PROINSULIN AND CHANGES IN GLUCOSE TOLERANCE IN
A NATIVE CANADIAN COMMUNITY EXPERIENCING AN
EPIDEMIC OF TYPE 2 DIABETES MELLITUS

by

Anthony James Gordon Hanley

A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy
Graduate Department of Community Health
University of Toronto

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Abstract

Title: Proinsulin and Changes in Glucose Tolerance in a Native Canadian Community Experiencing an Epidemic Type 2 Diabetes Mellitus

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The objectives of this thesis were to (1) identify the distribution and determinants of proinsulin concentration in a Native Canadian population with high rates of obesity and glucose intolerance, and (2) assess the relationship between metabolic and lifestyle factors and change in glucose tolerance over a period of 4 years among individuals from this population who were at high risk for diabetes at baseline. High-risk subjects were defined as those with impaired glucose tolerance (IGT) or normal glucose tolerance (NGT) with an elevated post-challenge glucose concentration (≥7.0 mmol/l). The research was carried out using data from the cross-sectional (1993-1995) and prospective (1998) phases of a population-based study conducted in partnership with an isolated First Nation community in northern Ontario. In each phase, samples for glucose, insulin, C-peptide, lipids and proinsulin were drawn after an overnight fast, and percent (%) body fat, waist circumference and blood pressure were measured. Diabetes and IGT were diagnosed using standard criteria after a 75g oral glucose tolerance test. Information regarding parity and lifestyle risk factors was collected using standardized questionnaires.

After adjustment for covariates including C-peptide, proinsulin concentration was significantly elevated in subjects with both diabetes and IGT compared to those with NGT, and proinsulin increased across the spectrum of NGT. Waist circumference and percent body fat were significantly positively associated with proinsulin concentration. In addition, both baseline and change in waist circumference were positively associated with follow-up concentrations and change
in proinsulin. Nulliparity was associated with diabetes risk as well as insulin resistance and increased proinsulin concentration. Proinsulin showed significant associations with concurrent concentrations of lipids in subjects with NGT. Further, elevated triglyceride and reduced high-density lipoprotein cholesterol concentrations were prospectively associated with follow-up and change in proinsulin concentration. Cigarette smoking, change in proinsulin and change in insulin resistance were prospectively associated with deterioration to diabetes.

These results demonstrate that (1) proinsulin is an important indicator of the stage of diabetes pathophysiology (2) proinsulin is associated with modifiable metabolic and lifestyle factors that may be indicative of elevated free fatty acid concentrations, and (3) smoking and changes in beta cell function and insulin resistance are associated with diabetes risk.
For my family - Jane, Bill and Ed;
and for Christine.
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Chapter 1

Background and Literature Review

1.1 INTRODUCTION

Type 2 diabetes mellitus (Type 2 DM) is a public health problem of increasingly serious proportions. It has been estimated that 100 million people currently suffer from this condition across the globe, and that by the year 2010 this number will increase to 215 million [McCarty and Zimmett 1994]. In addition, an increasing prevalence of obesity, the most important risk factor for type 2 DM identified to date, has been documented in both adults and children [Heini and Weinsier 1997, Troiano et al. 1995]. This development is likely to further increase the burden of diabetes.

Type 2 DM exacts its toll on the health of communities primarily through the induction of a number of late stage micro- and macro-vascular complications. In the U.S. it is the leading cause of lower extremity amputations and new cases of adult blindness [Reiber et al. 1995, Klein and Klein 1995], and accounts for approximately 35% of incident cases of end stage renal disease [Nelson et al. 1995]. Individuals with diabetes are at markedly increased risk for cardiovascular morbidity (CVD). Recent research has demonstrated that the risk of a first myocardial infarction (MI) among subjects with diabetes approximates that for re-infarction among non-diabetic individuals who have had a previous MI [Haffner et al. 1998]. The economic costs of type 2 DM are enormous: in Canada, for example, they were estimated to be greater than $400 million in 1997 [Birmingham et al. 1999]. Finally, despite the clearly demonstrated underestimation of mortality attributable to diabetes [Andresen et al. 1993], this disease is among the most common causes of death for men and women in the majority of developed nations [Zimmett et al. 1997].
One distinctive characteristic of the epidemiology of type 2 DM is the wide geographic and ethno-cultural variation in prevalence. While some populations have very low rates of diabetes, there are others for which the disease is remarkably burdensome [King and Rewers 1993]. Over the past decade it has become clear that First Nations communities in Canada experience diabetes prevalence rates that are among the highest in the world [Montour and Macaulay 1985, Brassard et al. 1993, Delisle et al. 1993, Harris et al. 1997]. There is additional evidence to suggest that this pattern of high disease frequency is a relatively recent phenomenon, with increases in prevalence of 45% between 1985 and 1994 among the aboriginal population of the Sioux Lookout Zone in Northwestern Ontario [Fox et al. 1994]. Further, the onset of diabetes among Native Canadians occurs at a much younger age than in most other populations [Harris et al. 1997], and pediatric diagnosis of type 2 diabetes is becoming more common [Dean et al. 1992, Harris et al. 1996]. The combination of both the advancing age of this relatively young population and the progression of a disease process that may still be in its early stages in most individuals will pose an extremely serious challenge to health systems in Canada in the coming years.

In this context, the development of strategies for the primary and secondary prevention of type 2 DM is of substantial public health importance [WHO 1994]. Epidemiologic research has the capacity to inform public health policy through the identification of factors that are involved in the evolution of disease. However, despite some promising advances in the epidemiology of type 2 DM over the past decade [Rewers and Hamman 1995], major gaps remain in the understanding of the etiology of this condition. The picture is even less complete regarding diabetes risk factors among Native Canadians.

Two relatively recent developments offer the opportunity to enhance the understanding of the causes and correlates of type 2 DM. First, there is increasing consensus that circulating concentrations of proinsulin, the physiologic precursor to insulin, represent a reliable surrogate indicator of pancreatic beta-cell dysfunction [Rhodes and Alarcon 1994, Kahn and Halban 1997], which is an important predictor of diabetes. Delineating the factors that are related to variation in proinsulin concentration may help to illuminate the mechanisms involved in beta cell injury and decompensation in the intermediate and late stages of diabetes pathogenesis. Second, the widespread application during the past 2 decades of the World Health Organization (WHO) diagnostic criteria for diabetes [WHO 1985] has confirmed that a diagnosis of impaired glucose tolerance (IGT) carries a significant increased risk for the subsequent development of diabetes [Alberti 1996]. Identification of the metabolic and lifestyle factors that contribute to improvement
or deterioration in glucose tolerance among nondiabetic subjects at high risk for diabetes (including those with IGT) will provide a valuable contribution to the understanding of the natural history of the disease.

This thesis will attempt to contribute to the knowledge-base regarding the epidemiology and natural history of type 2 DM among Native Canadians by addressing 2 research questions related to these recent developments. First, what are the distribution and determinants of elevated proinsulin concentration, an indicator of beta cell dysfunction, and how is proinsulin related to variation in cardiovascular disease risk factors? Second, what metabolic and lifestyle factors determine improvement or deterioration in glucose tolerance among nondiabetic subjects who are at high risk for the development of type 2 DM?

1.2 BACKGROUND AND REVIEW OF THE LITERATURE

1.2.1 BRIEF OVERVIEW OF THE EPIDEMIOLOGY AND NATURAL HISTORY OF TYPE 2 DIABETES MELLITUS

Comprehensive reviews of the epidemiology and natural history of type 2 DM are available in previous publications [Jarrett 1989, Zimmett 1992, 1995, Rewers and Hamman 1995] and only a very concise overview will be presented here.

1.2.1.a Definition, Classification and Diagnosis

Diabetes mellitus (DM) is a heterogeneous group of disorders characterized by elevated blood glucose concentrations and disturbances in carbohydrate, fat and protein metabolism [WHO 1994]. There are a number of types of DM, including type 1 (insulin dependent), type 2 (non-insulin-dependent), gestational, and “other specific types”, which include genetic syndromes and diabetes induced by drugs and infections [American Diabetes Association (ADA) 1997]. This classification is based on differences between the categories in etiology, natural history, and clinical features, including presentation and treatment modality. Type 2 DM represents over 85% of the total DM morbidity in most populations. Since 1980, DM has been diagnosed for research purposes using blood glucose concentrations in both the fasting state and 2 hours after a 75g oral glucose tolerance test (OGTT) [WHO 1980]. These criteria arose from prospective data that documented
high risk of subsequent diabetes-related complications among individuals with post-challenge (PC) blood glucose concentrations above the 2-hour diagnostic level [WHO 1985, Alberti 1996b]. This system also allows for the diagnosis of IGT, which captures the grey area between normal glucose tolerance and diabetes; IGT is reviewed in detail in Section 1.2.4.

1.2.1.b Prevalence, Incidence and Ethnocultural / Geographic Distribution

Documenting the prevalence of type 2 DM is a major challenge given the substantial proportion of asymptomatic and/or undiagnosed cases of this condition. The Second National Health and Nutrition Examination Survey (NHANES II, 1976-1980) reported rates of undiagnosed diabetes that were as high or higher than rates of previously diagnosed diabetes in both black and white members of the US population [Jarrett 1989]. However, in research settings there has been widespread application over the past 2 decades of the WHO diagnostic protocol for type 2 DM (considered the "gold standard" method), which has allowed for global comparisons of age-standardized prevalence rates.

The international variation in the prevalence of Type 2 DM is enormous [King and Rewers 1993]. Age-adjusted prevalence rates are very low (under 1%) in rural Melanesians of Papua New Guinea, rural Bantus of Tanzania and Mapuche Indians of Chile. On the other hand, age-adjusted prevalence exceeds 40% among Nauruans and Pima Indians. Diabetes patterns among migrants and recently urbanized groups are notable. For example, the age-adjusted prevalence rate among Chinese in China is under 2% for both sexes, whereas it is greater that 10% and 15% for females and males, respectively, among Chinese in Mauritius. Similarly, prevalence of type 2 DM among migrant Asian Indians in South Africa, Tanzania, Mauritius and Fiji is notably higher than among rural Asian Indians in India [King and Rewers 1993].

Less information is available regarding the incidence of type 2 DM, although studies have been conducted in a limited number of jurisdictions [Knowler et al. 1981, Haffner et al. 1991, Charles et al. 1991, Dowse 1996]. Incidence rates range from under 5 per 1000 person years (PY) in Papua New Guinea Highlanders and white Americans to approximately 30 per 1000 (PY) among the Pima. Patterns of international variation tend to reflect those seen in the prevalence data.

Prevalence of Diabetes among Native Canadians: Evidence is emerging which suggests that Native Canadian communities are experiencing an extremely high prevalence of diabetes. Earlier studies that relied on chart reviews, and thus clinical diagnoses of diabetes, indicated that age
standardized prevalence rates ranged from 6.6-14.7% [Evers et al. 1987, Brassard et al. 1993]. More recent studies that used WHO diagnostic criteria reported age standardized rates of 9-14% among the Algonquin of northern Quebec [Delisle et al. 1993], and 26.1% among the Oji-Cree of northwestern Ontario [Harris et al. 1997]. When rates from the later study were standardized and truncated using the methods of King and Rewers [1993], the resulting diabetes prevalence rate of 36.4% ranked as the 3rd highest reported to date.

1.2.1.c Risk Factors

*Obesity, Insulin Resistance, and Related Issues:* Obesity is the strongest and most uniformly implicated risk factor for Type 2 DM [Jarrett 1989, Barrett-Connor 1989]. Cross-sectional analyses have consistently demonstrated significant relationships between both total body and abdominal obesity and the prevalence of NIDDM [Jarrett 1989, Zimmett 1992], and this association has been confirmed in a large number of population-based prospective studies [see, for example, Haffner et al. 1990, Cassano et al. 1992, Chan et al. 1994, Carey et al. 1997]. Recent evidence has also indicated that both increases in weight and the duration of obesity are associated with increased risk for the development of diabetes [Chan et al. 1994, Wannamethee and Shaper 1999, Brancati et al. 1999, Sakurai et al. 1999].

Despite these consistent data implicating obesity in diabetes risk, the exact mechanism through which excess adiposity exerts its harmful effects is unknown. There is a large body of evidence which suggests that obesity-induced insulin resistance (IR) is probably playing an important role [Reaven 1988]. IR has been defined as the cellular resistance to the action of insulin, or an insufficient metabolic response to a given plasma concentration of insulin [Reaven 1988, DeFronzo and Ferrannini 1991]. Detailed metabolic studies have documented IR in subjects with type 2 DM, and IGT, as well as in those with normal glucose tolerance (NGT) who have abdominal obesity and/or a positive family history of DM [Zimmett 1993, Haffner et al. 1988]. In addition, large prospective studies have reported that IR is a significant independent risk factor for the subsequent development of type 2 DM [Skarfors et al. 1991, Haffner et al. 1992, Perry et al. 1999].

Recent developments have raised questions about the "primacy" of insulin resistance in the natural history of type 2 DM, however. Several studies have reported that beta cell dysfunction occurs earlier in the disease course than originally thought, and may either precede or exacerbate existing IR [Ferrannini 1998, Gerich 1998]. Related to this issue is the recognition that conventional radioimmunoassays used to measure insulin concentrations display substantial cross-reactivity with
proinsulin, the physiologic precursor of insulin and a surrogate indicator of beta cell dysfunction; this topic is reviewed in detail in Section 1.2.2. In addition, other metabolic characteristics of obesity have been identified, including elevated circulating levels of leptin and TNF-α [Considine et al. 1996, Zinman et al. 1999]. The importance of these molecules in the pathogenesis of type 2 DM remains to be elucidated.

**Physical Activity, Diet, Smoking and Alcohol:** A growing body of evidence indicates an important role for physical activity in the prevention of Type 2 DM [Rewers and Hamman 1995]. Early ecologic and cross-sectional associations suggesting reduced risk for physically active populations and individuals have been supported by the findings of prospective studies. This literature is reviewed in detail elsewhere [Rewers and Hamman 1995]. Physical activity has also been associated with improved insulin sensitivity in a cohort study of the elderly [Feskens et al. 1994], and experimental studies have demonstrated that physical activity and exercise training improve insulin sensitivity, glucose tolerance and carbohydrate metabolism [Ronnemaa et al. 1986, Regensteiner et al. 1995, Perseghin et al. 1996].

For many years ecological studies have indicated that dietary intake might be associated with risk of diabetes, although findings from analytic epidemiologic studies have yet to identify specific unequivocal dietary risk factors [Rewers and Hamman 1995]. Positive cross-sectional associations have been demonstrated between diabetes risk and diets high fat and low in carbohydrate and fibre [Rewers and Hamman 1995, Wolever et al. 1997]. This evidence is in line with the findings of short term dietary trials reporting the reduction of insulin levels after increased carbohydrate and reduced saturated fat consumption [see, for e.g. Fugakawa 1990, Swinburn 1991]. Although results from earlier population-based prospective studies were much less consistent regarding the role of these nutrients [Rewers and Hamman 1995], recent evidence from three large cohort studies supports the hypothesis that cereal fibre reduces the risk of both diabetes and hyperinsulinaemia [Salmeron et al. 1997 a, b; Ludwig et al. 1999]. In addition, Salmeron et al. [1997 a, b] found that diets characterized by high glycaemic load were positively associated with risk of diabetes. Protective effects have been reported for fish intake [Feskens et al. 1991, 1995] and for the consumption of a number of micronutrients, including calcium, magnesium and vitamin C [Colditz et al. 1992], although these associations are based on only a limited number of studies. Finally, in a recent prospective study, low baseline lipid-standardized vitamin E concentration was associated with a significant increased risk of diabetes development after 4 years of follow-up [Salonen et al. 1995]. This result and findings from in vitro studies demonstrating the susceptibility of beta cells to free radical damage
[Burkart et al. 1995] raises the possibility of a role for oxidative stress in diabetes etiology [Guigliano et al. 1995].

There is inconsistency in the literature regarding the role of cigarette smoking in the etiology of glucose intolerance. While several early studies reported no relationship [Butler et al. 1982, Wilson et al. 1986, Perry et al. 1995], recently four more detailed investigations have found significant positive associations [Feskens and Kromhout 1989, Rimm et al. 1993, 1995, Kawakami et al. 1997]. In addition, Rimm and coworkers [1993, 1995] have documented dose-response relationships with smoking in two U.S. populations. Smoking may increase the risk for diabetes through a number of mechanisms. Several studies have reported that smoking induces insulin resistance [Faccini et al. 1992, Atvall et al. 1993, Zavaroni et al. 1994], although others have not supported this finding [Godsland et al. 1992, Nilsson et al. 1995, Clausen et al. 1996], and it has been suggested that the opposite might be true [Wareham et al. 1996]. It is also possible that the chemicals in tobacco smoke may damage insulin producing beta cells through the action of free radicals [Pryor 1997].

The results of previous studies of alcohol consumption and diabetes development have been mixed, with the suggestion of both detrimental [Holbrook et al. 1990, Tsumura et al. 1999] and protective [Perry et al. 1995, Rimm et al. 1995] relationships, and in some cases, no effect [Hodge et al. 1993]. While moderate alcohol consumption has been shown to improve insulin sensitivity [Facchini et al. 1994], a recent rodent model study has demonstrated that elevated plasma levels of two diols associated with alcoholism can reduce whole body glucose uptake [Xu et al. 1998]. It is conceivable, then, that frequent and/or heavy alcohol consumption is harmful to glucose metabolism.

**Parity:** The relationship between parity and risk of diabetes has been examined in a large number of published studies [Boyko et al. 1990]. As pointed out by others [Boyko et al. 1990, Manson et al. 1992], the reported associations between parity and diabetes in the majority of these previous investigations were not adjusted for age or body adiposity, both of which are likely to be important confounding factors in this relationship. In the nine studies which have presented results adjusted for these factors, the findings have been highly inconsistent: three reported a positive relationship [O'Sullivan and Gordon 1966, Martin et al. 1984, Kritz-Silverstein et al. 1989], five found no effect [Boyko et al. 1990, Manson et al. 1992, Collins et al. 1991, Alderman et al. 1993, Cowan et al. 1997],
and one demonstrated a protective association with parity [Charles et al. 1997]. The role of childbearing in the pathogenesis of diabetes thus remains controversial.

**Genetic Factors:** Familial aggregation and twin studies have long implicated a role for genetic susceptibility in type 2 DM [Elbein et al. 1994]. There is currently an enormous amount of research activity targeted at elucidating the genetic determinants of this condition [Gosh and Schork 1996]. While a number of promising results of linkage analyses and candidate gene association studies have been reported, genes explaining large proportions of the global diabetes burden have yet to be identified. Indeed, Ghosh and Schork [1996] have pointed out that over 250 candidate genes have been tested for linkage or association, “but none have shown consistently significant results across different study populations”. It appears likely that type 2 DM is determined by a number of genes exerting small effects in the presence of environmental factors [Hegele 1999].

**Emerging issues:** Several issues in the epidemiology of type 2 DM have emerged over the past decade. The most controversial is the “thrifty phenotype hypothesis”, which suggests that risk of glucose intolerance and related disorders is increased among low birth weight cohorts [Hales and Barker 1992]. Low birth weight in this case is interpreted to reflect nutritional deficiencies in utero which result in abnormal islet cell structure and vascularization.

**Risk Factors among Native Canadians:** Very little is known about the etiology of type 2 DM among Native Canadians. Young et al. [1990] reported that age, triglyceride level, parental diabetes history, BMI, and waist-to-hip ratio (WHR) were associated with increased risk of diabetes in northern Ontario and Manitoba. In the population which is the focus of this investigation, obesity, and diets low in fibre and high in protein and added fat were associated with increased diabetes risk [Harris et al. 1997, Wolever et al, 1997, Gittelsohn et al. 1998]. Hegele et al. [1999] have reported that the hepatic nuclear factor-1α G319S variant is strongly associated with early onset type 2 diabetes in Sandy Lake, the population which is the focus of the studies outlined below.

