THE EMERGING EPIDEMIC OF TYPE 2 DIABETES MELLITUS IN FIRST NATION CHILDREN AND YOUTH: ISSUES RELATED TO DIAGNOSIS, ETIOLOGY, COMPLICATIONS AND TREATMENT

By Elizabeth AC Sellers

A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirement for the degree of Master of Science

Department of Community Health Sciences
Faculty of Medicine
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Chapter 1 Introduction

1.1 Brief Description of the Research Problem

Diabetes mellitus is defined as the failure of normal intracellular glucose transport resulting from an absolute or relative deficiency of functional insulin. Clinically, diabetes is characterized by hyperglycemia, glycosuria, and long-term micro- and macrovascular complications [1]. The diagnosis of diabetes is based on biochemical criteria (see appendix 1.1). Diabetes is classified into several subtypes.

Type 2 diabetes mellitus is a polygenic disorder with the underlying pathophysiologic derangement ranging from primarily insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance [2]. This is distinct from type 1 diabetes, which results from beta cell destruction (autoimmune or idiopathic) leading to absolute insulin deficiency. Gestational diabetes and diabetes resulting from single gene disorders or secondary to other disease processes are classified separately (see appendix 1.2) [2].

There is not however, a definitive diagnostic biochemical test to distinguish type 2 diabetes from the other subtypes of diabetes. Confirmation of the diagnosis depends on the evolution of the clinical course. In adults, the type of diabetes is usually evident by clinical characteristics of the patient.

Type 2 diabetes affects over 100 million people worldwide [3]. The prevalence is increasing and there are projections that over 250 million individuals will be affected by the year 2020 [4]. Classically considered a disease of adults, it is now becoming clear that

type 2 diabetes is affecting children and youth [5]. The earlier age of onset magnifies the potential socioeconomic and health impact at both an individual and societal level.

To combat this emerging problem, we need a better understanding of the epidemiology, diagnosis, complications, treatment, and prevention of type 2 diabetes in youth. Health care providers, policy makers and administrators face a major challenge in the 21st century to gain a better understanding of this new phenomenon in pediatrics.

This thesis represents several projects linked by the common theme of type 2 diabetes in youth. The unifying objective is to improve our understanding of this new disorder so we can ultimately improve prevention, detection and treatment in order to impact positively on the health of children and youth at risk for the development of type 2 diabetes.

1.2 Population of Interest

This work deals specifically with the issue of type 2 diabetes in First Nation children and youth (defined as 18 years of age and under). In Manitoba and Northwestern Ontario, the vast majority of children and adolescents with type 2 diabetes are of First Nation Origin. 138/141 (97.9 %) of the young people seen at the only tertiary pediatric center providing service to this area over the past 15 years have been of First Nation origin. First Nation origin was determined on a self-declared basis by the individual or by parental proxy.

The majority of First Nation people in Manitoba and Northwestern Ontario are of the Algonquian language family and speak Cree, Ojibway and Saulteaux. Traditionally, these populations were hunter-gathers expending large quantities of energy in daily activities [6]. This pattern has dramatically changed over that last half century and the culture is

now undergoing epidemiologic transition. There is a trend towards increasingly large quantities of carbohydrates and fats in the diet, and a relative overabundance of calories. Food gathering no longer demands a large investment of time and expenditure of energy. This has resulted in a significant decrease in physical activity.

Demographic data is available only for those of First Nation origin who are considered "registered Indians" according to section 10 of the *Indian Act*. This is determined primarily by historical events and ancestry. The demographic data thus presented here represents minimum total numbers and excludes those not registered.

As of December 31, 1999 the registered Indian population in Canada was 659,890. 54.5% (360,707/659,890) were living on reserve. Of those on reserve, 83.4% live in communities of less than 1000 people. 104,099 were residents of Manitoba and 150,236 residents of the Sioux Lookout District (Northwestern Ontario). Thus, 38.5% of the Canadian registered Indian population live in the Manitoba and Sioux Lookout regions. In Manitoba, 66% of the registered First Nation population live on reserve. 50.3% are female (49.7% male) and 45.3% are19 years of age and under. In the Sioux Lookout Region, 49.5% of the registered Indian population lives on reserve, and 51.6% were female. Age distribution on a regional basis was not available [7].

1.3 Study Region

Manitoba and Northwestern Ontario are situated in central Canada. The majority of the land is in the subartic zone, covered by lakes, rivers and boreal forest. The Great Plains area encompasses the most southern regions of Manitoba [6].

1.4 The Manitoba Diabetes Education and Resource for Children and Adolescents (DER-CA)

The Diabetes Education and Resource Center for Children and Adolescents is a program funded by Manitoba Health to provide care to the pediatric diabetic population in Manitoba. The DER-CA also provides consultative services to children and adolescents of Northwestern Ontario, as the next closest specialty pediatric diabetes care center is in Toronto. The DER-CA is based at the only tertiary care center providing services to these geographic areas (Winnipeg Children's Hospital, Health Sciences Center). The DER-CA team is comprised of a pediatric endocrinologist, a dietitian, and a nurse educator, a social worker and a secretary. The DER-CA provides the clinical setting for the work presented in this thesis.

1.5 General Research Objectives

Etiology

1) To determine if a specific mutation (HNF-1α G319S) is present with increased frequency in First Nation children and youth with type 2 diabetes compared to individuals of First Nation origin without diabetes.

Diagnosis

2) To investigate whether diabetes associated autoantibodies are associated with type 2 diabetes in First Nation children and youth.

Complications

3) To estimate the prevalence of an acute diabetic complication, diabetic ketoacidosis, in type 2 diabetes in First Nation children and youth.

Treatment

4) To determine if an oral antidiabetic agent, metformin, is effective in a population of First Nation children and youth with type 2 diabetes.

Appendix 1.1 Criteria for the Diagnosis of Diabetes Mellitus

Source: Canadian Clinical Practice Guidelines. [2]

Symptoms of diabetes plus casual plasma glucose concentration > 11.1 mmol/L. Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydypsia, and unexplained weight loss.

or

Fasting plasma glucose >6.9 mmol/L. Fasting is defined as no caloric intake for at least 8 hours

or

2h post prandial glucose >11.1 mmol/L during OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

Appendix 1.2 Classification of Diabetes

Type 1 diabetes mellitus (beta-cell destruction, usually leading to absolute insulin deficiency)

- Immune mediated
- Idiopathic

Type 2 diabetes mellitus (may range from predominantly insulin resistance with relative insulin deficiency to predominantly secretory defect with insulin resistance)

Gestational diabetes mellitus (onset or recognition of glucose intolerance in pregnancy)

Other specific types

Genetic defects of beta cell function

- Chromosome 12, HNF (MODY 3)
- Chromosome 7, glucokinase (MODY 2)
- Chromosome 20,HNF-4 (MODY 1)
- Mitochondrial DNA
- Others

Diseases of the endocrine pancreas

Infections
e.g. congenital rubella

Drug or chemical induced

Genetic defects in insulin action

Endocrinopathies

Uncommon forms of immune-mediated diabetes

Other genetic syndromes sometimes associated with diabetes

Adapted from: 1998 clinical practice guidelines for the management of diabetes in Canada CMAJ; 159 (8 Suppl) S1-S29.

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Chapter 2 Review of the Literature

2.1 Epidemiology

Type 2 diabetes currently affects over 100 million people worldwide. The prevalence is increasing and there are projections that by the year 2020 250 million people will be affected [1].

While no ethnic group is immune to type 2 diabetes, it is found much more commonly among some racial groups. These include Micronesians, Polynesians, Pacific Islanders, Australian aboriginal, and in North America, Native Americans, Hispanics, and African – Americans [1]. In Canada, people of First Nation origin are at specific risk. The factor uniting these diverse groups is the change from a traditional to a more westernized lifestyle. This typically involves a trend towards less physical activity and a change in dietary composition and caloric intake.

The epidemic of type 2 diabetes among many Aboriginal populations in Canada and the United States over the past several decades has been recognized for some time [2-4]. A more recent development is the diagnosis of type 2 diabetes among children. It has been slowly recognized in children and youth over the past 20 years. The initial report of type 2 diabetes in the pediatric age group occurred in 1979 [5]. In the past decade clinicians have diagnosed this disease in Aboriginal children as young as 7 years of age [6, 7]. There is now evidence that the prevalence of type 2 diabetes in children and youth is increasing and is not merely the result of improved detection [8].

The cause of the epidemic is not completely understood. Based on studies on adults, obesity has been demonstrated to be one of the most important risk factors for type 2 diabetes [9]. It is likely that the emerging pattern and burden of childhood type 2 diabetes is associated with an increasing prevalence of obesity among Aboriginal children, a result of changing lifestyles in terms of diet and physical activity level [10, 11].

Type 2 diabetes among youth is not a phenomenon unique to the Aboriginal population. As recently as 1994, type 2 diabetes was thought to account for only 1-2 % of all diabetes in childhood [12]. It is now recognized to account for at least 10% of youth onset diabetes in the United States [13]. In a population-based study in the Cincinnati region, the incidence of type 2 diabetes in youth increased more than ten-fold over a 13-year period [14]. Scott et al., reported a similar increase in Arkansas [15].

2.2 Diagnosis

The diagnosis of diabetes is made using biochemical criteria according to the criteria set by the American Diabetes Association June 1997 and adopted by the Canadian Diabetes Association in October of 1998 (see appendix #2.1). The distinction between type 1 and type 2 diabetes in First Nation youth is currently made using clinical criteria [16].

The clinical characteristics of First Nation youth with type 2 diabetes include age greater than 6 years and usually greater than 9 years and obesity (weight greater than 120% ideal body weight for height or BMI greater than the 85^{th} percentile for age and gender). Typically, there is no history of recent weight loss or of acute symptoms of hyperglycemia (polyuria, polydypsia). Acanthosis nigricans, a hyperpigmentation of the

skin in aposed and flexural areas, is associated with hyperinsulinemia and is frequently seen in this population. A positive family history for type 2 diabetes, in particular maternal diabetes, is also frequently found (see Table 2.1).

The majority of First Nation youth with type 2 diabetes are asymptomatic at presentation and are diagnosed by screening using a fasting blood glucose. This typically occurs through community wide screening programs or at an unrelated medical encounter when it is recognized that the child is at risk (e.g. First Nation origin, family history of diabetes, obesity). A significant number of individuals are also diagnosed by family members who, recognizing that the child is a risk, obtain a blood glucose reading using a home glucose monitor. An episode of diabetic ketoacidosis, typically associated with type 1 diabetes, does not rule out the diagnosis of type 2 diabetes (see chapter 5). The lack of diabetes associated autoantibodies may also aid in the distinction of type 2 diabetes (see chapter 3).

2.3 The Manitoba / Northwestern Ontario Experience

The first clinical description of youth onset type 2 diabetes in Canada was reported in 1992, based on cases among Aboriginal children in Manitoba [7]. In the Island Lake region of Manitoba, the prevalence of type 2 diabetes in Aboriginal children aged 5-14 years is seven times greater than type 1 diabetes among non-aboriginal children in Manitoba of the same age group, a rate comparable to that reported among the Pima Indians [17]. More recently, a screening study in one Island Lake community revealed a prevalence of 1.1% among children aged 4-19 overall, and more specifically 3.6% among girls aged 10-19 [18]. This finding is similar to that reported for adolescent girls in

another northern Ojibwa-Cree community in northwestern Ontario [19]. Type 2 diabetes can no longer be considered to be a rare entity in childhood.

From 1984 to May 1, 2000 141 individuals less than or equal to 18 years of age have been seen at the DER-CA with the diagnosis of type 2 diabetes mellitus. Of these, 138/141 are of self-declared First Nation origin (98.6%) and 104/141 are female (73.8%). For the total group, mean BMI at diagnosis was 30.2 kg/m^2 (+/-6.7). The mean BMI at diagnosis in males was 33.2 kg/m^2 +/- 6.5 and in females, 29.3 kg/m^2 +/- 6.5. As a group, mean age at diagnosis was 12.9 years (+/- 2.5, range 6-18 years). The mean age of diagnosis in the females versus the males (12.47 +/- 2.43; 13.97 +/- 2.22 respectively) differed significantly (p= 0.001) (Table 2.2). From 1984 to October 1, 2000, the number of new diagnoses of type 2 diabetes has steadily increased (Figure 2.1). In this current calendar year (2000) 34 new patients with type 2 diabetes have been seen. This represents 40.5 % of all new diagnoses of diabetes referred to the DER-CA in 2000 (34/84. Approximately 50% of the cases (8-18 years) currently being followed at the DER-CA have a HbA1c > 7.0%. This is above the target set by the Canadian Diabetes Association for optimal diabetes control [20].

2.4 – Pathophysiology [1]

Type 2 diabetes results from two physiologic defects, insulin resistance and a relative insulin deficiency [21]. Insulin resistance is defined as a steady-state plasma glucose that is higher than would be expected for the prevailing insulin level. There is much debate in the literature concerning whether insulin resistance or deficiency represents the primary

defect in type 2 diabetes. Despite this ongoing controversy there are some basic points of agreement. First, genetic factors are a major determinant of the risk of developing type 2 diabetes. Second, insulin resistance predicts development of type 2 diabetes. Third, early in the process the beta cells of the pancreas are able to compensate for insulin resistance by increasing insulin secretion thus maintaining normal levels of fasting glucose. Diabetes develops if the beta cells fail to compensate resulting in a relative or absolute insulin deficiency [1, 22].

Twin studies have provided evidence of a strong genetic component in the pathogenesis of type 2 diabetes. Newman et al., demonstrated a 58% concordance for type 2 diabetes by age 52-65 in monozygotic twins [23]. However, concordance was not 100%, suggesting that environmental factors play an important role in the expression of diabetes in the genetically susceptible individual. Multiple loci have been associated or linked with type 2 diabetes. However, these loci are not uniformly found in all populations [24].

The genetics of early onset type 2 diabetes has not been extensively investigated [24]. There is evidence of racial differences in insulin sensitivity in non-diabetic pediatric subjects. In the Bogalusa heart study, African American children 11-19 years of age had 30% lower insulin sensitivity than Caucasian children (corrected for obesity). African American children also had a higher insulin response to elevations in plasma glucose than comparable Caucasian children [25-27]. Of note, African American children are at higher risk for the development of type 2 diabetes, both in the pediatric period and as adults. Recently, a mutation in the HNF-1α gene has been associated with early onset type 2

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[1] This discussion excludes the rare, single gene disorders resulting in specific subtypes of type 2 diabetes mellitus.

diabetes in a specific Ojee-Cree population in North western Ontario (see chapter 5) [28, 29].

Type 2 diabetes has emerged in all societies as the prevalence of obesity increases. This, combined with a strong familial tendency towards the development of type 2 diabetes suggests that there may have been an advantage to a metabolic phenotype that is now detrimental [24]. This is the basis of the classic "thrifty gene" hypothesis proposed by Neel in 1962 [30]. This hypothesis proposes that in populations that historically experienced periods of food abundance and scarcity (hunter-gatherer populations) a genotype that promoted energy storage would be at a selective advantage. This advantage is now lost with an abundance of energy dense foods and a more sedentary lifestyle [30]. While this hypothesis is intriguing, no such gene, or collection of genes, has been found [1].

The prenatal environment may also impact on the risk of development of diabetes during post utero life. An association between low birth weight, thinness at birth and type 2 diabetes has been described in adults. This has lead to the speculation that beta cell capacity and insulin resistance is programmed in-utero. This is supported by studies that have shown that glycemic response to insulin is reduced in those thin at birth, suggesting insulin resistance.

These observations have lead to the "thrifty phenotype hypothesis" [31]. This hypothesis proposes that poor fetal nutrition is detrimental to both the development and function of beta cells and to the development of insulin sensitive tissues, leading to insulin resistance. An overabundance of nutrients later in life leads to obesity and increasing insulin

resistance and subsequently the development of type 2 diabetes [31]. However, the exact pathophysiologic mechanism to explain the "thrifty phenotype" hypothesis has yet to be elucidated

In Pima Indians of the Southwestern United States both low and high birth weight have been associated with risk of diabetes [32]. High birth-weight may reflect maternal diabetes. Maternal diabetes may have similar effects on the fetus as under-nutrition [24].

In summary, type 2 diabetes mellitus is a polygenic disorder resulting from a combination of physiologic defects (primarily beta cell dysfunction and insulin resistance). The exact pathophysiologic mechanism or mechanisms remain an area of controversy. There is a strong genetic component to the development of type 2 diabetes with expression of disease likely mediated by environmental factors.

2.5 – Complications

Type 2 diabetes is associated with characteristic long-term microvascular complications including neuropathy, retinopathy, nephropathy and other, macrovascular complications. Cardiovascular disease is the major cause of mortality in diabetes, and diabetes is the most common cause of end stage renal failure in North America [33]. We assume that children affected with type 2 diabetes will be at risk for the same complications associated with adult onset disease. The rate of complications increases with duration of diabetes. Yokoyama et al. reported a subgroup of Japanese with relatively early-onset type 2 diabetes (<30 years) who experienced severe complications before the age of 35 [34]. In Manitoba, severe complications, blindness and end stage renal disease, have now

occurred in one individual at the age of twenty-three after a 14 year history of diabetes. Sudden death occurred at 25 years of age. The population with youth onset type 2 diabetes is thus at risk for significant long-term morbidity and mortality and presents a major health care concern for the future.

