysfunction is characterized by an imbalance of vasodilators and vasoconstrictors and a decreased bioavailability of nitric oxide (NO) (Tesfamariam, 1992; Tesfamariam, 1994; Caballero, 2003; Shaul, 2003).

Ox-LDL has been previously implicated in the initiation and progression of macrovascular complications, primarily atherosclerosis (Steinberg, 1989, Esterbauer, 1992; Steinberg, 1997). Oxidative modification of LDL occurs through the loss of endogenous lipoprotein antioxidants, particularly α -tocopherol, which in turn leads to degradation of surface membrane PUFAs and formation of lipid peroxides (Esterbauer, 1990). Formation of lipid peroxides has been previously correlated to the occurrence of atherosclerotic lesions (Glavind, 1952; Goto, 1982). Oxidation causes alterations to the apoB moiety of LDL, which in turn promotes rapid recognition by macrophage scavenger receptors (Esterbauer, 1990). The increased uptake of macrophages into the subendothelial space leads to the formation of foam cells and the development of fatty streaks, which is the hallmark of atherosclerotic lesions (Steinbrecher, 1990; Virella, 1995).

Antioxidant supplementation has been suggested as a possible method to counteract the process of lipid peroxidation and ultimately progression of atherosclerosis. Many epidemiological and clinical studies support the link between dietary indicators and the decreased risk of cardiovascular events (Frankel, 1992; Block, 1994; Halliwell, 1994; Rice-Evans, 1996; Enstrom, 1992; Hu, 2000). These and numerous other studies have outlined a plausible means of protection against atherosclerotic events.

In order to develop a culturally applicable strategy to contend with the rising DM2 epidemic among Canadian Aboriginals, plants that were identified as nutritionally relevant according to the ethnobotanical survey were evaluated for their protective effects *in vitro*. Protection is viewed within a cellular system, with the use of bovine aortic endothelial cells (BAEC). The antioxidant ability of the plant extracts were assessed by their free radical scavenging activity, cytoprotective effects against ox-LDL, and inhibition of lipid peroxidation. This study is the first to evaluate the protective effects of Cree medicinal plants against endothelial damage.

6.2 MATERIALS AND METHODS

6.2.1 Plant Selection and Acquisition

Plants most frequently used by study participants for treatment of symptoms of DM2 were chosen for further laboratory analysis (Chapter 5). To ensure accuracy in plant identification, a community member assisted in the gathering. The traditional gift of tobacco was respected at each gathering site in accordance with local traditions. Approximately 500g of plant specimens were collected a day prior to departure to Montreal and stored in cold boxes. Once in the laboratory, plants were freeze-dried using a Flex-Dry Mp freeze-dryer and stored in Teflon®-capped amber vials at -20°C . Percent yields were calculated for plant materials dried ground versus extract (Appendix 5).

6.2.2 Preparation of plant extracts

Freeze-dried plant material was ground in a Wiley mill through an 850 μm sieve. Crude plant samples were extracted with ethanol using Soxtec HT extraction and vacuum evaporated using a Brinkman Büchi 461 Rotovapor evaporator. Residual ethanol was removed by overnight freeze-drying. Concentrated plant extracts were stored in amber vials at -20°C to avoid any potential photo-oxidation.

6.2.3 Free radical scavenging activity (DPPH)

The ability of the plant extracts to reduce the 1,1 diphenyl-2-picryl-hydrazyl (DPPH) radical was assessed using the methods of Cotelle et al. (1996). DPPH (Sigma Chem. Co.) was dissolved in ethanol to give a 100 μM solution. 3.0mL of the ethanolic DPPH solution was added to 0.5mL of plant extract dissolved in ethanol at various concentrations. Absorbance was read at 517nm and expressed as inhibitory concentration at 50% (IC₅₀) using the linear portion of the curve obtained using ascorbic acid. Three flavonoids commonly found in foodstuffs: quercetin, epicatechin and catechin (Sigma Chem. Co.) were used as additional standards due to their pre-established potent antioxidant effects.

6.2.4 LDL preparation and oxidation

Human LDL (5mg/mL) stored in a solution of 0.15M NaCl-0.01% EDTA, pH 7.2 (Intracel, MD) was diluted in 1mM phosphate buffer solution (PBS, pH 7.4) and passed through a Sephadex PD-10 column (Pharmacia Biotech) to remove EDTA and NaCl. Protein determination of LDL in solution was determined using the modified Lowry's method (Lowry, 1951) with bovine serum albumin (Sigma Chem. Co.) as the standard.

In vitro oxidation of LDL was performed using a modified protocol by Myers (1996). Briefly, LDL dissolved in PBS was oxidized with a 15 μ M copper sulphate (CuSO₄) solution (Sigma Chem. Co.) for 12-16 hours at 37⁰C. Oxidization of LDL was verified by monitoring the formation of conjugated dienes at 234nm (Esterbauer, 1990). Native low-density lipoprotein (n-LDL) was removed prior to addition of CuSO₄ and kept at 4⁰C until use.

6.2.5 Conjugated dienes formation

Conjugated diene formation was visualized using the modified protocol of Esterbauer (1990). LDL (100 μ g/mL, dissolved in PBS) and CuSO₄ (15 μ M) were added to a UV sensitive microtiter plate made to a final volume of 200 μ l. Readings were taken at 234nm every 10 minutes for an 8-12 hour period. The time lapsed before the appearance of conjugated dienes was determined from the intersection between the propagation and the lag phase.

6.2.6 Cell preparation

Bovine aortic endothelial cells (BAEC) were isolated from freshly harvested bovine aortas and cultured in Dulbecco's Modified Eagle Medium (DMEM- Sigma Chem. Co.) supplemented with essential amino acids, 5% Fetal Bovine Serum (FBS; Hyclone Lab) and 1% penicillin (100kU/l)/streptomycin (100mg/l) (Sigma Chem. Co.). Cells were characterized by their cobblestone morphology. Cells between passages 1-4 were used for experimental analyses.

6.2.7 Trypan Blue viability assay

The viable concentrations of plant extracts were deciphered using the Trypan Blue dye exclusion assay. Cells were treated with various concentrations of plant extracts. Following incubation, the media was removed and cells were lysed using a Trypsin solution (Gibco) and incubated at 37°C for 2 minutes. A 1:1 (v/v) solution of lysate to Trypan blue dye (0.4%; Sigma Chem.Co.) was injected into a hemacytometer for cellular count. Cell viability was determined by the percentage of cells having incorporated the Trypan blue dye. (data not shown)

6.2.8 Incubation with extracts and addition of ox-LDL

Post-confluent BAECs (in 12-well plates) were incubated with plants, *Abies balsamifera* (10µg/ml), *Alnus incana* (25µg/ml), *Larix laricina* (10µg/ml), *Picea banksiana* (5µg/ml), *Picea mariana* (5µg/ml), *Rhododendron groenlandicum* (10µg/ml), *Sorbus decora* (15µg/ml), *Sarracenia purpurea* (10µg/ml) for 24 hours at 37°C. Solvents and controls, dimethyl sulfoxide (DMSO- 0.1% final concentration), ethanol/DMSO and butylated hydroxytoluene (BHT- 10µM; positive control; Sigma-Aldrich) were also added to BAEC and incubated for 24 hours at 37°C. All plants were dissolved in dimethyl sulfoxide (DMSO, Sigma Chem. Co.), with the exception of *R. groenlandicum*, which was dissolved in a 50/50 (v/v) solution of ethanol (EtOH) and DMSO. *R. groenlandicum* was dissolved in 50/50 EtOH/DMSO due to its inability to dissolve completely in DMSO. DMSO never exceeded a final concentration of 0.1%. Our control, PBS, was incubated with basic DMEM for similar time intervals as extracts. Cells were subsequently stimulated with ox-LDL (100µg/ml) and incubated for 6 hours. Cytoprotective effects were measured at both the baseline (0 hours; pre-incubation for 24-hours) and the 6-hour (incubation with ox-LDL) stage.