1.2.1.d **Models of Diabetes Natural History**

Diagramatic models of the etiology and natural history of type 2 DM are useful in synthesizing existing information, as well as in focussing attention on etiologic factors that operate at different stages of the evolution of disease. Several of these models have been proposed over the past decade [Rewers and Hamman 1995]. In 1991, Saad and colleagues [1991] presented a two step model for the development of type 2 DM based on available evidence from studies of the Pima
Indians (Figure 1). Step 1 of this model, the transition from NGT to IGT, was thought to be largely related to insulin resistance brought on by obesity. Beta cells in this phase are healthy and able to compensate for insulin resistance by secreting larger amounts of insulin, thus maintaining glucose levels in the non-diabetic range. Step 2, on the other hand, is characterized by the inability of the beta cells to compensate for insulin resistance (beta cell failure or decompensation) which results in increases in glucose levels and the development of diabetes.

As mentioned above, the primacy of insulin resistance in the etiology of type 2 DM has been questioned [Ferrannini 1998]. There is emerging evidence which suggests that beta cell dysfunction and insulin resistance not only coexist in the prediabetic state, but that they exacerbate each other through multiple feedback mechanisms. This notion is reflected in Figure 2, which has been adapted from models presented by Ferrannini [1998] and Rewers and Hamman [1995].

The chronology of beta cell dysfunction and its role in diabetes risk can be further explored by examining prospective associations between proinsulin concentration and diabetes risk, as well as by delineating the factors that are related to variation in proinsulin concentration among non-diabetic subjects. In addition, identification of the metabolic and lifestyle factors that contribute to improvement or deterioration in glucose tolerance among nondiabetic subjects at high risk for diabetes (including those with IGT) will provide a valuable contribution to the understanding of the evolution of the disease. The literature regarding both proinsulin concentration and glycaemic progression among high-risk non-diabetic subjects is reviewed in greater detail in the following sections.
FIGURE 1. Saad et al.'s two-step model for the natural history of type 2 diabetes.

STEP 1: Insulin Resistance

Contributing factors:
- Genotype
- Other Factors
  - Age
  - Obesity
  - Physical inactivity

STEP 2: Beta Cell Defect

Contributing factors:
- Genotype
- Age
- Declining beta cell mass
- Glucose toxicity
- Beta cell exhaustion

FIGURE 2. Early beta-cell dysfunction model for the natural history of type 2 diabetes.

1.2.2 CIRCULATING PROINSULIN CONCENTRATION: BIOLOGY, ASSOCIATED FACTORS AND RELATIONSHIP WITH DIABETES AND CARDIOVASCULAR DISEASE

1.2.2.a Introduction and Overview of Proinsulin Biology

The molecular physiology of proinsulin processing has been reviewed in previous publications [Porte and Kahn 1989, Halban 1991, 1994 Rhodes and Alarcon 1994], and a detailed presentation is beyond the scope of this thesis. The sequence of events is depicted in Figures 3-5 (adapted from Halban 1991), and is briefly summarized below. The initial precursor molecule, preproinsulin (Figure 3), is converted to proinsulin by a signal peptidase “shortly after (or during) translocation into the lumen of the rough endoplasmic reticulum” [Halban 1991, 1994]. After subsequent transportation to the trans-cisternae of the Golgi complex, there are two possible secretory pathways (Figure 4). In healthy individuals, more than 99% of proinsulin is directed toward the regulated secretory pathway, in which conversion of proinsulin to insulin and C-peptide occurs within secretory granules. In this process, the proinsulin molecule is sequentially cleaved by endoproteases at two possible sites (Figure 5), which results in the split proinsulin conversion intermediates des 31, 32 proinsulin and des 64, 65 proinsulin. Insulin and C-peptide are generated by further endoproteolytic cleavage of these split products. The contents of the secretory granules are then “discharged by exocytosis in response to an appropriate stimulus” [Halban 1991, 1994]. The alternative route, known as the constitutive secretory pathway, is characterized by “rapid transfer of products from the Golgi complex to the plasma membrane for immediate release, with ... little occasion for prohormone conversion” [Halban 1991, 1994].

The suspicion that proinsulin may be important in chronic disease etiology has emerged relatively recently, and is related in part to the discovery that the conventional radioimmunoassays used for many years to determine circulating insulin concentrations display a high degree of cross-reactivity with proinsulin [Temple et al. 1989]. Thus, the interpretation of results of studies that have used conventional (non-specific) assays to document associations between “hyperinsulinaemia” and chronic diseases (including Type 2 DM, ischaemic heart disease, dyslipidaemia, hypertension and cancers of the colon and breast) is complicated by the limited current knowledge of the relative importance of proinsulin and specific insulin concentrations in these relationships.
**FIGURE 3.** The linear arrangement of the constituent peptides of preproinsulin (Source: Halban PA. Structural domains and molecular lifestyles of insulin and its precursors in the pancreatic beta cell. Diabetologia; 1991;34:767-778).

**FIGURE 4.** The insulin biosynthetic pathway, showing both the regulated and constitutive pathways. RER, rough endoplasmic reticulum.

**FIGURE 5.** Processing of insulin precursors.
1. Conversion of preproinsulin to proinsulin.
2. Conversion of proinsulin to two possible split proinsulin intermediates.
3. Further conversion of split proinsulin, resulting in mature insulin and C-peptide.

1.2.2.b Proinsulin and Glucose Tolerance Status

It has been suggested that raised circulating proinsulin concentration may be an indicator of \( \beta \)-cell dysfunction [Rhodes and Alarcon 1994], an hypothesis that was originally based on the observation of elevated proinsulin levels and proinsulin/insulin ratios (P/I) in individuals with Type 2 DM and gestational DM (Table 1). While elevated proinsulin and P/I have been reported for other Type 2 DM-related phenotypes such as IGT, the evidence for hyperproinsulinaemia early in the natural history of diabetes is much less consistent (reviewed in detail below).

Two general mechanisms have been proposed to explain these elevated circulating proinsulin concentrations in Type 2 DM [Porte and Kahn 1989, Rhodes and Alarcon 1994, Shiriashi et al. 1991, Kahn and Halban 1997]. It is possible that the metabolic clearance rate of proinsulin in the diabetic state is altered, although the recent work of Kahn and Halban [1997] suggests that this is unlikely. The more widely accepted mechanism involves the secretion of larger amounts of proinsulin relative to insulin in those with diabetes. There are several reasons why this might occur [Yoshioka et al. 1988, Kahn and Halban 1997]: (1) the beta cells are hyper-stimulated in the face of the insulin resistance of Type 2 DM, and thus are forced to mobilize proinsulin-rich immature secretory granules; (2) conversion of proinsulin to insulin may be impaired due to metabolic alterations; and (3) a primary islet lesion is responsible for impaired proinsulin processing [Porte and Kahn 1989]. Recent *in vitro* studies have documented increased proinsulin release by human \( \beta \)-cells after chronic exposure to glucose [Usac et al. 1998, Bertuzzi et al. 1999, Zambre et al. 1998, Hostens et al. 1999, Bjorklund et al. 1999]. The glucose concentrations used in these experiments were high (\( \geq 20 \text{ mmol/l} \) in most cases), and the findings lend support to the notion of that chronic hyperglycaemia in diabetic subjects is toxic to the beta cell. However, these results do not appear to shed light on the mechanisms responsible for elevated proinsulin concentration in states characterized by lower glucose levels (for example, IGT).

A number of publications have reported proinsulin levels in human subjects at various stages of glucose intolerance (Table 1). While many of these studies have employed small, clinical samples, several groups of investigators have examined this issue in larger, population-based studies [Saad et al. 1990, Haffner et al. 1994, 1995, Birkeland et al. 1994, Kahn et al. 1995, Iochida 1996, Snehalatha et al. 1998]. Participants were generally middle aged or older (mean ages 45+), although Yoshioka et al. [1988], Saad et al. [1990], and Shiriashi et al. [1991] examined younger subjects (mean ages 20-30s).
TABLE 1. Summary of published studies examining circulating proinsulin concentrations among human subjects at various stages of glucose tolerance.

<table>
<thead>
<tr>
<th>First author, date;</th>
<th>Ethnic group, nature of sample</th>
<th>Sample size, glucose tolerance status (male/female)</th>
<th>Age range, or mean ± SD</th>
<th>Proinsulin assay; components measured;</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duckworth 1972</td>
<td>Information not provided</td>
<td>NGT=55 &quot;borderline&quot;=8 DM=32</td>
<td>21-60 20-60 21-60</td>
<td>Insulin-specific protease and immunoassay; total proinsulin-like molecules</td>
<td>- higher fst PI in obese DM vs NGT (p&lt;0.05, t-test)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DM=26/33</td>
<td></td>
<td></td>
<td>- higher proinsulin and PI/IRI response in DM (compared to NGT)</td>
</tr>
<tr>
<td>Mako 1977</td>
<td>Information not provided</td>
<td>NGT=23/21 DM=26/33</td>
<td>21-65 21-77</td>
<td>RIA (Starr 1974); total proinsulin-like molecules</td>
<td>- positive relationship between fst glucose and fst PI (r=0.47) and PI/IRI (r=0.46), both p&lt;0.001; no categorical statistical comparisons</td>
</tr>
<tr>
<td>Ward 1987</td>
<td>Clinical sample</td>
<td>NGT=23/5 DM=21/1</td>
<td>52±4 58±2</td>
<td>In-house RIA (Ward 1986); total proinsulin-like molecules</td>
<td>- higher fst PI/IRI in DM vs NGT (32 vs 15%, p&lt;0.001)</td>
</tr>
<tr>
<td>Deacon 1988</td>
<td>Clinical sample</td>
<td>NGT=13/4 DM=11/5</td>
<td>~52 58±13</td>
<td>RIA (Deacon and Conlon 1985)</td>
<td>- higher fst PI in OHA-treated DM vs NGT (p&lt;0.05)</td>
</tr>
<tr>
<td>Yoshioka 1988</td>
<td>Japanese*, clinical sample</td>
<td>NGT=14/11 IGT=16/5 DM=22/18</td>
<td>23-66 40-71 23-76</td>
<td>In-house RIA; Total proinsulin-like molecules</td>
<td>- higher fst PI in IGT (p&lt;0.05) and DM (p&lt;0.001) vs NGT during 100g OGTT; - higher fst PI/I in DM vs NGT (p&lt;0.05) - higher ΣP/I and PI/I in IGT vs control (p&lt;0.01) - steeper Σinsulin - Σproinsulin slope in DM group (no statistical test)</td>
</tr>
<tr>
<td>Temple 1989</td>
<td>Clinical sample</td>
<td>NGT=8 NGT=8 IGT=8 DM=20 DM=29</td>
<td>54±8 51±9 59±11 52±12</td>
<td>RIA (Sobey &quot;in press&quot;); can distinguish split proinsulin, but totals used in analysis</td>
<td>- higher fst intact PI and fst PI/RI in DM vs NGT in both obese and non-obese (all p&lt;0.01)</td>
</tr>
<tr>
<td>Saad 1990</td>
<td>Pima Indians, clinical sample</td>
<td>NGT=31/46 IGT=11/35 DM=21/25</td>
<td>35 34 39</td>
<td>in-house RIA (Ward 1986); total proinsulin-like molecules</td>
<td>- higher fst PI in IGT and DM groups (vs NGT: both p&lt;0.001) - higher fst PI/IRI in DM group only (vs NGT: p&lt;0.001) - higher fst PI and fst PI/IRI in DM (vs IGT, both p&lt;0.001)</td>
</tr>
<tr>
<td>First author, date;</td>
<td>Ethnic group, nature of sample</td>
<td>Sample size, glucose tolerance status (male/female)</td>
<td>Age range, or mean ± SD</td>
<td>Proinsulin assay; components measured;</td>
<td>Main results</td>
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</tbody>
</table>
| Shiraishi 1991      | Japanese*, clinical sample      | NGT<sup>nm</sup>=5/10 NGT<sup> obese</sup>=7/11 IGT<sup>nm</sup>=24/15 IGT<sup> obese</sup>=26/13 DM<sup>nm</sup>=14/6 DM<sup> obese</sup>=12/4 | 41±16 45±11 45±16 48±12 58±12 53±17 | in-house RIA (Yoshioka 1988); total proinsulin-like molecules | - higher fst PI and fst PI/I in "IGT" and DM groups, stratified by obesity (vs NGT, all p<0.001)  
- higher fst PI and fst PI/I in DM (vs "IGT", all p<0.01)  
- "IGT"="borderline DM" - definition less strict than WHO |
| Williams 1991       | British (ethnicity unknown), general practice registry | NGT=51 IGT=7 | 50-64 | in-house RIA (?) (Sobey 1989); specific proinsulin & split products | - higher fst PI/IRI in IGT vs NGT (p=0.04);  
- those with IGT significantly shorter than NGT |
| Krentz 1993         | British (ethnicity unknown)     | NGT=8 IGT=8 (non-obese) | 48±4 51±5 | Sobey 1989 (specific and split proinsulin) | - higher intact and split proinsulin levels in IGTs vs. NGTs (p=0.005, p=0.02, respectively) |
| Reaven 1993         | Clinical sample                 | NGT=5/9 IGT=5/7 DM=13/3 | 39-54 45-59 51-61 | in-house ELISA (Hartling 1986); total proinsulin-like molecules | - higher full day PI in IGT and DM groups (vs NGT: all p<0.002)  
- higher full day PI in DM (vs IGT: p<0.002)  
- no assessment of PI/IRI |
| Birkeland 1994      | Finnish, population sample      | NGT<sup>b</sup>=21/10 NGT<sup>male</sup>=20/14 IGT=11/15 DM=6/24 | 64.5±2 57.2±3 50.2±3 48.7±2 | IMFA (Novo-Nordisk); total proinsulin-like molecules | - higher PI<sub>ave</sub> and fst PI/I in DM group only (p-val not indicated) |
| Haffner 1994        | Mexican-American, Non-HispanicWhite pop'n sample | NGT=64/92 IGT=10/40 NGT=52/10 IGT=43/18 | 48±1 a 52±2 b 50±5 c 52±4 d | RIA (Bowsher 1992); total proinsulin-like molecules | - higher fst PI in IGT and DM groups (vs NGT, all p<0.001)  
- higher fst PI/I in IGT and DM (IGT vs NGT: p=0.048, DM vs NGT p<0.001)  
- higher fst PI and fst PI/I in DM (vs IGT: both p<0.001) |
| Kahn 1995           | Japanese-American; Pop'n sample | NGT=58 IGT=55 DM=57 (all male) | 45-74 | RIA (Ward 1986); total proinsulin-like molecules | - higher fst PI and fst PI/IRI in DM (vs NGT and IGT, both p<0.05)  
- association between fst glucose and fst PI/IRI in DM (r=0.43, p<0.001) |
<table>
<thead>
<tr>
<th>First author, date;</th>
<th>Ethnic group, nature of sample</th>
<th>Sample size, glucose tolerance status (male/female)</th>
<th>Age range, or mean ± SD</th>
<th>Proinsulin assay; components measured;</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haffner 1995</td>
<td>Mexican-American, pop’n sample</td>
<td>NGT+IGT=166/411</td>
<td>48.2-51.2 e</td>
<td>RIA (Bowsher 1992); total proinsulin-like molecules</td>
<td>- higher fst PI and fst P/I in IGT vs NGT (all p&lt;0.02)</td>
</tr>
<tr>
<td>Iochida 1996</td>
<td>Japanese-Brazilian, pop’n sample</td>
<td>NGT=166/185; IGT=37/49; DM=60/33</td>
<td>40-79</td>
<td>IMFA (Dalbosco 1994); total proinsulin-like molecules</td>
<td>- higher fst PI in IGT and DM groups (vs NGT, all p&lt;0.01) - no assessment of PI/IRI</td>
</tr>
<tr>
<td>Nagi 1996</td>
<td>S. Asians, Whites</td>
<td>NGT=82; IGT=16; NGT=67; IGT=13</td>
<td>35-70</td>
<td>Soby 1989 - specific and split proinsulins</td>
<td>- NGT vs IGT - no difference in whites, borderline sig in Asians (p=0.052) for intact proins. No difference for other PI measures - NGTs - Asians higher intact PI compared to whites</td>
</tr>
<tr>
<td>Wang 1997</td>
<td>Clinical sample</td>
<td>NGT=9/16; IGT=7/14; IGT=6/13</td>
<td>53±2; 56±2; 57±2</td>
<td>RIA kit (Bowsher 1992; Linco); total proinsulin-like molecules</td>
<td>- higher fst PI in IGT group (vs NGT p&lt;0.01), but fst P/I differences =n/s</td>
</tr>
<tr>
<td>Snehala-atha 1998</td>
<td>South Indians (Asian), pop’n based</td>
<td>NGT=122; IGT=29</td>
<td>42.7±1; 49±1.5</td>
<td>RIA kit (Bowsher 1992; Linco); total proinsulin-like molecules</td>
<td>- higher fst and 2h PI in IGT (both p&lt;0.05). No difference for PI/IRI</td>
</tr>
</tbody>
</table>

abbreviations used: NGT = normal glucose tolerance; IGT = impaired glucose tolerance; DM = diabetes mellitus; RIA = radioimmunoassay, ELISA = enzyme-linked immunosorbent assay; PI = total proinsulin like material; IRI = total immunoreactive insulin (i.e. non-specific, including proinsulin); I = specific insulin; OHA = oral hypoglycaemic agent; OGTT = oral glucose tolerance test; IR = insulin resistance/resistant; n/s=not significant; fst= fasting;

a= Mexican American male, b=Non-Hispanic White male, c=Mexican American female, d=Non-Hispanic White female, e=means for subgroups

Both males and females were included in all but one study [Williams et al. 1991], and members of East Asian, South Asian, Native American, northern European, and Mexican American ethnocultural groups are represented. In the majority of the investigations, proinsulin level was determined using radioimmunoassays or immunofluorometric assays that also detected proinsulin split products (especially the major split product des 31, 32 proinsulin) at moderate or high levels, and thus these studies report levels of total proinsulin-like molecules. However, 3 studies (Williams et al. 1991, Krenz et al. 1993, Nagi et al. 1996) employed assays that were able to detect both intact proinsulin and des 31, 32 split proinsulin.

Table 1 presents a summary of results of studies examining proinsulin levels and glucose tolerance status. Proinsulin concentrations and proinsulin-to-insulin (PI/I) ratios were consistently elevated among individuals with Type 2 DM relative to subjects with NGT, and, in some cases, relative to those with IGT [Saad et al. 1990, Shirasashi et al. 1991, Reaven et al. 1993, Haffner et al. 1994]. The role of proinsulin levels in IGT is less clear, however: Caucasians, Mexican Americans and Japanese with this metabolic disorder had elevated proinsulin levels and P/I ratios relative to NGT controls, while Pima Indians, Japanese-Americans and Fins with IGT did not. In addition, Beer and colleagues [1990] reported that, among first degree relatives of individuals with type 2 DM, those who were glucose intolerant based on "achieved" glucose levels (FPG > 9.3 one hour after IV infusion of 5ml of glucose per kilogram of ideal body weight) had higher fasting and "achieved" proinsulin/fasting C-peptide ratios compared to glucose tolerant subjects.