Of particular concern in the First Nation population is an increased overall incidence of renal disease reported in children [35]. Among adults, the overall risk of end-stage renal failure from all causes in the Canadian Aboriginal population nationally is 2.5 to 4 times higher, and the risk of end stage renal disease (ESRD) due to diabetes specifically is at least 3 times higher [36]. Similarly high rates of ESRD have been reported among Saskatchewan aboriginal patients with diabetes compared to non-aboriginal patients [37]. Diabetic complications, and in particular, nephropathy, present a significant threat to the well being of this population. Hyperinsulinemia, a characteristic of type 2 diabetes, is also a risk factor for hypertension, an independent risk factor for nephropathy.

The Diabetes Control and Complications Trial (DCCT) established that a causal relationship exists between hyperglycemia and microvascular disease in type 1 diabetes [38]. The UK Prospective Diabetes Study final report has now supplied this evidence for type 2 diabetes [39, 40]. The Canadian Diabetes Association recommends a fasting blood glucose <6.7 mmol/L and hemoglobin A1c <7% as target values in type 2 diabetes to limit the risk of the development of complications [20].

Diabetic ketoacidosis, an acute complication of diabetes (typically associated with type 1 diabetes) has been described in adults with type 2 diabetes. There has been one report of

DKA in African American children with typical insulin resistant type 2 diabetes [41]. This complication has not been reported in the First Nation population (see chapter 5).

2.6 Treatment

Current treatment strategies for type 2 diabetes focus on lifestyle modification and, in adults, on pharmacologic agents that improve glycemic control. Weight loss and/or weight maintenance, and exercise are both known to decrease insulin resistance. Mixed results were reported in a study by Erikson et al that assessed the effects of dietary advice and exercise on type 2 diabetes [42]. A small, sustained weight loss was achieved but there was no significant difference in the mean two-hour glucose values following OGTT at five-year follow up. A behavioral weight loss program in adult patients with type 2 diabetes has been shown to be less effective in a minority African American population versus a Caucasian population [43]. The reason for this difference is not known.

Unfortunately, modification of diet for health-related problems in children and adolescence is rarely successful [13]. In the Manitoba experience, lifestyle modification within the structured setting of a summer camp, has been successful in short-term improvement of glycemic control in First Nation youth with type 2 diabetes [44]. Within this setting of structured meals and high energy camp activities, normalization of pre-meal blood sugars is achievable within one week. Unfortunately, these efforts have not translated to long-term glycemic control once the children are out of the structured setting and back into the community [17, 44]. However, this experience does demonstrate the potential for adequate control through lifestyle modification.

The use of oral hypoglycemic agents in type 2 diabetes, inadequately controlled by diet, improves mean fasting blood glucose and HbA1c values in adults' [45]. The definitive results of the large community-based United Kingdom Prospective Diabetes Study demonstrated improvements in mean blood glucose and glycated hemoglobin values in subjects with type 2 diabetes allocated to pharmacologic treatment (oral antidiabetic agent or insulin), compared to diet alone [39, 40]. The results indicate that the majority of adults with type 2 diabetes will require drug treatment in order to achieve fasting blood glucose values below 7.8 mmol/L [39, 40, 46].

Insulin therapy and sulphonylurea oral hypoglycemic agents, while of demonstrable benefit in type 2 diabetes in adults, promote weight gain and are associated with hypoglycemic episodes [40]. While there are many years of experience with the use of insulin in the pediatric age group, it is unlikely to be a feasible long term alternative. Insulin therapy is not well tolerated by asymptomatic adolescents with type 2 diabetes for several reasons. These include the unpleasant mode of administration, the risk of hypoglycemia, the potential for abuse, the tendency to promote weight gain, the perception of its use only in end-stage diabetes and the lack of perceived benefit in the absence of symptoms [47].

To date, there have been no published trials to ensure the efficacy and safety of oral antidiabetic agents in the pediatric age group. Insulin remains the only pharmacologic agent licensed for the treatment of diabetes in children in both the United States and in Canada. Despite this lack of evidence, the American Diabetes Association recently issued a consensus statement that states that pharmacologic therapy is warranted in children not

achieving blood glucose control with lifestyle changes alone. They suggest that most children will need pharmacologic therapy in order to achieve adequate control. The statement also suggests adding a second pharmacologic agent if adequate control not achieved in 3-6 months with monotherapy [48].

Table 2.1 Clinical Characteristics of Type 2 Diabetes in First Nation Youth

1. Age > 6 years and usually >9 years
2. Centripetal obesity (weight >120% ideal body weight for height or BMI > 85th percentile for age and gender)
3. No recent weight loss
4. No acute symptoms of hyperglycemia
5. Family history of type 2 diabetes

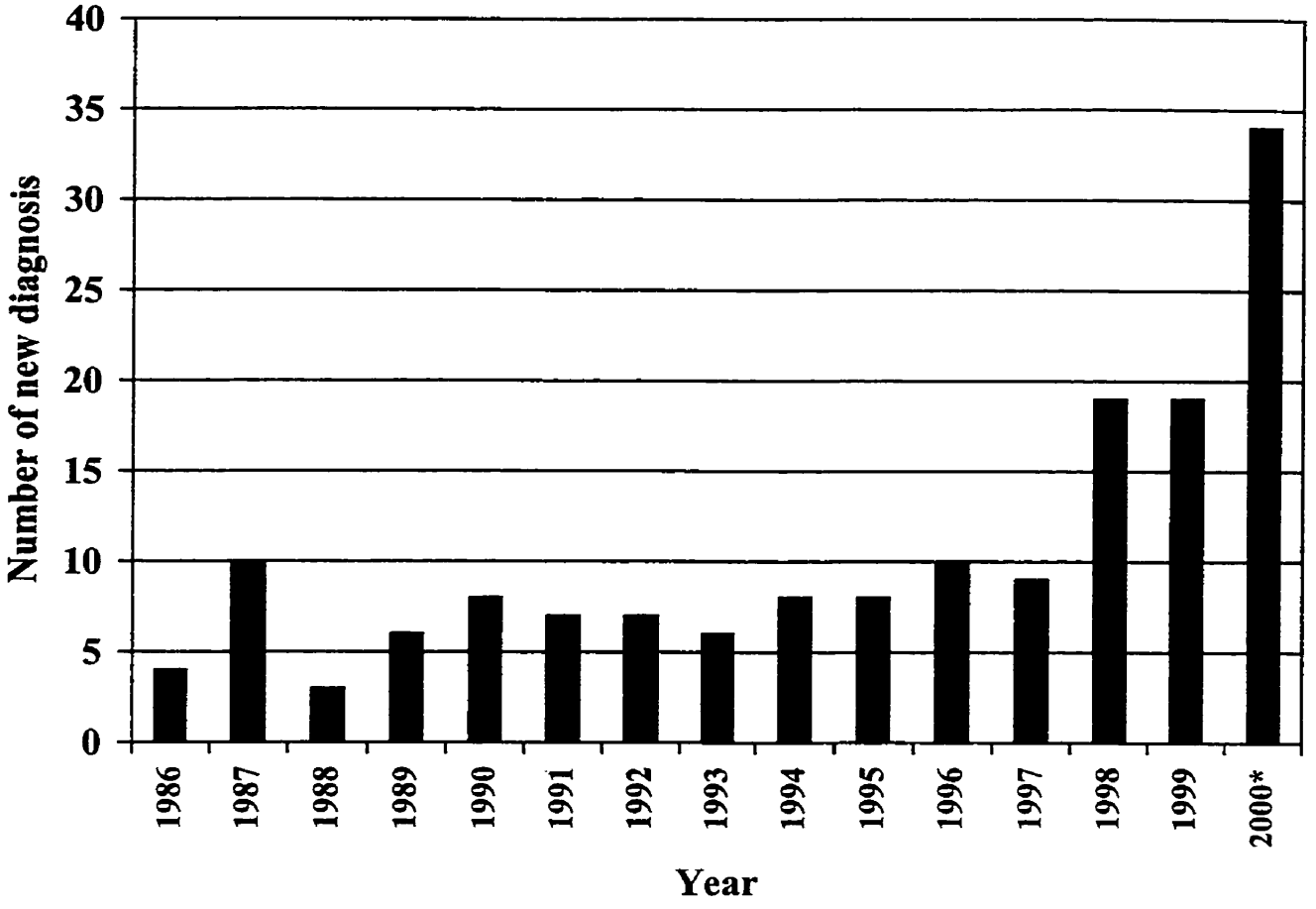
Adapted from Dean HJ., *Diagnostic criteria for non-insulin dependent diabetes in youth (NIDDM-Y)*. Clinical Pediatrics 1998; 37: 67-72.

Table 2.2 The Manitoba/ Northwestern Ontario experience: gender distribution, age and body mass index (BMI) at diagnosis

	N (%)	BMI +/- SD (kg/m^2)	Age +/- SD (years)
Female	104 (73.8)	29.3 +/- 6.5	12.47 +/- 2.43*
Male	37 (26.2)	33.2 +/- 6.5	13.97 +/- 2.22*
Total	141 (100)	30.23 +/- 6.7	12.87 +/- 2.46

* $p = 0.001$

Figure 2.1 New Diagnoses by Year



* until Oct. 1, 2000

Appendix 2.1 Criteria for the Diagnosis of Diabetes Mellitus

Source: Canadian Clinical Practice Guidelines [20]

Symptoms of diabetes plus casual plasma glucose concentration > 11.1 mmol/L. Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydypsia, and unexplained weight loss.

or

Fasting plasma glucose >6.9 mmol/L. Fasting is defined as no caloric intake for at least 8 hours

or

2h post-prandial glucose >11.1 mmol/L during OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

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Chapter 3 Diagnosis
Diabetes-associated autoantibodies in First Nation Youth: a population at risk for type 2 diabetes mellitus.

3.1 Introduction

A fasting blood glucose of >7.0 mmol/L or a two hour post-prandial blood glucose of >11.3 mmol/L are the biochemical criteria required for the diagnosis of diabetes [1]. These criteria do not distinguish between the subtypes of diabetes. Historically, age of onset less than 20 years was used to support the diagnosis of type 1 diabetes. However, it is now clear that type 2 diabetes occurs not infrequently in youth less than 20 years of age [2-8]. Currently, the differentiation between type 1 and type 2 diabetes is based on clinical impression [9]. This can be a difficult distinction to make in childhood and adolescence. The distinction is important because of different education, treatment and prevention strategies.

Typically, youth with type 2 diabetes are members of high-risk populations and have a strong family history of diabetes. Obesity, defined as a body mass index >95th percentile for age and gender, is common. Acanthosis nigricans is also frequently found. Acanthosis nigricans is a dermatologic condition characterized by papillomatosis and hyperkeratosis of the skin. It has distinct clinical features as well as distinct histopathological findings. Histologically, the lesion is characterized by papilloma formation at the dermal-epidermal junction, hyperkeratosis with thickening of the stratum corneum and an increase in melanocyte number [10]. Clinically, it appears as hyperpigmented areas of skin, particularly in flexural and apposed locations (e.g. neck, axilla, knuckle, and groin).

The diagnosis of type 2 diabetes is more firmly established on an individual basis by following the natural history of the disease (e.g. ability to be maintained off insulin therapy). The presence

or absence of episodes of diabetic ketoacidosis has not been found to be helpful as this complication has been observed in both type 1 and type 2 diabetes in children [9].

Antibodies are proteins that are formed in response to an antigen and react specifically with that antigen. They are a fundamental component of the acquired immune response. In the normal state, antigens are substances that are recognized as foreign by the immune system. Antibodies are synthesized by B lymphocytes or their derivatives, plasma cells [11]. Autoimmune diseases are those attributable to a failure to discriminate "self" from "foreign" material and results in an immune response of the host to its own tissues. Autoantibodies are antibodies formed against "self" antigens [12].

Autoimmune destruction of the β-cells of the pancreas is the major cause of type 1 diabetes [1]. Diabetes-associated autoantibodies have been identified in patients with type 1 diabetes mellitus with increased frequency. Autoantibodies to islet cell cytoplasm (islet cell autoantibodies or ICA), insulin (insulin autoantibodies or IAA) and to glutamic acid decarboxylase (glutamic acid decarboxylase autoantibodies or GADA) have been described [13, 14]. In one large, cross-sectional study involving over 3000 school children in Great Britain diabetes-associated autoantibodies were present in 97% of those with type 1 diabetes, 92% had increased levels of two or more markers [13]. This is similar to the findings of the Finnish diabetes study [14]. In contrast, diabetes-associated autoantibodies were absent in 90.6% of the non-diabetic schoolchildren. Less than 1% of non-diabetic school children had elevated levels of two or more autoantibodies [13]. The presence of serum antibodies has also been found to be helpful in the prediction of type 1 diabetes in both populations at risk and in the general population [13].

Diabetes-associated antibody positivity has been studied in type 2 diabetes in adults. In a large study of patients with newly diagnosed type 2 diabetes, 5.8% were positive for islet cell antibodies, 9.8% for glutamic acid decarboxylase antibodies and 3.9% were positive for both. The presence of autoantibodies was predictive of the need for insulin therapy within 6 years. It was most predictive in those under 35 years of age and with a leaner phenotype more suggestive of type 1 diabetes. It is possible that this group do not have type 2 diabetes mellitus but a latent form of autoimmune type 1 [15].

3.2 Hypothesis and Objectives

Objective: to determine whether the absence of diabetes associated autoantibodies could be used to support the diagnosis of type 2 diabetes in aboriginal youth.

We hypothesize that evidence of autoimmunity will be absent in this cohort of youth with type 2 diabetes mellitus (based on clinical criteria). This will differ significantly from the published data on autoimmunity in children with type 1 diabetes.

3.3 Design and Methods

3.3.1 Design

A case-control study

3.3.2 Population

The study population consisted of 20 First Nation children and youth with type 2 diabetes and 40 age and gender matched First Nation controls. The 40 control samples were randomly selected from 717 samples collected as part of a screening study involving aboriginal children from a remote northern Manitoba community [16]. All children (cases and controls) were between 10-

17 years of age, were of aboriginal origin and resided in either Northwestern Ontario or Manitoba, Canada. The affected children clinically had classic type 2 diabetes with obesity, acanthosis nigricans and a positive family history of type 2 diabetes in first or second-degree relatives. All these children had fasting glucose levels of 7.0 mmol/L or higher in accordance with the diagnostic criteria adopted by the Canadian Diabetes Association [1]. All could be maintained off exogenous insulin therapy without acute decompensation.

3.3.3 Procedure

Serum samples for three diabetes associated-autoantibodies - islet cell antibodies (ICA), glutamic acid decarboxylase (GAD) antibodies and insulin autoantibodies (IAA) - were drawn on the 20 children affected with type 2 diabetes and 40 age and gender matched controls. Autoantibodies were assayed in a single laboratory. Positive cut offs for IAA, GAD and ICA were 0.01, 0.032 and 0.071 respectively [17].

3.3.4 Analysis

Data are presented as prevalence *n* (%).

3.4 Results

Of the affected cases, only 1 child was weakly positive for ICA (0.073; 1/20=5%). There were no positives for either GAD or IAA in the affected group. Thus 95% of cases were negative for all three diabetes-associated autoantibodies (CI 0.93 – 0.98). From the control group, there were no positives for any of the three antibodies (ICA, GAD, IAA). Overall, this gives a positivity of ICA of 1.7%, GAD 0% and IAA 0% for the controls and cases combined (Table 3.1). While the control sample is small, the prevalence of diabetes associated autoantibodies does not appear to

differ significantly from previously published control data derived primarily from Caucasian children

3.5 Discussion

Increased levels of diabetes associated antibodies were not found in this group of aboriginal children who clinically have type 2 diabetes mellitus or in their age and gender matched aboriginal controls. This supports the clinical impression of non-autoimmune diabetes in these children and youth. The absence of antibody markers for type 1 diabetes mellitus may provide a useful biochemical tool to aid in the distinction between type 1 and type 2 diabetes mellitus in aboriginal youth in Canada.

Table 3.1. Antibody markers above the 99thcentile in 20 cases and 40 controls

	Present Study		Study of Bingley et al.*	
	Type 2 diabetes (n=20)	**Controls (n=40)**	**Type 1 diabetes (n=256)**	**Controls (n=2855)**
No antibody markers	19 (95)	40 (100)	8 (3)	2587 (90.6)
ICA512 (IA-2)	1 (5)	0	193 (75)	60 (2.1)
GAD antibodies	0	0	190 (74)	63 (2.2)
IAA	0	0	177 (69)	57 (2.0)
Two or more antibodies	0	0	237 (92)	20 (0.7)

Data are prevalence *n* (%)

* Bingley PL, Bonifacio E, Williams AJK, Genovese S, Bottazzo GF, Gale EAM. Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. Diabetes 1997; 46: 1701-1710.

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Chapter 4 Etiology

The Prevalence of HNF-α (G319S) Mutation in First Nation Youth with type 2 diabetes.

4.1 – Introduction

Identification and understanding of the factors involved in the development of type 2 diabetes in the First Nation population may better direct our efforts to develop therapeutic and preventative strategies specific to this population.

Lifestyle changes have been identified as one factor involved in the development of type 2 diabetes. Specifically, an increase in caloric intake coupled with a decrease in physical activity has led to an increase in obesity among the First Nation population [1, 2]. However, obesity is on the rise in childhood throughout North America [3]. If obesity was the sole factor involved in the development of type 2 diabetes we would expect to see substantial numbers of non-native children affected with type 2 diabetes. This does not appear to be the case. Of the 141 children and youth who have been seen at the Winnipeg Children's Hospital between 1986-1999, 138 (98.6%) have been of First Nation origin. This marked predilection for a specific racial group suggests that genetic factors may be involved in the development of type 2 diabetes in the First Nation population of Northwestern Ontario and Manitoba.