6.2.9 Ox-LDL cytotoxicity via Lactate Dehydrogenase (LDH) release

The cytoprotective effects of crude extracts against ox-LDL were viewed using the LDH Assay Kit (Sigma Chem. Co., TOX-7). LDH is an intracellular enzyme found to leak upon damage to the membrane. High % LDH release would therefore represent increased cellular damage. BAECs in 12-well plates were incubated for 24 hours with standards and/or plant extracts. Thereafter, supernatants were collected and 0.5ml of cold PBS was added to lyse cells. Following 5-minute incubation on ice, cells were scraped and lysates collected and sonicated at 4⁰C for 10 minutes to ensure membrane rupture. Supernatants and lysates were centrifuged at 2,500g for 10 minutes at 4⁰C and plated on a microtiter plate. The levels of LDH release were determined spectrophotometrically at a wavelength of 490 and subtracted background at 690nm. Levels of LDH release were determined at baseline (24-hour pre-incubation with plant extracts and/or standards) and 6 hours post-incubation with ox-LDL. A 24-hour pre-treatment with plant extracts allowed for potential integration of phytochemicals into the intracellular matrix. A 6-hour incubation time with ox-LDL aims to simulate chronic exposure to oxidative stress and the ability of plant extracts to provide prolonged protection.

6.2.10 Inhibition of Lipid Peroxidation (TBARS)

Post-confluent BAECs were incubated in 12-well plates with standards and/or plant extracts for a 24-hour period at 37⁰C. Ox-LDL was added thereafter and incubated for an additional 6 hours. Supernatants and lysates were collected at baseline (pre-treatment with extracts for 24 hours) and 6 hours post-incubation with ox-LDL and solutions of EDTA (40µm; Sigma Chem. Co.) and BHT (10µM) were added to halt oxidation. Samples were stored at -80⁰C until further analysis.

Lipid peroxidation was determined using a modification of the protocol of Sobal et al. (2000). Briefly, samples were defrosted and solutions of 1.3% (w/v) thiobarbituric acid (TBA) (Sigma Chem. Co.) and 50% (w/v) trichloroacetic acid

(TCA) (Sigma Chem. Co.) were added. The mixture was vortexed and heated in a 60°C water bath for 40 minutes. Tubes were immediately cooled on ice for 5 minutes and centrifuged at 2000g for 10 minutes. The samples were plated on a microtiter plate and read fluorometrically at an excitation wavelength of 510nm and an emission wavelength of 553nm. The concentration of TBARS produced was determined using a standard curve of malondialdehyde (MDA), derived from the acid hydrolysis of 1,1,3,3-tetraethoxypropane (Sigma-Aldrich). The results are expressed as nmol MDA equivalents/1g LDL protein. BHT (10µM) was used a positive control.

6.2.11 Statistical Analysis

The results of the DPPH, LDH and TBARS analyses are expressed as means \pm standard deviations (SD) of at least 3 independent experiments performed in triplicate. A one-way ANOVA was used to test significance and post-hoc comparisons were made using Tukey's test (SAS, version 9.1) Pearson correlations were performed between LDH and TBARS assays. Significance was set at $p < 0.05$.

6.3 RESULTS

6.3.1 Free Radical Scavenging Ability of Plant Extracts

The free radical scavenging ability of crude ethanol plant extracts is depicted in Figure 6.3.1.1. The antioxidant activity of the plant extracts is represented as the increase in the inhibitory concentration at 50% (IC₅₀) in relation to ascorbic acid. The results of the plant extracts and standard flavonoids, ascorbic acid, quercetin, catechin, and epicatechin are represented in descending order of antioxidant activity. Among the 8 extracts tested, *P. mariana*, *A. rugosa*, *P. banksiana*, and *R. groenlandicum* were found to have antioxidant activity comparable to ascorbic acid and standard flavonoids and significantly different from the remainder of the plants ($p < 0.05$).

6.3.2 Characterization of LDL Oxidation (Conjugated Dienes)

The conjugated dienes' assay performed on n-LDL and ox-LDL demonstrated the copper-mediated oxidation of LDL. The increase in lag time of ox-LDL in comparison to n-LDL indicated an increase in the level of lipid peroxidation and thus verification for oxidative modification (Figure 6.3.2.1).

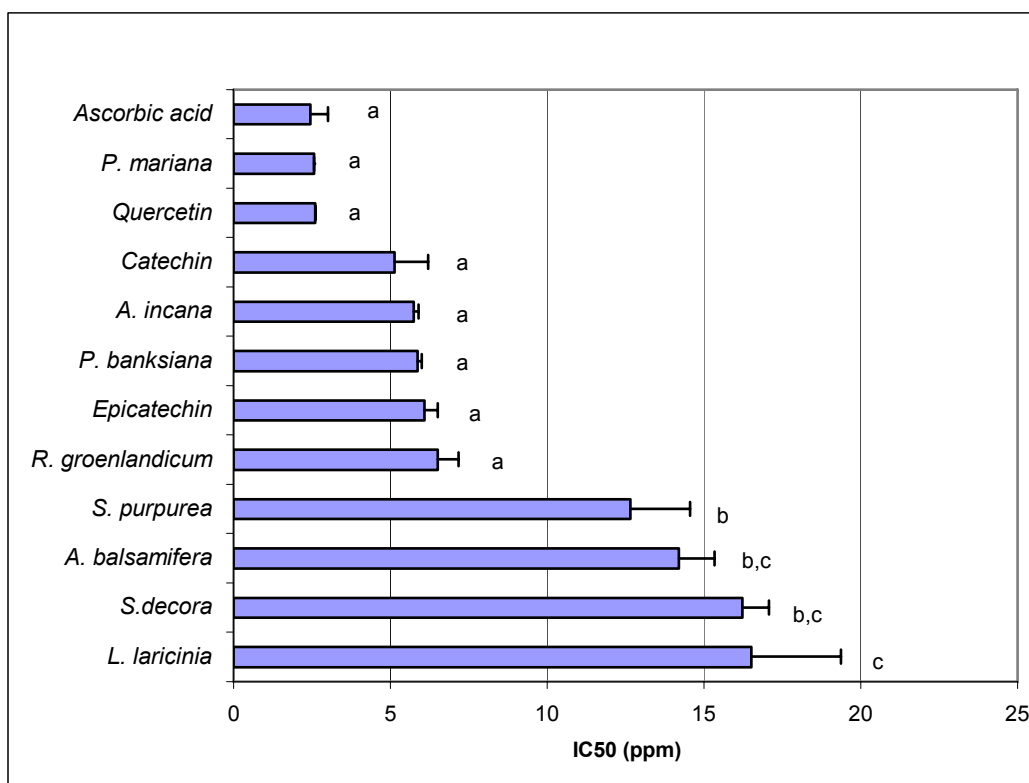


Figure 6.3.1.1: 1, 1 diphenyl-2-picryl-hydrazyl (DPPH) scavenging ability of crude ethanol plant extracts and standards. Results represent means \pm SD from at least 3 independent experiments performed in triplicates. Varying letters denote a significant difference between respective treatments ($p < 0.05$).