*Discussion and Rationale for the Present Studies:* It is clear that a number of issues regarding the relationship between proinsulin and glucose tolerance require further examination. While proinsulin concentrations are consistently elevated in subjects with type 2 DM (Table 1), controversy exists in the literature regarding the presence of elevated proinsulin among individuals with IGT. In addition, there is limited information regarding factors associated with proinsulin within glucose tolerance categories. Recent evidence suggests that the metabolic profile worsens continuously across the spectrum of nondiabetic glucose intolerance [Meigs et al. 1999], although proinsulin concentration has not been examined in this regard. In addition, there have been relatively few population-based studies of proinsulin distribution and determinants, and only one that has examined proinsulin levels in North American Native people [Saad et al. 1990]. Thus a number of
important research questions can be addressed in the context of the Sandy Lake Health and Diabetes Project (SLHDP).

Specific Thesis Objective (I(a)): To determine whether proinsulin levels are elevated among individuals with glucose tolerance abnormalities, including Type 2 DM and IGT, relative to those with NGT in a Native Canadian population with high rates of glucose intolerance.

1.2.2.c Proinsulin and Adiposity

Scientific evidence is accumulating regarding the role of modifiable factors in insulin resistance and hyperinsulinaemia [Fukagawa et al. 1990, Lovejoy and DiGirolamo 1992, Feskens et al. 1994, Clausen et al. 1996]. Of particular public health concern are relationships with obesity, parity, physical activity/fitness level, dietary intakes of fat and fibre and tobacco use. As outlined above, many of the studies that have reported these associations have used insulin radioimmunoassays that display strong cross-reactivity with proinsulin and its split products. In light of this methodological limitation, and given that obesity, parity, physical activity, dietary intake and smoking affect insulin resistance, it is of interest to determine whether proinsulin levels are associated with similar modifiable environmental factors.

The relationship between proinsulin levels and obesity in human subjects has been assessed in a limited number of papers (Table 2), many of which also examined glucose tolerance status (reviewed above). Several studies used population-based samples, and a fairly broad spectrum of age and ethnicity is represented. Most groups employed body mass index (BMI) as a measure of obesity, either dichotomized using conventional cutoffs or treated as a continuous variable. Only three groups examined abdominal adiposity, using either waist-hip ratio (WHR) [Nagi et al. 1990, Haffner et al. 1993] or intraabdominal fat area (IAF) measured by magnetic resonance imaging [Kahn et al. 1995] as continuous variables.

Table 2 presents a summary of results of studies examining proinsulin levels and obesity. Both total proinsulin-like molecules and specific proinsulin were positively and significantly correlated with BMI in most studies, with coefficients ranging from 0.19 to 0.41. Split proinsulin was measured in 3 studies and was also positively and significantly correlated with BMI (range of correlations: 0.32-0.43). When obesity was treated as a dichotomous variable, individuals classified
as obese had significantly higher proinsulin levels than controls. WHR was a significant positive correlate of total and specific proinsulin (r=0.19-0.35) [Nagi et al. 1990, Haffner et al. 1993, Snehalatha et al. 1998], as was IAF, although this was examined in only one paper (r=0.37) [Kahn et al. 1995].

Findings are much less consistent, however, regarding the relationship between measures of obesity and PI/I ratio. While the majority of studies reported no association (Table 2), a significant inverse correlation with BMI was reported among the Pima Indians, and the PI/I ratio was lower in a group of obese Mexican Americans compared to controls. In addition, WHR was inversely correlated with PI-specific/immuno-reactive insulin (IRI) (r=-0.21, p<0.05) in a population sample of Finns (Table 2).

Recent work by Unger's group has suggested a mechanism whereby excess adiposity might lead to increased proinsulin concentrations [Unger 1995]. The adipocytes of obese individuals release high concentrations of free fatty acids (FFA) [Unger 1995] which, in the short term, causes beta cell hyperplasia and hyperinsulinaemia, but with chronic exposure (and subsequent increase in FFA levels) leads to functional and morphologic changes in beta cells and consequent diabetes [Unger 1995]. This notion has been supported with the documentation of substantial fat deposition in islets of obese Zucker rats, and the demonstration of FFA-induced loss of glucose-stimulated insulin secretion [Lee et al. 1994]. Increased FFA also induce nitric oxide synthase, and Shimabukuro et al. [1997, 1998] have shown that elevated FFA in rat beta cells cause increases in both nitric oxide levels and ceramide-mediated beta cell apoptosis (programmed cell death). The consequent reduction in the number of beta cells may result in elevated proinsulin concentration, in that the rate of secretion by remaining cells is increased, thereby decreasing the intracellular stores and forcing the release of incompletely processed materials [Bollheimer et al. 1998, Furukawa et al. 1999].

**Discussion and Rationale for the Present Studies:** The effects of both total body and regional obesity on proinsulin concentration require further examination. In most of the studies reviewed here, the effect obesity was not the primary focus, and thus analyses of these variables were often lacking in detail. For example, obesity was often defined as a simple dichotomy of BMI. It would
TABLE 2. Summary of published studies examining the association of obesity and circulating proinsulin concentrations.

<table>
<thead>
<tr>
<th>First author, date;</th>
<th>Ethnic group, nature of sample</th>
<th>Sample size, glucose tolerance status (male/female)</th>
<th>Age range, or mean ± SD</th>
<th>Proinsulin assay; components measured;</th>
<th>Definition of obesity</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koivisto 1986</td>
<td>Clinical sample</td>
<td>NGT&lt;sup&gt;++&lt;/sup&gt;=8/3 NGT&lt;sup&gt;+++&lt;/sup&gt;=5/2</td>
<td>30±10 37±24</td>
<td>ELISA (Hartling 1986)</td>
<td>Non-obese: mean RBW=103±33%; Obese: mean RBW=214±32%</td>
<td>- fst PI level higher in obese vs non-obese (26 vs 6.3 pmol/L, p&lt;0.01); - fst PI/IRI slightly lower in obese vs non-obese (13 vs 19%, ns)</td>
</tr>
<tr>
<td>Nagi 1990</td>
<td>Caucasian/Asian; clinical sample</td>
<td>Asian=17/8 Cauc.=12/14 (all DM)</td>
<td>50±9 55±8</td>
<td>IRMA (Sobey 1989); specific PI, 32-33 split PI</td>
<td>BMI and WHR treated as continuous variables</td>
<td>- BMI and WHR correlated with fst PI&lt;sup&gt;++&lt;/sup&gt; (r=0.14 and 0.18, both p=ns) - BMI and WHR correlated with fst PI&lt;sup&gt;+++&lt;/sup&gt; (r=0.22 and 0.19, both p=ns)</td>
</tr>
<tr>
<td>Saad 1990</td>
<td>Pima Indians, clinical / pop'n sample (unclear)</td>
<td>NGT=31/46 IGT=1/35 DM=21/25</td>
<td>35 34 39</td>
<td>in-house RIA (Ward 1986); total proinsulin-like molecules</td>
<td>Obese: BMI ≥ 27, Also BMI as continuous variable</td>
<td>- fst PI level higher in obese vs non-obese (17 vs 11 pmol/L, p=0.010) - PI/IRI lower in obese vs non-obese (10.3 vs 18.7%, p&lt;0.001), - inverse correlation between PI/I and BMI (r=-0.34, p=0.002)</td>
</tr>
<tr>
<td>Shiraishi 1991</td>
<td>Japanese* clinical sample</td>
<td>NGT&lt;sup&gt;++&lt;/sup&gt;=5/10 NGT&lt;sup&gt;+++&lt;/sup&gt;=7/11 IGT&lt;sup&gt;++&lt;/sup&gt;=24/15 IGT&lt;sup&gt;+++&lt;/sup&gt;=26/13 DM&lt;sup&gt;++&lt;/sup&gt;=14/6 DM&lt;sup&gt;+++&lt;/sup&gt;=12/4</td>
<td>41±16 45±11 45±16 48±12 58±12 53±17</td>
<td>in-house RIA (Yoshioka 1988); total proinsulin-like molecules</td>
<td>Obese: BMI &gt; 25</td>
<td>- elevated fst PI in obese vs non-obese in all GT strata (all p&lt;0.05) - no significant differences in fst PI/I between obese and non-obese within GT strata</td>
</tr>
<tr>
<td>Haffner 1993</td>
<td>Mexican-American, Non-Hispanic White, pop'n sample</td>
<td>NGT=100/101 IGT=18/41</td>
<td>Male: 49±1 Female: 49±1</td>
<td>Bowsher 1992; total proinsulin-like molecules</td>
<td>BMI and WHR treated as continuous variables</td>
<td>- fst PI correlated with BMI (r=0.19, p&lt;0.05) and WHR (r=0.35 p&lt;0.001)</td>
</tr>
<tr>
<td>Proudler 1994</td>
<td>Clinical sample</td>
<td>Control=0/12 Obese=6/6</td>
<td>31±4 50±11</td>
<td>Sobey 1989</td>
<td>Obese &gt; 120% ideal body weight</td>
<td>- fst PI&lt;sup&gt;++&lt;/sup&gt; higher in obese vs controls (3.7 vs 1.4 pmol/L, p&lt;0.001) - no difference between obese and controls in (PI&lt;sup&gt;++&lt;/sup&gt; + PI&lt;sup&gt;+++&lt;/sup&gt;)/total insulin</td>
</tr>
<tr>
<td>First author, date;</td>
<td>Ethnic group, nature of sample</td>
<td>Sample size, glucose tolerance status (male/female)</td>
<td>Age range, or mean ± SD</td>
<td>Proinsulin assay; components measured;</td>
<td>Definition of obesity</td>
<td>Main results</td>
</tr>
<tr>
<td>---------------------</td>
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<td>------------------------</td>
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<td>-------------</td>
</tr>
<tr>
<td>Birkeland 1994</td>
<td>Finnish, population sample</td>
<td>NGT&lt;sup&gt;+&lt;/sup&gt;=21/10</td>
<td>64.5±2</td>
<td>IMFA (Novo-Nordisk); total proinsulin-like molecules</td>
<td>BMI treated as continuous variable</td>
<td>- BMI significantly correlated with log-fst PI in all subjects (r=0.37, p&lt;0.01) and non-diabetic subjects (r=0.37, p&lt;0.05)</td>
</tr>
<tr>
<td>Mohammed-Ali 1995</td>
<td>Europid, general practice sample</td>
<td>NGT=120 male NGT=150 fem.</td>
<td>53.9±10 54.5±10 (40-75)</td>
<td>in-house IMRA, specific PI, 32-33 split PI</td>
<td>BMI treated as continuous variable</td>
<td>- BMI correlated with fst PI&lt;sup&gt;SEC&lt;/sup&gt; (r=0.35 p&lt;0.001)</td>
</tr>
<tr>
<td>Kahn 1995</td>
<td>Japanese-American; Pop'n sample</td>
<td>NGT=58 IGT=55 DM=57 (all male)</td>
<td>45-74</td>
<td>RIA (Ward 1986); total proinsulin-like molecules</td>
<td>BMI and intra-abdominal fat (IAF) (cm²) treated as continuous variables</td>
<td>- fst PI correlated with both BMI (r=0.36, p&lt;0.0001) and IAF (r=0.37, p&lt;0.0001)</td>
</tr>
<tr>
<td>Haffner 1995</td>
<td>Mexican-American, pop'n sample</td>
<td>NGT+IGT=166/411</td>
<td>48.2-51.2 *5</td>
<td>RIA (Bowsher 1992); total proinsulin-like molecules</td>
<td>non-obese: mean BMI=26.3 obese: mean BMI=33.2</td>
<td>- elevated fst PI in obese vs non-obese (p=0.040); lower PI/I in obese vs non-obese (p=0.012)</td>
</tr>
<tr>
<td>Hochida 1996</td>
<td>Japanese-Brazilian, pop'n sample</td>
<td>NGT=166/185 IGT=37/49 DM=60/33</td>
<td>40-79</td>
<td>IMFA (Dalbosco 1994); total proinsulin-like molecules</td>
<td>Obese: BMI ≥ 25</td>
<td>- fst PI higher in obese vs non obese in NGT and IGT (p&lt;0.01)</td>
</tr>
<tr>
<td>Yudkin 1997</td>
<td>South Asian (SA), Europid (E), pop'n and general practice</td>
<td>NGT(S. Asian): 132/125 (38 IGT); NGT (Europid): 464/570 (47 IGT)</td>
<td>46±11</td>
<td>in-house IMRA, specific PI, 32-33 split PI</td>
<td>BMI treated as continuous variable</td>
<td>- BMI correlated with fst PI&lt;sup&gt;SEC&lt;/sup&gt;: SA (r=0.33 p&lt;0.001); E (r=0.40, p&lt;0.001)</td>
</tr>
</tbody>
</table>

**Note:** Fst PI = fasting proinsulin; BMI = body mass index; IAF = intra-abdominal fat; IRT = insulin resistance; SA = South Asian; E = Europid; NS = not significant.
<table>
<thead>
<tr>
<th>First author, date;</th>
<th>Ethnic group, nature of sample</th>
<th>Sample size, glucose tolerance status (male/female)</th>
<th>Age range, or mean ± SD</th>
<th>Proinsulin assay; components measured;</th>
<th>Definition of obesity</th>
<th>Main results</th>
</tr>
</thead>
</table>
| Mykkanen 1997     | Finns, pop'n sample             | NGT=61/77                                         | 57±2                    | Sobey 1989; specific PI, 32-33 split PI | BMI and WHR treated as continuous variables | - BMI correlated with fst Pi^{sec} (r=0.37, p<0.001), fst Pi^{sec} (r=0.43, p<0.001), fst Pi^{sec}/IRI (r=-0.10, n/s) and Pi^{sec}/IRI (r=0.13, n/s)  
- WHR correlated with Pi^{sec} (r=0.34, p<0.001), Pi^{sec} (r=0.45, p<0.001), Pi^{sec}/IRI (r=-0.21, p<0.05), Pi^{sec}/IRI (r=-0.09, n/s) |
| Snehala-ath 1998  | South Indians (Asian), pop'n based | NGT=122 IGT=29                                    | 42.7±1 49±1.5          | RIA kit (Bowsher 1992; Linco); total proinsulin-like molecules | BMI and WHR treated as continuous variables | - BMI correlated with fst Pi (r=0.41, p<0.001)  
- WHR correlated with fst Pi (r=0.19, p<0.05)  
- in multiple regression: both BMI and 2h gluc associated with fist Pi |

Abbreviations used: RBW = relative body weight; BMI = body mass index (weight/height^2 in kg/m^2); WHR = waist-to-hip ratio; IAF = intraabdominal fat area; for others, see footnote to Table 1.
be of interest to examine these relationships in more detail given the substantial overlap among glucose tolerance categories in the distributions of both adiposity and proinsulin concentration. In particular, there has been no analysis of the relationship between proinsulin and percent body fat, a measure of total body adiposity that has better criterion validity than BMI against gold standard measures [Gallagher et al. 1996, Curtin et al. 1997, Durenburg et al. 1999]. It would also be of value to examine the relationship of proinsulin with waist circumference alone, given the work of Despres’ group who have shown it to be superior to WHR as a surrogate measure of intraabdominal fat [Lemieux et al. 1996]. Finally, the prospective relationships between measures of obesity and change in proinsulin concentrations have not been examined. The SLHDP offers an ideal opportunity to address these important research questions.

**Specific Thesis Objective (I(b))**: To investigate the relationship between proinsulin concentration and concurrent measures of anthropometry, including percent body fat and waist circumference;

**Specific Thesis Objective (I(c))**: To determine whether baseline levels and change over time in these anthropometric variables are associated with follow-up and change in proinsulin levels.

1.2.2.d Proinsulin and Parity

To date, no information is available regarding the effect of parity on proinsulin levels, and discordant results have been reported regarding the effect of parity on insulin levels. After adjustment for obesity, Kritz-Silverstein coworkers [1994] found that the number of pregnancies had a significant positive effect on fasting insulin levels and an inverse relationship with an index of insulin sensitivity after adjustment for waist-hip ratio and other covariates. Reports from the Pima and San Luis Valley diabetes studies, however, documented inverse associations between parity and insulin levels [Alderman et al. 1993, Charles et al. 1994]. A similar inverse association between parity and C-peptide concentration has been reported, although this has been examined in only one study [Alderman et al. 1993]. Recently, Cowan et al. found that parity was not related to variation in fasting insulin levels among American Indian women participating in the Strong Heart Study [Cowan et al. 1997].

**Discussion and Rationale for the Present Studies**: The relationship between parity and proinsulin concentration is of interest given the equivocal results presented to date regarding associations of parity with both insulin concentration and diabetes risk. The demonstration of either a detrimental or protective role for parity in diabetes risk would be of substantial public health importance. Rates of diabetes and impaired glucose tolerance (IGT) among women participants in
the SLHDP were very high, and the age of onset of glucose intolerance is relatively early. Further, families in Sandy Lake are large, and women tend to initiate childbearing at an early age. The SLHDP thus provides an informative setting in which to examine these relationships.

**Specific Thesis Objective (I[d]):** To evaluate the association of parity with proinsulin concentration and with risk of glucose intolerance.

1.2.2.e Proinsulin and Cardiovascular Disease Risk Factors

Ischaemic heart disease and stroke are the first and fourth leading causes of death, respectively, among individuals with Type 2 DM, and together they account for half of the total mortality among individuals with this condition [Geiss et al. 1995]. Further, the risk of a first myocardial infarction among subjects with diabetes approximates that for reinfarction among non-diabetic previous MI patients [Haffner et al. 1998]. This highly unfavourable cardiovascular profile is foreshadowed by a period during which individuals with both diabetes and IGT are dyslipidaemic and hypertensive [Meigs 1998, Alberti 1996]. It has been suggested that these abnormalities might be a consequence of the extended period of exposure to insulin resistance-related hyperinsulinaemia [Reaven 1988, DeFronzo and Ferrannini 1990]. Insulin resistance reduces lipoprotein lipase activity, which may reduce VLDL catabolism and, in turn, induce hypertriglyceridemia as well as reduced HDL cholesterol [Garg 1996]. Insulin and lipid concentrations are associated cross-sectionally [Zavaroni 1985, Burchfiel et al. 1998], and baseline insulin has been shown to predicted 8-year changes in lipid levels and blood pressure [Mitchell et al. 1992]. Although insulin levels were associated with atherosclerosis in one study [Folsom et al. 1994] this finding has not been replicated subsequently [Niskanen et al. 1996, Katz et al. 1996]. The role of insulin resistance in cardiovascular disease (CVD) thus remains controversial [Jarrett 1994].

The assessment of this relationship is complicated by the fact that, as mentioned above, conventional insulin radioimmunoassays display a high degree of cross-reactivity with proinsulin and its split products. Given that proinsulin levels are disproportionately elevated in subjects with diabetes and (in some studies) impaired glucose tolerance (IGT) (Table 1), it is possible that this prohormone may have particularly detrimental metabolic effects in the pathogenesis of cardiovascular disease. Support for this theory emerged during the 2-year interim analysis of a clinical trial comparing human proinsulin and human insulin [Galloway 1992]. Six myocardial infarctions (including 2 deaths) occurred in the human proinsulin group, and none in the comparison group. It should be noted, however, that individuals in this arm of the trial were
receiving doses of proinsulin that were many times higher (1400 pmol/l) than normal physiologic levels (<100 pmol/l).