Hepatic Nuclear Factor 1-α (HNF-1α) is a transcription factor involved in the transcription of hepatic genes including albumin, 1-antitrysin, apolipoproteins and several clotting factors [4]. It is also expressed in pancreatic β cells [5], kidney and intestine [6]. Heterogenous mutations of the HNF-1α gene have been identified as the cause of

maturity onset diabetes of the young type 3 (MODY 3) [7, 8]. MODY 3 is an autosomal dominant form of type 2 diabetes with a high degree of penetrance resulting primarily from a defect in pancreatic β-cell function. It is associated with early age of diabetes onset, typically less than 25 years of age [4]. These individuals are not obese and lack features suggestive of insulin resistance. Thus they do not have the typical phenotype of classic type 2 diabetes mellitus [8]. More recently, small numbers of individuals with typical insulin resistant type 2 diabetes have been described with mutations of the HNF 1-α gene [9, 10].

HNF-1 α is a transactivator of the insulin 1-gene in the mouse model [4]. In experimental studies involving a HNF-1α knock-out mouse, several possible mechanisms for the development of diabetes have been proposed. Insulin responses to glucose and arginine are reduced suggesting that there is a primary defect in the insulin secretory pathway. The insulin content of the pancreatic beta cells of these mice is also decreased suggesting an effect of HNF-1α on insulin synthesis [4]. Furthermore, the beta cell mass of these mice did not increase appropriately to compensate for hyperglycemia. Diabetes in the HNF-1α(-/-) mice likely results from several factors including a insulin secretory defect, decreased beta cell mass and decreased insulin synthesis [4].

The human insulin gene has been proposed as a target gene of HNF-1α. In vitro, mutations in the HNF-1α gene result in abnormal transcription of the insulin gene and consequent reduction in the insulin content of the pancreatic β-cells. Thus, decreased insulin production may play a role in the pathogenesis of diabetes in humans with HNF-1 α mutations [5].

Recently, a previously undescribed mutation in the HNF-1 α gene has been identified in the Oji-Cree of Sandy Lake, Ontario. This population has one of the highest prevalence of type 2 diabetes in the world [11]. The diabetic phenotype in this population is of typical, insulin resistant type 2 diabetes. Dr. Hegele and his group identified a substitution of serine for glycine at codon 319 in the HNF-1α gene (HNF-1α G319S)[10]. Heterozygotes for the mutation are thus identified as 'S319/G319'and homozygotes as 'S319/S319'. 'G319/G319' is used to describe the wild type or those homozygous for the absence of the mutation. Almost 40% of the Sandy Lake population with type 2 diabetes were either hetero- or homozygous for this mutation. This was significantly higher than the frequency in non-diabetic members of this population. Of the population with diabetes, those with the identified mutation were characterized by a lower BMI [10] and, in women, a younger age of onset [12]. Women with the mutation had the onset of diabetes in the third and fourth decade of life compared to the fifth decade in those without the mutation.

The G319S mutation has also been reported in young people with diabetes from the Sandy Lake community (aged 10-25 years). In this small study (n=16) the frequency of the mutant allele was 0.34[13].

In subjects without diabetes, lower insulin levels were associated with the G319S mutation. This suggests that an insulin secretory defect may play an important role in the development of diabetes in those with this mutation [10]. No difference was found in the insulin levels after developing diabetes. However, a hyperglycemic milieu can directly

alter insulin secretion. An understanding of the pathophysiology is important when choosing a therapeutic intervention for type 2 diabetes in those with the mutant allele.

A strong association between diabetes and HNF-1α G319S was identified in this population. However, linkage analysis failed to identify HNF 1α as a determinant of diabetes. It is possible that this reflects the heterogeneity of type 2 diabetes, even within an isolated population [14]

The G319S mutation has not been found in the Pima Indians who have a prevalence of type 2 diabetes in youth similar to the Island Lake and Sandy Lake regions of Manitoba and Northwestern Ontario [15]. The presence or absence of this mutation in other Canadian aboriginal populations has been a question raised at both the Expert Committee of the Diabetes Section of the Center for Disease Control (Atlanta, Georgia) and the Diabetes Interagency Coordinating Committee of the National Institute of Health (Bethesda, Maryland).

This study may have direct application to the individual and/or the community. It may allow us to better direct our efforts to develop therapeutic and preventative strategies for type 2 diabetes in this population. The knowledge gained through this study may impact on recommendations for public health screening of populations at risk.

4.2 Study Objectives

Primary objective: to investigate the prevalence of the HNF 1-α (G319S) mutation in a population of youth of First Nation origin with type 2 diabetes mellitus from Manitoba

and Northwestern Ontario and to compare this prevalence to a population of First Nation people without diabetes.

Secondary objective: to investigate the relationship between clinical and historical characteristics and the presence or absence of the HNF 1-α (G319S) mutation. Characteristics to be examined include BMI, maternal diabetes during gestation, acanthosis nigricans, age at diagnosis and family history of diabetes.

4.3 Methods

4.3.1 Study Design

The population of children and youth seen at the Diabetes Education and Resource for Children and Adolescents (DER-CA) with type 2 diabetes was screened for the HNF-1α G319S mutation. Recruitment occurred between March and August 2000. Participation in this study did not require an additional visit. Blood for genetic analysis (approximately 5 cc's) was drawn when blood work for clinical care purposes was done. The project coordinator (EACS) obtained informed consent for participation in this study. Clinical and historical data was gathered on each participant via chart review (age at diagnosis, current BMI, current HbA1c, family history of diabetes, history of maternal diabetes, community of origin, current acanthosis nigricans and mode of presentation of diabetes).

The results of another study were used as control data for this study. This study investigated vitamin D receptor (VDR) polymorphisms and serum vitamin D levels in 109 pregnant women of First Nation origin. 41of the participants were from Norway

House, and 68 from the Island Lake communities of St. Theresa Point and Garden Hill. Participants were recruited at their initial prenatal visit in a consecutive manner until the required sample size was achieved. There was no known history of diabetes in this group though diabetes was not formally ruled out.[1].

4.3.2 Population

All patients with type 2 diabetes mellitus of self-declared First Nation origin seen at the DER-CA were offered participation in this study.

4.3.3 Procedure

Informed consent was obtained from potential participants by the project coordinator (EACS) who did not have a clinical relationship with this population. Once informed consent was obtained a sample of blood (approximately 5 cc's) was drawn.

Preparation of cells for DNA extraction

Whole blood was collected in EDTA vacutainer tubes. For each 1 cc of blood collected, the volume was made up to 5 cc using NH4Cl:Tris. The sample was then incubated at 37 C for 10 minutes and centrifuged at 1000 RCF for ten minutes (room temperature). The supernatant was than discarded. The remaining pellet was washed with 5 cc of saline (37°C) and centrifuged at 1000 RCF for ten minutes (room temperature). The supernatant was then discarded. This procedure was repeated once. Following the washings, the cells

[1].Fischer A., Greenberg C. and Taback, S (unpublished data)

were re-suspended using 0.3 ml of high TE for each 1 ml of whole blood used. The cells were then lysed by adding an equal volume of lysis mixture.

DNA Extraction

Equal volumes of TE saturated phenol and the lysed cell mixture were mixed for 10 minutes and then centrifuged at 2000 RCF for 5-10 minutes to separate the phases. The aqueous phase was then removed and transferred to a clean centrifuge tube. A half volume of High TE was added and re-extracted with an equal volume of TE saturated phenol. The aqueous layer was then extracted with an equal volume of chloroform:isoamyl acohol.

DNA Precipitation

The volume of the aqueous phase was measured and 1/50 volume of 5 M NaCl added. An equal volume of 100% ethanol was then added to precipitate the DNA. This caused clumping of the DNA filaments. The solution was poured off and 100% ethanol added to give a final concentration of 70%. At this point any remaining solution was poured off and 2 ml of 100% ethanol added to condense the DNA pellet. The DNA pellet was then transferred to a sterile Eppendorf tube and allowed to air dry (≈10 minutes). Once dry, the pellet was re-suspended overnight in 0.3 ml Low TE at 4C.

Polymerase Chain Reaction (PCR) Amplification

PCR Conditions: 10 mM Tris (pH 8.3), 50 mM KCL, 1 mM MgCl, 0.01% gelatin, 200 µM NTPs, 300nM forward primer, 300 nM reverse primer, 1,25 units Taq, 1 µl DNA (not quantitiated), 50 µl total volume.

PCR Program:

95°C 3 minutes
95°C 1 minute
60°C 1 minute
72°C 2 minutes
} repeat for a total of 35 cycles
72°C 8 minutes
4°C

Restriction Enzyme Digestion

20 µl of PCR reaction was removed to a fresh tube. 5 units of *Bse*DI (Fermentas) was then added to the PCR reaction and incubated at 55°C for 4 hours. 6 volumes of loading dye was added and the samples were separated on 10% PAGE gels in 1X TBE running buffer and visualized by staining with ethidium bromide. The expected banding pattern of the PCR product after digestion with *Bse*DI is:

G319/G319 (wild type): 189, **82,39**,30,25,14,12,6 base pairs
S319S/S319: 189, **121**,30,25,14,12,6 base pairs
G319/S319 189,**121,82,39**,30,25,14,12,6 base pairs

4.4 Sample size and Statistical Analysis

At the time of study conception (January 1, 2000) there were 55 active patients with type 2 diabetes being followed at the DER-CA of self declared aboriginal origin. A sample size of 40 - 50 was chosen for this study. This is a sample size of convenience given the

small population available and the unknown prevalence of the mutation. Much of the data collected was analyzed with descriptive statistics. Between group differences in genotype and allele frequencies were compared using the chi-square test for proportions employing Yates correction. 95% confidence intervals were also calculated. The student t-test was used to test for differences between means for normally distributed continuous variables. (BMI, Hba1c, age at diagnosis). Fisher's exact test for proportions was used to compare distribution of acanthosis nigricans and mode of presentation.

4.5 Ethical considerations

Involvement in this study took place only after informed written consent was obtained. This study did not require an additional venipuncture to be performed and there were therefore no identified additional risks involved. The additional 5 cc of blood needed for this study is not thought to be of clinical significance to the individual.

The results of this study will be held in the strictest confidence. Any publications resulting will bear no individual identifying features. DNA extracted for this study will be dealt with according to the wishes of the individual (see appendix 4.1). Composite results will be communicated to the study participants.

4.6 Results

Frequency of the G319S allele / Genotype Frequency (Table 4.1)

A total of 40 individuals participated in this study. One individual approached refused entry. Twenty-three mutant alleles (of 80) were found giving an allele frequency of 0.29

(95% CI 0.2-0.38). This is significantly different from the allele frequency of 0.13 in the control population (χ^2=8.65, p=0.003). The allele frequency in published data from the Sandy Lake First Nation in Northwestern Ontario (in whom the G319S mutation was first identified) is 0.087 and 0.209 in the non-diabetic and diabetic populations respectively. Among our study participants a frequency of 0.15 was found for the S319/S319 genotype, higher than has previously been reported.

Regional Origin

17 participants in this study had at least one copy of the mutant allele. 14 of the 40 participants were from the Island Lake communities of Garden Hill, Ste. Theresa Point and Wasagamak. Of these 14, 11 (78.5%) had the mutant allele. 6/11 were homozygous for the mutant allele (all homozygotes were from the Island Lake communities). Therefore the frequency of the mutant allele in youth from the Island Lake region was 0.61 and the frequency of the SS genotype in participants from this region was 0.49 (6/14). Of the 5 other individuals with the mutant allele, 3 were from Northwestern Ontario (Sandy Lake, Cat Lake, Bearskin Lake) 1 from Shamattawa and one from Norway House (see appendix 4.2 and 4.3).

Clinical and Historical Characteristics (Tables 4.2 and 4.3)

The age of diagnosis of diabetes was significantly older in those with the mutant allele compared those without (14.7 ± 2.1 years and 13.0 ± 2.1 years respectively; p = 0.02). BMI (at study entry) was significantly lower in those with the mutation compared to those without (mean BMI with mutation 25.78 ± 5.3 kg/m^2, without mutation 32.14 ±

6.55 kg / m^2 p= 0.002; mean age did not differ significantly at study entry). Mean HbA1c (an indicator of diabetes control) also differed between the groups significantly. The mean HbA1c in the group with the mutant allele was 10.38 ±3.50, in those without 7.80 ± 2.20 (p = 0.01). Acanthosis nigricans, a marker of insulin resistance, was more frequently found among those with the wild type allele compared to those either heterozygous or homozygous for the S319 mutation (87 vs 59%, p = 0.067 non-significant). This meets statistical significance when comparing the wild type to the homozygous mutant (S319/S319)(87% vs 33%, p = 0.02).

For each of these characteristics (mean of diagnosis, mean HbA1c, mean BMI, and acanthosis nigricans), the heterozygous genotype (S319/G319) was intermediate between the wild type (G319/G319) and homozygous mutant (S319/S319) genotypes (table 4.3).

A history of maternal diabetes during pregnancy was found in 50% of both those with the mutation and those without. A positive family history of diabetes (first degree relative) was also similar in the two groups; 14/17 (82.4%) and 17/22 (77.3%) for those with and those without the mutation respectively. (Note that for family characteristics n = 22 for the group without the mutation as one individual was adopted and this information was not available).

Classical symptoms of hyperglycemia were significantly more frequent in those with the wild type allele (10/22, 45.5%, data unavailable on one participant). Only one individual with the mutation had symptoms of hyperglycemia at presentation (p = 0.01)

4.7 Discussion

Allele and Genotype frequency

The increased frequency of the G319S allele found in this study supports the positive association proposed by Hegele between this mutation and type 2 diabetes [10].

The Sandy Lake report involved participants with a mean age of onset of diabetes in the 40's. Our study involved a younger group of subjects, all in the pediatric years (age ≤ 18 years). Of note, homozygote S319/S319 individuals in the Sandy Lake study had the youngest age of diagnosis of diabetes (compared to G319/G319 and G319/S319 individuals). The Sandy Lake study included a small number (n = 16) of individuals with diabetes between 10-25 years of age [13]. The allele frequency within this group was 0.34, similar to 0.29 found in this study. This suggests that the mutant allele is associated not only with type 2 diabetes but also with onset of the disease at a younger age.

In our study, the mutant allele was found most frequently in participants from the Island Lake communities (frequency = 0.61). These are Ojee-Cree communities with close links (geographic, cultural and linguistic) to the community of Sandy Lake, Ontario. It is likely that these communities share a significant common genetic heritage.

Clinical and Historical Characteristics

The mean age of diagnosis of diabetes was older in the group with the mutant allele compared to the wild type. We do not know if the age of onset of diabetes differs similarly between these two groups. This will only be answered with regular, community based screening for diabetes within this population. A screening program will provide

more accurate data on the age of onset of diabetes, while, at the present, we are only able to determine the age of diagnosis of the disorder. The sub-population with the mutant allele had a lower frequency of acanthosis and lower mean BMI, both indicators of insulin resistance. It is possible that individuals who are asymptomatic, non-obese and lack acanthosis nigricans are less likely to be screened for diabetes when in contact with the health care system. This may result in a delay in diagnosis.

Participants with the wild type allele (G319/G319) were more likely to have symptoms of hyperglycemia at presentation (45.5% vs. 5.9%). We do not have an explanation for this difference beyond an artifact of relatively small numbers. The lack of symptomatology may, however, be a factor contributing to the older age of diagnosis seen in the group with the mutant allele.

The lower average BMI and decreased frequency of acanthosis nigricans suggests that insulin resistance is not the major pathologic factor in the development of diabetes in the sub-population with the G319S mutation. In the Sandy Lake study, the non-diabetic population with the G319S mutation had significantly lower insulin levels than the non-diabetic population with the wild type allele [10]. This, coupled with our data, suggests that an insulin production and /or secretion defect may play an important role in the pathogenesis of diabetes in those with the G319S allele. Evidence of decreased production and secretion of insulin in the HNF-1α (-/-) knockout mouse lends support to this proposed mechanism [4]. This has potentially important clinical/treatment implications. Insulin and/or pharmacologic agents that promote insulin secretion maybe more appropriate than sensitizing agents in this sub-population.

Diabetes control was significantly poorer in those with the mutant allele as evidenced by a higher average HbA1c in this group. This finding requires further investigation, but may also reflect a difference in the pathophysiology of diabetes in those with the G319S mutation. It is possible that lifestyle interventions (exercise, weight control) aimed at improved insulin sensitivity are not adequate in this population and that a more aggressive use of insulin or pharmacologic agents that promote insulin secretion would be of benefit.

Using cross-sectional data, the S319 HNF 1-α allele has a specificity of 97.0% (91.2-99.9) and a positive predictive value of 93.8% (88.5-99.1%) for the detection of diabetes in the Sandy Lake population over 50 years of age [16]. 68 individuals in our control population were from the Island Lake communities. A high frequency of the S319 allele was found in this population (0.19). These were all young, pregnant women (<50 years of age) without any known history of diabetes. This group represents the ideal population to follow prospectively to determine if the S319 allele can be used to identify individuals who are at very high risk of developing diabetes by the age of 50. Time to disease onset could also be determined. Genotype testing has the potential to identify those at high risk at a young age, allowing for the implementation of an intervention program to prevent or delay the onset of diabetes. Ideally, any intervention should be evaluated within the framework of a randomized controlled trial.