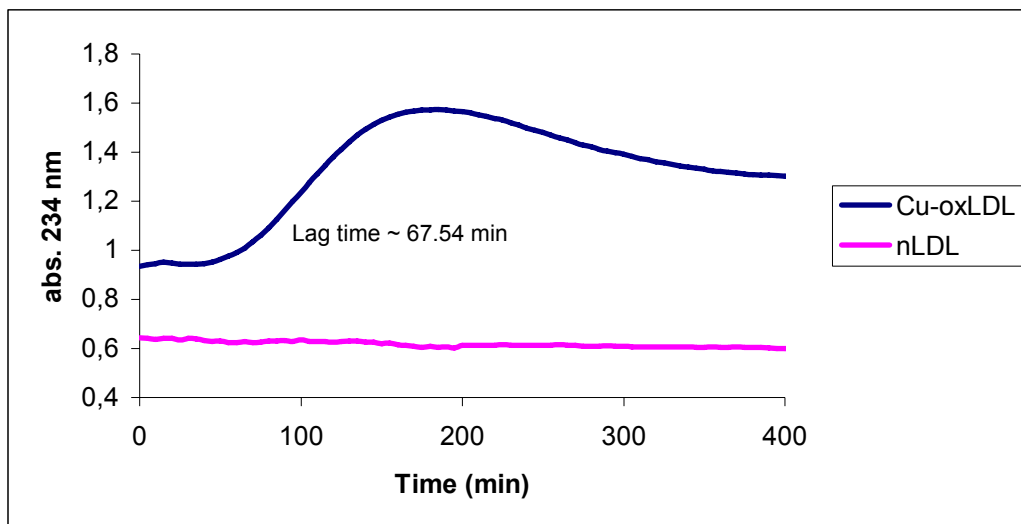


Figure 6.3.2.1: Lag time (minutes) of ox-LDL (oxidized with 15 μ M of Cu²⁺) and n-LDL. Absorbance was monitored every 10 minutes at 234nm for an 8-12 hour period. Results represent means \pm SD from at least 3 independent experiments performed in triplicates.

6.3.3 Cytoprotective Effects

All plant treatments showed cytoprotective effects in comparison to ox-LDL ($p < 0.05$; Figure 6.3.3.1). No significant differences existed between the % LDH release of plant treatments at baseline and 6 hours post-incubation with ox-LDL.

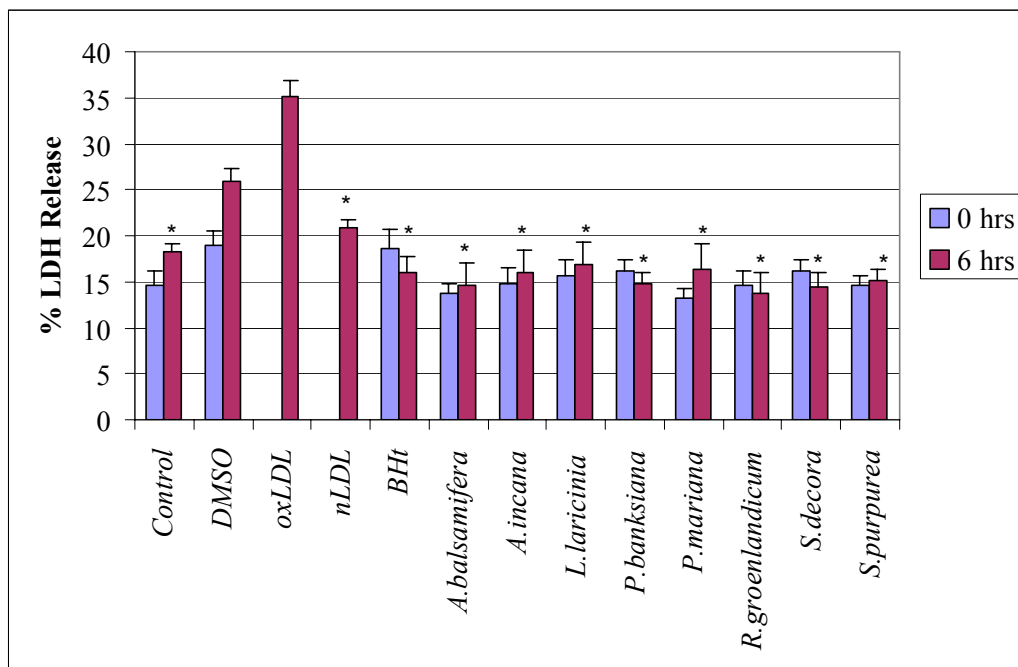


Figure 6.3.3.1: Cytoprotective effects of plant extracts against ox-LDL using the LDH assay. Our control was incubated with DMEM alone at the various time intervals. Results represent means \pm SD from at least 3 independent experiments performed in triplicates. * Denotes a significant difference of the treatments at 6 hours from ox-LDL ($p < 0.05$). No differences existed between the % LDH release of treatments at baseline and 6 hours post-incubation with ox-LDL. NB: 6 hour values denote a pre-treatment with vehicle (DMSO), standard (BHT) or plant treatments followed by a subsequent treatment with ox-LDL for 6 hours.

6.3.4 Inhibition of Lipid Peroxidation

In the supernatant, all plants showed a significant difference in the level of TBARS from ox-LDL and no difference in comparison to BHT ($p < 0.05$) (Figure 6.3.4.1). *R. groenlandicum* demonstrated notable protection being the only plant to significantly decrease the level of lipid peroxidation at 6-hours from baseline.

In the lysates, all plants showed a significant difference in the level of TBARS produced in comparison to our control (PBS) and vehicle (DMSO) ($p < 0.05$) (Figures 6.3.4.2). No significant differences existed between treatments and ox-LDL ($p < 0.05$) (Figure 6.3.4.2). The level of TBARS for ox-LDL treatment was

low and could be a result of compromised membrane integrity due to accumulation of intracellular lipid peroxidation by-products (Esterbauer, 1990; Martin-Nizard, 2003). *A. balsamifera*, *A. incana*, *P. banksiana*, *P. mariana*, *S. decora* and *R. groenlandicum* levels of TBARS at 6 hours showed significantly different results from baseline.