A summary of the studies that have examined the relationship between proinsulin and cardiovascular risk factors is presented in Table 3. Most of these analyses were conducted with non-diabetic subjects, although Nagi et al. [1990] studied a small group with Type 2 DM. Moderate (r=0.10-0.46) but statistically significant univariate correlation coefficients between proinsulin and/or split proinsulin and CVD risk factors, including cholesterol, triglyceride, LDL, HDL, systolic BP, and diastolic BP, were reported in all investigations. The magnitude of the relationships between proinsulin and triglyceride and/or HDL cholesterol tended to be stronger than associations with other variables. When the correlations were adjusted for potentially confounding variables including age, gender, and measures of anthropometry, coefficients were attenuated (r=0.02-0.32), although many of the relationships maintained statistical significance, particularly those between proinsulin and triglyceride and/or HDL. Multiple linear regression modeling was conducted in most studies, and the findings were similar to those from the partial correlation analyses: among subjects with type 2 DM, proinsulin was significantly independently associated with diastolic blood pressure in the presence of sex, race, age and BMI [Nagi 1990]; among nondiabetics, proinsulin was independently associated with triglyceride level in 4 studies after adjustment for covariates. Findings for other endpoints varied, although independent relationships between proinsulin and HDL [Mohamed-Ali et al. 1995], and proinsulin and systolic BP [Haffner et al. 1993] are notable.

In addition to total proinsulin levels, Grootenhuis et al. [1998] examined the relationship between the PI/IRI ratio and CVD risk factors. They found weak but significant associations with triglyceride, HDL and systolic and diastolic blood pressure. When the coefficients were partialled for age, sex, BMI, WHR, and specific insulin level, associations with triglyceride and systolic BP remained significant.

Intermediate categorical traits associated with CVD have also been examined in relation to proinsulin concentration. A recent study of Japanese Brazilians [Ferreira et al. 1997] reported a significant independent association between hypertension and fasting proinsulin level (OR=1.14, 95% CI 1.00-1.31) after adjustment (using logistic regression) for insulin level, sex, age, family history of hypertension, WHR, IGT, and creatinine. Using data from the San Antonio Heart Study, Haffner et al. [1994b] examined the relationship of proinsulin level and PI/I ratio with metabolic conditions thought to be related to the insulin resistance syndrome, including IGT (yes vs. no),
hypertension (yes vs. no), low HDL (vs. high) and high triglyceride (vs. low). Both proinsulin and the PI/I ratio increased significantly with the increasing number of disorders in nondiabetic subjects.

Despite positive associations with CVD risk factors, inconsistent evidence has emerged from 4 studies of the role of proinsulin in documented cardiovascular disease. Katz and colleagues [1996] defined CAD status using catheterization and/or thallium stress studies, and found no independent association between CAD and proinsulin or PI/I ratio after logistic regression models were adjusted for BMI. Yudkin et al. [1997] reported that neither intact nor split proinsulin was a significant risk factor for prevalent or incident coronary heart disease (diagnosed by ECG) after adjustment for age, sex and BMI. Contrary to these studies, however, Bavenholm et al. [1995] have documented higher adjusted proinsulin concentrations in young men presenting with a first MI compared to controls, as well as significant independent associations between proinsulin concentration and global coronary atherosclerosis score. In addition, Lindahl and coworkers [1999] recently reported the results of a nested case-control analysis which indicated that subjects in the highest quartile of proinsulin concentration had a 3-fold increased risk of acute MI compared to those in the lowest quartile, after adjustment for cholesterol, smoking, blood pressure and antihypertension medication. Although 95% confidence intervals for this estimate included unity after adjustment for BMI, this study had relatively limited power for detecting such an effect.

A number of recent papers have further contributed to this body of literature. Du et al. [1996] demonstrated the induction of apoptosis by high glucose and proinsulin concentrations in human umbilical endothelial cells. They suggest that increased endothelial cell death might partially explain the enhanced thrombogenic risk in diabetics. However, it is also possible that increased apoptosis might “eliminate ... damaged cells to prevent the fixation of vascular defects ... and delay the development of vascular complications of diabetes”. This latter mechanism would appear to contradict the proinsulin-atherosclerosis hypothesis. Haffner et al. [1998] reported weak but significant associations between proinsulin level and intima-media (carotid artery) wall thickness (IMT). While the relationship was stronger than that between true insulin and IMT, it was not independent of plasminogen activator inhibitor-1 level, which suggested to the authors that “proinsulin may represent a marker of atherosclerosis rather than a causal factor”. Finally, three studies have reported associations between proinsulin concentration and higher concentrations of small, dense LDL particles, which are thought to be more atherogenic compared to larger LDL particles [Haffner et al. 1995, Tan et al. 1995, Festa et al. 1999].
TABLE 3. Summary of published studies examining the association of fasting proinsulin concentrations and cardiovascular risk factors.

<table>
<thead>
<tr>
<th>First author, date; [reference]</th>
<th>Ethnic group, nature of sample</th>
<th>Sample Size, glucose tolerance status (m/f)</th>
<th>Age Range, or mean</th>
<th>Proinsulin assay; components measured;</th>
<th>Unadjusted correlation coefficients between proinsulin (total, or intact and split, as indicated) and cardiovascular risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nagi 1990 Caucasian, Asian; clinical sample</td>
<td>Asian=17/8 Cauc.=12/14 (all DM)</td>
<td>50±9 55±8</td>
<td>IRMA (Sobey 1989); specific PI, 32-33 split PI</td>
<td>PI\textsuperscript{total}</td>
<td>Chol 0.21 Trig 0.29 LDL -0.29 BP\textsuperscript{m} 0.17 BP\textsuperscript{s} 0.33\textdagger</td>
</tr>
<tr>
<td>Haffner 1993 Mexican-American, Non-HispanicWhite, pop’n sample</td>
<td>NGT=100/101 IGT=18/41</td>
<td>Male: 49±1</td>
<td>Bowsher 1992; total proinsulin-like molecules</td>
<td>PI 0.03</td>
<td>Chol 0.41\textdagger Trig -0.11 LDL -0.05 BP\textsuperscript{m} 0.19\textdagger BP\textsuperscript{s} 0.15\textdagger</td>
</tr>
<tr>
<td>Mohammed-Ali 1995 Caucasian, general practice sample</td>
<td>NGT=120 male NGT=150 fem.</td>
<td>53.9±10 54±10 (40-75)</td>
<td>in-house IMRA, specific PI, 32-33 split PI</td>
<td>PI\textsuperscript{total} 0.26\textdagger</td>
<td>Chol 0.32\textdagger Trig -0.36\textdagger LDL 0.28\textdagger BP\textsuperscript{m} 0.32\textdagger BP\textsuperscript{s} 0.26\textdagger</td>
</tr>
<tr>
<td>Yudkin 1997 South Asian (SA), Europid (E), pop’n and general practice</td>
<td>NGT(S. Asian): 132/125 (38 IGT); NGT (Europid): 464/570 (47 IGT)</td>
<td>46±11 54±10</td>
<td>in-house IMRA, specific PI, 32-33 split PI</td>
<td>PI\textsuperscript{total} (Europid) 0.25\textdagger</td>
<td>Chol 0.25\textdagger Trig -0.27\textdagger LDL 0.21\textdagger BP\textsuperscript{m} 0.25\textdagger BP\textsuperscript{b} 0.18\textdagger</td>
</tr>
<tr>
<td>Grootenhuis 1998 Europid, pop’n sample</td>
<td>NGT=132/117 IGT=54/62</td>
<td>63±7 74±7</td>
<td>RIA kit (Bowsher 1992)</td>
<td>PI -</td>
<td>Chol 0.36\textdagger Trig -0.25\textdagger LDL 0.23\textdagger BP\textsuperscript{m} 0.24\textdagger</td>
</tr>
<tr>
<td>Snehalatha 1998 South Indians (Asian)</td>
<td>NGT=122 IGT=29</td>
<td>42.7±1 49±1.5</td>
<td>RIA kit (Bowsher 1992)</td>
<td>PI -</td>
<td>Chol 0.06 Trig 0.46\textdagger LDL -0.14 BP\textsuperscript{m} 0.19\textdagger BP\textsuperscript{b} 0.08</td>
</tr>
<tr>
<td>Reference</td>
<td>chol</td>
<td>trig</td>
<td>HDL</td>
<td>LDL</td>
<td>BP&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
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<td>-----------</td>
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<tr>
<td>Nagi 1990</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Haffner 1993</td>
<td>PI</td>
<td>-0.02</td>
<td>0.26</td>
<td>-0.03</td>
<td>-0.11</td>
</tr>
<tr>
<td>Mohammed-Ali 1995</td>
<td>PI&lt;sup&gt;±±&lt;/sup&gt;</td>
<td>0.20</td>
<td>0.22</td>
<td>0.20</td>
<td>-0.31</td>
</tr>
<tr>
<td></td>
<td>PI&lt;sup&gt;±±&lt;/sup&gt;</td>
<td>0.22</td>
<td>0.26</td>
<td>0.23</td>
<td>-0.32</td>
</tr>
<tr>
<td>Yudkin 1997</td>
<td>PI&lt;sup&gt;±±&lt;/sup&gt; (Europe)</td>
<td>-</td>
<td>0.16</td>
<td>0.13</td>
<td>-0.20</td>
</tr>
<tr>
<td></td>
<td>PI&lt;sup&gt;±±&lt;/sup&gt; (Europe)</td>
<td>-</td>
<td>0.20</td>
<td>0.15</td>
<td>-0.20</td>
</tr>
<tr>
<td></td>
<td>PI&lt;sup&gt;±±&lt;/sup&gt; (Asia)</td>
<td>-</td>
<td>0.31</td>
<td>0.08</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>PI&lt;sup&gt;±±&lt;/sup&gt; (Asia)</td>
<td>-</td>
<td>0.29</td>
<td>0.11</td>
<td>-0.06</td>
</tr>
<tr>
<td>Grootenhuis 1998</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Snehalatha 1998</td>
<td>-</td>
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</tr>
</tbody>
</table>

Abbreviations: chol = total cholesterol; trig = triglyceride; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; BP = blood pressure; for others, see footnote to Table 1.

† p<0.05, ‡ p<0.01, †† p<0.001
Discussion and Rationale for the Present Studies: While there have been fairly consistent associations of proinsulin with cardiovascular risk factors in various populations, the relationship has not been examined in Native North Americans. This issue is of public health interest in that, until recently, the Ojibway, Cree, and Oji-Cree of central Canada have had low risk for CVD relative to the general population of Canada [Young et al. 1993]. However, recent evidence indicates that hospitalizations for acute myocardial infarction among Native Canadians from this region have increased over the past 15 years, a period that follows the dramatic increases in the prevalence of diabetes [Shah et al., 2000]. In addition, all studies published to date have employed the cross-sectional design, a method which complicates the interpretation of cause-effect relationships. The SLHDP database offers the opportunity to assess the prospective relationship between change in proinsulin level and change in CVD risk factors in a cohort of participants at high risk for diabetes.

Specific Thesis Objective (I(c)): To assess the relationship between proinsulin concentration and concurrent measures of the following cardiovascular disease (CVD) risk factors: cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) levels, and systolic and diastolic blood pressure.

Specific Thesis Objective (I(f)): To determine whether baseline levels and change over time in proinsulin concentration are associated with follow-up and change in the above-listed CVD risk factors.

1.2.2.f Methodological Issues Regarding the Analysis of Proinsulin, Insulin and C-peptide

The parameter of primary interest in most of the papers reviewed here was the “proinsulin-to-insulin ratio” (PI/I ratio), essentially proinsulin as a percentage of total insulin. In healthy individuals, small amounts of proinsulin are secreted proportionately with true insulin [Halban 1991]. Thus hyperinsulinaemic normoglycaemic individuals will have higher amounts of circulating insulin and proinsulin relative to their normoinsulinaemic normoglycaemic counterparts. Elevated proinsulin levels alone, therefore, may not necessarily be indicative of abnormal beta-cell physiology, and this has motivated the use of PI/I ratio to parameterize “disproportionately elevated” proinsulin. The use of this variable is problematic, however, for two reasons:

(1) Kronmal [1993], has pointed out that the use of “ratio standards” can lead to spurious associations in correlation and regression, and has suggested that they be avoided in favour of the use of the denominator of the ratio in question as an independent variable in linear regression. In
the case of the relationship between fasting blood glucose (FBG) and proinsulin concentration (PI), for example, the model

\[ \text{PI} = \text{intercept} + \beta_1 \text{FBG} + \beta_2 \text{insulin} \]

is preferable to

\[ \text{PI}/\text{I} = \text{intercept} + \beta_1 \text{FBG} \]

Other than in the work of Yoshioka et al. [1988], this issue has not been taken into account.

(2) Peripheral insulin levels do not adequately represent insulin secretion given that "insulin undergoes a large and variable hepatic extraction as well as peripheral clearance that varies under different physiological circumstances" [Van Cauter et al. 1992]. In this thesis, therefore, analyses of proinsulin levels were conducted after adjustment for C-peptide, which is "co-secreted with insulin in an equimolar ratio, is not extracted by the liver, and has a constant peripheral clearance" [Van Cauter et al. 1992].

1.2.2.g Proinsulin and Inheritance

The role of inheritance in the variation of proinsulin concentration is unclear. Mutations in the insulin gene that impair the conversion of proinsulin to insulin have been reported to result in hyperproinsulinaemia [Collinet et al. 1998]. However, subjects with this mutation do not uniformly have hyperglycaemia [Roder et al. 1996]. Further, results have been inconsistent regarding proinsulin concentrations in subjects with a strong family history of type 2 diabetes. Compared to controls, proinsulin was significantly elevated in healthy first-degree relatives of Mexican American, European and South Asian subjects with diabetes [Gelding et al. 1995; Haffner et al. 1995; Ramachandran et al. 1995], although this was not the case among Asian relatives [Gelding et al. 1995], or among those from a study with multi-ethnic representation [Gelding et al. 1994]. Finally, in a study of identical twins discordant for type 2 diabetes, Roder and colleagues [1999] concluded that hyperproinsulinaemia was not a genetically determined trait per se. Rather, variation appeared to be related directly to diabetes pathogenesis. Genetic and inherited aspects of proinsulin will not be considered in this thesis.
1.2.3 IMPAIRED GLUCOSE TOLERANCE: NATURAL HISTORY AND FACTORS RELATED TO DETERIORATION OR IMPROVEMENT IN GLUCOSE LEVELS

1.2.3.a Introduction

The term impaired glucose tolerance (IGT) was introduced in 1979 by the National Diabetes Data Group [NDDG 1979] as an "intermediate category covering the grey area between unequivocal diabetes mellitus and risk free ... normal glucose tolerance" [Alberti 1996a]. Previously this grey area had been termed "borderline" or "chemical diabetes". IGT is defined in the 1985 World Health Organization (WHO) and ADA diagnostic systems as a normal fasting blood sugar level (<7.8 mmol/l) and a level two hours after an 75g OGGT of between 7.8 and 11.1 mmol/l [WHO 1985, ADA 1997]. The identification of the upper bound of this category was based on data from prospective studies that had documented high risk of subsequent diabetic complications among individuals with 2-hour PC blood glucose concentrations above this level [WHO 1985, Alberti 1996b]. IGT is asymptomatic, and thus remains undetected outside of population or clinical screening programmes.

The prevalence of IGT varies widely across the globe, with rates ranging from under 5% among urban Egyptian males, rural Koli of Papua New Guinea, and white Australians, to greater than 25% among females in Naru and Tanzania (Muslim Indian) and elderly Finnish males [King and Rewers 1993, Alberti 1996a]. Striking migration and urbanization effects have been reported: for example, rates approach 20% among Chinese in Mauritius, whereas among rural Chinese in China, the condition is extremely rare [Harris 1995].

The literature regarding risk factors for the development of IGT was recently reviewed by Alberti [1996a] and Nijsps [1998], and will be discussed only briefly here. In cross-sectional studies, individuals with IGT were more obese than those with normal glucose tolerance; this was the case in terms of both total body mass [Modan 1986, Cedarholm et al. 1991, Fujimoto et al. 1994] as well as central obesity estimated using the waist-hip circumference ratio [Mooy et al. 1995, Herman et al. 1995]. In addition, it was demonstrated that risk of IGT was related to higher levels of thoracic and visceral adiposity as measured by computed tomography [Fujimoto et al. 1994]. Relative to controls, individuals with IGT had higher insulin and lipid values, and reported lower levels of physical activity and a stronger family history of diabetes [Annuzzi 1985, Cedarholm et al. 1991, Schranz 1991, Fujimoto et al. 1994]. Prospective studies among the Pima Indians have demonstrated that
baseline obesity and elevated insulin concentrations are significant risk factors for the transition from normal glucose tolerance to IGT [Saad et al. 1988, 1991]. In addition, Charles et al. [1993] reported that high baseline fasting insulin concentration was a significant predictor of IGT independent of weight gain, baseline BMI and glucose, and age and sex.

There is a notable lack of consensus in the literature regarding pathophysiological mechanisms associated with IGT. Saad and colleagues' [1990] classic two-step model for diabetes (Figure 1) hypothesizes that obesity and insulin resistance characterize the transition from normal glucose tolerance to IGT, and that individuals with IGT have marked insulin resistance but normal beta cell function. Under this model, beta cell decompensation occurs later in the natural history and characterizes the IGT-diabetes transition. This hypothesis is supported by the findings of some studies that have reported insulin insensitivity and hyperinsulinaemia in individuals with IGT [Lilioja et al. 1988, Reaven et al. 1989], including one recent investigation that employed a true insulin-specific assay [Goetz et al. 1995]. Others, however, have documented reduced first phase insulin secretion in IGT [Walker et al. 1995, Byrne et al. 1996, Pimenta et al. 1996], suggesting that impaired beta cell function may occur earlier in the disease course. Interestingly, Davies et al. [1994] reported that the loss of first phase response occurred primarily in those with persistent IGT (positive on more than one occasion), which might imply that specific phenotypic subtypes or the duration of IGT influence the results of these studies. Advocates of the early beta cell dysfunction theory also point to the documentation of elevated proinsulin levels among individuals with IGT in certain populations [Yoshioka et al. 1988, Shirashi et al. 1991, Williams et al. 1991, Haffner 1994], although this is not a consistent finding, as discussed in Section 1.2.2.b [Saad 1990, Birkeland 1994, Kahn 1995]. In light of these divergent results, Lilioja [1996] hypothesized that the relative importance of insulin resistance and beta-cell function depended on both the phenotype and ethnicity of the population. Among individuals with type 1 DM, MODY, and lean Caucasians with Type 2 DM, beta cell dysfunction is the dominant initial lesion, with comparatively little contribution from insulin resistance. At the opposite end of the spectrum (Pima Indians and obese individuals from other ethnic groups), insulin resistance plays a much more important role.

The usefulness of IGT as an epidemiological or clinical category has been hotly debated in the literature almost since its creation. IGT has been christened by some as "the unwanted legacy of screening for" type 2 DM [Warram et al. 1996] and "a diagnostic ragbag" [Yudkin et al. 1990]. Critics draw particular attention to the low short-term reproducibility of a single diagnosis of this condition. Yudkin et al. [1990] presented a review of reports that examined this issue. In these
studies, time between initial and second OGTTs ranged from 7 days to 1 year, and subjects were aged 15 and older. Twenty-eight to 76% of re-screened subjects reverted to normal glucose tolerance, while 21 to 56% remained IGT and only 3 to 16% progressed to diabetes. This phenomenon is related to the relatively high coefficient of variation for the 2 hour PC glucose level, as well as the narrow 2-hour glucose range for defining IGT [Alberti 1996a]. Several approaches have been suggested to mitigate or evade the reproducibility problem, including the use of average values from two or more OGTTs, diagnosing IGT only after two positive OGTTs, and the use predictive models employing combinations of risk factors routinely measured in clinical settings [Bourne et al. 1992, Stern et al. 1993]. To date, none of these recommendations has achieved wide acceptability in the diabetes research community.