4.8 Summary

This data suggests that a population based screening program within the Island Lake communities for the presence of the G319S allele may identify a subgroup of the

population at particular risk for the development of diabetes. This group may not be as readily identifiable as 'at risk for diabetes' as they lack clinical characteristics associated with insulin resistance (obesity and acanthosis nigricans). The lack of clinical characteristics of insulin resistance suggests that an insulin secretory and/or production defect plays an important role in the development of diabetes in this group. The pathophysiology of diabetes in this population needs further investigation in order to develop and implement appropriate treatment strategies.

Table 4.1 Genotype and Allele Frequencies

Genotype	Study Participants (n=40)	Controls (n=109)	Controls Island Lake (n=68)	Sandy Lake– non-diabetic (n=334)*	Sandy Lake– Diabetic (n=117)*	Sandy Lake– Diabetic <25 years (n=16)**
G319/G319	0.575	0.771	0.680	0.829	0.624	0.437
G319/S319	0.275	0.193	0.260	0.168	0.333	0.437
S319/S319	0.150	0.037	0.059	0.003	0.043	0.125
Allele						
S319	0.288	0.133	0.188	0.087	0.209	0.343

* [10]
**[13]

Table 4.2 Clinical Characteristics by Genotype

Genotype	BMI (kg/m^2)	Acanthosis Nigricans (%)	Mean age at diagnosis (years)	HbA1c (%)
GG (G319/G319)	32.1 (29.5-34.8)	87	13.0 (12.2-13.9)	7.80 (6.8-8.7)
GS (G319/S319)	28.1 (25.4-30.7)	72.7	14.0 (12.7-15.2)	9.94 (6.4-13.5)
SS (S319/S319)	21.6 (17.32-25.92)	33.3	16.0 (14.7-17.2)	11.0 (7.3-14.7)

Table 4.3 Clinical Characteristics by Presence or Absence of Mutant Allele

	Mutant Allele (G319/S319 or S319/S319)	Wild Type (G319/G319)	
Age at Diagnosis (years)	14.7 (13.7-15.7)	13.0 (12.2-13.9)	p=0.02
Body Mass Index	25.8 (23.3-28.3)	32.1 (29.5-34.8)	p=0.002
HbA1c	10.4 (8.6-12.2)	7.8 (6.8-8.7)	p=0.002
Presence of Acanthosis Nigricans (%)	59 %	87%	NS

Appendix 4.1 Consent Form

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: The Prevalence of the HNF-1α (G319S) Mutation in First Nation Youth with Type 2 Diabetes

Protocol number: IE00:11

Principal Investigator: Dr. Elizabeth Sellers, Rm 512E Buhler Centre, 725 McDermot Street, Winnipeg,. MB 204-789-3564

Co-Investigator: Dr. Heather Dean, Rm 325 Community Services Building, 685 William Street, Winnipeg, MB 204-787-3011

Sponsor: The Networks of Centers of Excellence: Canadian Genetic Diseases Network, 351-2125 East Mall (UBC), Vancouver, BC.

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

Purpose of Study
This Clinical Trial is being conducted to study possible genetic factors involved in type 2 diabetes in youth. You are being asked to take part in this study because you have type 2 diabetes. A total of 50 participants will participate in this study

The purpose of this study is to look for a mutation ("a change") in the DNA (the building blocks of our cells) that has been identified in a population of Canadian aboriginal people with diabetes. It is possible that this change may predispose, or make a person more susceptible to diabetes. This mutation was more common in younger people with

diabetes. We would like to look for this mutation in the children with type 2 diabetes seen at the Winnipeg Children's Hospital. If it is more common in our population with diabetes it may help us identify those people at high risk for developing diabetes. It may also help us to better select treatment choices for those who already have diabetes.

Study procedures.
If you take part in this study, you will have the following tests and procedures:
An additional sample of blood (about 1 tablespoon) will be taken at the same time as other blood work that is part of your routine diabetes care. Participation in this study will not require extra clinic visits.

Participation in the study will be completed at one, regularly scheduled clinic visit

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff and your regular doctor first.

Risks, Discomforts, and Benefits
Participation in this study does not require any additional testing. Therefor, we do not anticipate any additional risks or discomforts. There may or may not be direct medical benefit to you from participating in this study. We hope the information learned from this study will benefit other participants with type 2 diabetes in the future.

Costs and Payment for participation
All clinic and professional fees, diagnostic and laboratory tests that will be performed as part of this study are provided at no cost to you. You will receive no payment or reimbursement for any expenses related to taking part in this study.

Confidentiality
Information gathered in this research study may be published or presented in public forums, however your name will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include the University of Manitoba Faculty of Medicine Research Ethics Board.

Voluntary Participation/Withdrawal From the Study
Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision to not participate or to withdraw from the study will not effect your other medical care at this site. If your study doctor feels that it is in your best interest to withdraw you from the study, your study doctor will remove you without your consent.

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

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact the study doctor and the study staff: Dr. E. Sellers 204-789-3564 or Dr. HJ Dean 204-787-3011

For questions about your rights as a research participant, you may contact The University of Manitoba Faculty of Medicine Research Ethics Board at (204) 789-3389

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

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Sellers or Dr. Dean and or his/her study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my medical records by The University of Manitoba Faculty of Medicine Research Ethics Board.

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

CONSENT –Sign only one of the sections below.

OPTION A

I am only interested in determining if I/my child has the G319S mutation of HNF 1-α. I DO NOT want any further testing done. After this testing the blood sample and DNA obtained from it will be destroyed.

Parent/legal guardian's signature_____ Date _____

Parent/legal guardian's printed name: _____

Child's signature _____Date _____

Child's printed name: _____

OPTION B

Option B means that the blood sample and any DNA obtained from me/my child may be saved and may be tested in the future for other genetic factors thought to contribute to type 2 diabetes. Samples will be stored without my/my child's identity. Samples will be stored indefinitely. If I chose to withdraw from this study the sample will be destroyed. For the following statements please indicate your choice by circling Yes or No.

Specifically:

1. I wish to be re-contacted regarding the results of any new tests that are performed on my DNA in the future Yes No

2. Samples may be used in this laboratory or sent to other laboratories for research on type 2 diabetes or other genetic conditions after all the identifying information has been removed.
   Yes No

3. Prior to my death, members of my family are allowed access to my stored DNA only if I give my written permission Yes No

4. My first degree relatives will be allowed access to my stored DNA after my death
   Yes No

Parent/legal guardian's signature _____Date _____

Parent/legal guardian's printed name: _____

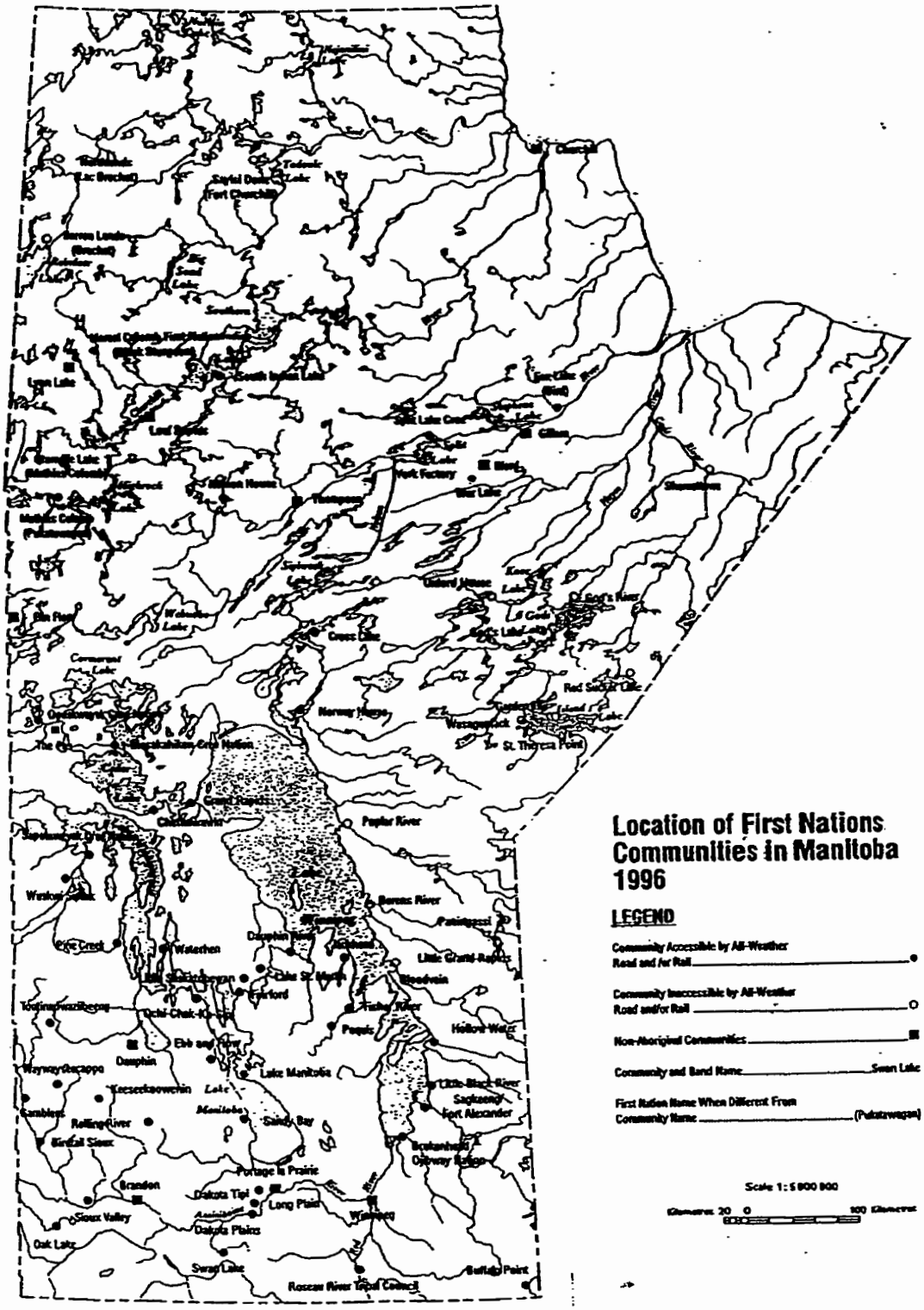
Child's signature _____Date _____

Child's printed name: _____

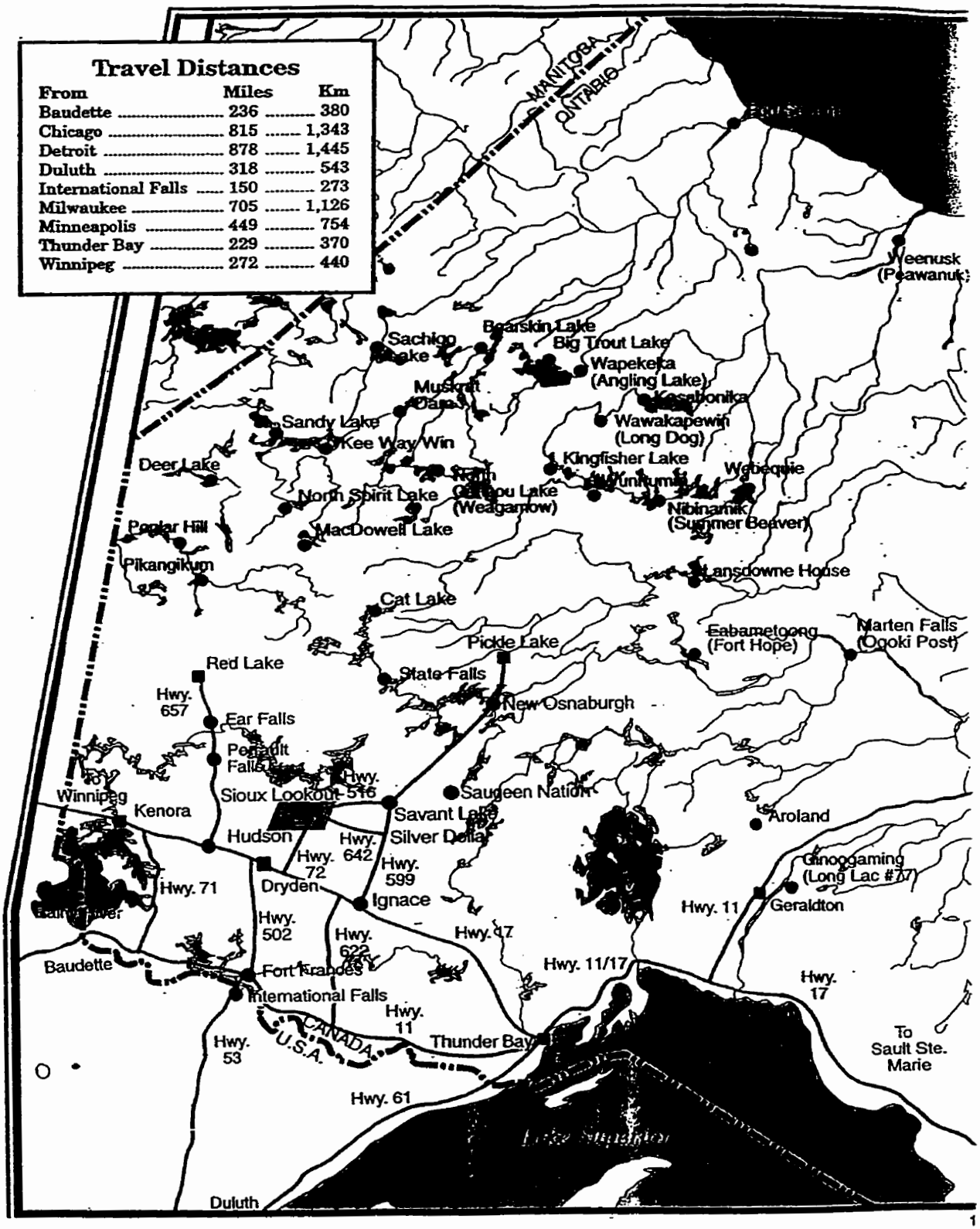
Investigator's signature _____ Date _____

Investigator's printed name _____

Appendix 4.2 Map of Manitoba



Appendix 4.3 Map of Northwestern Ontario



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Chapter 5 Complications

Diabetic ketoacidosis: a complication of type 2 diabetes in First Nation Youth

5.1 – Introduction

Diabetic ketoacidosis (DKA) is characterized by the biochemical features of hyperketonaemia, metabolic acidosis and hyperglycemia [1]. It is classically considered an acute complication of type 1 diabetes mellitus and can cause severe morbidity and mortality from volume depletion and cerebral edema if not recognized and treated in a judicious manner. DKA is precipitated by an absolute or relative lack of insulin in combination with an increase in the catabolic hormones, which leads to an increased production of ketone bodies and glucose by the liver [1].

There have been several recent reports of DKA in adults with type 2 diabetes [2-6]. Pinhas-Hamiel and colleagues in Cincinnati have reported its occurrence among obese African-American youth with typical insulin-resistant type 2 diabetes [7]. There are also several reports describing the increasing problem of type 2 diabetes in youth that include mention of DKA at diagnosis in some of these youth. These reports do not however, define DKA and/or include pH in their criteria for DKA [8-10]. We present our experience with DKA in Canadian Aboriginal children and youth with type 2 diabetes mellitus.

5.2 – Objectives

To determine the prevalence of diabetic ketoacidosis in children and youth with type 2 diabetes mellitus seen at the Winnipeg Children's Hospital for the period 1986-1999 inclusive.

5.3 - Research Design and Methods

5.3.1 – Study Design

A retrospective chart review was performed for all individuals diagnosed with type 2 diabetes at the Winnipeg Children's Hospital, Winnipeg, Manitoba, Canada during the fourteen year period between January 1986 and December 1999 inclusive. It is possible that mild cases of DKA were treated in peripheral hospitals and not referred to the Children's Hospital. Thus, this report generates a minimum prevalence for DKA in youth with type 2 diabetes in these regions.

5.3.2 – Population

All patients were 18 years of age or less, had the diagnosis of type 2 diabetes established by a single pediatric endocrinologist and resided in Manitoba or Northwestern Ontario. These regions are serviced by a single tertiary care pediatric center (Winnipeg Children's Hospital, Winnipeg, Manitoba, Canada).

5.3.3 – Procedure

Each chart was searched for the presence of an episode of diabetic ketoacidosis. In all individuals, diabetes was diagnosed according to the guidelines of the Canadian Diabetes Association [10]. Type 2 diabetes was diagnosed in individuals who were able to be maintained without exogenous insulin for periods of time greater than six months and

who had clinical features consistent with the diagnosis of type 2 diabetes. These included a positive family history, obesity, acanthosis nigricans, and absence of any medication or underlying illness that might predispose to secondary diabetes.

Diabetic ketoacidosis was defined as a pH ≤7.35 and a HCO3 ≤15 meq/L in the presence of hyperglycemia. Total glycosylated hemoglobin was measured by an affinity chromatography method (Isolab) from 1986-1996 and by the Abbott Imx Analyzer from 1996 onwards. Results are reported as a calculated HbA1c (normal range 4.4 –6.4%).

5.3.4. Analysis

Descriptive analysis was undertaken. Data are presented are percentages of the population with type 2 diabetes and as means +/- 2 standard deviations.