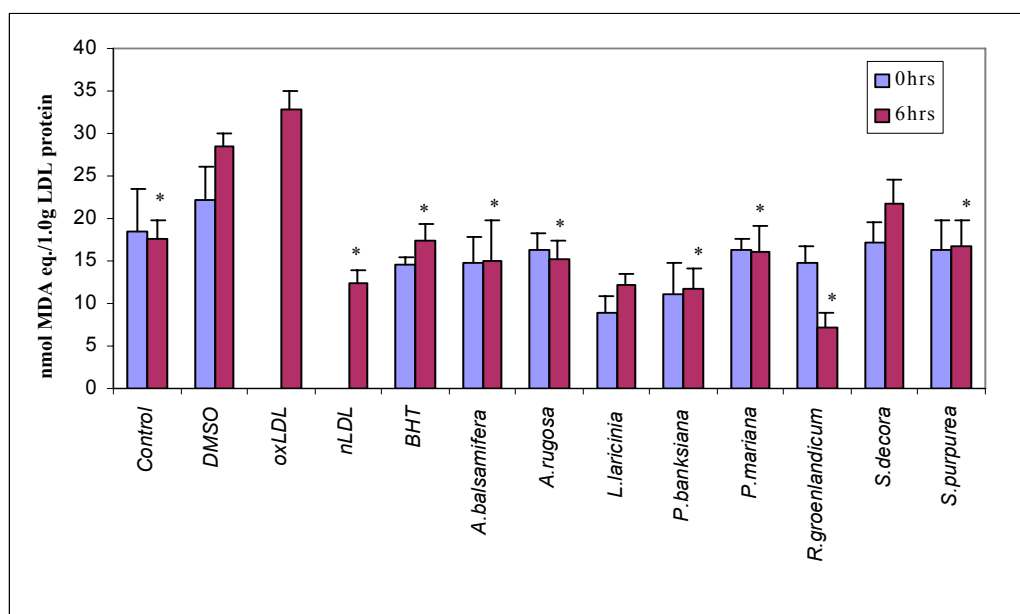


Figure 6.3.4.1: Inhibition of lipid peroxidation by plant extracts found within the supernatant. Our control was incubated with DMEM alone at the various time intervals. Results represent means \pm SD from at least 3 independent experiments performed in triplicates. * Denotes significant difference from ox-LDL and vehicle (DMSO) ($p < 0.05$). NB: 6 hour values denote a pre-treatment with vehicle (DMSO), standard (BHT) or plant treatments followed by a subsequent treatment with ox-LDL for 6 hours.

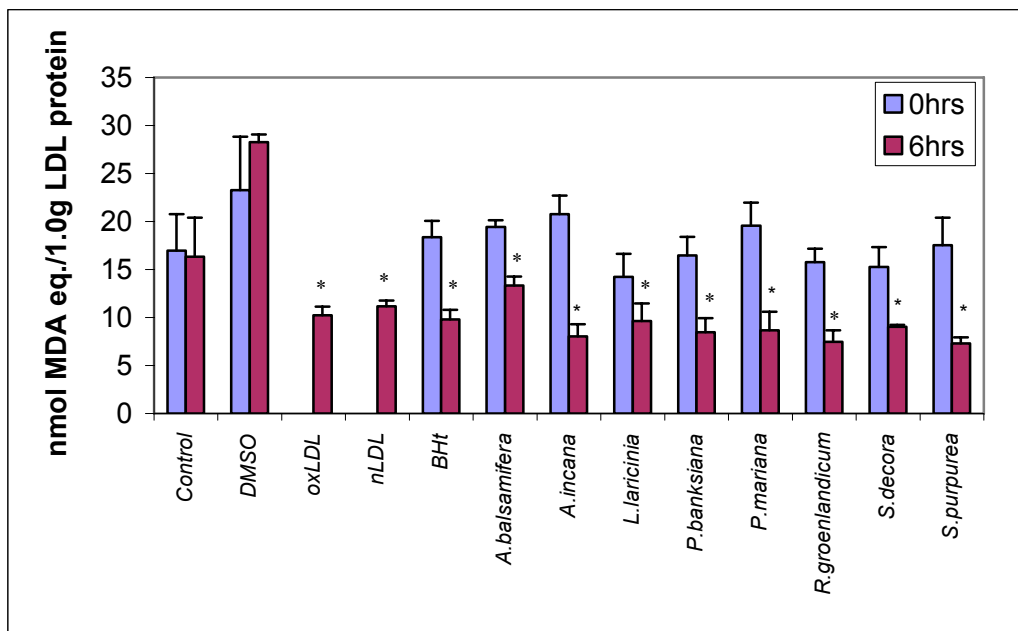


Figure 6.3.4.2: Inhibition of lipid peroxidation by plant extracts found within the cellular lysate. Our control was incubated with DMEM alone at the various time intervals. Results represent means \pm SD from at least 3 independent experiments performed in triplicates. * Denotes a significant difference from control (PBS) and vehicle (DMSO) ($p < 0.05$). NB: 6 hour values denote a pre-treatment with vehicle (DMSO), standard (Bht) or plant treatments followed by a subsequent treatment with ox-LDL for 6 hours.

Based on results for both supernatants and lysates we can conclude that *A. balsamifera*, *L. laricina*, *P. banksiana* and *R. groenlandicum* effectively protect BAEC from oxidative stress caused by modified LDL. *R. groenlandicum* showed the most potent antioxidant capacity in being the sole plant able to reduce levels of TBARS both in the supernatant and the lysate.

6.3.5 Correlation between TBARS and LDH

Levels of TBARS and % LDH release were measured at two-time points, baseline and 6 hours incubation using ox-LDL. The DPPH assay gives an absolute measure of antioxidant potential upon reaction completion. As such, correlations were valid between results of TBARS and LDH assays. A significant positive correlation was found between TBARS (lysate) and LDH at 6

hours ($r=0.2936$, $p=0.043$) (Table 6.3.4.1). Variability would be a probable cause for the low correlation coefficient seen between these two experiments and the non-significant correlation at 0 hours.

	Correlation Coefficient			
	TBARS-Supernatant (0hrs)	TBARS-Supernatant (6hrs)	TBARS-Lysate (0hrs)	TBARS-Lysate (6hrs)
LDH (0hrs)	$r = -0.1006$ $p = 0.2350$		$r = -0.0894$ $p = 0.4395$	
LDH (6 hrs)		$r = 0.1433$ $p = 0.0615$		$r = 0.2936$ $p = 0.043^{**}$

Table 6.3.4.1: Correlation coefficients and p-values for TBARS and LDH results.

******Denotes a statistically significant value ($p < 0.05$).

6.4 DISCUSSION

Oxidative modification of LDL plays a pivotal role in the pathogenesis of atherosclerosis (Esterbauer, 1990; Esterbauer, 1992; Steinberg, 1997). In this study, we demonstrated the potential of certain plant extracts to inhibit the damaging effects of oxidatively modified LDL to endothelial cells.

Initial *in vitro* screening of crude plant extracts using the DPPH assay showed 4 plants, *A. incana*, *P. mariana*, *P. banksiana* and *R. groenlandicum* having free-radical scavenging activity comparable to our control flavonoids. DPPH is a stable radical that changes colour (purple to yellow) upon reaction with an antioxidant. The extent of the colour change and thus, antioxidant potential, is dependent on the hydrogen donating ability of the respective treatment (Bondent, 1997). *P. mariana* and *R. groenlandicum* have been previously tested for their antioxidant potential with DPPH (McCune, 2003). Our results however, show enhanced effects in relation to those obtained by McCune (2003) with antioxidant potential similar to standard flavonoids.