Despite the limitations of the IGT category, however, there is a notable body of evidence to defend its use. Risk of progression to diabetes among individuals with IGT is high, with a relative risk of 6.3 compared to normoglycemic controls reported among the Pima Indians [Saad et al. 1988]. Rates of conversion range from 1.5% [Keen et al. 1982] to 13.8% [Heine et al. 1996] per year, depending on the age and ethnicity of the study population (Figure 6, Table 4; reviewed in detail below). In addition, individuals with IGT are often dyslipidaemic [Reaven 1988, DeFronzo and Ferrannini 1991, Zimmett 1993] and have been demonstrated to have increased risk of cardiovascular disease (CVD) [Melandor 1996, Pyorala et al. 1987, Jarrett 1996]. Its usefulness is further supported by the simple fact that even a single positive result indicates increased risk for developing diabetes over the next 5-10 years [Alberti 1996a].

The body of literature examining rates of progression from IGT to Type 2 DM and factors associated with risk of this outcome is large and dates to the late 1960s [Yudkin et al. 1990]. Many of the studies published prior to 1980 are not directly comparable to more recent work, given that the WHO criteria were not employed (e.g. use of glucose challenge other than the 75g load, and/or different diagnostic criteria). Papers that were published between 1968 and 1989 have been reviewed by Yudkin and colleagues [1990], and the reader is referred to their paper for details on this material. In the interests of clarity and brevity, information from this time period regarding rates of progression and associated risk factors will be included only for those papers that employed WHO criteria. Later work has been reviewed by Alberti [1996a], Harris [1996], and Niipels [1998]. Edelstein et al. [1997] recently published an updated prospective analysis of the Naru Study and several of the larger US-based longitudinal studies (Pima, San Antonio, San Luis Valley, Rancho Bernardo, and Baltimore). In this latter publication, information for the calculation of annual rates
of progression is available for only the Rancho Bernardo and San Antonio Heart Studies; comparable information for other populations was obtained from earlier papers.

1.2.3.b Rate of Progression from IGT to Type 2 DM and Associated Risk Factors

Figure 6 depicts average annual rates of progression from IGT to type 2 DM for studies that employed WHO/NDDG criteria. Rates were low (~2-3% per year) for Scandinavians and South Asian Hindus living in Tanzania, while Pacific populations, Maltese, Pima Indians, and American Hispanics and non-Hispanic Whites had intermediate levels of progression (~3-6% per year). The highest rates were found among South Asians, elderly Dutch, and residents of Da Qing, China (11-13% per year).

1.2.3.c Metabolic and Anthropometric Risk Factors Associated with Progression from IGT to Type 2 DM

Table 4 presents a summary of studies that have examined factors influencing risk of progression to DM among individuals with IGT. While the natural history of IGT could have been assessed with different statistical approaches, including the analysis of factors associated with returning to NGT, or the use of continuous measures of glucose, progression to DM (yes/no) is the parameter most often presented.
FIGURE 6. Rates of progression from IGT to diabetes: summary of studies that have used World Health Organization criteria.
<table>
<thead>
<tr>
<th>Study Name or Location [reference]</th>
<th>Age (mean or range)</th>
<th>Sample size</th>
<th>Follow-up period (yrs)</th>
<th>Rate of progression (%/year)</th>
<th>Statistically significant factors associated with progression in univariate analysis</th>
<th>Significant independent variables in multivariate model</th>
<th>Variables not related to progression in full or stepwise models (unless indicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark [Agner et al 1982]</td>
<td>73</td>
<td>39</td>
<td>10</td>
<td>2.0</td>
<td>Risk factors for progression not presented</td>
<td></td>
<td>Sex, exam date, BMI, chol, trig, uric acid, creatinine, smoking, fam Hx</td>
</tr>
<tr>
<td>Naru [King et al 1984]</td>
<td>38</td>
<td>51</td>
<td>6.2</td>
<td>4.0</td>
<td>Fst gluc, 2h gluc, BMI (n/s: triceps, chol, trigly, uric acid, sys BP, creatinine, smoking, fam Hx)</td>
<td>2h gluc, age, SBP (-)</td>
<td></td>
</tr>
<tr>
<td>India [Ramachandran et al 1986]</td>
<td>35-72</td>
<td>107</td>
<td>4.3</td>
<td>8.4</td>
<td>Obesity, weight gain, diet adherence (n/s: family Hx, insulin response)</td>
<td>No use of multivariate methods</td>
<td></td>
</tr>
<tr>
<td>Pima [Saad et al 1988]</td>
<td>32</td>
<td>384</td>
<td>3.3 (5)</td>
<td>6.1</td>
<td>Age, BMI, fst gluc, 2h gluc, fst ins, 2h ins (-) (n/s: sex, fam Hx, cholesterol, skinfolds)</td>
<td>Fst gluc., 2h gluc., fst ins., 2h ins (-), age</td>
<td>Sex, family history, BMI, skinfolds, BP, cholesterol*</td>
</tr>
<tr>
<td>Malta [Schranz 1989]</td>
<td>35-74</td>
<td>75</td>
<td>6</td>
<td>5.1</td>
<td>No statistical testing by GT status at baseline</td>
<td>Unclear statistical analysis; no use of multivariate methods</td>
<td></td>
</tr>
<tr>
<td>Niue [Tukuitonga 1990]</td>
<td>44</td>
<td>48</td>
<td>5</td>
<td>6.1</td>
<td>Age (-), occupation, SES, modernity, fst gluc, 2h gluc (n/s: sex, family Hx, parity, education, phys act, BMI, total caloric intake)</td>
<td>No use of multivariate methods</td>
<td></td>
</tr>
<tr>
<td>Tanzania [Swai et al 1990]</td>
<td>25-65</td>
<td>49</td>
<td>1</td>
<td>2.0</td>
<td>Risk factors for progression not presented</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Name or Location [reference]</td>
<td>Age (mean or range)</td>
<td>Sample size</td>
<td>Follow-up period (yrs)</td>
<td>Rate of progression (%/year)</td>
<td>Statistically significant factors associated with progression in univariate analysis</td>
<td>Significant independent variables in multivariate model</td>
<td>Variables not related to progression in full or stepwise models (unless indicated)</td>
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<tr>
<td>Japanese-American men [Tsunehara 1991] (abstract)</td>
<td>n/a</td>
<td>66</td>
<td>5</td>
<td>3.64</td>
<td>Caloric intake, amt of animal fat, % animal fat calories, amt of cholesterol</td>
<td></td>
<td>Abstract only - use of multivariate methods not clear</td>
</tr>
<tr>
<td>South Africa [Motala et al 1993] b</td>
<td>&gt;15</td>
<td>113</td>
<td>4</td>
<td>12.6</td>
<td>Fst gluc, 2h gluc, BMI, sex (f), dias BP (n/s: age, family Hx)</td>
<td>Fst gluc, 2h gluc</td>
<td>Sex, BMI, (dias BP not included due to missing values)</td>
</tr>
<tr>
<td>Eastern and Southern Finland [Stengard et al 1993]</td>
<td>65-84</td>
<td>147 (men only)</td>
<td>5</td>
<td>2.3</td>
<td>2h gluc (n/s: age, first gluc, BMI, chol, HDL, BP)</td>
<td>No multivariate analysis</td>
<td></td>
</tr>
<tr>
<td>San Luis Valley [Marshall et al 1994]</td>
<td>30-74</td>
<td>123</td>
<td>2</td>
<td>8.1</td>
<td>Fst gluc, 1h gluc, 2h gluc, first ins, sex, BMI, education (-), family Hx, total and mono fat (n/s: age, phys activity)</td>
<td>Fst ins, 1h ins, fist gluc, sex, skinfolds, total dietary fat (energy adjusted)</td>
<td>Age, ethnicity, BMI, WHR</td>
</tr>
<tr>
<td>Sweden (Malmo) [Eriksson et al 1996]</td>
<td>48-54</td>
<td>322</td>
<td>6</td>
<td>2.7</td>
<td>HT, trig, uric acid, gamma-glut, BMI, FVC, VO2max, fist gluc, 40-min gluc, 2h gluc, fist ins, 40-min ins increment, 2h ins (n/s: age, family Hx, BP, chol, creatinine, ht, triceps, hrt rate, phys act, smoking)</td>
<td>Fst gluc</td>
<td>BMI, FVC, 40-min ins increment, 2h ins</td>
</tr>
<tr>
<td>Northern Finland [Qiao et al 1996]</td>
<td>55-57</td>
<td>187</td>
<td>2.1</td>
<td>3.9</td>
<td>Fst ins, BMI (≥ 30), ΔBMI (n/s: sex, chol, HDL, trig, smoking, family Hx, phys act, Δ fist ins, fist gluc, 2h gluc, HT meds)</td>
<td>BMI (≥ 30), ΔBMI</td>
<td>sex, chol, HDL, trig, smoking, family Hx, phys act, fasting ins, Δ fist ins, fist gluc, 2h gluc, HT meds</td>
</tr>
<tr>
<td>Netherlands (Hoorn) [Nijpels et al 1997]</td>
<td>50-75</td>
<td>158 (Dx based on 2 OGGT)</td>
<td>3</td>
<td>11.3</td>
<td>none (note: glucose levels not assessed)</td>
<td>Age, sex, months of follow-up</td>
<td>trig, HDL, fist ins (specific), 2h ins (specific), HT, WHR</td>
</tr>
<tr>
<td>Study Name or Location [reference]</td>
<td>Age (mean or range)</td>
<td>Sample size</td>
<td>Follow-up period (yrs)</td>
<td>Rate of progression (%/year)</td>
<td>Statistically significant factors associated with progression in univariate analysis</td>
<td>Significant independent variables in multivariate model</td>
<td>Variables not related to progression in full or stepwise models (unless indicated)</td>
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<tr>
<td>Rancho Bernardo [Edelstein et al 1997] +</td>
<td>52-82</td>
<td>186</td>
<td>8.2</td>
<td>3.2</td>
<td>BMI, waist, WHR, fst gluc, 2h gluc (n/s: age, sex, family Hx)</td>
<td>No multivariate analysis</td>
<td></td>
</tr>
<tr>
<td>San Antonio Heart Study [Edelstein et al 1997] +</td>
<td>25-65</td>
<td>353</td>
<td>8.2</td>
<td>3.7</td>
<td>BMI, fst gluc, 2h gluc (n/s: age, sex)</td>
<td>No multivariate analysis</td>
<td></td>
</tr>
<tr>
<td>Da Qing, China [Pan et al 1997] c</td>
<td>56.5±9.3</td>
<td>133</td>
<td>6</td>
<td>11.2</td>
<td>Intervention study - data not provided on factors related to progression among controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kin-Chen, China [Chou et al 1998]</td>
<td>30+</td>
<td>131</td>
<td>2</td>
<td>8.8</td>
<td>Results presented for NGTs and IGTs combined</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a South Asian Hindus, b South Asians, c control group
* assumed from table 1
^gamma-glut = serum gamma-glutamyltransferase (ukt/l)
+ data gleaned from recently published combined study [Edelstein et al 1997]
abbreviations used: NGT = normal glucose tolerance; IGT = impaired glucose tolerance; DM = diabetes mellitus; OGTT = oral glucose tolerance test; IR = insulin resistance/resistant; n/s=not significant; fst= fasting; BMI = body mass index (weight/height² in kg/m²); WHR = waist-to-hip ratio; chol = total cholesterol; trig = triglyceride; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; BP = blood pressure, HT = hypertensive, Hx = history
Baseline blood glucose levels, both fasting and at various times after a 75g OGTT, have been the most consistently examined factor in determining progression to DM [Alberti 1996a]. The positive relationship was first documented in the early Bedford [Keen et al. 1982] and Whitehall [Jarrett et al. 1984] projects, and results from subsequent studies (using both WHO/NDDG and alternative criteria) have been uniformly supportive of this finding [Alberti 1996a]. In univariate analyses, most studies report significantly increased risk of progression with elevations in both fasting and 2-hour glucose levels (Table 4). Interestingly, the only exceptions were two studies from Finland; in the northern Finnish population, neither fasting nor 2-hour glucose levels were related to progression, while in southern and eastern Finland, only 2-hour glucose level was a significant univariate risk factor. Results from multivariate analysis are slightly less consistent, although in most papers that employed this analytic technique either fasting or 2-hour glucose levels (or both) are independently associated with progression in the presence of other factors (Table 4). In the recent update of six large prospective studies [Edelstein et al. 1997], univariate analysis indicated that fasting and 2-hour glucose levels were significantly related to progression in all populations, with odds ratios ranging from 1.3-1.8 per 0.56 mmol/l increase in fasting glucose level, and from 1.2 to 1.5 per 0.56 mmol/l increase in 2-hour glucose level.

Although less regularly measured in studies from this body of literature, elevated fasting insulin level has also been documented as a risk factor for progression (Table 4). Significant univariate relationships were reported in the Pima [Saad et al. 1988] and San Luis Valley Diabetes Studies [Marshall 1994], as well as in two Scandinavian populations [Eriksson et al. 1996, Qiao et al. 1996], although only in the North American groups were the associations significant in multivariate analysis. Findings regarding 2-hour insulin levels are less consistent. In the Maalmo study [Eriksson et al. 1996], the positive univariate association was not maintained during multivariate modeling, while among the Pima, the association was in the opposite direction (i.e. lower levels were related to progression), and remained significant in the presence of age, fasting and 2 hour glucose levels, and fasting insulin [Saad 1988].

While measures of obesity show consistent relationships with insulin resistance and the development of IGT, the role of total and regional adiposity in the further deterioration to diabetes is less clear. The large majority of papers reviewed in Table 4 reported that BMI was related to progression in univariate analysis, although, in the six positive studies that further evaluated the association using multivariate analysis, BMI was a significant independent predictor in only one. Edelstein et al's [1997] recent update confirms the significant univariate association (5 of 6
populations, range of ORs 1.2-1.5 per 4kg/m² increase), although their paper did not employ multivariate methods. The role of abdominal obesity has received only limited attention. Significant univariate relationships are reported for both waist circumference waist-hip ratio in the recent prospective studies update [Edelstein et al. 1997], whereas only one project assessed the independent contribution of this risk factor (San Luis Valley Diabetes Study [Marshall et al. 1994]: ratio of subscapular to triceps skinfold thicknesses; see Table 4 for variables in model).

As was mentioned briefly above, there is controversy in the literature regarding the stage at which beta cell abnormality is manifest in the natural history of Type 2 diabetes. Circulating proinsulin levels are elevated in individuals with type 2 DM, and this situation is thought to reflect beta cell dysfunction. Proinsulin concentration in individuals with IGT has been examined cross-sectionally in a number of studies, with conflicting results (reviewed in section 1.2.2). Seven recent papers have reported on the prospective relationship between baseline proinsulin concentration and subsequent risk of diabetes in nondiabetic cohorts (Table 5).

The methodological aspects and results of these studies are summarized in Table 5. Most were prospective cohort studies of relatively short duration (2.5-5 years), and 2 groups employed nested case-control analyses. Participants tended to be middle-aged or older, and Europeans, East Asians, Japanese-Americans and urban Mexicans were represented. Nijpels et al. [1996] followed only subjects with IGT. In the remainder of the investigations, participants consisted of subjects with both NGT and IGT, although the high proportion of individuals with IGT in 4 studies is notable. In all studies, either fasting proinsulin (split, specific or total) or the PI/I ratio were significant risk factors for progression to diabetes after adjustment for covariates, including glucose levels and measures of obesity and insulin secretion. Shin et al. [1997] did not evaluate the association with proinsulin using multivariate statistical methods.

The insulin response 30 minutes after an OGTT is an alternative surrogate measure of beta cell function. Kadowaki et al. [1984] and Nagi et al. [1995] have demonstrated that individuals with a reduced early insulin response are at increased risk of deterioration in glucose tolerance. Finally, it was reported that hyperglycaemic progression in a small sample of subjects with IGT was associated with a decline in beta-cell function as estimated using the continuous infusion of glucose with model assessment (CIGMA) method [Cook et al. 1993].
Discussion and Rationale for the Present Studies: Evidence from the literature published to date indicates that elevated baseline concentrations of fasting and 2-hour glucose are well-established risk factors for progression from IGT to type 2 DM. The roles of insulin concentrations and obesity, however, require clarification. In particular, risk associated with elevated waist circumference and waist-hip ratio (estimates of excess abdominal adipose tissue) has not been assessed adequately, and, to our knowledge, percent body fat, a better measure than BMI of total body adiposity, has not been studied in this context. Further, although baseline proinsulin concentration has been shown to be a significant predictor of diabetes development in a number of previous studies, this association has yet to be established in a population indigenous to the Americas. Finally, the prospective relationship of baseline concentrations and change in proinsulin with follow-up and change in glucose concentrations would be of interest. The SLHDP offers the opportunity to explore these research questions.

Specific Thesis Objective (II(a)): To determine whether baseline levels and changes over time in proinsulin, insulin resistance, and adiposity are associated with the development of diabetes and changes in glucose concentrations among Native Canadian subjects at high risk for the development of diabetes.

1.2.3.d Lifestyle Factors Associated with Progression from IGT to Type 2 DM

Limited information is available in the literature regarding the role of lifestyle factors in determining progression to diabetes from IGT. Marshall et al. [1994] identified 123 subjects with IGT (via OGTT) in a population-based survey in Colorado. Diet was assessed at baseline by 24-hr recall, and information was collected on other demographic, anthropometric, and biochemical variables. Subjects were followed for 1-3 years, and a second OGTT administered. Increased baseline total fat intake was an independent risk factor for the development of Type 2 DM, with an OR of 6.0 (95% CI 1.2-29.8, adjusted for energy intake, gender, central obesity, and glucose and insulin concentrations (fasting and 1h)) per 40g of fat intake per day.

Eriksson et al. [1996] examined the role of fitness level (measured using bicycle ergometry) as a predictor of diabetes in a cohort of middle-aged Swedish men. Fitness level was a significant
TABLE 5. Summary of papers examining proinsulin concentration as a risk factor for progression to diabetes in studies that have employed WHO/NDDG diagnostic criteria.