5.4 Results

During the period 1986-1999, 120 children and youth age 6-18 years have been seen at the DER-CA with type 2 diabetes. 118/120 were children of self declared aboriginal origin (98%) and 90/120 were female (75%). Of the 120 youth with type 2 diabetes, 13 had episodes of DKA (10.8%). All 13 episodes of DKA were in children of aboriginal origin. 5/13 (38.5 %) occurred at diagnosis of type 2 diabetes. A female predominance was seen (11/13, 84.6%). Mean age at DKA was 14.2 +/- 1.8 years. As a group, the subjects were obese with a mean BMI of 28.8 (+/-5.0 kg/m^2).

Obvious precipitants for the episode of DKA were found in three individuals (pneumonia, gonoccoccal septicemia, and a severe culture-negative systemic illness

resembling sepsis). One young woman was pregnant at the time of DKA and subsequently had a spontaneous miscarriage. Glycemic control at the time of DKA was uniformly poor in all patients in whom a HbA1c was available (mean HbA1c 13.9+/-2.6%). 5/13 (38.5%) individuals (all female) had a second documented episode of DKA.

A positive family history of type 2 diabetes was found in 100 % of this case series. 11/13 patients had an affected first-degree relative; all 13 subjects had many affected second-degree relatives.

Details of their clinical presentation are shown in table 5.1.

5.5 – Discussion

Type 2 diabetes is increasing in the youth and children of Canada's Aboriginal People [11, 12]. There is evidence that the prevalence, and not just the detection of this problem, is increasing [13]. In our institute, The majority of cases of type 2 diabetes in youth seen at the DER-CA are of aboriginal origin. The diagnosis of type 1 diabetes is very rare in this group and occurs in young children of mixed ancestry.

The distinction between type 1 and type 2 diabetes can be difficult in the pediatric population particularly when DKA is the presenting feature. However, this distinction is important because of differing education and long term treatment strategies. The implications for family members for the risk of diabetes also differ. Those young people with type 2 diabetes may well be able to discontinue insulin once stabilized and therefore not have to contend with injections and the side effects of insulin in the long term (e.g. weight gain, hypoglycemia).

DKA has been previously reported in type 2 diabetes, predominantly in adults [2-6]. Many of the previous reports demonstrate a predominance of males [4-6, 14]. A lower prevalence of obesity has been noted in some reports [2, 4, 5]. In our population females predominate (reflecting the predominance of females with the diagnosis of type 2 diabetes in aboriginal youth) and the majority of BMI's were in the obese range (>85th%ile for age and gender).

Pinhas-Hamiel et al., have reported the occurrence of DKA among obese African American youth with type 2 diabetes [7]. Our population is also obese and had their episode of DKA at a mean of 14.0 years, similar to the African American adolescents. The sex distribution in our population is more skewed with a greater female predominance. Four older adolescents (aged 15-17 years) with type 2 diabetes presenting in DKA have been reported from one study in Japan, all of these were obese males with a history of exceptionally large intakes of sugared drinks [14].

A precipitating illness was found in the minority (3/13) of our population contrary to other reports [3, 4, 15]. This is similar to the population reported by Pinhas-Hamiel et al., who found an acute illness in only 4/12 episodes of DKA in their series [7]. Glycemic control in our population was uniformly poor (mean HbA1c=13.79+/- 2.6%) and likely is a contributing factor to DKA.

Five of the subjects in this report had at least one documented repeat episode of DKA. Despite this we remain confident that they have type 2 diabetes on the basis of clinical criteria and the significant periods of time (greater than six months) without insulin

therapy without weight loss, symptoms of hyperglycemia or acute metabolic decompensation. Continued poor long-term glycemic control was the factor common to all these cases.

The occurrence of DKA in type 2 diabetes in aboriginal youth emphasizes the importance of screening youth at risk for diabetes (e.g. aboriginal origin, positive family history, obese). 38.5% of patients in this report had DKA at presentation of their diabetes. Screening at risk populations may prevent presentation of individuals in DKA and thus prevent a potentially fatal complication of diabetes. Screening will also provide for earlier diagnosis allowing introduction of education and treatment at an earlier stage and potentially decrease the chronic complications of diabetes.

In summary, diabetic ketoacidosis occurs in aboriginal children and youth with type 2 diabetes and represents a potentially life threatening complication of this disorder. DKA may occur at the presentation of the disease or during the disease course. Thus, the presence of an episode of DKA can not be used to support the diagnosis of type 1 diabetes in this population or alternatively, used as evidence against the diagnosis of type 2 diabetes.

Table 5.1 Clinical Presentation of Diabetic Ketoacidosis

Pt.#	Sex	Age at Dx (yrs)	Age at DKA (yrs)	BMI * (%ile) at DKA	pH	Serum HCO_3 (mmol/L)	BHOB§ (normal 0.0-0.3 mmol/L)	HbA1c (mmol)	HbA1c (%)
1.	F	9	12	24 (>95th)	7.35	13.0	1.9	27.0	12.6-15.3†
2.	F	12	14	28 (>95th)	6.90	N/A	N/A	32.0	10.9-12.2†
3.	F	11	14	23 (>75th)	7.10	N/A	N/A	22.2	16.2
4.	F	16	16	36 (>95th)	7.02	N/A	N/A	N/A	N/A
5.	F	14	17	35 (>95th)	7.25	7.7	6.1	22.5	14.6
6.	F	15	15	35 (>95th)	7.10	15.0	6.2	32.6	9.5
7.	F	11	13	22 (>75th)	7.00	2.7	15.0	31.0	14.3
8.	F	13	15	28 (>95th)	7.00	1.0	8.0	16.8	18.6
9.	F	15	15	27 (>95th)	7.31	14.7	6.9	28.8	N/A
10.	F	12	12	29 (>95th)	7.17	3.0	8.3	37.7	N/A
11.	M	12	12	26 (>95th)	7.28	9.9	8.9	58.2	13.8
12.	M	13	13	34 (>95th)	7.09	5.8	5.4	54.9	N/A
13.	F	10	17	27 (>90th)	7.22	7.8	8.8	24.1	12.7

§ Betahydroxybutarate
* BMI percentiles reference [16]
† HbA1c unavailable at time of DKA, values obtained in the six months prior to episode of DKA
Cases 1-3 1986-1990
Cases 4-9 1991-1995
Cases 10-13 1996-1999

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Chapter 6 Treatment

A Pharmacologic Intervention Trial for the Treatment of Type 2 Diabetes in First Nation Youth (research in progress)

6.1 Introduction

Type 2 diabetes has emerged as a significant cause of morbidity and mortality among Aboriginal people in Canada. It is now being diagnosed with increased frequency among children and adolescents, especially in the Ojibwa-Cree population in Manitoba and Northwestern Ontario. Available data indicate that long term complications of diabetes are becoming a major health concern for this population. There is evidence that early intervention and adequate glycemic control may slow or prevent the progression of complications. The impact of this disease is tremendous, to the individual, the community, and our health care system. A therapeutic intervention is essential in order to improve the long-term health of this population.

The development of effective interventions is urgently needed. A search of PUBMED did not reveal any randomized controlled trial of an oral antidiabetic agent for the treatment of type 2 diabetes in the pediatric age group (<18 years of age) (for strategy see appendix 6 .1). In this context of an advancing health care crisis, we propose a pharmacologic intervention trial for the treatment of type 2 diabetes in First Nation youth. We have chosen metformin (Glucophage ®) as the study drug.

6.2 Metformin: an oral antihyperglycemic agent

Introduction

Metformin, a di-substituted biguanide, was introduced into clinical practice in 1957 as an oral antihyperglylcemic agent [1]. It is structurally related to guanidine, the active ingredient found in the French Liliac (Galega officinalis) which has been used as a traditional remedy for diabetes since the middle ages (Fig.6.1–structure of metformin). The biquanide class of drugs was initially synthesized in the 1920's but their clinical potential was not pursued at the time, likely as insulin was becoming more widely available at the time [2].

Two other biguanides have been previously marketed as antihypergylcemic agents, phenformin and buformin. These were both withdrawn from the market in the late 1970s because of a significant risk of associated lactic acidosis. Metformin differs in both its chemical structure and pharmacologic profile from these agents.

There are three major clinical differences between metformin and the sulphonylureas, the other major class of oral agents used to treat diabetes for which there is long term experience. First, therapeutic doses do not cause hypoglycemia. Second, metformin does not lower glucose in non-diabetic individuals. Third, metformin has direct beneficial effects on serum lipids and lipoproteins [3].

Absorption, Distribution and Metabolism

Oral bioavailablitity of metformin is 50-60%. Absorption is relatively slow and is the rate-limiting step in the disposition of the drug. Absorption occurs predominantly across the small intestine and is complete in approximately 6 hours [4]. Co-administration of food slightly decreases the rate and extent of absorption. The drug is rapidly distributed and accumulates in the

esophagus, stomach, duodenum, salivary glands and kidneys. It is not thought to bind to plasma proteins and is not metabolized in either healthy volunteers or diabetics. No metabolites or conjugates have been identified [4].

Metformin is renally excreted with a half-life of 4-8.7 hours. Renal clearance is greater than GFR indicating active tubular secretion [1]. Little is known about placental transfer of metformin or of its excretion into breast milk.

Sites and Mechanisms of Action of Metformin (Figure 6-2).

Despite many years of clinical use the precise sites and mechanisms of action of metformin remain unclear [4]. Metformin has been shown to inhibit intestinal absorption of glucose in diabetic and non-diabetic rats though the effect is not great enough to account for the majority of the antihyperglycemic effect of the drug [3]. This finding has not been substantiated in human studies at therapeutic doses [4].

Administration of metformin does not increase circulating levels of c-peptide or insulin indicating that metformin does not stimulate insulin secretion [3, 4]. In vivo studies have shown improved glucose tolerance with normal or reduced insulin concentrations. This suggests that improved glucose sensitivity is a predominant feature in metformin action. In vitro studies have demonstrated an enhancement of the function of transmembrane glucose polypeptide transporters by metformin. This is the proposed mechanism for the increased peripheral glucose uptake by muscle and adipose tissue in the presence of metformin [3, 4]. Metformin also acts at the level of the liver, reducing hepatic gluconeogenesis. The precise mechanism, however, remains unclear [4, 5].

Clinical Effects of Metformin

Metfomin lowers HbA1c 1-2 % and fasting plasma glucose by up to 4.9mmol/L [6-9]. The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that this is similar to the changes achieved with sulphaureas or insulin [10].

Metformin does not promote weight gain [6, 9, 10]. The final results of the UKPDS indicated that after ten years of therapy, weight change in metformin group was the same as the conventionally treated group; both significantly less than in the insulin or sulphonylurea groups [10]. The weight loss associated with the use of metformin is likely attributed to a decrease in appetite [9]. In a randomized, single-blind, placebo controlled trial, metformin decreased caloric intake [11]. Interestingly, the weight loss associated with metformin use appears to be preferentially from adipose tissue [9].

Metformin does not cause hypoglycemia [4, 10]. This is in contrast to insulin or the sulphaurea drugs that are both associated with significant risks of hypoglycemia. The final report of the UKPDS reported a 0.0% rate of major hypoglycemic episodes in the metformin group. This is significantly less than that of the insulin or sulphaurea groups and also less then the conventionally (non-pharmacoloic treatment) treated group [10].

In the UKPDS, the metformin group had a lower risk of developing any diabetes-related end point compared to conventional treatment (lifestyle intervention), and compared to intensive treatment using either insulin or sulphonylureas. The metformin group also had the greatest risk reduction for all-cause mortality [12].

In patients with type 2 diabetes, both obese and non-obese, TG levels were reduced by metformin by up to 45% [6], the result of a decrease in hepatic synthesis of VLDL. Other studies have been unable demonstrate changes to TG levels, however, no increases in TG levels have been reported with metformin therapy [4]. Total serum levels of cholesterol have been shown to decrease with metformin therapy [6]. The lowering of cholesterol levels is thought to be secondary to a decrease in VLDL (very low density lipoprotein) and LDL (low density lipoprotein) production [1]. Modest increases in HDL cholesterol have also been reported with metformin therapy [6]. The benefits on lipid profile appear to be independent of improvements in metabolic control [13].

Metformin may also have beneficial effects on vascular properties. An increase in fibrinolysis has been shown in patients treated with metformin as indicated by an increase in tissue plasminogen activator, a reduction in tissue plasminogen antigen activity and a reduction in plasminogen activator inhibitor (PAI-1). PAI-1 inhibits fibrinolysis, therefor a decrease in PAI-1 may decrease the likelihood of extension of a thrombus [4, 12].

Side Effects/Tolerability

Acute, transient and reversible side effects of metformin occur in 5-20% of individuals [1]. The most common complaints are anorexia, nausea, diarrhea and a metallic taste. These are usually transient and can be minimized by slow titration of the dose and by taking the medication with meals. Symptoms typically decrease with time and/or dose lowering. Inability to tolerate the drug occurs in less than 5% [1].

Metformin is thought to decrease gluconeogenesis from alanine, pyruvate and lactate. This may allow the accumulation of lactic acid under specific conditions. The reported incidence of lactic acidosis associated with metformin use is 0-0.084 per 1000 patient years. Mortality risk

associated with lactic acidosis is 0-0.024 per 1000 patient years. Of note, this is lower than the risk of major hypoglycemic episodes reported with the use of sulphonylureas. The risk of mortality associated with lactic acidosis is about the same as the risk of mortality from hypoglycemia associated with the use of sulphonylureas [14]. In most cases of lactic acidosis, one or more of the contraindications to the use of metformin has been overlooked [13, 15].

Impaired gastrointestinal absorption of vitamin B12 and folate are described with the use of metformin. However, these effects are not thought to be clinically significant [4].

Contraindications/Precautions to the Use of Metformin

Renal impairment, significant liver dysfunction, and any condition (acute or chronic) resulting in tissue hypoxia are contraindications to the use of metformin [4]. Examples of conditions causing tissue hypoxia include heart failure, respiratory failure and severe systemic illness leading to hypoxemia and/or renal failure. A recent cross-sectional study investigated current metformin treatment practice detected major contraindications to the use of metformin in 51.8% of the patients surveyed (n=308; mean age 66 ±11.3 years). However, despite the presence of major contra-indications, no episodes of lactic acidosis occurred [16].

The use of contrast media while on metformin therapy has traditionally been listed as one of the contraindications for its use. This was based on reports of lactic acidosis precipitated by contrast media. However, more recent reviews indicate that the problem arises with the use of metformin in renal failure associated with the contrast media [17]. It is now recommended that that metformin be held for 48 hours after administration of contrast media. If significant renal failure does not ensue, metformin can then be restarted [17].

The use of metformin in pregnancy is controversial and will be dealt with in detail in the subsequent section (6.3)

In summary, metformin is an antihyperglycemic agent that appears to exert its actions by decreasing hepatic glucose production, and enhancing peripheral glucose uptake. Fasting blood glucose is decreased by up to 4.9 mmol/L and HbA1c by 1-2 % on metformin therapy. Beneficial effects on serum lipids (decreased total cholesterol, increased HDL) and potentially beneficial vascular effects have also been noted. The risk of serious side effects when used in the absence of known contradictions is rare.

6.3 Pregnancy, diabetes and metformin

Strict glycemic control, before conception and throughout gestation is the standard of care for the patient with diabetes [18, 19]. Hyperglycemia is a known teratogen to the developing fetus [20-22]. Gylcemic control beginning in the preconception period is critical to the prevention of congenital anomalies in the offspring of diabetic mothers' [20]. The impact of glycemic control on major congenital malformations occurs during the first trimester, the period of organogenesis [20-22] The major congenital malformations associated with DM are anencephaly, spina bifida, great vessel abnormalities and caudal regression (sacral agenesis) [23]. Glycemic control throughout gestation remains important for the prevention of other fetal-maternal problems (e.g. macrosomia).

The effect of oral antidiabetic agents on the developing fetus is an area of controversy. Piacquadio et al., reported an increase in congenital malformations associated with oral hypoglycemic use in diabetic women (N=20)[24]. However, the confounding effect of poor

metabolic control was not excluded. This study was limited to Mexican American women and did not include any individuals on metformin. Hellmuth et al., found no major congenital malformations and only one minor malformation in the offspring of diabetic mothers who were treated with oral hypoglycemics at conception and throughout the first trimester (N=25)[25]. 7 of these women were treated with metformin. Coetzee and Jackson report a study of the use of metformin in 60 women and their offspring. No increase in congenital malformations (major or minor) was seen in the offspring of this group. However, only 12 of these women received treatment with metformin during the first trimester with the remainder commencing therapy during the second or third trimester [26].

In 1995, Towner et al., confirmed an increased rate of congenital malformations in the infants of diabetic mothers but this was not associated with treatment modality (non-pharmacologic therapy, insulin or oral hypoglycemic agents)[27]. In the absence of good metabolic control, the risks of an infant with congenital malformations in type 2 patients approximated those with type 1 diabetes. Thus, the common suggested teratogen is hyperglycemia, not treatment modality [27]. However, until oral hypoglycemic agents have been adequately been shown to be safe in pregnancy, it is currently recommended that diabetic women treated with oral antidiabetic agents discontinue their use prior conception [18].

6.4 Rationale for the Use of Metformin in this Proposal

The only currently licensed pharmacologic agent for the treatment of diabetes in the pediatric age group is insulin though it has not been specifically studied in children with type 2 diabetes. It is has been our experience that the long-term use of insulin is not well tolerated in this group [28].