Incubation of BAEC with ox-LDL showed significant cell damage as evidenced by increased LDH release in comparison to n-LDL. Permeability is affected by increased oxidation to cellular membranes, which in turn increases the leakage of LDH (Martin-Nizard, 2003). The inhibition of oxidative stress induced by ox-LDL by the plant extracts can be suggested to be due to the significant reduction in LDH release in comparison to ox-LDL alone.

MDA is an unstable compound shown to produce mutagenic and cytotoxic events (Zin, 2002). In the TBARS assay, MDA binds TBA to form a red chromagen quantifiable at 510nm. Ox-LDL's ability to induce cellular injury was substantiated by the increased level of TBARS formed in the supernatant and high LDH release. The low level seen within the lysate of ox-LDL treated cells can be attributed to the possible loss of membrane integrity resulting in increased release of reactive oxygen species in the supernatant. This assumption is validated by the high % LDH release, which is reflective of cellular damage resulting in leakage of macromolecules across the membrane (Martin-Nizard, 2003). This is further substantiated by previous studies, which have shown alterations in endothelial permeability and vascular tone as a result of detectable cellular damage (De Cheng Ren, 2002).

The high levels of TBARS of plant treatments following pre-incubation for 24 hours could be a result of the inherent oxidizing ability of endothelial cells (Steinbrecher, 1984; Morel, 1984). The sequential lowering of TBARS levels by plants following addition of ox-LDL provides promising effects of these plants to counter further damage to membrane integrity and thus oxidative damage. The high level of TBARS in the lysate of *A. incana* alone at 24 hours shows a pro-oxidant effect within the endothelial cell system. Its ability however to diminish the level of TBARS after a 6-hour incubation with ox-LDL may demonstrate an ability of *A. incana* to halt the propagation phase of free radical generation (Rasmussen, 2005). *A. incana* has been suggested for use as a topical reagent for

sores and rashes therefore these results may support its anti-bacterial capacity. It has however also been implicated for diarrhea and heart ailments for which it is taken orally. The *A. incana* preparation for oral ingestion is primarily a water infusion and thus may not be as concentrated as the ethanolic extract used within our bioassays.

Oxidation occurs as a result of depletion of endogenous antioxidants within the LDL particle. Previous studies have shown the ability of probucol and BHT to effectively reduce lipid peroxidation via incorporation into the LDL particle (Carew, 1987; Kita, 1987). The plant extracts may possibly act in the same manner by stabilization of the modified LDL particle to prevent further damage to the cellular membrane. They could also act within the cellular membrane to prevent infiltration of damaging by-products into the intracellular matrix (Mabile, 1995; Zapolska-Downar, 2002; Martin-Nizard, 2003).

Overall, we note the antioxidant potential in all three tests of *R. groenlandicum* and *P. banksiana*. These two extracts exemplified antioxidant potential comparable to standard controls alongside their ability to effectively prevent oxidative stress both intra- and extracellularly upon incubation with ox-LDL. The antioxidant and anti-atherogenic potential of these plants can most likely be attributed to their major tannin and flavonoid constituents (Duke, 1985; Farnsworth, 1999; Marles, 2000). Tannins and flavonoids are known for their antioxidant and antiradical potential due to their highly reactive hydroxyl group and their ideal positioning at the aqueous surfaces of phospholipids (Brandi, 1992; Cotellet, 1996). Polyphenols and more specifically proanthocyanidins found within red wine, tea, grapes, chocolate, cranberries, and crude plants have been shown to positively alter fatty acid profiles, reduce blood pressure, and improve endothelial function by increased production of vasodilators and inhibition of inflammatory responses (Auger, 2002; Badia, 2004; Stocker, 2004; Waddington, 2004).

Pyconogenol®, a proanthocyanidin found within the bark extract of French maritime pine (*Pinus maritima*), has been found to improve endothelial function by its ability to decrease endothelin-1 levels and increase prostacyclin levels (Liu, 2004). This phytochemical has also been found to increase nitric oxide production by upregulation of nitric oxide synthase leading to improvements in vascular homeostasis (Liu, 2004; Rasmussen, 2005).

Due to the implication of ox-LDL in the progression of oxidative stress, induced by DM2, antioxidant treatment could provide an effective method to counter the progression of this disease state. Our results highlight the ability of the plant extracts used in Cree traditional medicine to scavenge free radicals and reduce oxidative stress resulting in effective protection in the endothelial cell system. Use of these plant treatments, primarily *P.banksiana* and *R.groenlandicum*, may provide essential resistance to oxidative stress for DM2 patients. Future research is needed, but these plants may, in the least, contribute positively to a health plan targeting DM2 alleviation.

7.0 CONCLUSIONS

7.1 LIMITATIONS

7.1.1 Nutritional Assessment

The use of a non-random sample introduced a degree of bias to our results and thus limits our ability to draw precise conclusions about characteristics of the general population. This sampling strategy was used due to time and monetary constraints. The findings do however provide an overview of the effect of acculturation on the use of traditional medicines and foods and a foundation for future implementation strategies integrating traditional medicines for treatment of DM2.

The study may have also benefited from the use of a quantitative nutritional assessment tool. The use of a quantitative FFQ or 24-hour recall administered four times yearly would have captured a more precise measurement of consumption trends and would have allowed for in depth analysis of nutritional contribution of traditional food sources to the daily diet.

7.1.2 Bioactivity of plant extracts

The DPPH assay was limited in that it shows absolute, non-specific antioxidant potential. The procedure is such that it is not able to decipher the destabilization of the DPPH radical by the plant extracts. This assay, however, is an effective screening tool for observing trends in antioxidant potential in relation to standards controls.

The TBARS assay is limited such that it is not a specific measure of lipid peroxidation by-products. The process of lipid peroxidation releases numerous lipid by-products of which MDA is but one. This limitation can be overcome by the use of the HPLC technique.

The use of cell culture introduces a margin of error within-treatments due to the variability of cellular conditions inherent in each well. The inability to control for this factor leads to variability in experimental results. This restriction can be alleviated by increasing the number of trials or by further investigation of trends seen within the cellular system using *in vivo* animal models and clinical trials. Overall, the cellular system offers reliable measure for preliminary identification of characteristic behaviours of phytoactive constituents.

The within-treatment variability may also be a result of the array of constituents within the plants. Past research looking at similar parameters, as those viewed in this study, have focused on isolated plant constituents rather than crude plant extracts (Martin-Nizard, 2003; Tsai, 2003; Miyazawa, 2004). Our review of plant extracts may have lead to the inability of one or several plants to show exceptional antioxidant effects. Isolation of plant components would also facilitate the identification of individual or synergistic effects of these compounds.

The degree of oxidation of LDL and the variability inherent in LDL samples introduces an additional source of error. The degree of oxidation can affect the rate of uptake by macrophages, which could affect the activity of ox-LDL and the plant extracts (Esterbauer, 1990). Previous studies have outlined the variability inherent in the chemical properties of LDL, which in turn affects its action *in vitro* and *in vivo* (Esterbauer, 1990). This, therefore, could explain variability and non-specific effects observed for plant extracts.