(a) IGT at baseline only

<table>
<thead>
<tr>
<th>Study Name or Location [reference]</th>
<th>Age (mean or range)</th>
<th>Sample size</th>
<th>Follow-up period (yrs)</th>
<th>Rate of progression (%/year)</th>
<th>Statistically significant factors associated with progression in univariate analysis</th>
<th>Significant independent variables in multivariate model</th>
<th>Variables not related to progression in full or stepwise models (unless indicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands (Hoom) [Nijpels 1996]</td>
<td>50-75</td>
<td>158 (Dx based on 2 OGTT)</td>
<td>3</td>
<td>11.3</td>
<td>Fst gluc, 2h gluc, fst proins</td>
<td>2h gluc, fst proins</td>
<td>age, sex (stepwise elim: BMI, WHR, HbA1c, fst gluc, fst (spec) ins, 2h (spec) ins, 2h proins, HT, sex*WHR)</td>
</tr>
</tbody>
</table>

(b) IGT and NGT at baseline

<table>
<thead>
<tr>
<th>Study Name or Location [ref]</th>
<th>Age (mean or range)</th>
<th>Sample size</th>
<th>Follow-up period (yrs)</th>
<th>Rate of progression (%/year)</th>
<th>Statistically significant factors associated with progression in univariate analysis</th>
<th>Significant independent variables in multivariate model</th>
<th>Variables not related to progression in full or stepwise models (unless indicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan [Inoue 1995]</td>
<td>45-55 (range of medians)</td>
<td>22 NGT 37 IGT</td>
<td>2.5</td>
<td>5.4 (IGT only)</td>
<td>fast proins, 2h proins, 2h C-peptide</td>
<td>fast proins, 2h proins, fasting C-peptide</td>
<td>fst gluc, 2h gluc, fst ins, 2h ins, 2h C-peptide (model also adj for age, sex, and BMI, but data not shown)</td>
</tr>
<tr>
<td>Finland [Mykkanen 1995]</td>
<td>65-74</td>
<td>69 cases 138 controls (108 IGT)</td>
<td>3.5 years nested case-control study</td>
<td>IGT, BMI, fst gluc, 2h gluc, fst intact proins, fst split proins, PI/I, split PI/I</td>
<td>split PI/I (adjusted for fst gluc and BMI)</td>
<td>(in individual models adjusted for fst gluc and BMI): fst IRI, fst specific ins, fst proins, fst split proins, PI/I</td>
<td></td>
</tr>
<tr>
<td>Japanese-American men [Kahn 1995]</td>
<td>60.9 61.4</td>
<td>49 NGT 38 IGT</td>
<td>5 years</td>
<td>6.3 (IGT only)</td>
<td>fst gluc, 2h gluc, fst C-pep, fst PI, fst PI (adj for IRI)</td>
<td>fst gluc, 2h gluc, fst C-pep, fst PI, fst PI (adj for IRI), PI/IRI (individual models adjusted for BMI and IAF)</td>
<td>fst IR, fst specific ins</td>
</tr>
<tr>
<td>Study Name or Location [ref]</td>
<td>Age (mean or range)</td>
<td>Sample size</td>
<td>Follow-up period (yrs)</td>
<td>Rate of progression (%/year)</td>
<td>Statistically significant factors associated with progression in univariate analysis</td>
<td>Significant independent variables in multivariate model</td>
<td>Variables not related to progression in full or stepwise models (unless indicated)</td>
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<tr>
<td>Mexico City Diabetes Study [Haffner 1997]</td>
<td>47.4 46.6</td>
<td>85 cases 85 controls (57 IGT)</td>
<td>3.25 years</td>
<td>nested case-control study</td>
<td>IGT, BMI, IRI, fst gluc, 2h gluc, fst PI, PI/l</td>
<td>IGT, BMI, PI/l</td>
<td>BMI, specific ins, WHR</td>
</tr>
<tr>
<td>Korea [Shin 1997]</td>
<td>30+</td>
<td>67 cases 66 controls</td>
<td>2 years</td>
<td>n/a</td>
<td>fasting proinsulin</td>
<td>Information not provided</td>
<td>Information not provided</td>
</tr>
<tr>
<td>Isle of Ely [Wareham 1999]</td>
<td>54.3 (m) 53.4 (f)</td>
<td>767 NGT 96 IGT</td>
<td>4.5</td>
<td>2.2</td>
<td>fst gluc, fst ins, fst intact PI, fst split PI, fst PI,</td>
<td>fst glucose, family Hx, PI</td>
<td>age, BMI, WHR, phys act, sex, fst ins, fst intact PI, fst split PI</td>
</tr>
</tbody>
</table>

Abbreviations used: NGT = normal glucose tolerance; IGT = impaired glucose tolerance; DM = diabetes mellitus; OGTT = oral glucose tolerance test; BMI = body mass index (weight/height² in kg/m²); WHR = waist-to-hip ratio; PI = total proinsulin-like material; IRI = total immunoreactive insulin (i.e. non-specific, including proinsulin); I = specific insulin; Hx = history.
independent protective factor in the entire group (both NGT and IGT), although the estimate of the
effect was weaker and non-significant when the analysis was restricted to only those men with IGT
at baseline (n=191). Similarly, self-reported physical activity was not associated with progression to
diabetes in this study, nor in 3 others (univariate analysis only: Nuie study, San Luis Valley, northern
Finland (Table 4)). A lack of association with smoking has been reported in 3 studies (Naru,
Maalmo, Northern Finland), although the exposure was treated as simple dichotomous variable in
these analyses. In a published abstract, Tsunehara et al. [1991] reported that total energy intake as
well as consumption of animal fat and cholesterol were associated with progression to diabetes
among a cohort of 2nd generation Japanese-American men with IGT. It is not clear whether these
estimates were adjusted for confounding factors such as adiposity.

Discussion and Rationale for Present Studies: The body of knowledge regarding lifestyle
exposures and diabetes progression is sparse. Results from clinical trials of diet and exercise
interventions have been promising [Alberti 1996a, Melander 1996, Bourn 1996], particularly the
recently completed Da Quing IGT Study, in which diet and exercise, as well as a combination of the
two, resulted in significantly reduced 6-year incidence of diabetes relative to controls [Pan et al.
1997]. Effects of specific food groups and nutrients are difficult to assess from these studies,
however. The work of Marshall et al. [1994] is the only published observational study reporting an
independent significant risk associated with a particular dietary behaviour. And although no
significant risk has been reported for smoking, this exposure has usually been modeled as a simple
yes/no dichotomous variable, an analytic approach that may mask an association at the high end of
the exposure spectrum. Finally, the role of alcohol consumption and risk of progression to diabetes
among high-risk subjects does not appear to have been evaluated.

Gaps in the literature regarding diet and smoking are notable in light of the recent work of
Vijayalingam and colleagues [1996], who reported that antioxidant status, which is known to be
compromised in subjects with diabetes [Giugliano et al. 1995], is also poor among individuals with
IGT. Previous research has demonstrated that the beta cell is highly susceptible to oxidative stress
[Halliwell et al. 1989], and that alpha-tocopherol (vitamin E) protects beta cells from NO2 damage
[Burkart et al. 1995]. Among subjects with type 2 DM, vitamin E supplementation reduces
oxidative stress and improves insulin action [Paolisso et al. 1993]. Similar benefits have been
reported with chronic vitamin C supplementation [Paolisso et al. 1995]. In a recent prospective
study, Salonen and colleagues [1995] reported that low baseline lipid-standardized vitamin E
concentration was associated with a 3.9 fold (95% CI 1.8-8.6) increased risk of incident diabetes
after 4 years of follow-up. Dietary intake is responsible for a substantial proportion of the variation in serum antioxidants [Stryker et al. 1988], whereas smoking is a source of exogenous free radicals and is known to reduce serum antioxidants [Pryor 1997]. The SLHDP dataset, which contains information dietary intake and cigarette smoking, offers the opportunity to explore the role of oxidative stress in the natural history of IGT.

*Specific Thesis Objective (II(b)): To assess the importance of smoking, alcohol consumption, and dietary intakes of fat and fiber in determining the development of diabetes and changes in glucose concentrations among Native Canadian subjects at high risk for the development of diabetes.*

### 1.3 SUMMARY OF OBJECTIVES

The objectives of this thesis are summarized below:

(I) **To identify the distribution and determinants of proinsulin concentration in a Native Canadian population with high rates of obesity and glucose intolerance.** Specifically, the study will:

(a) Determine whether proinsulin levels are elevated among individuals with glucose tolerance abnormalities, including Type 2 DM and IGT, relative to those with NGT;

(b) Investigate the relationship between proinsulin level and concurrent measures of anthropometry, including percent body fat and waist circumference;

(c) Determine whether baseline levels and change over time in these anthropometric variables are associated with follow-up, and change, in proinsulin levels;

(d) Evaluate the association of parity with proinsulin concentration and risk of glucose intolerance;

(e) Assess the relationship between proinsulin concentration and concurrent measures of the following cardiovascular disease (CVD) risk factors: cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) levels, and systolic and diastolic blood pressure;

(f) Determine whether baseline levels and change over time in proinsulin concentration are associated with follow-up, and change, in the above-listed CVD risk factors.
To assess the relationship between metabolic and lifestyle factors and change (improvement or deterioration) in glucose tolerance among individuals at high risk for diabetes over a period of 4 years. Specifically, the study will:

(a) Determine whether baseline levels and change over time in proinsulin, insulin resistance, and adiposity are associated with the development of diabetes and changes in glucose concentrations;

(b) Assess the importance of smoking, alcohol consumption, and dietary intakes of fat and fiber in determining the development of diabetes and changes in glucose concentrations.

Figure 7 presents the thesis objectives as well as the arrangement of the thesis papers diagramatically in the context of the broader concepts of the natural history and etiology of type 2 diabetes that were discussed in section 1.2.1.d.
FIGURE 7. Diagramatic summary of thesis objectives and arrangements of thesis papers in the context of the natural history of type 2 DM.

**Paper 1.**

![Diagram of thesis objectives and arrangements](image)

**Background:** Results from previous studies have consistently indicated that proinsulin concentrations are elevated in diabetic subjects compared to those with NGT. Whether proinsulin concentrations are also elevated among subjects with IGT is unclear. In addition, little is known about the association between glucose and proinsulin concentrations among subjects with normal glucose tolerance. Finally, the relationship between proinsulin concentration and glucose tolerance among Native Canadians has not been explored.

**Objective I(a):** To determine whether proinsulin levels are elevated among individuals with glucose tolerance abnormalities, including Type 2 DM and IGT, relative to those with NGT.

**Background:** Despite clear associations between adiposity and risk of glucose intolerance, the relationship between adiposity and proinsulin concentration is equivocal.

**Objective I(b):** To investigate the relationship between proinsulin level and concurrent measures of anthropometry, including percent body fat and waist circumference.

**Objective I(c):** To determine whether baseline levels and change over time in these anthropometric variables are associated with follow-up and change in proinsulin levels.
Background: Results from previous studies have been highly inconsistent regarding the association between parity and both risk of diabetes and insulin concentrations / insulin resistance. In addition, the association between parity and proinsulin concentration has not been evaluated.

Objective 1(d): To evaluate the association of parity with proinsulin concentration and risk of glucose intolerance.
Background: Both lipid and proinsulin concentrations are elevated in subjects with type 2 DM, and there is evidence to suggest that this may also be the case for subjects with IGT. Lipid and proinsulin concentrations have been shown to be cross-sectionally associated in a number of populations, although it is unknown whether this is the case among native Canadians. Further, the prospective association between proinsulin and lipid concentrations has not been evaluated.

Objective 1(e): To assess the relationship between proinsulin concentration and concurrent measures of the following cardiovascular disease (CVD) risk factors: cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) levels, and systolic and diastolic blood pressure.

Objective 1(f): To determine whether baseline levels and change over time in proinsulin concentration is associated with follow-up and change in the above-listed CVD risk factors.
Background: Previous studies have indicated that elevated glucose concentration is a well-established risk factor for progression from IGT to type 2 DM. The roles of insulin concentrations and obesity, however, require clarification. In particular, risk associated with elevated levels of waist circumference and percent body fat have not been assessed adequately. Further, although baseline proinsulin concentration has been shown to be a significant predictor of diabetes development in a number of previous studies, this association has yet to be established in a population indigenous to the Americas. Finally, the prospective relationship of baseline concentrations and change in proinsulin with follow-up and change in glucose concentrations would be of interest.

Objective II(a): To determine whether baseline levels and change over time in proinsulin, insulin resistance, and adiposity are associated with the development of diabetes and changes in glucose concentrations.
Background: The body of knowledge regarding lifestyle exposures and risk of diabetes progression among high-risk subjects is sparse. The work of Marshall et al. [1994] is the only published observational study reporting a significant independent risk associated with a particular dietary behaviour. And although no significant risk has been reported for smoking, this exposure has usually been modeled as a simple yes/no dichotomous variable, an analytic approach that may mask an association at the high end of the exposure spectrum.

Objective II(b): To assess the importance of smoking, alcohol consumption, dietary intakes of fat and fiber, physical activity, and fitness level in determining the development of diabetes and changes in glucose concentrations.
1.4 REFERENCES


Alberti KGMM. Impaired glucose tolerance - fact or fiction? Diabetic Med 1996(b);13:56-58.


Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ. Chronic exposure to free fatty acid reduces pancreatic beta-cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. J Clin Invest 1998;101:1094-1101.


Bourne DM. The potential for lifestyle change to influence the progression of impaired glucose tolerance to non-insulin dependent diabetes mellitus. Diabetes Care 1996;13:938-45.


Charles MA, Pettitt DA, Saad MF, Nelson RG, Bennett PH, Knowler WC. Development of impaired glucose tolerance with or without weight gain. Diabetes Care 1993; 16:593-6.


Collins VR, Dowse GK, Zimmet PZ. Evidence against association between parity and NIDDM from five population groups. Diabetes Care 1991;14:975-81.


Rhodes CJ, Alarcon C. What β-cell defect could lead to hyperproinsulinemia in NIDDM? Some clues from recent advances made in understanding the proinsulin processing mechanism. Diabetes 1994;43:511-17.


Chapter 2

Subjects and Methods

2.1 BACKGROUND

2.1.1 Sandy Lake First Nation: Historic and Cultural Setting, and Recent Epidemiologic Transition

Sandy Lake First Nation (Ontario) is located roughly 2000 km northwest of Toronto in the subarctic boreal forest region of central Canada. Approximately 1800 people live in this community, located at the western end of a large lake connected to the Severn River system, which drains north into Hudson Bay. The community is isolated and accessible only by light aircraft for most of the year, except during a six-week period in February and March when a winter road system allows access to permanent roads in the south. The population is stable, with a small amount of in- and out-migration. The unemployment rate is high (~60-70%), with limited full-time work available in band office administration, community services, and retail sales. Some seasonal employment is obtainable in construction and fire fighting. The indigenous language of the community is Oji-Cree (also called Severn River Ojibway), a member of the Algonkian family of languages. Most adults currently speak both English and Oji-Cree, although older community members communicate entirely in the native language.

The history of Sandy Lake people has been chronicled in the 1985 book, “Killing the Shamen” [Fiddler and Stevens 1985], and only a brief summary will be presented here. The ancestors of the
present day community members lived in a number of small clans (Sucker, Sturgeon, Pelican, and Crane) of 10-25 individuals that hunted and trapped along river systems for the majority of the year. The clans were closely associated through periodic cooperative hunting, trading and intermarriage, and would agglomerate in larger groups in the summer months for these activities. These people lived a nomadic hunting and gathering subsistence similar to other indigenous groups of the eastern subarctic. The lifestyle was extremely physically active, and the diet was high in protein from wild meat and fish, with seasonal subsistence from berries and roots. In 1926, a treaty was signed with the federal government that established the present day reserve at the western end of Sandy Lake. While aspects of the traditional culture were maintained in subsequent decades, the reserve and residential school systems gradually altered the lives of the members of this community. The past few decades, in particular, have witnessed dramatic lifestyle changes in this region, with a marked decrease in physical activity and an alteration in diet to one characterized by excess consumption of saturated fat and processed foods [Young 1988a]. This population is consequently undergoing an epidemiologic transition [Young 1988b], with a marked increase in morbidity related to chronic diseases, such as obesity and Type 2 DM [Montour et al. 1985, Delisle et al. 1993, Brassard et al. 1993, Harris et al. 1997].

2.1.2 The Sandy Lake Health and Diabetes Project (SLHDP):

A Partnership between the First Nation and Health Researchers

The SLHDP was conceived in 1991 after initial discussions between the Sandy Lake Band Council and the study investigators, Drs. Stewart Harris and Bernard Zinman. The investigators and community leaders have met regularly since that time to discuss all aspects of the project, including the study protocol, funding, new initiatives, personnel, and publications [Hanley et al. 1995].

2.1.3 Ethical and Community Approval

Both the baseline and follow-up protocols were approved by the Sandy Lake First Nation Band Council and the University of Toronto human subjects review committee.
2.2 Baseline Survey, 1993-1995

2.2.1 Survey Design and Training of Community Surveyors

The objectives of the baseline survey were to determine the true prevalence of type 2 DM as well as the risk factors associated with this condition. The study employed a cross-sectional design, with both concurrent and (in some cases) retrospective measurement of risk factors [Hanley et al. 1995, Harris et al. 1997], as described below.

In May of 1993, five local women who were fluent in both Oji-Cree and English were hired to work as Community Surveyors. They participated in a comprehensive 2-month training programme which provided detailed instruction in each of the data collection instruments and techniques, as well as guidance in proper questionnaire administration and the assurance of confidentiality. Reliability was assured using standardization exercises, including multiple recording of sample interviews and review of inter-surveyor variability.

Questionnaires were either developed specifically for the SLHDP or modified from previously published instruments. In either case, the results of qualitative research exercises were used in item generation, reduction and/or modification [Streiner and Norman 1995]. These exercises, which have been described previously [Gittelsohn et al. 1996], included key informant interviews, free-lists, pile sorts and participant observation. Questionnaires were translated into Oji-Cree by the surveyors, and then back-translated by an independent third party. All survey procedures were pre-tested in a small representative sample of community members to assure acceptability and ease of use by the surveyors.

2.2.2 Eligibility, Recruitment and Participation

Individuals were considered eligible to participate in the survey if they were registered members of the Sandy Lake band and had lived in Sandy Lake for at least 6 months of the past year. Registered members of other bands who were living in the households of Sandy Lake band members were also included. The most recent copy of the Sandy Lake band list was used to determine the population base. In addition, a community household mapping survey was conducted prior to the initiation of data collection. Recruitment was carried out systematically using these
resources. Individuals age 10 years and older were requested to participate in the full survey protocol. Those under 10 years of age took part in anthropometric assessment only.

Approximately 73% (728/1018) of eligible community members age 10 years and older participated in the prevalence screening and risk factor assessment. Participation varied by age and sex (50-84%), with the lowest participation rate among males aged 40-49 years [Hanley et al. 1995].

2.2.3 Blood Sampling and Metabolic Testing

Participants arrived at the Diabetes Project House between 08:00 and 09:30 after an 8-12 hour overnight fast. Fasting blood samples were drawn for glucose, insulin, C-peptide, lipids and lipoproteins. A 75g oral glucose tolerance test (OGTT) was administered (Glucodex, Rouguer Inc, Chanbley, Quebec), and a second sample for glucose was drawn after 120 minutes. Individuals were excluded from the OGTT if they had physician diagnosed diabetes and were (a) currently receiving treatment with insulin or oral hypoglycemic agents, or (b) if they had a fasting blood glucose concentration exceeding 11.1 mmol/l. Diabetes and impaired glucose tolerance were diagnosed according to World Health Organization (WHO) criteria [WHO 1980, 1985]. Women who were pregnant at the time of recruitment had their OGTT deferred until 3-months post-partum.

2.2.4 Sample Processing and Laboratory Assays

Blood samples drawn for glucose, insulin, C-peptide were allowed to clot at room temperature in serum-separation tubes for 20 minutes, and were then centrifuged for 10 minutes at 3000 rpm. Single 5-ml aliquots for determination of fasting and 2-hour post-challenge glucose were refrigerated and transported by air the following day to the Sioux Lookout Zone Hospital (SLZH). Four 5-ml aliquots of fasting serum (2 for insulin and C-peptide, and 2 saved specimens) were transferred to plastic cryovial tubes and stored at -70°C in Sandy Lake until shipment to Toronto. EDTA-containing tubes drawn for lipids and lipoproteins were gently inverted and refrigerated for 20 minutes until centrifugation, which took place for 30 minutes at 2000 rpm. A single 5ml and three 2ml aliquots were transferred to cryovial tubes and frozen as described above. At 3-4 month intervals, samples were packed in dry ice and shipped by air to Toronto.
Glucose concentration was determined at the SLZH using standard laboratory procedures. Insulin, C-peptide, proinsulin, and leptin were measured at the University of Toronto Banting and Best Diabetes Centre Core Lab. Insulin concentration was determined using a radioimmunoassay (Pharmacia, Inc.) which has a lower detection limit of 22 pmol/l, and an interassay coefficient of variation (CV) of 7.2-8.8%. This assay displays a very high degree of cross-reactivity with proinsulin (100%), and thus values refer to total immuno-reactive insulin (IRI) [Jeremy Kwan, Banting and Best Diabetes Centre Core Lab, personal communication; Morgan and Lazarow 1963]. C-peptide level was measured using a radioimmunoassay (Diagnostic Products Corporation, Los Angeles) which has minimal detection limit of 43 pmol/l, and cross-reactivities of 0% with insulin and <13% with proinsulin.