There are several potential reasons for this including the risk of hypoglycemia associated with insulin use, the unpleasant mode of administration, a lack of perceived benefit and weight gain. There also is a perception within this population that insulin is a "last effort" and is associated with terminal diabetes [29]. The sulphonylurea class of antidiabetic agents may also cause hypoglycemic episodes and weight gain. The relatively new class of insulin sensitizing agents, the thiazolidinediones, has not been as well studied in the adult population and may have significant hepatic toxicity.

Metformin has a long-term history of use in the adult population, does not promote weight gain (and may in fact promote modest weight loss) and is not associated with hypoglycemia. It is also in oral form, elevating the need for multiple daily subcutaneous injections. In Canada, Hoeschst Marion Roussel Canada Research Inc., Laval, Quebec, markets metformin under the trade name of Glucophage®.

6.5 Study Objective and Hypothesis

The objective of this research project is to determine if treatment with metformin will improve glycemic control in a population of Canadian First Nation youth with type 2 diabetes mellitus.

We hypothesize that the treatment group will have lower fasting blood glucose and hemoglobin A1c values than the control group.

6.6 Design and Methods

6.6.1 Overview

A double blind, randomized, placebo-controlled trial is to be conducted among First Nation children and adolescents between the ages of 8 and 17 years with type 2 diabetes mellitus (the target population). Each subject will be randomly assigned to treatment with metformin or placebo and followed for a 12-month period. Biochemical markers of diabetes control, fasting serum insulin levels, and body mass index will be assessed at entry into the study, and at three, six, nine and 12 months. Side effects and compliance will be further assessed by pill count at clinical encounters.

6.6.2 Subject Selection

Study subjects will be composed of members of the target population who reside in Manitoba or Northwestern Ontario who meet the inclusion and exclusion criteria outlined below. Only prevalent cases will be eligible for inclusion. It is known that females predominate in this population. The diagnosis of type 2 diabetes will be established according to the criteria set by the Canadian Diabetes Association (appendix 6.2), and confirmed by one of us (HD), a pediatric endocrinologist.

All subjects will also have received 'usual care' at diagnosis which consists of approximately two hours with the physician, 6-8 hours of education with the nurse educator, 3-4 hours of dietary counseling with the dietitian and a one hour meeting with the social worker. This is typically divided over a 3 to 4-day initial education period. All subjects will have received at least two follow-up assessments and counseling sessions after diagnosis before being eligible for inclusion in this study. This is being done to allow each individual a trial of non-pharmacologic treatment.

The inclusion criteria are:

- 8 to 17 years of age at time of recruitment
- self declared First Nation origin (declared by parent or guardian
- if female and sexually active, willing to take an approved method of contraception
- fasting blood glucose > 6.7 and/or HbA1c >7.0% after at least 3 months of lifestyle modification (These values are chosen as these are the target values for diabetes control set by the CDA [18])
- signed informed consent

The exclusion criteria are:

- inability to attend regular follow-up
- pregnancy (all female participants must have a undetectable serum HCG at the time of entry)
- primary renal disease (creatinine values greater than the upper limits of the reference range for age)
- liver disease (defined as alanine aminotransferase [ALT] and asparatate aminotransferase [AST] greater 200IU)
- history of significant psychiatric disorder (e.g. previous suicide attempts, drug or substance abuse, including alcohol abuse)
- insulin or oral hypoglycemic use in the previous three months

6.6.3 Sample Size

Sample size is based on the primary outcome measure (HbA1c). It is not feasible to use side effects as the basis for calculation of sample size, given the rarity of significant side effects. If the use of metformin can be shown to be effective in this population, a larger, multi-center trial would then be warranted to assess side effects. Observation for side effects will be a component of this study.

A 1% change in HbA1c is a clinically significant difference (δ), given the relationship between HbA1c and the risk of developing long term complications of diabetes [12]. The standard deviation (σ) of HbA1c of 1.2% is derived from the literature. Using the formula $N= n_1+n_2 = 4[(z_\alpha + z_\beta)^2 \sigma^2 / \delta^2]$, a total sample size of 45 is calculated when using means derived from

independent groups and a two-tailed test (α= 0.05, β= 0.20, σ= 0.012, δ= 0.01). A total of 45 patients are needed to attain a power of 80% to demonstrate a change in HbA1c of 1.0% at a significance level of 0.05. Thus, 27 patients will be needed in each group, allowing for a dropout rate of 20% (The detailed calculation can be found in appendix 6.3).

There are currently approximately 100 individuals (age 8-17) being followed in the study region with type 2 diabetes for more than three months. Of the 27 who have had a hemoglobin A1c done in the past six months, 14 (52%) were >7.0%. Thus, we expect 50% of the whole group to have HbA1c >7.0% and need more intensive treatment than at present. Three communities within the study region are currently undergoing screening for type 2 diabetes in their youth, it is anticipated that this will result in a doubling of the current case load. Incident cases will require a three-month trial of non-pharmacologic treatment prior to being eligible for the study. Given the current caseload, and the ongoing screening studies, a six-month recruitment period is anticipated.

6.6.4 Recruitment Area

Subjects will be recruited from Manitoba and Northwestern Ontario. These are regions where an increased incidence of early onset type 2 diabetes has been recognized in Aboriginal youth [28, 30-32]. These areas are all within the referral area for the Winnipeg Health Sciences Center, where there is currently a single pediatric endocrinologist providing consultative care to the diabetic population (HD).

6.6.5 Study Team

The functions of each collaborator and co-investigator in the study team are as follows:

Principal Investigator/Project Director (Dr. Elizabeth Sellers): Responsible for overall scientific direction of project. Responsible for administrative aspects of project and liaison with collaborating local physicians. She will have access to blood and urine results, and will be responsible for monitoring for abnormalities in renal and liver function. She will have no clinical contact with the participants. She will be blind to patient assignments.

Co-investigator/Consultant pediatric endocrinologist (Dr. Heather Dean "HD"): Responsible for providing consultative care to the diabetic population in Manitoba and Northwestern Ontario and direct diabetic care and evaluations at study visits for those individuals who have been referred to Winnipeg.

Co-investigator (Dr. T. Kue Young): Collaboration in terms of study design, data collection, analysis and reporting.

Manitoba DER–CA: The Diabetes Education and Resource Center for Children and Adolescents is funded by Manitoba Health to provide care to the pediatric diabetic population in Manitoba. The DER also provides consultative services to children and adolescents of Northwestern Ontario, as the next closest specialty pediatric diabetes care center is in Toronto. The DER-CA is based at the only tertiary care center providing services to these geographic areas (Winnipeg Children's Hospital, Health Sciences Center). The DER team is comprised of a pediatric endocrinologist (HD), a dietitian, a nurse educator, a social worker and a secretary.

Sioux Lookout Diabetes Program: The Sioux Lookout Diabetes program provides diabetes education coverage in the Sioux Lookout Zone, a regional health service for 29 First Nations with a combined population of 15,000. They also provide service to the towns of Sioux Lookout,

Savant and Pickle Lake. It is staffed by 3.0 FTE registered nurses, 2.0 FTE registered dietitians, 1 chiropodist and 1 relaxation therapist.

Local Physicians: These are the local physicians providing general medical care in the individual communities from which patients will be recruited. They will continue to provide medical care as required for individuals in these communities.

Research Pharmacist: The research pharmacist at Health Sciences Center, Winnipeg will be responsible for the preparation and distribution of the study medication under the direction of the Project Director. The pharmacist will also prepare the placebo dose.

Data Monitoring Committee: This will be chaired by a pediatric endocrinologist-clinical epidemiologist (Dr. S. Taback) who has not been involved in the development of this protocol and has no clinical involvement with the study participants. Dr. Taback also has formal training in biostatistics. The committee will review the reports of side effects at 3, 6 and 9 months to ensure there are no safety issues that would warrant discontinuation of the trial.

6.6.6 Experimental Maneuver

The consultant pediatric endocrinologist, in consultation with the local physicians, will identify patients from the target population who meet the inclusion and exclusion criteria. She will request permission from the individual for the project director to discuss the trial with them. The project director will then approach patients and their guardians for informed consent.

Once informed consent is obtained, subjects will be randomly allocated to receive either the active treatment medication (metformin) or placebo. The randomization schedule will be based on a computer-generated random numbers list. Within each successive block of six random

numbers, the three highest numbers will be assigned to the treatment arm and the three lowest to placebo. This will be done to ensure that there will be comparable distribution of the patients from month to month.

Study participants will be evaluated at a study visit at the time of entry, and at 3, 6, 9, and 12 months (see Appendix 6.4). A study visit within 3 weeks of the target time will be acceptable. The following data will be collected at study entry:

- baseline anthropometric characteristics: height, weight, BMI, waist circumference[1]
- baseline metabolic studies: HbA1c, fasting serum glucose, fasting serum insulin levels, creatinine, AST, ALT, random urine for albumin / creatinine ratio
- baseline historical data: duration of diabetes, previous treatment, age of menarche, menstrual history, contraceptive history

All females must have a negative serum and urine HCG to ensure that they are not pregnant at the time of entry into the study. The metformin and placebo will be provided in tablet form with no identifying marks. For those receiving the study drug, a standardized starting dose of 250 mg daily will be used. The manufacturer of metformin will provide the treatment drug and a placebo identical to the treatment drug with the exception of the active ingredient (metformin).

Follow-up will be standard for both groups and consist of:

- A study visit every six months (time 0, 6 and 12 months) conducted by the diabetes teams in Winnipeg or Sioux Lookout Zone coordinated by the project director. At each of these visits the participant will receive "usual care" which consists of the following:

[1] Waist circumference will be performed supine at the umbilicus.

1. Clinical evaluation and physical examination conducted by the consultant pediatric endocrinologist this will include blood pressure and height, weight and waist circumference measurements) (appendix 6.5).
2. 10-15 minute session with a nurse educator, during which the importance of exercise and weight control will be reviewed with both patient and primary care givers
3. 15-20 minute session with the dietician for review of healthy eating patterns with patient and primary care givers
4. 20-30 minute counseling session regarding barriers to optimum therapy with lifestyle changes including discussion of risk-taking behaviors
5. serum samples for fasting blood glucose, fasting insulin, HbA1c and creatinine, urine albumin/creatinine, lipid profile
6. review of home blood glucose monitoring records (as part of ongoing usual care, individuals will be asked to perform home blood glucose monitoring - specifically they will be requested to perform a minimum of a daily am fasting blood glucose)

In addition to 'usual care' all participants will undergo the following at each study visit:

1. serum sample for ALT,AST
2. serum βHCG
3. consultant endocrinologist (as part of the evaluation) will elicit responses to questionnaire re: potential side effects of study medication (appendix 6.6)

The study visits at 3 and 9 months with the consultant pediatric endocrinologist will be done in the local community when feasible. This is being done to avoid the need for further travel to Winnipeg for the Manitoba participants. While in the communities, the consultant pediatric

endocrinologist will use the opportunity to increase community awareness of diabetes and to increase contact with the local health professionals. It is hoped that this will have long term positive effects at a community and professional level.

At these visits the following will be performed:

- clinical evaluation and physical examination conducted by the consultant pediatric endocrinologist/medical director of the SLZ diabetes program (this will include blood pressure[2] and height, weight and waist circumference measurements)
- serum samples for fasting blood glucose, fasting insulin, HbA1c and creatinine, urine albumin / creatinine, lipid profile
- review of home blood glucose monitoring records
- serum sample for ALT,AST
- serum βHCG
- side effect questionnaire (appendix 6.6)

The consultant endocrinologist, or the local physician in consultation with the consultant endocrinologist, will be free to withdraw the participant and unblind individual subjects' treatment status if there are compelling clinical reasons to do so. If a patient becomes metabolically unstable (significant weight loss and/or ketosis) or if the patient's HbA1c exceeds 10 % the study medication will be stopped at the discretion of the consultant endocrinologist and short term insulin therapy instituted (usual care in this situation). After stabilization and after a period of three months off of insulin, the patient may resume the study medication if the 12-month study period has not been completed.

The consultant endocrinologist and / or the local physician will be free to see the participant on a more frequent basis should medical reasons necessitate more frequent evaluation. The number of clinic visits and telephone contacts with members of the diabetes teams and/or the local physician-

[2] BP to be performed by the physician at the end of the clinical encounter in the recumbent position

for issues concerning diabetic management will be recorded to monitor for potential co-intervention effect.

Participants, the consultant endocrinologist, local physicians and members of the DER-CA team and the SLZ diabetes team will be blinded as to the treatment status of the patient. They will also be blind to the results of the urine and blood testing at entry and at subsequent follow up study visits with the exception of the HbA1c results and home glucose monitoring records. This will be available to the investigators and participants, as it is a standard of monitoring ongoing diabetes control. The treatment status of participants will be available only when the analysis is complete.

6.6.7 Exposure and Outcome Measurement

The exposure to be studied in this clinical trial is the drug metformin. All patients randomized to the treatment arm will receive a standard starting dose of metformin 250 mg orally daily. This will be increased according to a set schedule until 750 mg twice a day is achieved. Participants will receive a calendar to follow for dose adjustment (appendix 6.7). The consultant pediatric endocrinologist will be available by telephone if there are questions arising during dose adjustment. Further dose adjustments will be on the basis of HbA1c and fasting blood glucose results to a maximum of 750 mg 3 times a day. The consultant endocrinologist will adjust the dose (active drug or placebo) on the basis of these results. The project director will monitor HbA1c, fasting blood glucose levels, renal and liver function tests and side effect questionnaire.

The research pharmacist, working in collaboration with the project director, will prepare both the study drug and the placebo for dispensing. In order to maintain blinding, placebo patients will also have their 'dose' adjusted according to the set schedule and HbA1c results. Participants will also be asked to record on a calendar provided the number of pills taken each day (appendix 6.8).

The consultant pediatric endocrinologist/medical director of the SLZ diabetes program or local physician, in consultation with the consultant endocrinologist, may reduce the dose (minimum 250 mg daily) if it is felt to be necessary based on clinical assessment. This will be done only if there are compelling medical reasons to do so.

The outcome measures are related to the degree of glycemic control as assessed by HbA1c and fasting blood glucose. Secondary outcome measures include (1) BMI (2) fasting insulin levels (3) compliance and (4) side effects.

Hemoglobin A1c

The use of glycated hemoglobin in the management of diabetes has been an important tool since the early 1980's [33]. The measurement of hemoglobin A1c provides a measure of glycemic control over the proceeding 2-3 months [34]. All samples will be analyzed at the laboratory of the Health Sciences Centre, Winnipeg.

Fasting blood glucose

Fasting glucose is used as a predictor of HbA1c and thus, glycemic control. It also provides a measure of safety to ensure that overnight fasting is tolerated without hypoglycemia. While the study drug is not reported to cause hypoglycemia, we feel this is an important safety check given that its use is untested in the pediatric population. All samples will be analyzed using the Hitachi 705 Analyzer at the Health Sciences Centre clinical chemistry laboratory.

Side Effects

Clinical and biochemical side effects will be monitored clinically at each study visit.

(1) The consultant endocrinologist will be asked to elicit responses to specific questions aimed at symptoms that may result from the use of the study drug. They will be asked to fill out a questionnaire with these responses. This will ensure that the systematic search for potential side effects is done (appendix 6.6).

(2) Serum samples for renal and hepatic function will be drawn as well as a sample for fasting blood glucose (as previously described). The subject will be automatically unblinded and treatment stopped if any abnormality of renal or liver function is detected (defined as elevation of creatinine above the normal for age, AST or ALT >200 IU).

Body Mass Index (BMI)

Given the relationship between increased BMI, insulin resistance and type 2 diabetes, BMI will be assessed at each visit. BMI will be calculated using the formula BMI = kg/m^2. Weight will be measured in kilograms (to 1 decimal point), using a standard office scale. The mean of three measurements will be recorded. Height will be measured (in centimeters) using a stadiometer; again, the mean of three measurements will be recorded. All instruments will be calibrated on a monthly basis. The same instruments will be used throughout the study period.

Fasting Insulin Levels

Fasting insulin levels will be used as a marker of insulin resistance, the underlying pathologic mechanism of type 2 diabetes. The study drug improves insulin resistance, and thus would be expected to decrease insulin levels. Blood for fasting insulin levels will be assessed by radioimmune assay using the Pharmacia kit at the Health Sciences Centre clinical chemistry laboratory.

Compliance

Patients will be asked to bring their pill bottles to each study visit and pill counts will be performed (The consultant pediatric endocrinologist will not be blinded to these results as it is standard practice to asses compliance in this way if compliance is a concern). Counts will be performed by the diabetes clinic nurses at study entry, 6 and 12-month visits and by the consulting endocrinologist at the 3 and 9-month study visits.

6.6.8 Reporting of Adverse Events

It will be the responsibility of the collaborating endocrinologist to report any unexpected and/or serious clinical adverse events immediately to the Project Director and to the Chair of the Data Monitoring Committee. Local health care providers (physicians/nurses) will be sent covering letter outlining specific concerns with the use of the study drug that they need to be aware of (appendix 6.9). They will be asked to report any concerns arising in a study patient with the collaborating endocrinologist, if the concern is an adverse event the collaborating endocrinologist will be responsible for reporting it. The Project Director will have access to biochemical data and will be responsible for reporting a change in renal of hepatic function to the Data Monitoring Committee and for informing the clinicians (patient to be unblinded and medication stopped).