7.2 GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

7.2.1 General Conclusions

The continual rise in DM2 within the Cree Nation and the complications inherent in this disease state has supported the need for culturally appropriate measures. The low rate of chronic disease before the urbanization of the Cree people substantiates the plausibility of an alternative intervention strategy integrating

traditional remedial practices. The intake survey established the frequency of use of traditional medicines among community members of varying ages alongside the perceptions underlying the use of the plants for treatment of DM2. The laboratory analysis authenticated the traditional knowledge obtained from the intake surveys. This project is the first of its kind to show a link between the traditional knowledge of the James Bay Cree and the biologically active phytochemicals of the plant species mentioned.

This study exemplifies the decreased use of traditional medicines and foods, which can be consequently linked to the decreased transmission of traditional knowledge. The eagerness of the participants to return to past traditions favours the successful integration of traditional medicines into the western healthcare system.

Scientific evaluation of plant extracts most frequently cited for symptoms of DM2 showed antioxidant and anti-atherogenic potential. We noted a protective effect of two plants, *P. banksiana* and *R. groenlandicum*, within all three assays. Their ability to effectively scavenge free radicals and prevent damage incurred by oxidative stress could be attributed to their phenolic constituents. Due to the lipophilic nature of phenols they are able to interfere with the propagation of free radicals at the surface of the membrane. The ability of *P. banksiana* and *R. groenlandicum* to conserve endothelial function in light of oxidative stress induced by ox-LDL could be attributed to the proanthocyanidins within these plants.

No analysis of consumption of traditional medicines and the factors underlying this consumption been previously undertaken. This study is among the first to assess the repercussions of modernization on cultural transmission and the willingness of reintegration of past traditions. The bioassays provide a basis for therapeutic relief of symptoms related to DM2.

These findings support the traditional knowledge of medicinal plant uses and the applicability of this knowledge in modern medicine. Moreover, the study findings highlight the importance of cultural transmission and the pertinence of culturally appropriate health interventions in Aboriginal communities.

7.2.2 Future Research

As part of a more extensive integrative project involving collaboration of several laboratories, this study offers a basis for future supplementation and nutritional intervention strategies. Findings from the laboratories of Dr. Pierre Haddad (University of Montreal) and Dr. John Thor Arnason (University of Ottawa) both *in vitro* and *in vivo*, alongside these findings, support hypoglycaemic, antioxidant and anti-atherogenic properties of the plants in question. This findings need to be further investigated in *in vivo* animal models to determine physiologically appropriate doses for administration in humans. The question of multiple plant combinations also needs to be addressed due to the complexity of constituents within these plants. The potential side effects of these plants and Western treatments currently in use need further investigation to avoid lethal repercussions. Randomized, double blind, placebo-controlled clinical trials would offer substantial support for the efficacy and safety of these remedies for use in humans.

Another important consideration is the possible synergistic effect of traditional foods and traditional remedies. The benefits of traditional foods have been established in past research (Kuhnlein, 1991, 1996, 2000; Delorimier, 1999; Dewailly 2002; Rosol, 2005) but the benefits in conjunction with traditional remedial treatment have not yet been viewed. *In vitro* studies investigating the potential synergism of traditional plants and PUFAs would, therefore, prove to be beneficial. Positive results would foster the need for *in vivo* animal model work, followed by human clinical studies. These types of studies would aid in promoting the already established benefits of traditional food consumption and

the benefits of adherence to a traditional lifestyle involving traditional medicines and foods.

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10.0 APPENDICES

Appendix 1: Food Frequency Questionnaire

Food Frequency Questionnaire by Cree Community Members

Respondent: _____

Date of Interview: _____

Age Group: 20-59 _____ >59 _____

Interviewer: _____

Gender: M _____ F _____

Length of Interview: _____

How often do you consume the following?

FOOD	Daily			Weekly			
	1-2	3-4	> 4	1-2	3-4	> 4	Never (in past year)
<i>Caribou</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/parts</i> Head Ribs Heart Tongue Liver Blood Stomach Intestine Kidney Bone Marrow Fat (in any form) Other (please specify)							
<i>Black Bear</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Blood Brain Fat/ grease Other (please specify)							
<i>Moose</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried							

FOOD	Daily			Weekly			
	1-2	3-4	> 4	1-2	3-4	> 4	Never (in past year)
<i>Organs/parts</i> Head Ribs Heart Tongue Liver Blood Stomach Intestine Kidney Bone Marrow Fat (in any form) Other (please specify)							
<i>Muskrat</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Liver Blood Brain Tail Other (please specify)							
<i>Lynx</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Liver Blood Brain Head Other (please specify)							
<i>Porcupine</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Liver Blood							

FOOD	Daily			Weekly			
	1-2	3-4	> 4	1-2	3-4	> 4	Never (in past year)
Brain Other (please specify)							
Otter <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Tail Liver Brain Blood Other (please specify)							
Canadian Goose <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/parts</i> Gizzard Liver Heart Eggs Fat (in any form) Other (please specify)							
Ptarmigan <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Gizzard Liver Heart Other (please specify)							
Beaver <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/parts</i> Liver Blood Tail & Feet Brain Castorium							

FOOD	Daily			Weekly			
	1-2	3-4	> 4	1-2	3-4	> 4	Never (in past year)
Other (please specify)							
<i>Rabbit</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/parts</i> Liver Brain Blood Head Other (please specify)							
Other Mammal or Part (please specify)							
<i>Lake Whitefish</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/parts</i> Head Eggs Fish-pipe Other (please specify)							
<i>Lake Trout</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Head Eggs Fish-pipe Other (please specify)							
<i>Speckled Trout</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Head Eggs Fish-pipe							

FOOD	Daily			Weekly			
	1-2	3-4	> 4	1-2	3-4	> 4	Never (in past year)
Other (please specify)							
<i>Sturgeon</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Head Eggs Fish-pipe Other (please specify)							
<i>Loche (burbot)</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Head Eggs Liver Other (please specify)							
<i>Northern Pike (jackfish)</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Head Eggs Fish-pipe Other (please specify)							
<i>Sucker Fish</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i> Head Eggs Fish-pipe Other (please specify)							
<i>Dove (walleye)</i> <i>Meat</i> Cooked (fresh or frozen) Smoked/ dried <i>Organs/ parts</i>							

FOOD	Daily			Weekly			
	1-2	3-4	> 4	1-2	3-4	> 4	Never (in past year)
Head Eggs Fish-pipe Other (please specify)							
Other Fish or Part (Please specify)							
Bannock (traditional)							
Bannock (non-traditional)							
<i>Uyooskan</i> (Wild Raspberries)							
<i>Sapoominak</i> (Gooseberries)							
<i>Otahimin</i> (Wild Strawberries)							
<i>Minsec</i> (Blueberries)							
<i>Chicoute</i> (Cloudberry)							
<i>Askiminasih</i> (Crowberries)							
<i>Wiisichiminaahtikw</i> (Mountain Cranberry)							
Jams or Preserves							
Frozen or Dried Berries (any type)							
Other Berry (please specify)							