Proinsulin was determined using a human proinsulin radioimmunoassay which has a laboratory sensitivity of 3.5 pM, and a CV of 6.2-21.0% (Linco Research Inc., St. Louis, MO). This assay displays 46% cross-reactivity with des 31,32 proinsulin, the major form of circulating split proinsulin, and thus reported values refer to total proinsulin-like materials [Bowshe et al. 1992]. Cross-reactivity of this assay with des 64, 65 proinsulin, insulin and C-peptide is very low (<0.1%). Serum leptin was measured with a radioimmunoassay prepared using recombinant human leptin as the standard. It has a minimum detectable concentration of 0.5 µg/l, a limit of linearity of 100 µg/l, and a CV of 3.4-8.3% (Linco Research Inc.) [Ma et al. 1996]. Both proinsulin and leptin were measured in serum specimens that had been stored at -70°C for between 3-5 years at the University of Toronto Banting and Best Diabetes Centre Core Lab.

Concentrations of lipids and lipoproteins were determined at the J. Alick Little Research Laboratory of St Michael's Hospital, Toronto, which is certified under the National Heart, Lung and Blood Institute Lipid Standardization Program [Lipid Research Clinics Program 1982, 1984]. Concentrations of total cholesterol and triglyceride were determined using procedures described in the Lipid Research Clinics manual of operations [Lipid Research Clinics Program 1984, Connelly et al. 1992]. Information on reliability of these procedures is contained in this document. High-density lipoprotein (HDL) cholesterol was measured using the methods described by Bachorik et al. [1984], and the concentration of low-density lipoprotein cholesterol (LDL) was estimated using the Freidwald formula [Freidwald et al. 1972].
2.2.5 Estimation of Insulin Resistance and Beta-Cell Function

In the present study, it was not possible to directly measure insulin resistance or beta-cell function, and thus simple indices of these parameters were used. Insulin resistance was estimated using the homeostasis model assessment (HOMA IR) method of Matthews and colleagues [1985], which is defined as follows:

\[
\text{fasting insulin (μU/ml) x fasting glucose (mmol/l)} \times \frac{22.5}{22.5}
\]

This index has shown acceptable criterion validity against gold-standard measures of insulin resistance, including the euglycaemic clamp (r=0.88,) and hyperglycaemic clamp (r=0.69) techniques, as well as estimates of insulin sensitivity from the frequently sampled intravenous glucose tolerance (FSIVGTT) test (r=-0.56) and insulin tolerance test (r=-0.61) [Matthews et al. 1985, Phillips et al. 1993, Anderson et al. 1995].

Fasting insulin has also been proposed as a valid surrogate estimate of insulin resistance. Laakso [1993] has reported results of criterion validation of fasting insulin against measures from the euglycaemic clamp technique (NGT, r=-0.68; IGT, r=-0.47; DM, r=-0.56, all p<0.05), and others have demonstrated validity for fasting insulin against measures of insulin sensitivity from the FSIVGTT (r=-0.63), and the insulin tolerance test (r=-0.57) [Phillips et al. 1993, Anderson et al. 1995].

Elevated proinsulin concentration has been proposed as an indicator of beta cell dysfunction. Five studies have presented information comparing proinsulin concentration with other measures of beta cell function (Table 6). In all but one study, proinsulin concentration displayed moderate to strong criterion validity against more detailed estimates.
TABLE 6. Summary of studies examining the correlation between proinsulin concentration and other measures of beta cell function.

<table>
<thead>
<tr>
<th>Author / Year</th>
<th>n</th>
<th>Comparison Measure</th>
<th>Results (correlation with proinsulin concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phillips 1993</td>
<td>85 NGT</td>
<td>3 minute insulin secretion during FSIVGTT</td>
<td>Intact: 0.09 (NGT), -0.11 (IGT)</td>
</tr>
<tr>
<td></td>
<td>23 IGT</td>
<td></td>
<td>Split: 0.11 (NGT), 0.00 (IGT)</td>
</tr>
<tr>
<td>Mykkanen 1997</td>
<td>138 NGT</td>
<td>AIR (10 minutes) during IVGTT</td>
<td>Intact: 0.35, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Split: 0.45, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PI/I: -0.40, p&lt;0.001</td>
</tr>
<tr>
<td>Roder 1998</td>
<td>9 NGT</td>
<td>AIR (incremental insulin response to arginine at maximal glucose)</td>
<td>PI (DM), r=-0.76, p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>10 DM</td>
<td></td>
<td>PI/I (DM), r=-0.48, p&lt;0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(&quot;not significant&quot; among NGTs; data not presented in paper)</td>
</tr>
<tr>
<td>Larson 1999</td>
<td>10 NGT</td>
<td>APIR (arg) / AIR (arg)</td>
<td>PI (glucose @ FPG (%)), r=0.84, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>13 IGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mykkanen 1999</td>
<td>182 newly diagnosed DM</td>
<td>AIR (average 2+4 minute insulin) during FSIVGTT</td>
<td>Intact: r=0.23, p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Split: r=0.42, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PI/I: r=-0.29, p&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus; FSIVGTT, frequently samples intravenous glucose tolerance test; AIR, acute insulin response; PI, proinsulin; PI/I, proinsulin/insulin ratio; APIR (arg) / AIR (arg), acute proinsulin response / acute insulin response to 5 g IV arginine stimulation at 3 glucose levels (fasting, 14, and >25 mmol/l).

Beta cell function can also be estimated using the homeostasis model assessment method (HOMA β-cell) [Matthews et al. 1985]. This index has been validated against the hyperglycaemic clamp technique (NGT: r=0.59, p<0.05; IGT: r=0.72, p<0.02), which is considered the gold standard technique for determining insulin secretion. HOMA β-cell is defined as follows:

\[
\text{HOMA-β} = \frac{20 \times \text{fasting insulin (µU/ml)}}{\text{fasting glucose (mmol/l)} - 3.5}
\]

2.2.6 Anthropometric Measurements and Blood Pressure Determination

Anthropometric measurements were performed with the volunteer wearing either undergarments and a hospital gown or light athletic clothing, and no shoes. Each measurement was performed twice, and the average was used in the analysis. Armstrong et al. [1992; pg. 115] have pointed out that, under the assumption of non-differential misclassification, using two or more
measures of exposure can be an effective method of decreasing measurement error compared with the use of a single measure.

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, with the feet together and the head held in the Frankfurt plane. Weight was measured to the nearest 0.1 kg using a hospital balance beam scale. Body mass index (BMI) was defined as weight/height$^2$ (kg/m$^2$). Waist and hip circumferences were measured to the nearest 0.5 cm using an inelastic metal tape. Two surveyors were involved in each measurement: one insured that the tape was parallel and held on the correct landmarks, and the other determined the circumference. The natural waist was measured at the point of narrowing between the umbilicus and xiphoid process as viewed from behind. The hips were measured at the maximum extension of the buttocks as viewed from the side. The waist-to-hip ratio (WHR) was calculated as the ratio of these two circumferences. Both the WHR and waist circumference have been shown to correlate strongly against direct quantification of abdominal visceral adipose tissue measured by computed tomography ($r=0.71$-$0.77$) [Pouliot et al. 1994; Lemieux et al. 1996]. However, in these studies waist circumference consistently demonstrated superior criterion validity compared to WHR in all age-sex groups, and thus waist circumference was used in the present study as a measure of abdominal obesity.

Percent body fat was estimated by bioelectrical impedance analysis (BIA) using the Tanita TBF-201 Body Fat Analyzer (Tanita Corp., Tokyo). This measurement was not performed if subjects had joint prostheses or pacemakers. High reproducibility of percent fat estimates using this machine in a sample from this population has been documented (intraclass correlation coefficient (ICC) = 0.99) [Hanley et al. 1994], and the instrument has been validated by others against dual energy x-ray absorptiometry (DEXA) in both non-diabetic and diabetic subjects [Sakamoto et al. 1994, Nunez et al. 1997, Tsui et al. 1998]. Percent body fat measured by BIA has shown criterion validity against DEXA that is superior to that for BMI [Gallagher et al. 1996]. In addition, the relationship between BMI and gold standard measures of body fat has been shown to be dependent on age, sex, ethnicity and body build, and thus BMI might not be a valid measure of total body adiposity [Curtin et al. 1997, Durenburg et al. 1999].

Blood pressure was measured in the right arm with the volunteer seated and the arm bared. Two measurements were taken using an aneroid sphygmomanometer, and the average was used in the analysis. Systolic pressure was recorded to the nearest 2 mmHg at the appearance of the first
Korotkoff sound (phase I), and the diastolic pressure was recorded to the nearest 2mmHg at the appearance of the fifth Korotkoff sound (phase V).

2.2.7 Risk Factor Questionnaire

The risk factor questionnaire was administered by trained Community Surveyors in the interval between the fasting and 2-hour blood samples. The following information was collected:

Smoking and Alcohol Use: Smoking history was determined, including time since cessation among former smokers, as well as duration in years among ever smokers and usual number of cigarettes per day among current smokers. Pack-years were calculated for current smokers as (years of smoking x (number of cigarettes/day/20)). Frequency of alcohol consumption (4-levels) as well as usual amounts of beer, wine and liquor consumed during each drinking occasion were determined. A drinking intensity scale was calculated as (frequency of drinking x usual amount per occasion).

Dietary Intake: A 24-hour dietary recall was administered during which participants were asked to report all foods consumed during the past 24-hour period; volumes and portion sizes were estimated using measuring cups and spoons, and 2- and 3-dimensional food models. The coding and preliminary analyses of these data have been reported elsewhere [Wolever et al. 1997a, b]. Briefly, the recalls were coded by a dietician at the Department of Nutritional Sciences, University of Toronto, using a database derived from the Canadian nutrient file [Health and Welfare Canada 1991] as well as other sources [Pennington 1989]. The validity and reproducibility of the 24-hour recall technique have been discussed by Willett [1990, pg. 53]. Estimates of validity vary depending on the “gold standard” that is utilized and the demographic profile of the target population, and few data are available regarding reproducibility given the day-to-day variability of individual diets. Indeed, it is generally accepted that the 24-hour recall provides a poor picture of an individual’s usual diet, unless there is little day to day variation in the foods eaten [Willett 1990, pg. 57]. This technique, however, provides a relatively good estimate of nutrient intake over the past 24-hour period, with criterion validation for specific nutrients ranging between 0.58 and 0.74 versus observed intake, between 0.20 and 0.50 versus diet histories, and between 0.33 and 0.97 versus specific biological measurements [Willett 1990, pg. 57]. Reproducibility and validity of the 24-hour recall method have not been established in the SLHDP study population.
A food frequency questionnaire was used to assess the frequency of consumption over the previous 3 months of 34 commonly eaten foods (both store bought and traditional). The 6 consumption frequency categories included: more than once per day, once per day, 3-6 times per week, 1-2 times per week, 1-3 times per month, rare or never. Additional questions inquired about added sugar, salt, fat, and preparation techniques. Portion sizes were not included in this instrument. Coding and preliminary analysis of these data have been reported elsewhere [Gittelsohn et al. 1998]. The validity and reproducibility of this instrument have not been established. Willett reports that the test-retest correlations for food frequency questionnaires generally range from 0.50-0.70; estimates of validity vary widely ($r = 0.04-0.90$) depending on the food item and choice of gold standard [Willett 1990, pg. 69].

Additional information: Date of birth and gender were determined. Subjects with previously diagnosed diabetes were asked about the duration of their diabetes and current method of treatment (diet, oral hypoglycaemic agents, insulin). For women, information on number of live births and the age of each child was determined. In addition, information regarding current use of oral contraceptives (OC) was obtained, with consent, from medical records maintained at the community clinic.

2.3 Follow-up Survey of High Risk Subjects, 1998

Participants in the baseline survey who were found to be at high risk for subsequent diabetes were invited to participate in a follow-up visit during the summer of 1998 to determine current glucose tolerance and risk factor status. High risk subjects were defined as baseline survey participants who had impaired glucose tolerance (IGT) ($n=74$) or normal glucose tolerance (NGT) with a 2 hour post challenge glucose level greater than or equal to 7.0 mmol/l ($n=51$). Of the 125 individuals in this follow-up cohort, 3 (all IGT) had died, 11 (9 IGT, 2 NGT) were no longer living in the community, 2 (1 IGT, 1 NGT) were too infirm to participate, and 14 (6 IGT, 8 NGT) refused to attend. Thus 95 (76%) members of this high-risk cohort participated in the follow-up examination. Non-participants did not differ significantly from participants in age, gender, anthropometry, metabolic or lifestyle variables (Table 7), although there was some suggestion that there were fewer non-drinkers among the non-participants.
The rationale for re-examination of subjects with IGT was based on (1) the documented high risk for subsequent progression to diabetes among individuals with this diagnosis, and (2) the unfavourable metabolic profile of subjects with IGT, especially regarding lipids and anthropometry [Alberti 1996]. The rationale for re-examination of subjects with high risk NGT was based on the documentation of worsening metabolic risk factors across the spectrum of NGT [Meigs et al. 1998], as well as evidence which indicates that elevated glucose levels in subjects with NGT are predictive of diabetes development [Rewers and Hamman 1995]. In addition, little evidence was available in the literature regarding risk factors that were related to glycaemic progression in high-risk nondiabetic subjects. Finally, on a number of occasions the Sandy Lake community leaders and grass-roots organizations had identified subjects with IGT as a priority for detailed follow-up and future study. Thus there were strong public health, epidemiologic and political bases for this follow-up study.

The laboratory procedures and protocols for glucose tolerance status determination, anthropometric and blood pressure measurements, and smoking status were identical to those described for the baseline survey (see Sections 2.2.1 to 2.2.7).
### TABLE 7. Characteristics of participants and non-participants in follow-up study of glucose tolerance status (GTS) and associated risk factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Participants (n=95)</th>
<th>Non-participants (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (% male / % female)</strong></td>
<td>29.47 / 70.53</td>
<td>26.67 / 70.33</td>
<td>0.767</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>35.10 (16.97)</td>
<td>37.72 (17.98)</td>
<td>0.469</td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>41.54 (10.55)</td>
<td>38.77 (13.63)</td>
<td>0.256</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.35 (11.37)</td>
<td>95.29 (16.54)</td>
<td>0.747</td>
</tr>
<tr>
<td><strong>Metabolic Variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.73 (0.55)</td>
<td>5.83 (0.72)</td>
<td>0.479</td>
</tr>
<tr>
<td>2-hour glucose (mmol/l)</td>
<td>8.28 (1.06)</td>
<td>8.37 (1.02)</td>
<td>0.672</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>161.55 (97.35)</td>
<td>164.62 (108.07)</td>
<td>0.756</td>
</tr>
<tr>
<td>Fasting C-peptide (pmol/ml)</td>
<td>0.83 (0.43)</td>
<td>0.77 (0.40)</td>
<td>0.324</td>
</tr>
<tr>
<td>Fasting proinsulin (pmol/l)</td>
<td>17.06 (11.60)</td>
<td>17.10 (7.71)</td>
<td>0.708</td>
</tr>
<tr>
<td>HOMA IR (units)</td>
<td>5.78 (3.71)</td>
<td>6.06 (4.00)</td>
<td>0.900</td>
</tr>
<tr>
<td>HOMA β-cell (%)</td>
<td>208.23 (123.94)</td>
<td>199.98 (145.64)</td>
<td>0.510</td>
</tr>
<tr>
<td>Leptin (units ng/ml)</td>
<td>20.89 (13.86)</td>
<td>21.56 (16.02)</td>
<td>0.683</td>
</tr>
<tr>
<td><strong>Baseline Alcohol Consumption</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% non-drinkers</td>
<td>57.5</td>
<td>40.0</td>
<td>0.144</td>
</tr>
<tr>
<td>% heavy drinkers</td>
<td>4.3</td>
<td>10.0</td>
<td>0.470</td>
</tr>
<tr>
<td># drinks / occasion</td>
<td>13.65 (12.45)</td>
<td>12.56 (21.34)</td>
<td>0.841</td>
</tr>
<tr>
<td>frequency x amount scale</td>
<td>233.27 (1132.17)</td>
<td>802.30 (3612.89)</td>
<td>0.402</td>
</tr>
<tr>
<td><strong>Baseline Smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% ever smokers</td>
<td>73.4</td>
<td>70.0</td>
<td>0.897</td>
</tr>
<tr>
<td>% current smokers</td>
<td>50.0</td>
<td>50.2</td>
<td>1.000</td>
</tr>
<tr>
<td># years of smoking</td>
<td>14.32 (11.77)</td>
<td>12.91 (8.93)</td>
<td>0.606</td>
</tr>
<tr>
<td># cigarettes / day</td>
<td>9.53 (8.53)</td>
<td>8.33 (7.31)</td>
<td>0.626</td>
</tr>
<tr>
<td><strong>Baseline Pattern of Food Consumption</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter and Lard (% consumption ≥ 1x/day)</td>
<td>69.47</td>
<td>70.00</td>
<td>0.956</td>
</tr>
<tr>
<td>Fish (% no or rare consumption)</td>
<td>26.42</td>
<td>30.00</td>
<td>1.000</td>
</tr>
<tr>
<td>Fruit Scale</td>
<td>5.20 (2.33)</td>
<td>5.17 (2.21)</td>
<td>0.943</td>
</tr>
<tr>
<td>Vegetable Scale</td>
<td>7.00 (3.64)</td>
<td>7.00 (3.44)</td>
<td>1.000</td>
</tr>
<tr>
<td>Combined Fruit &amp; Vegetable Scale</td>
<td>12.23 (5.85)</td>
<td>12.17 (3.76)</td>
<td>0.951</td>
</tr>
</tbody>
</table>
TABLE 7 (con't).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Participants (n=95)</th>
<th>Non-participants (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline Nutrient Intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>2171.31 (1168.74)</td>
<td>2219.43 (962.56)</td>
<td>0.386'</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>16.10 (7.16)</td>
<td>16.03 (6.98)</td>
<td>0.708'</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>35.66 (10.03)</td>
<td>35.11 (11.54)</td>
<td>0.803</td>
</tr>
<tr>
<td>Saturated Fat (%)</td>
<td>12.93 (4.27)</td>
<td>12.97 (4.66)</td>
<td>0.959</td>
</tr>
<tr>
<td>Polyunsaturated Fat (%)</td>
<td>4.77 (2.03)</td>
<td>4.86 (2.82)</td>
<td>0.796'</td>
</tr>
<tr>
<td>Monounsaturated Fat (%)</td>
<td>13.09 (4.52)</td>
<td>13.02 (5.39)</td>
<td>0.944</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>46.43 (11.27)</td>
<td>47.02 (12.83)</td>
<td>0.808</td>
</tr>
<tr>
<td>Starch (%)</td>
<td>25.56 (9.77)</td>
<td>24.12 (9.71)</td>
<td>0.483</td>
</tr>
<tr>
<td>Sugar (%)</td>
<td>20.67 (12.16)</td>
<td>22.81 (13.85)</td>
<td>0.420</td>
</tr>
<tr>
<td>Fibre (g/1000kcal)</td>
<td>5.33 (3.13)</td>
<td>5.19 (2.07)</td>
<td>0.635'</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>640.17 (823.62)</td>
<td>741.37 (737.61)</td>
<td>0.351'</td>
</tr>
<tr>
<td>Vitamin A (RE)</td>
<td>583.07 (1895.60)</td>
<td>568.60 (779.52)</td>
<td>0.571'</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>54.44 (52.30)</td>
<td>48.00 (51.90)</td>
<td>0.558</td>
</tr>
<tr>
<td>Niacin (NE)</td>
<td>38.97 (21.93)</td>
<td>38.58 (24.20)</td>
<td>0.883</td>
</tr>
<tr>
<td>Folate (mg)</td>
<td>167.84 (81.31)</td>
<td>150.87 (80.75)</td>
<td>0.321</td>
</tr>
</tbody>
</table>

Values are mean (±SD) or proportion
1 variable log-transformed for hypothesis testing
2 self-reported drinking more than 50-times per year
3 among drinkers only (non-participants, n=18; participants, n=40)
4 midpoint of frequency category x usual amount consumed per occasion (non-drinkers=0)
5 ever smokers only (non-participants, n=22; participants, n=72)
6 current smokers only (non-participants, n=15; participants, n=49)
7 subjects age 18 and older (non-participants, n=27; participants, n=78)
8 reduced sample (non-participants, n=20; participants, n=56)
9 from 3-month food frequency questionnaire
10 from 24 hour recall
2.4 Statistical Analysis

2.4.1 General Issues.

All analyses were carried out using SAS version 6.12 [SAS 1990] and PEPI [Abramson and Gahlinger 1999] version 3.0. The distributions of continuous variables were assessed for normality using the Shapiro and Wilk statistic ($W$) and distributional plots provided by SAS PROC UNIVARIATE. If the p-value of the Shapiro and Wilk statistic was <0.01, the variable was considered skewed and the natural log transformation ($\ln$) of the variable was used in subsequent multivariate analysis. Residuals were examined for normality to ensure that the log transformations had resulted in the desired normal distributions for most analyses.