6.6.9 Data Analysis

The primary outcome measure (HbA1c) is a continuous variable and will be analyzed as means. The exposure is dichotomous (drug or placebo). Univariate analysis will be performed using unpaired t-tests. Those secondary measures (BMI, fasting glucose, fasting insulin) which are continuous variables will also be analyzed using unpaired t-tests to compare means. Other variables (presence/absence of side effects, compliance) will be analyzed as dichotomous

variables using chi-square test and their association with the exposure variable summarized by the odds ratio. While randomization is intended to make the treatment and control groups comparable in terms of measured and unmeasured potential confounders, stratified analysis will be performed to control for age and duration of diabetes (analysis of variance for continuous outcome variables and Mantel-Haesnzel procedure for categorical outcome variables). Separate analyses will be performed for each sex, and if results are not significantly different, they will be combined to increase sample size. The 95% confidence intervals of all means and odds ratios will be calculated and reported. All data will be analyzed on an intent-to-treat basis. A "drop out" will be defined as only those individuals who did not meet the entry criteria (and were mistakenly enrolled) or those who withdraw consent for the use of their data.

6.7 Premature Closure of the Trial

The Data Monitoring Committee will recommend early termination of the trial if concerns arise due to serious side effects associated with the study drug. The Committee will not evaluate, nor recommend early termination of the trial on the basis of the HbA1c results. This decision has been taken as all patients will be receiving the current standard of treatment in this trial (lifestyle modification). While study groups may show between group differences in HbA1c prior to the trial end at 12 months it is the benefits of long term glycemic control that are of interest and are associated with a decrease in diabetic complications.

6.8 Ethical Considerations

This study involves a therapeutic maneuver (use of metformin) which has not been studied in the pediatric population. The safety of metformin has, however, been established within the adult type 2 diabetic population. Its safety in the context of pregnancy has not been established. For this reason all patients who are pregnant are excluded from this study and all sexually active females must agree to use contraception. These concerns will be discussed with the patient as part of the informed consent process. We feel that the study of this drug is justified in a population who has not responded to lifestyle intervention. This population is at risk for increased morbidity and mortality associated with poor diabetic control. Metformin is currently being prescribed in the USA and Saskatchewan off-label for youth with type 2 diabetes. We believe that a well designed randomized controlled trial is vital in this context.

Potential benefit to the individual, in the form of improved diabetic control, may be derived from participation in this study. While there is no anticipation of harm to the individual, patients will be monitored closely for adverse events associated with the therapeutic maneuver. An external data monitoring committee will be in place to monitor safety issues.

Subjects will only be entered into this trial after voluntary and informed written consent has been received from the subject or his/her parent with assent of the child if applicable (see appendix 6.10). The data gathered will be held in the strictest confidence.

6.9 Relevance and Significance

The health status of Canada's Aboriginal peoples remains an issue of concern as we approach the twenty-first century. In 1997, infant mortality in this group was twice the national average, and

life expectancy at birth was eight years below the national average [35]. The emergence of new health problems such as type 2 diabetes superimposed upon an already high burden of infectious diseases and social problems will put further demand on the health care system. Type 2 diabetes among children and adolescents is of great concern in the Aboriginal communities, many members of which are alarmed by the prevalence of the condition and the catastrophic impact on the quality of life of those who suffer from its long-term complications such as blindness, amputations and renal dialysis. There is an urgent need to develop and evaluate an effective intervention program to combat this problem.

6.10 Progress Report

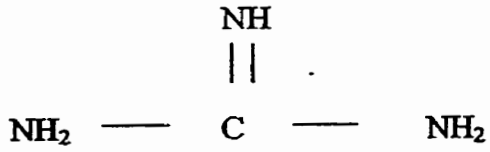
As of September 15, 2000 13 participants have been recruited into this study. Recruitment has been slower than anticipated for several reasons. First, the drug and placebo where not available from the manufacturer until December, 1999 as production of the placebo required a total shut down of the production of the marketable drug. This was done at the convenience of the manufacturer. Second, aggressive, short-term insulin is being used more frequently in this patient population in those with sub-optimal control. Typically, patients are receiving 1-3 months of insulin therapy. This has delayed potential enrolment for an individual 4-6 months, as they are required to be treatment naïve for a period of 3 months prior to enrolment. Third, inclusion of participants from Sioux Lookout has been on hold because of a delay in receiving local ethics approval. This delay has been a result of several factors including a restructuring of the medical system in Sioux Lookout. We have had to wait for the re-institution of a local ethics committee, which had become defunct. We anticipate approval within this calendar year.

No significant adverse effects or side effects have been reported to date. Tolerance of the study medication has been good with no participant yet reporting difficulties. No interim analysis was planned for this study. Therefor the data presented in table 6.1 represents the unanalyzed data that is available to the clinical investigator (blinding remains in effect).

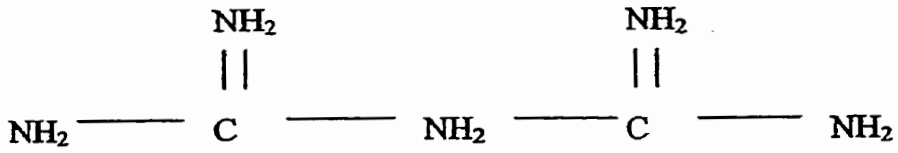
Table 1. Status report: HbA1c and Side Effects

Pt. #	HbA1c Study Entry	HbA1c 3 months	HbA1c 6 months	HbA1c 9 months	HbA1c 12 months	Reported Side Effects
1	10.5	12.3	10.1			nil
2	9.9	8.7	11.3			nil
3	10.2	7.1	6.0			nil
4	10.2	9.8	11.1			nil
5	7.1	7.1				nil
6	10.3	8.7				nil
7	12.9					
8	13.1					
9	11.6					
10	9.1					
11	10.1					
12	10.1					
13	8.4					

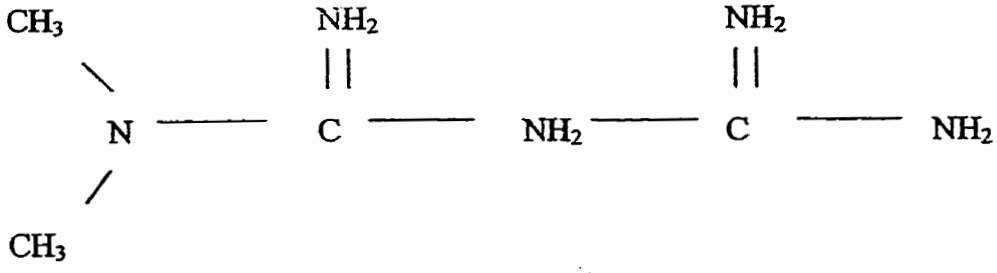
Figure 6.1 Structural formulae of guanidine, its derivative biguanide and metformin (dimethylbiguanide).



Guanidine

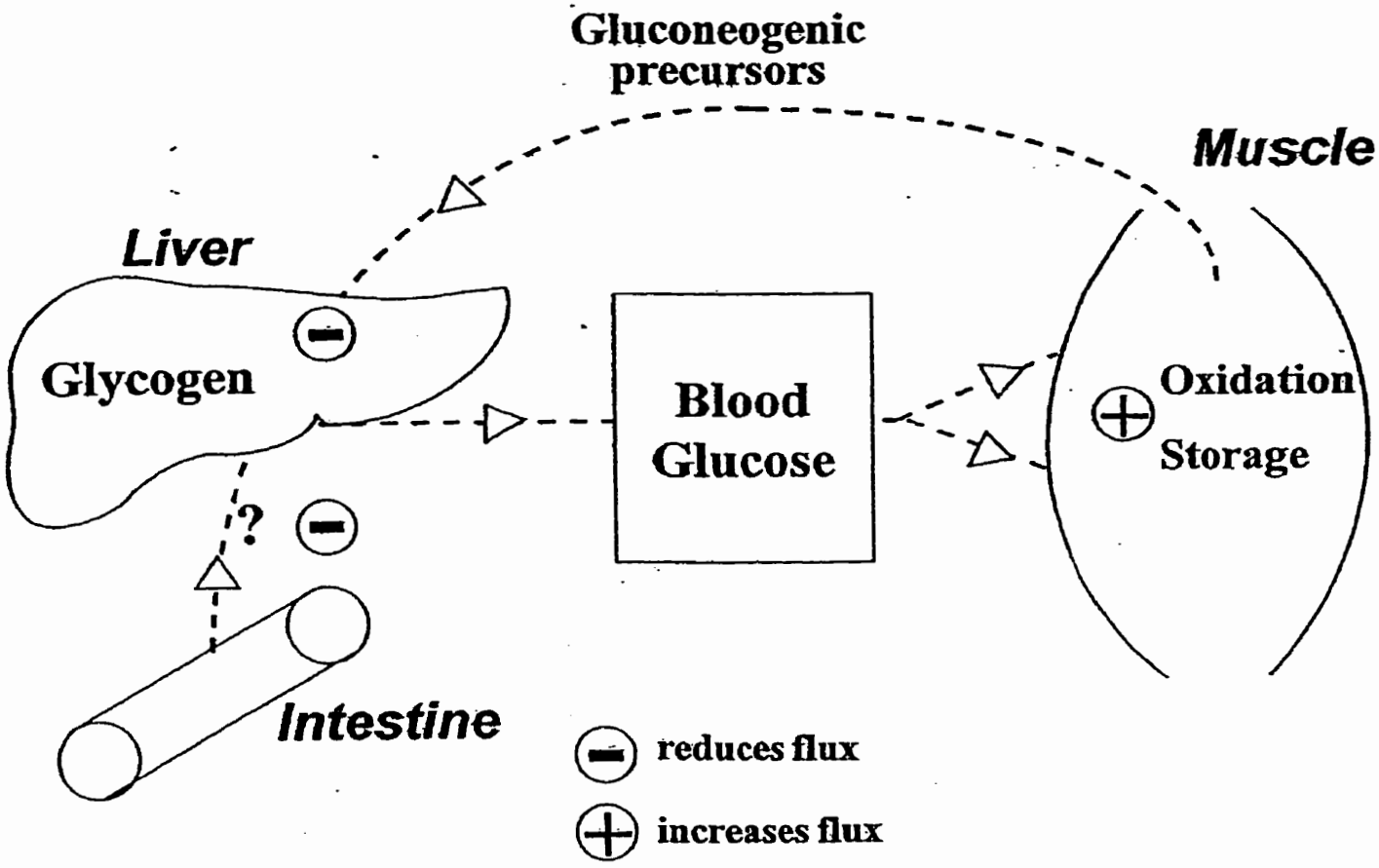


Biguanide



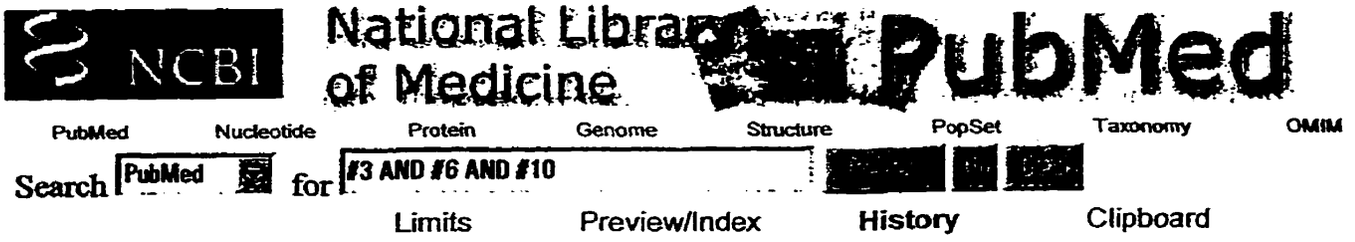
Metformin (dimethylbiguanide)

Figure 6.2 Scheme of Major Actions of Metformin



From Bell PM and Hadden DR, Metformin. Endocrinology and Metabolism Clinics of North America 1997; 26: p. 523-537.

Appendix 6.1 Strategy for PUBMED Search



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- To combine searches use # before search number, e.g., #2 AND #6.

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Search	Most Recent Queries	Time	Result
#11	Search **#3 AND #6 AND #10**	12:23:53	5
#10	Search **#7 OR #8 OR #9**	12:20:58	22168
#9	Search **(adult onset diabetes)**	12:20:19	233
#8	Search **(non-insulin dependent diabetes)**	12:19:38	21977
#7	Search **diabetes mellitus, type 2**	12:18:14	20091
#6	Search **#4 OR #5**	12:17:12	17512
#5	Search **RCT**	12:16:35	653
#4	Search **Randomized AND control AND trial***	12:15:47	16942
#3	Search **#1 OR #2**	12:14:30	1085201
#2	Search **pediatric***	12:13:44	171431
#1	Search **child***	12:12:49	1022852

Appendix 6.2

Criteria for the Diagnosis of Diabetes Mellitus

Source: Canadian Diabetes Association Guidelines. *CMAJ* October 20,1998

Symptoms of diabetes plus casual plasma glucose concentration > 11.1 mmol/L. Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydypsia, and unexplained weight loss.

or

Fasting plasma glucose >6.9 mmol/L. Fasting is defined as no caloric intake for at least 8 hours

or

2h post prandial glucose >11.1 mmol/L during OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

Appendix 6.3

Sample Size Calculation

$N = n_1 + n_2 = 4[(z_\alpha + z_\beta)^2 \sigma^2 / \delta^2$

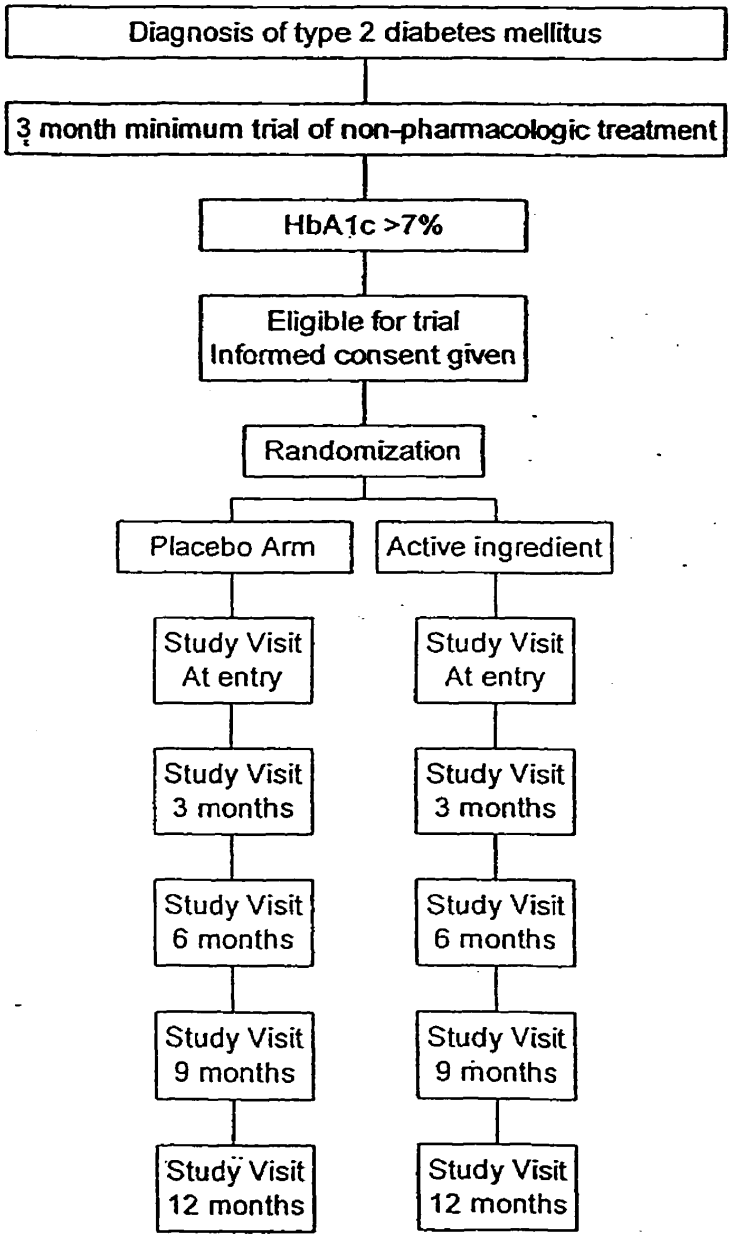
$z_\alpha = 1.96$ (for $\alpha = 0.05$, two sided)
$z_\beta = 0.84$ (for $\beta = 0.20$, power = 0.80)
$\sigma = 0.012$ (derived from previous studies)
$\delta = 0.01$ (judged to be a clinically significant change in HbA1c of at least 1.0%)

Therefore $N = 4[(1.96 + 0.84)^2 \times 0.012^2]/0.01^2 = 45.2$

Add 20% for the estimated number of drop outs

Total sample size = 54 (27 in each group)

Appendix 6.4 Study Flow Sheet



Appendix 6.5 Data Collection

Baseline Historical Data (Study visit #1 only)
Family History: Affected family members

Mother
Father
Sister
Brother
M. Grandmother
M. Grandfather
P. Grandmother
P. Grandfather
Other _____

Age at diagnosis_____ Duration of Diabetes_____
Previous Treatments_____
_____
Menstrual History
Age at menarche _____ LMP_____ Regular cycles Yes No
Contraceptive History_____

Data Collection Form (all study visits)
Patient Study #_____

Date_____ Study Visit Month # _____

Height_#1_____ #2 _____#3 _____ Weight #1____ #2_____#3_____ BP_____

Waist Circumference _____(cm)

Acanthosis nigricans Absent
Mild
Moderate
Severe

Tanner Stage (by photograph)
I
II
III
IV
V

Pill Count (remaining)_____

Additional Comments_____

Appendix 6.6 Side Effects Questionnaire

1. **Has the patient experienced any nausea?** Yes No

   If so, timing in relation to dose of study drug?
   - Within 1 hr prior to administration
   - Within 1 hr of administration
   - Within 4 hrs of administration
   - Other _____

   How long did it last?_
   - <1 hr
   - 1-2 hrs
   - >2 hrs

   How frequent are the episodes?
   - With every dose
   - Daily
   - Twice a week
   - Once a week
   - Less than once / week

2. **Has the patient had any diarrhea?** Yes No

   Duration
   - <1 day
   - 1-3 days
   - 4-7 days
   - >7 days

3. **Has the patient complained of an unusual taste in his/her mouth?** Yes No

   If so, how long did it last?_
   - <1 hr
   - 1-2 hrs
   - >2 hrs

   How is it described?_____

   Relation to dose
   - Within 1 hr of dose
   - 1-2 hrs after dose
   - >2 hrs after dose

4. **Has the patient had any vomiting?** Yes No

   Relationship to dose
   - Within 1 hr of dose
   - 1-2 hrs after dose
   - >2 hours after dose

   Frequency
   - With every dose
   - Daily
   - Twice/week
   - Once /week
   - < Once/ week

5. **Has the patient had any other symptoms/complaints?**_____

Appendix 6.7 Flow Sheet for Dose Adjustment (to be given to the participant)

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1	half pill at breakfast	half pill at breakfast	half pill at breakfast	half pill at breakfast	half pill at breakfast	half pill at breakfast	half pill at breakfast
Week 2	half pill at breakfast	half pill at breakfast	half pill at breakfast	half pill at breakfast	half pill at breakfast	half pill at breakfast	half pill at breakfast
Week 3	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner
Week 4	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner	half pill at breakfast half pill at dinner
Week 5	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner
Week 6	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner	1 pill at breakfast 1at dinner
Week 7	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner
Week 8	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner	1 and half pills at breakfast, 1at dinner
Week 9	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner
Week 10	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner
Week 11	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner
Week 12	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner	1 and half pills at breakfast, 1 and half at dinner

Appendix 6.7 (cont.)