Traditional Plants/ Medicines	Use & Method of Preparation	Currently In Use
<i>Kachebuk</i> (Labrador Tea)		
<i>Inaasht- Piichou</i> (Balsam Fir- sap)		
<i>Inaasht- Outkoun</i> (Balsam Fir- branches)		
<i>Waachinaakan- Wiicheskw</i> (Tamarack- inner bark)		
<i>Waachinaakan- Outkoun</i> (Tamarack- branches)		

Traditional Plants/ Medicines	Use & Method of Preparation	Currently In Use
<i>Waachinaakan- Stakoun</i> (Tamarack- needles)		
<i>Inaatuk- Outkoun</i> (Black Spruce- branches)		
<i>Inaatuk- Stakoun</i> (Black Spruce- needles)		
<i>Inaatuk- Ouchsketjoosh</i> (Black Spruce- cones)		
<i>Muskanaa Natuk- Wiicheskw</i> (Mountain Ash- inner bark)		
<i>Muskanaa Natuk- Outkoun</i> (Mountain Ash- branches)		
<i>Muikutushpi- Wiicheskw</i> (Speckled Alder- bark)		
<i>Muikutushpi- Outkoun</i> (Speckled Alder- branches)		
<i>Utushpi- Wiyyuuchii</i> (Willow- inner bark)		
<i>Ayigadash- Uskaataayaapiiy</i> (Purple Pitcher Plant- roots)		
<i>Ayigadash- Whole Plant</i> (Purple Pitcher Plant)		
<i>Ushchishk- Wiicheskw</i> (Jack Pine- inner bark)		
<i>Ushichishk- Ouchsketjoosh</i> (Jack Pine- cone)		
<i>Pasnacowon- Niipssii</i> (Stag's Horn Clubmoss- leaves)		
Bog Laurel		
<i>Minsec</i> (Blueberry)		
<i>Minsec- Niipssii</i> (Blueberry- leaves)		
<i>Minsec- Uskaataayaapiiy</i> (Blueberry- roots)		
<i>Miitus- Niipssii</i> (Poplar- leaves)		
<i>Miitus- Wiicheskw</i> (Poplar- bark)		
<i>Wiisichiminaahtikw</i> (Lingonberry/ Mountain Cranberry)		
<i>Wiisichiminaahtikw- Whole Plant</i>		

Traditional Plants/ Medicines	Use & Method of Preparation	Currently In Use
(Lingonberry/ Mountain Cranberry- Plant)		
<i>Minahiikw-Wiicheskw</i> (White Spruce- bark)		
<i>Minahiikw- Ouchsketjoosh</i> (White Spruce- cones)		
<i>Piyaaumin</i> (Creeping Snowberry)		
<i>Piyaaumin- Whole Plant</i> (Creeping Snowberry- Plant)		
<i>Wahotahuk</i> (Cattail)		
<i>Waahkunaapiskw</i> (Black Lichen)		
Other Traditional Plant/ Medicine (please specify)		

Appendix 2: Socio-Behavioural Questionnaire

Socio-Behavioural Questionnaire by Cree Community Members

Respondent: _____ Date of Interview: _____
 Age: 20-59 _____ >59 _____ Interviewer: _____
 Gender: M _____ F _____ Length of Interview: _____

Sociodemographics

- 1) Are you from Mistissini? Yes _____ No _____
- 2) In the last year, did you stay in Mistissini all the time? Yes _____ No _____
- 3) In the last year, did you stay at hunting or fishing camps at any time?
 Yes _____ No _____
- 4) If yes, for how long?
- 5) How many persons, including yourself, currently live in your household?
- 6) Is anyone employed outside the home? If yes, at what level (P/T, F/T, seasonal)?
- 7) What is the highest level of education you have received?
- 8) Was your education on the reserve or in an urban setting?
- 9) Do you still participate in traditional activities? (i.e. fish camps, geese hunting, caribou hunting, etc.) If yes, please specify the activities.
 Yes _____ No _____ Specific activities _____

Traditional Food Use

10) Does your family eat traditional foods in the house?

Yes _____ No _____

11) What kinds of traditional foods does your family eat?

Big Game _____

Small Game _____

Fish _____

Geese, Ducks, Ptarmigan _____

12) How do you store your food?

Chest freezer _____

Freezer in fridge _____

Smoking _____

Drying _____

Other (please specify) _____

13) Where do you get your traditional food?

Someone in family hunts or fishes _____

Relatives and/or friends _____

Cree trappers _____

Store _____

CTA _____

Other (please specify) _____

14) Does everyone in your house like to eat traditional food?

Yes _____ No _____

15) If no, why?

16) Has the amount of traditional food you eat changed over the past year?

Yes _____ No _____

17) Has the amount you eat changed since you were young?

Yes _____ No _____

18) Can you tell me the reason for the changes, if any?

19) The Cree have a long experience with mercury programs. Has this concern about mercury ever stopped you from eating fish?

Yes _____ No _____

20) Some hunters have had concerns about finding sick animals. In the past year, have you ever had any concerns about the traditional foods you have eaten?

Yes _____ No _____

21) If you don't consume traditional foods but your family does, does it affect the relationship among family members?

Yes _____ No _____

Herbal Medicine/ Traditional Remedy

22) Have you heard of traditional medicines?

Yes _____ No _____

23) Do you yourself use them?

Yes _____ No _____

24) What kind of traditional medicines do you use?

25) What do you use them for? (i.e. sicknesses, preventive teas, taste, etc.)

26) How do you take them?

27) Are these traditional plants taken primarily as medicines or do you consider them a normal part of your diet?

Yes _____ No _____

28) How often do you use them?

Daily _____

Weekly _____

Monthly _____

When needed _____

29) Has this changed over the years?

Yes _____ No _____

30) Where did you learn about them?

31) If you only consume traditional medicines, where do you get them?

32) If you provide traditional medicines, who taught you about them?

33) If traditional medicines were more available in the community, through the clinic or through local elders, do you think you would use them more?

Yes _____ No _____

34) If yes, why?

35) Do you think the clinic should be providing traditional medicines, as well as, modern medicines for Cree if it could help them?

Yes _____ No _____

36) If yes, what would be the best form to use these medicines?

37) Have your past experiences of illness influenced your attitude toward medicinal plant use?

Yes _____ No _____

38) If yes, how so?

39) Do you think a program involving the use of medicinal plants would be helpful for the Cree community?

Yes _____ No _____

Health and Diabetes

40) What do you feel is the general health status of the Cree?

Good _____ Average _____ Below Average _____

41) What do you think is good health?

42) Do you know anyone with diabetes who has used traditional medicines to help them with their symptoms?

Yes _____ No _____

Traditional Knowledge

43) What type of traditional knowledge have you learned from your elders?

44) Do you use this traditional knowledge in your daily life?

Yes _____ No _____

45) Do you think the transmission of traditional knowledge in your community has increased or decreased over the years?

Increased _____ Decreased _____

46) Is there any pressure to learn traditional knowledge (i.e. from parents or elders)?