INSTRUCTIONS

(1) Pills to be taken just before the meal
(2) Please cross off each box when the pills are taken
(3) Please keep this sheet and take to your next appointment
(4) If your blood sugar first thing in the morning is less than 6.7 on 3 of 5 consecutive days - do not increase the dose you are taking, continue to take the dose at which this occurred.
(5) If you have any questions, please call the Children's Hospital (204-787-2033) and ask for the doctor covering diabetes.

Appendix 6.8

Please mark the number of pills taken each day

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1							
Week 2							
Week 3							
Week 4							
Week 5							
Week 6							
Week 7							
Week 8							
Week 9							
Week 10							
Week 11							
Week 12							
Week 13							

Appendix 6.9

Dr. X
Address

Date
Dear Dr.----------,

We are conducting a randomized double blinded placebo controlled trial for the treatment of type 2 diabetes mellitus in First Nation youth (8-17 years). Metformin (glucophage) is the active study medication. My co-investigators are Dr Heather Dean (pediatric endocrinology) and Dr. Kue Young (Community Health Sciences). One of your patients,-------, has just been enrolled in this study.

To briefly summarize, this trial includes First Nation youth with the diagnosis of type 2 diabetes who have had a minimum 3 month trial of lifestyle modification but despite this have unacceptable blood glucose control (defined as per the Canadian Diabetes Association guidelines as a HbA1c >7% or fasting blood glucose >6.7). Patients with primary renal dysfunction or hepatic dysfunction will be excluded. They will also be monitored for the development of abnormalities of renal or liver function and, if this develops, will be immediately withdrawn from the study.

Given that hepatitis A, PIGN and IgA nephropathy are known to occur with higher than average frequency in this group, it is important that all health care workers involved with this potential group of patients be aware of the need to stop the study medication if any of these conditions were to occur in a study patient. We would also like to be informed if an outbreak of hepatitis were to occur in the community, we would then arrange for more frequent monitoring of liver function in participants from that community. The study medication should also be held if the patient were to need radio-contrast material for an imaging procedure, or to suffer from a severe systemic illness or accident associated with hypotension. The study team will be available 24 hours a day to the participant and to you if a problem were to arise in one of the participants (phone #204-787-2033).

We look forward to your collaboration with this study. As is our usual practice, you will receive a note following each clinical visit. Please do not hesitate to contact us is you have any questions.

I would be happy to send you a copy of the complete protocol if you are interested.

Sincerely,

Elizabeth Sellers, MD

Appendix 6.10 Consent Form
(Printed on letterhead)

The Treatment of Type 2 diabetes Mellitus in Canadian First Nation Youth

Study Description and Consent Form

Background
Your son/daughter has type 2 diabetes mellitus and is being followed at the diabetic clinic at the Winnipeg Children's Hospital and/or by the health care team in your community. As part of his/her routine care he/she is seen regularly and a hemoglobin A1c level is performed. Hemoglobin A1c is a measure of blood sugar control over the past two to three months. We know that good control of blood sugar levels with hemoglobin A1c values of <7.0% is associated with a significant decrease in diabetic complications such as kidney and eye problems. This is the goal of diabetes treatment. Sometimes this goal can be achieved without pills or insulin, in other cases these medications are needed.

Purpose of the Study
Your son/daughter's diabetes is currently being treated with an effort to encourage a healthy diet and increased exercise. We are conducting a one year study to see whether the use of a pill, metformin, will improve the control of diabetes. This pill is used in adults with type 2 diabetes but has not been tested in children or teenagers. It is thus not licensed for use in children or teenagers. In adults, metformin may decrease hemoglobin A1c by 1-2% when used together with a healthy diet and exercise.

Risks and Discomforts
A few patients may have nausea, vomiting or a metallic taste in their mouth when starting the medication (<10%). These side effects are usually temporary and are decreased by taking the medication with meals. An increase in a substance in the blood called lactic acid is a potentially serious side effect that may be life threatening. This is extremely rare and occurs most often in people with liver or kidney problems. Your son/daughter will have blood tests every three months to look for problems with the function of the liver or kidney. If this occurs, they will be immediately withdrawn from the study. Your son/daughter will be monitored carefully for the development of side effects.

There have not been any reported effects of metformin on growth. However, as metformin has not been adequately studied in growing children we will be monitoring your son/daughter's growth carefully.

We do not know if this pill is safe in pregnancy. Therefore, if your daughter is sexually active a form of contraception must be used during this study. Acceptable methods of birth control include oral contraceptives ("the pill"), an intrauterine device ("IUD"), and conscientious use of condoms and spermicidal foam. Your daughter's doctor can discuss this with

Participation in the Study
If your son/daughter agrees to participate in this study he/she will continue to receive the routine care currently being provided by the diabetes team. In addition, your son/daughter will be randomly assigned (like flipping a coin) to receive the study drug (metformin) or a placebo (this is a pill without any medication in it). Neither you, your son/daughter or your doctor will know whether your son/daughter is receiving the drug or placebo. One investigator will know the test results and whether or not your son/daughter is receiving the medication. If there are any results that are of concern, this physician will notify the diabetic team immediately.

If you agree to participate in this study, your child will have a hemoglobin A1c drawn at the time of the clinic visit. This does not differ from the routine care your son/daughter is already receiving. At the same time, blood will also be taken for a fasting sugar level, insulin levels and tests to monitor the function of the kidneys and the liver. As this will be done at the same time as the hemoglobin A1c it will not require additional blood sampling. This sample will be taken every three months. As is routine at each visit, your son/daughter will have his/her height, weight and blood pressure taken.

Confidentiality of Records
All information gathered during the course of the study will be confidential. Data used for publication purposes will not identify individual participants. You are free to withdraw your son/daughter from the study at any time. This will not influence the medical care your child is receiving in Winnipeg or in your community. Blood samples taken as part of this study will not be used for other analysis without specific consent. Individuals do not waive any legal rights by participating in this study.

Costs
If extra expenses occur because of your participation in this study, these will be covered by the study (eg telephone calls).

Drs. E. Sellers, H. Dean and TK Young of the University of Manitoba are conducting this study. They can be contacted through the diabetic team at 204-787-3011. If you have any questions, please feel free to contact them. If there is an urgent concern, please call the Winnipeg Children's Hospital at 787-2071 and ask for the doctor covering diabetes (available 24 hours a day).

I give permission to allow my child to be enrolled in this study. This consent form has been explained to me and I have been assured that personal records relating to this study will be kept confidential. I understand that I may withdraw my child/myself from this study at any time. I also understand that if I choose to withdraw, the quality of care my child receives will not be altered in any way.

Name of patient	Signature of patient if age of assent

Name of parent	Signature of parent	Date

This study has been explained to the person authorized to sign above and I am satisfied that it is understood.

Name of investigator	Signature of investigator	Date

Appendix #6.11

Detailed Budget (estimated)

A. Personnel

Subtotal: 0

B. Equipment

Subtotal: 0

C. Materials and Supply

Study Meeting with Collaborating Physicians/Local Diabetes Teams

Study Meeting #1	Location: Sioux Lookout
Plane fares	Winnipeg – Sioux Lookout return (x2 persons) = $1620
Accommodation	1 night [x2 persons] = $160
Meals/incidentals	@$40/person/day [2 persons x 2 days] = $160
Local transportation	$50

Study meeting #2	Location: Winnipeg
Plane fares	Sioux Lookout – Winnipeg return (1 person) = $810
Accommodation	1 night = $ 80
Meals/incidentals	@$40/person/day = $40
Local transportation	$50

Subtotal: $2,970

Transportation Costs Associated with Study Visits

Transportation and accommodation expenses for consulting pediatric endocrinologist (for study visits in local setting at months 3 and 9)

Island Lake $400 x 2 trips =$800.00
Accommodation/meals $60.00 x 2 trips =$120.00
Peguis $100 x 2 trips=$200.00
Sioux Lookout Zone $810 x 2 trips=$$1600.20
Accommodation/meals $80 x 2 trips=$160.00
North Eastern Region $100 x 2 trips=$200.00
Norway House/Cross Lake $400 x 2 trips=$800.00
Accommodation/meals $$60.00 x 2 trips $120.00
North Western Region $ 400 x 2 trips=$800.00
Accommodation/meals $60.00 x 2 trips=$120.00

Subtotal: $4920.20

Estimated costs for study visit to Winnipeg for participants unable to be seen in local setting (estimate 10% of visits for month 3 and for month 9 or 12 visits) = $10,000 (given that this involves participants of the pediatric age group this estimate includes the costs of an accompanying adult)

Subtotal: $10,000

Pharmacy Charge

Basic charge applied to all studies = $200
Charge per Rx dispensed (including placebo) $8.50 x 4 x 54 = $1836
Yearly chare per patient enrolled $31.50 x 54 = $1701

Subtotal: $3,737

Clinical Chemistry Laboratory Charges

Serum HCG $8.50 x 54 x 4= $1836
AST/ALT $11 x 54 x 5 = $2970
Urine marker for compliance $10 x 54 x 12 =$6480
Urine HCG $6.70 x 54 = $361.80

Subtotal: $11,647.80

Other Supplies/Services

Office Supplies = $200
Telephone/Courier/Postage = $500
Photocopying/Printing = $500
Clerical Support = $2000
Statistical Consultation @$65/hr x 20 hours = $1300
Publication costs $50.00 US/page x 5 pages = $250 US ≈ $360.00 CAN

Subtotal: $4,860

D. Travel

Subtotal: $1,500

Note: We include under this item travel costs associated with presentation of results at scientific conferences. Expenses related to two meetings with collaborating physicians from northwestern Ontario are included under Materials and Supply

GRAND TOTAL: $39,635

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Chapter 7 Summary and implications of original work.

7.1 - Summary of Original Work

The differentiation between type 1 and type 2 diabetes is important as the pathophysiology differs and there are differing educational and treatment strategies. We have shown that First Nation youth with diabetes lack diabetes-associated antibodies supporting the diagnosis of type 2 diabetes in this population. In the population of First Nation youth with diabetes, diabetes-associated autoantibody status will serve as a useful adjunct to clinical impression when trying to differentiate between type 1 and 2 diabetes.

The recognition that diabetic ketoacidosis occurs in type 2 diabetes in youth may potentially prevent significant morbidity and/or mortality that can arise from this acute complication. It is important to disseminate this information, particularly to those most likely to be confronted with this situation (e.g. patients and their families, primary health care providers).

The association between the G319S mutation of the HNF-1alpha gene and youth onset type 2 diabetes is supported by our data. It may allow identification of individuals at particular risk for developing diabetes and potentially allow for interventions to prevent or delay the onset of diabetes in these individuals. The presence of this allele is also associated with a phenotype less suggestive of insulin resistance. This remains an important feature to be investigated as it may direct therapeutic interventions.

There remains little experience with oral antidiabetic agents in the pediatric population. Despite this lack of evidence, the American Diabetes Association is now recommending

the use of metformin in youth with type 2 diabetes. This recommendation is based on consensus and not on evidence derived from well designed clinical trials. It is imperative that we pursue our randomized double blind trial of metformin to ensure the safety and efficacy of metformin in this population. The timing of this trial is critical, as it will become more difficult to undertake as clinicians adopt the use of metformin in youth with type 2 diabetes.

7.2 - Implications

The discrepancy between the health status of Canada's aboriginal peoples and the general Canadian public persists despite some improvements over the last quarter of the 20th century [1]. Infant mortality remains twice the national average and life expectancy is 8 years shorter in the aboriginal population. Diabetes is one of the health concerns that affect aboriginal people at disproportionately high rates [1].

The emerging epidemic of type 2 diabetes in youth presents a major potential health crisis. It is estimated that the aboriginal population in Canada will exceed 1,000,000 by 2016 [2]. Currently, more than 50 % of the aboriginal population is under 24 years of age. Less than <1/3 of these young people live in large urban centres, compared to approximately 2/3 of all young Canadians [3]. Manitoba has the highest number of aboriginal youth (aged 5-24 years) in Canada [3]. In the First Nation population, type 2 diabetes in youth has been demonstrated to be a significant problem. The potential social, cultural and economic impact of the disease and its complications on the individual, their families and their communities is great.

In 1994-95 the financial cost of treatment for both direct and indirect ill health effects of diabetes was estimated at $4 billion [2]. This figure is now outdated and will likely increase exponentially as increasing numbers of youth are diagnosed with diabetes. The diagnosis of diabetes in youth and the potential for complications is a particular concern for several reasons. First, the development of complications is associated with duration of disease. Youth are thus at risk by virtue of the potential for many years of life with diabetes. Second, there is evidence that complications may be more aggressive in those diagnosed within the pediatric years [4]. Anecdotal data from Manitoba supports this as we have seen several individuals with end-stage complications in their early 20's. We have seen one individual with end-stage renal disease and blindness who died from cardiovascular complications at age 24.

The costs associated with diabetes are of course not just financial, though these may be the easiest to estimate. The development of end-stage renal disease for example, may necessitate a move to a major medical center. Such a move is disruptive to the family and may also cause significant disruption to the community and to the community structure and support systems. This may result in a loss of culture and tradition. The value of these losses are impossible to quantitate but may have a wide impact on the health and well being of individuals and communities.

Lifestyle interventions are the mainstay of treatment and prevention of type 2 diabetes. Access to healthy dietary options and opportunities for physical activity are critical. There are however, barriers to this within many remote communities. Healthy food options, especially fresh produce, are costly. Many communities have single food

suppliers who have a monopoly on price and choices available. Practical opportunities for increased physical activity in a harsh environment may be limited. Recreational facilities require financial resources for their establishment. The high rates of poverty within many of these communities may thus have a direct impact on diabetes prevention and management.

In 1991, Canada ratified the United Nations Convention on the Rights of the Child (1989) [5]. As a signatory, the Canadian government made a commitment to progress to full implementation of the articles of the convention. Article 24 of the convention provides for the provision of the highest attainable standard of health for all children. Article 27 recognizes the right of all children to a standard of living adequate for their physical, mental, spiritual, moral and social development. Canada has been criticized for failing to meet these standards particularly within the aboriginal population.

The National Forum on Health in their final report identifies aboriginal children and youth as a population at risk and in particular identifies the issue of poverty as a priority [6]. The Canadian Institute of Child Health in their recent report on the health of Canada's children also identifies child poverty as a concern and urges the federal government to invest in long term income supports and services for children and youth given the current growing federal surplus [7].

An understanding of the issues surrounding the epidemiology, diagnosis, etiology, complications and treatment of type 2 diabetes in children and youth is necessary to improve treatment and to develop successful prevention strategies. Type 2 diabetes in youth must be a research priority. Equally, attention must be paid to societal factors such

as poverty that may have profound impact on the prevention and management of this disease.

Programs focusing on lifestyle interventions to modify diabetes risk factors have not been met with much success [8]. It is likely that for such programs to be successful it will require the marriage of scientific rigor with community involvement. For this to happen, Aboriginal communities must be active participants in the design and implementation of strategies to prevent and control this emerging epidemic. This is the challenge that must be met in the 21st century.

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