Yes _____ No _____

Appendix 3: Letter of Consent



Letter of Consent

Introduction

Diabetes mellitus is a disease, which has reached serious levels within your community. The rates of obesity and glucose intolerance have also increased over the years and are beginning to affect the younger generations.

We will be doing research to evaluate the use of medicinal plants and the views toward use of these plants for symptoms of diabetes mellitus. The findings of the study will help to put in place a supplementation program using the medicinal plants identified by the elders within the community. All Cree people aged 20 years and older who identify as Cree and live in Mistissini will be invited to participate in the study.

Please read the details of the consent form carefully. If you would like more information, please feel free to contact Sonia Grandi or Paul Linton. A copy of this consent form will be given to you, please keep of a copy of it.

Purpose

We hope to determine the use of medicinal plants in your community, as well as, the beliefs toward the use of these plants. We will also test the ability of the important plants for use against symptoms of diabetes mellitus. The main goals are to give a more holistic approach to treatment of diabetes mellitus and to emphasize the need to conserve traditional knowledge.

Study Description

Participants, you will be visited in your home or wherever you feel comfortable in meeting the researcher for the interview. You will be able to have the interview conducted in Cree, by a community member. The interview may be taped.

Interview

The interview will involve two questionnaires. The first will be a food frequency questionnaire, which is a list of traditional foods and traditional plants. You will be asked to check the number of times you have eaten or drank the foods or plants over the last week. The second questionnaire has questions concerning your everyday habits, (i.e. education, work, home), use of medicinal plants and general beliefs toward medicinal plants.

Plant collection

The plants identified by the elders to be most often used for symptoms of diabetes mellitus will be collected for analysis in the laboratory. The plant extracts will be prepared according to methods described by elders.

Right to Refuse Participation

The choice to participate in the study is based on your own free will. You have the right to withdraw at any time from the study. You will not be punished for not participating in the study and will continue to receive services from the Cree Board of Health and Social Services of James Bay. If at any time, you feel the questions violate your privacy you have the right not to answer. If at any time, you feel uncomfortable with the interview, please feel to speak to any of the study contacts.

Benefits and Risks

The study interviews will be scheduled at your convenience with the researcher. There is little risk involved in participating in the study. At the end

of the study you will receive a newsletter or an update at a community meeting about the study results. No results will be presented or published before the community is made aware of the findings. Your community will be able to benefit from the use of traditional medicines for treatment of symptoms of diabetes mellitus.

Confidentiality

The information obtained about you and your family will only be used for the purpose of this study. Your identity and personal information will be kept confidential. The information will be presented as group results, by age and gender, with no association to personal identity. The Cree Board of Health, as well as, our research team will keep a copy of the list of participants. We will keep the findings locked in a file cabinet in Dr. Johns' laboratory in the School of Dietetics and Human Nutrition at McGill University.

Study Participants and Contacts

This study is a part of a larger study on traditional treatments for diabetes, funded by the Canadian Institutes of Health Research and headed by Dr. Pierre Haddad of University of Montreal. The following groups are involved with this study, the Cree Board of Health, the University of Montreal, the Montreal Botanical Gardens, the University of Ottawa, McGill University and the Centre for Indigenous Peoples' Nutrition and Environment (CINE).

If you wish to speak to someone about this project or the larger study, please do not hesitate to contact one of the following people,

Jane Blacksmith, Public Health Officer (Mistissini)

Solomon Awashish, Cree Board of Health

Paul Linton, Regional Diabetes Initiative, Cree Board of Health

Timothy Johns, PhD. Tel: (514) 398-7847

Alain Cuerrier, PhD. Tel: (514) 872-3182

Sonia Grandi, BSc. Tel: (514) 845-7114

Consent

I agree to participate in the study and my signature means that I understand the above information.

Name of Participant (print clearly)
Date

Signature of Participant and

Researcher (print clearly)

Signature of Researcher

Participant's address where possible newsletter can be sent

A copy of this consent form will be provided for you. Please keep it for your personal record and future reference.

Consent explained by: _____ Date: _____

Questions answered by: _____ Date: _____

Appendix 4: Ethics Approval Form

**MCGILL UNIVERSITY
FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES**

**CERTIFICATE OF ETHICAL ACCEPTABILITY FOR
RESEARCH INVOLVING HUMANS**

Approval Period: June 1'04 - May 31-04 REB #: #845-0403

The Faculty of Agricultural and Environmental Sciences Ethics Review Committee consists of 4 members nominated by the Faculty of Agricultural and Environmental Sciences Nominating Committee and elected by Faculty, an appointed member from the community and an individual versed in ethical issues.

The undersigned considered the application for certification of the ethical acceptability of the project entitled:

Assessment of the Importance of
Potential Antidiabetic Plants in the
Health of the James Bay Cree.

as proposed by:

Applicant's Name Sonia Grandi Supervisor's Name Dr. Timothy Johns

Applicant's Signature Sonia Grandi Supervisor's Signature Timothy Johns

Degree / Program / Course MSc Nutrition Granting Agency (ies) CIHR
Grant Title(s): Thesis (Canadian Institutes for Health Research)

Rigorous scientific evaluation of selected anti-diabetic plants: Towards an alternative therapy for diabetes in the Cree of Northern Quebec.

The application is considered to be:

A Full Review ☐ An Expedited Review ☒

A Departmental Level Review

Signature of Chair / Designate

Peter Jones
Chair, Faculty of Agricultural and Environmental Sciences Ethics Review Committee
School of Dietetics and Human Nutrition
Tel: (514) 398-7547; Fax: (514) 398-7738

Peter Jones

Signature / date

Appendix 5a: Plant Percent Yields (Post Lyophilization)

Plant	Part	Original Weight	Final Weight	% Yield
<i>Abies balsamifera</i>	Inner bark	N/A	N/A	N/A
<i>Alnus incana ssp. rugosa</i>	Inner bark	N/A	N/A	N/A
<i>Larix laricina</i>	Inner bark	242.82g	127.10g	52.34%
<i>Pinus banksiana</i>	Inner bark	N/A	N/A	N/A
<i>Picea mariana</i>	Inner bark	N/A	N/A	N/A
<i>Rhododendron groenlandicum</i>	Leaves	115.72g	49.59g	42.85%
<i>Sarracenia purpurea</i>	Whole Plant	258.50g	39.27g	15.19%
<i>Sorbus decora</i>	Inner bark	75.11g	57.70g	76.82%

Appendix 5b: Plant Percent Yields (Post-Extraction)

Plant	Part	Original Weight	Final Weight	% Yield
<i>Abies balsamifera</i>	Inner bark	N/A	N/A	N/A
<i>Alnus incana ssp. rugosa</i>	Inner bark	N/A	N/A	N/A
<i>Larix laricina</i>	Inner bark	10g	0.0275	0.275%
<i>Pinus banksiana</i>	Inner bark	N/A	N/A	N/A
<i>Picea mariana</i>	Inner bark	N/A	N/A	N/A
<i>Rhododendron groenlandicum</i>	Leaves	10g	0.0280g	0.280%
<i>Sarracenia purpurea</i>	Whole Plant	10g	0.0275g	0.275%
<i>Sorbus decora</i>	Inner bark	10g	0.0301g	0.301%