Associations between continuous variables were evaluated using both the Spearman correlation analysis provided by SAS PROC CORR, and simple and multiple linear regression provided by SAS PROC REG. Scatterplots of the variables of interest were examined prior to analyses to scan for outliers and departures from linearity. Given the distributional assumptions of linear regression analysis, log transformations of skewed variables were used when necessary [Weisberg 1980]. Collinearity of multiple linear regression models was assessed using the variance inflation factor (VIF) method described by Freund and Littell [1986]. When specified in the model statement, the VIF is calculated for each independent variable in the model, and the statistic indicates how multicollinearity has increased the instability of the coefficient estimates. For the $i$th independent variable, the VIF is defined as $1/(1-R^2_i)$, where $R^2_i$ is the “coefficient of determination for the $i$th independent variable on all other independent variables” [Freund and Littell 1986]. While there are no formal criteria for determining a statistically important VIF, Freund and Littell suggest that independent variables with a VIF > $1/(1-R^2)$, where $R^2$ is the full model coefficient of variation, are cause for concern.

Differences between categories in concentrations or levels of continuous variables were assessed using analysis of covariance (ANCOVA) in SAS PROC GLM. ANCOVA also allowed for the estimation of least-square means, also known as population marginal means, which are the average levels of the dependent variable for each category after adjustment for covariates in the model. P-values for pairwise comparisons between individual least-square means of interest were calculated both with and without adjustment for multiple comparisons. The Tukey-Kramer method was employed for pairwise multiple comparison adjustment because it is considered more powerful.
for this application than the Bonferroni, Sidak or Scheffe approaches, and has performed well in Monte Carlo studies [SAS 1990, pg. 944; SAS 1996, pg.49]. The natural logarithms of least square means were back-transformed for presentation in the tables, and 95% confidence intervals were calculated.

Risk estimates for two-level outcomes were evaluated using both contingency table analyses and the maximum likelihood logistic regression procedure provided by SAS PROC LOGISTIC. Odds ratios were calculated by taking the exponent of the beta coefficient for the independent variable of interest, and 95% confidence intervals were calculated.

In all correlation and regression analyses proinsulin concentration was analyzed after linear adjustment for C-peptide, a reliable surrogate indicator of insulin secretion. The rationale for this approach is described in Section 1.2.2.f.

Change over the follow-up period in continuous variables, including % body fat, waist circumference, blood pressure, and concentrations of proinsulin, glucose, lipids and lipoproteins were calculated as the 1998 follow-up concentration minus the 1993-1995 baseline concentration. In correlation and regression analyses that evaluated change variables, the baseline value of the given variable was included in the model in addition to other independent variables to adjust for the correlation between the initial and follow-up levels.

Duration of follow-up was defined as the time period in years between the baseline and follow-up survey examinations (median 4.2 years, range 3.0-5.2 years). We included duration of follow-up as a continuous variable in all fully adjusted logistic regression models examining metabolic, anthropometric or lifestyle variables and risk of progression. This variable had no effect on the magnitude or significance of any of the ORs (data not shown), and it was thus not included in the models presented in the tables of papers 4 and 5.

The identification of potential confounding factors was carried out using information from the current metabolic and epidemiologic literature, as well as by employing the criteria outlined by Rothman and Greenland [1998], which include:

(1) A confounding factor must be a risk factor for the disease;
A confounding factor must be associated with the exposure under study in the source population (the population at risk from which the cases are derived);

A confounding factor must not be affected by the exposure or the disease. In particular, it cannot be an intermediate step in the causal path between the exposure and the disease.

Criterion (3) is difficult to apply in light of the relatively limited understanding of the sequence of pathophysiological events in the natural history of type 2 DM. Indeed, it would be a dangerously over-simplistic approach to make expansive declarations about confounder relationships in many of these analyses. For example, in examining the relationship between proinsulin and lipid concentrations, it is unclear at this point in time whether adiposity is a confounder (and would thus be adjusted for) or is on the pathway either before or in between proinsulin and lipids (and would thus not be adjusted for). Therefore, crude models as well as one or more adjusted models are presented in subsequent papers. Justification for the variables included in individual models is provided in the descriptions of specific analyses that follow.

Biologically plausible effect modifications were identified based on information from the current metabolic and epidemiologic literature, and were assessed by examining stratum specific results and by including interaction terms in regression models. If this interaction term was statistically significant, or if the stratum-specific results indicated clinically or biologically significant differences, an effect modification was considered to be present, and stratum-specific results were presented.

2.4.2 Objective-Specific Analyses

Below the statistical analyses are presented in more detail and are organized according to both the specific objectives and the papers in which they appear in Chapter 3.

Paper 1.

Objective 1 (a): To determine whether proinsulin levels are elevated among individuals with glucose tolerance abnormalities, including Type 2 DM and IGT, relative to those with NGT:

Differences in proinsulin concentration between glucose tolerance categories were tested using analysis of covariance (ANCOVA). The dependent variable was ln proinsulin concentration, and the independent variables included GTS (3-level) and ln C-peptide as an estimate of insulin
secretion. Ln age, sex, and waist circumference were included as independent variables in a subsequent model as potential confounders. To further explore variation in proinsulin concentration across the spectrum of glucose tolerance, subjects with NGT were categorized using quartiles of both fasting and 2-hour glucose concentration. Thus, in subsequent ANCOVA models, GTS was modeled as a 6-level categorical variable: NGT quartile (N)1, N2, N3, N4, IGT, and type 2 DM, with similar crude and adjusted modeling as described above. Finally, the association between proinsulin and glucose concentrations was assessed using continuous variables in linear regression and correlation models.

**Objective I (b): To investigate the relationship between proinsulin level and concurrent measures of anthropometry, including percent body fat and waist circumference:**

Correlation and multiple linear regression were employed to determine the independent contribution of anthropometric factors to variation in ln fasting proinsulin concentration within glucose tolerance categories. In lieu of BMI and WHR, % body fat and waist circumference were used as measures of total and abdominal adiposity, respectively. The rationale for the use of these measures was presented in Section 2.2.6. The dependent variable in these models was ln fasting proinsulin, and independent variables were either waist circumference or % body fat, with adjustment for insulin secretion using ln C-peptide concentration. Both crude and adjusted (for sex and ln age) models are presented. It was anticipated that gender-adiposity and waist circumference-percent body fat effect modifications were plausible, and these interactions were evaluated by examining stratum-specific results and by including cross-product terms in adjusted models. These analyses indicated that the association between waist circumference and proinsulin concentration was stronger among males with NGT, and among females with type 2 DM. In addition, the sex-waist interaction term was statistically significant (p<0.05). It was thus concluded that a gender-waist circumference effect modification existed, and stratum-specific results were presented for these variables.

**Objective I (c): To determine whether baseline levels and change over time in these anthropometric variables are associated with follow-up and change in proinsulin levels:**

The effect of both baseline levels and changes in % body fat and waist circumference on both follow-up and change in proinsulin concentrations were evaluated using Spearman correlation analyses. Both crude and adjusted associations were examined. Crude coefficients were adjusted for
C-peptide, and partial coefficients were adjusted for C-peptide and the potential confounders age, sex and baseline and follow-up diabetes status. Models analyzing the effect of change in the independent variable (% body fat or waist circumference) also included the baseline of that variable.

**Paper 2.**

**Objective I (d): To evaluate the association of parity with proinsulin and risk of glucose intolerance:**

These analyses were restricted to female participants aged 12 years and older, given that this is the earliest age of childbearing in this population. For women with previously diagnosed diabetes, live births that occurred after the onset of the disease were excluded from the calculation of number of live births to ensure that risk estimates related to exposures that occurred prior to the onset of disease. The association between parity and risk of diabetes was assessed using multiple logistic regression. The risk associated with parity was evaluated using 3 different approaches: parity modeled (1) as a continuous variable (risk per 1 additional live birth); (2) as a dichotomous variable (nulliparous vs. parous); (3) as a 4-level categorical variable with nulliparity as the reference category (vs. 1-2 births, 3-4 births, and 5 or more births). Crude odds ratios were examined, as well as odds ratios adjusted for ln age, waist circumference, and use of oral contraceptives.

ANOVA was employed to estimate, for each 4-level parity category, age-adjusted mean levels of % fat, waist circumference, HOMA IR, HOMA β-cell function, and concentrations of glucose, insulin, C-peptide, proinsulin and leptin. Among non-diabetic women, mean levels of % fat, waist circumference, HOMA IR, HOMA β-cell function, and concentrations of proinsulin, insulin, C-peptide and leptin by 4-level parity category were estimated and compared after adjustment for age, waist circumference and GTS (NGT vs. IGT).

It was suspected that age might modify the effect of parity on both diabetes risk and proinsulin concentration. The analyses described above were thus conducted within age strata (12-19, 20-29, 30 and older), and effect differences were examined. In addition, age-parity interaction terms were included in models and the magnitude and significance of these variables were evaluated. Based on these unadjusted analyses, it was concluded that the effect of parity on diabetes risk was different between young (age 19-29: strong significant inverse association) and older women (age 30+: weaker, non-significant inverse association), and results are presented separately for these age strata. Conversely, age did not modify the associations between parity and metabolic and
anthropometric variables among non-diabetic women, and thus these results are presented with the age groups pooled.

**Paper 3.**

**Objective I (c): To assess the relationship between proinsulin concentration and concurrent measures of the following cardiovascular disease (CVD) risk factors: cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL), and high density lipoprotein cholesterol (HDL) concentrations, and systolic and diastolic blood pressure:**

These analyses were restricted to subjects with NGT and IGT. Crude and adjusted associations between concurrent measures of proinsulin and cardiovascular risk factors (lipids and blood pressure) were assessed using correlation coefficients and multiple linear regression within categories of glucose tolerance (NGT or IGT). Analyses were conducted for six dependent variables: concentrations of total cholesterol, triglyceride, LDL and HDL, and systolic and diastolic blood pressure. For each dependent variable, three models with the following independent variables were constructed: (i) proinsulin alone; (ii) proinsulin, age, sex, and C-peptide, and (iii) proinsulin, age, sex, C-peptide and waist circumference. Statistical interactions between sex and proinsulin were considered plausible, and these were evaluated using stratified analyses and by adding a sex x proinsulin term to each of the final models. None of the interaction parameter estimates were significant at the 5% level, and the parameters for proinsulin were similar between the sexes.

**Objective I (f): To determine whether baseline levels and change over time in proinsulin concentration is associated with follow-up and change in the above-listed CVD risk factors.**

The effect of both baseline levels and changes in proinsulin concentration on both follow-up and change in cardiovascular risk variables were evaluated using Spearman correlation analyses. Both crude and adjusted associations were examined. Models assessing the effect of baseline proinsulin concentration were adjusted for age, sex, C-peptide concentration, change in waist circumference, baseline and follow-up diabetes status, and baseline level of the dependent variable. Models assessing the effect of change in proinsulin concentration were adjusted for age, sex, change in C-peptide concentration change in waist circumference, baseline and follow-up diabetes status, and baseline level of the dependent variable.
Paper 4.
Objective II (a): To determine whether baseline levels and change over time in proinsulin, insulin resistance, and adiposity are associated with the development of diabetes and changes in glucose concentrations;

Risk factors for progression to diabetes were evaluated using logistic regression analyses. The dependent variable in these models was development of diabetes (yes/no). Separate models for each independent variable of interest were constructed; these variables included presence of IGT at baseline (yes/no), baseline values of fasting and 2-hour glucose, and baseline values and change over the follow-up period in concentrations of fasting insulin, HOMA IR, HOMA β-cell, fasting C-peptide and fasting proinsulin, as well as baseline and change over the follow up period in percent body fat and waist circumference. Crude models were examined, as well as models adjusted for age, sex, waist circumference, and baseline GTS, which were included as potential confounding factors. Finally, duration of follow-up was included as a continuous variable in all fully adjusted models. This variable had no effect on the magnitude or significance of the ORs (data not shown), and it was thus not included in the models presented in the tables.

Spearman correlation analyses was employed to evaluate deterioration or improvement of glucose levels as continuous outcome variables. Independent variables in these models were similar to those outlined above for the logistic regression analyses: baseline values and change over the follow-up period in concentrations of fasting and 2-hour glucose, fasting IRI, HOMA IR, fasting C-peptide and fasting proinsulin, as well as baseline and change over the follow up period in percent body fat and waist circumference.

Paper 5.
II (b): Assess the importance of smoking, alcohol consumption, dietary intakes of fat and fiber in determining the development of diabetes and changes in glucose concentrations.

Risk factors for progression to diabetes were evaluated using logistic regression analyses. The dependent variable in these models was development of diabetes (yes/no). Separate models were constructed for each independent variable of interest, including baseline cigarette smoking, alcohol consumption, nutrient intake from the 24-hour recall and patterns of food consumption from the food frequency questionnaire. When independent factors were modeled as continuous variables, the odds ratio (OR) refers to an increase in risk per 1 SD change among subjects who did
not progress to diabetes. Both crude and adjusted odds ratios were examined. Potential
confounding factors included age, sex, baseline diabetes status, change in waist circumference,
smoking, alcohol consumption, and energy intake. Finally, duration of follow-up was included as a
continuous variable in all fully adjusted models. This variable had no effect on the magnitude or
significance of the ORs (data not shown), and it was thus not included in the models presented in
the tables.
2.5 References


Chapter 3

Results

3.1 INTRODUCTION

This thesis has been written in the “journal format”, and the results are presented as 5 short papers assembled using the general aspects of style and organization for manuscripts submitted to peer-reviewed scientific journals. To ensure coherence between the individual papers, each is followed by a brief linking section, which will provide a conceptual bridge and rationale leading to the subsequent paper.
PROINSULIN, GLUCOSE TOLERANCE STATUS AND OBESITY IN A NATIVE CANADIAN COMMUNITY EXPERIENCING AN EPIDEMIC OF TYPE 2 DIABETES MELLITUS

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ABSTRACT

An understanding of the natural history of type 2 diabetes mellitus (Type 2 DM) is complicated by the lack of reliable measures of beta cell function. Proinsulin is emerging as a surrogate indicator of beta cell decompensation, although little information is available regarding factors that contribute to proinsulin variation within glucose tolerance (GT) categories. The objectives of the present study were (1) to assess differences in fasting proinsulin concentration between subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 DM in a population with high rates of glucose intolerance, (2) to determine the relationship between glucose and proinsulin concentrations within glucose tolerance categories, and (3) to examine the contribution of adiposity to variation in fasting proinsulin within GT categories.

Between 1993 and 1995, 728 (72%) of eligible members of an isolated Native Canadian community participated in a population-based survey to determine the prevalence and risk factors for type 2 DM. Samples for determination of serum concentrations of glucose, C-peptide and proinsulin were drawn after an overnight fast, and a 75g oral glucose tolerance test was administered with a second sample for glucose drawn after 120 minutes. Type 2 DM and IGT were diagnosed according to WHO criteria. Waist circumference was measured, and percent (%) body fat was determined using bioelectrical impedance analysis. In 1998, 95 individuals who at baseline had IGT or NGT with an elevated 2-hour glucose level ($\geq 7.0$ mmol/l) participated in a follow-up evaluation using the same protocol employed at baseline.

After adjustment for age, sex, C-peptide, % body fat and waist circumference, proinsulin level was found to be significantly elevated in diabetic subjects relative to subjects with both IGT and NGT (both $p<0.0001$), and the concentration in those with IGT was higher compared to NGTs ($p<0.0001$). In addition, proinsulin increased across quartiles of normal glucose tolerance ($p_{trend}<0.0001$). After similar adjustment, both fasting and 2 hour (hr) glucose levels were independently associated with variation in proinsulin concentration in subjects with NGT (fasting glucose: partial $r=0.32$, $p<0.0001$; 2hr glucose: partial $r=0.12$, $p=0.0063$) and Type 2 DM (fasting glucose: partial $r=0.33$, $p=0.0003$; 2hr glucose: partial $r=0.40$, $p=0.0007$). Further, waist circumference was an independent determinant of variation in proinsulin in NGT and Type 2 DM after adjustment for covariates. After further adjustment for % body fat, waist circumference was significantly related to proinsulin concentration in NGT (males: partial $r=0.23$, $p=0.0005$, females: partial $r=0.17$, $p=0.0046$) and Type 2 DM (females only: partial $r=0.33$, $p=0.0058$). In the
prospective analysis, baseline waist circumference was positively associated with follow-up and change in proinsulin concentration (both p<0.05).

These data highlight the detrimental effects of abdominal obesity on beta cell function, and support the hypothesis that beta cell dysfunction occurs early in the natural history of glucose intolerance.
INTRODUCTION

In 1971, Saad and colleagues hypothesized a two step model for the natural history of type 2 diabetes mellitus (type 2 DM) based on research results from studies of the Pima Indians [1]. In this model, the transition from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) (step 1) was largely characterized by insulin resistance, while the further deterioration to diabetes (step 2) was thought to be a consequence of beta cell decompensation [1]. While a large volume of research in the subsequent period has generally supported the notion that both insulin resistance and beta cell dysfunction are components in the pathogenesis of type 2 DM, the specific sequence of events remains highly controversial [2]. In fact, recent literature syntheses highlight the close physiological connection between insulin resistance and insulin deficiency [2, 3]. They further suggest that beta-cell dysfunction may be present much earlier in diabetes pathophysiology, and that this condition is exacerbated by intra-abdominal obesity-related insulin resistance, glucose toxicity and other beta-cell stressors [2, 3].

An understanding of the etiology of beta cell failure is complicated by the fact that there is currently no agreed-upon “gold standard” technique for its measurement [2], although fasting proinsulin concentration is emerging as a possible surrogate indicator of beta cell dysfunction for use in larger, population-based studies [4, 5]. Both proinsulin and the proinsulin-to-insulin ratio (PI/I) have been reported to be increased in subjects with type 2 DM relative to controls with NGT, a finding that has been confirmed in several populations [6-19]. The evidence for elevated proinsulin is much less consistent, however, in other conditions that are thought to be related to the development of diabetes, including impaired glucose tolerance (IGT) [8, 10, 11, 12, 14-19]. Further, the relationship between glucose and proinsulin concentrations within categories of glucose tolerance requires more specific investigation. While fasting glucose has been shown to correlate with proinsulin among diabetic subjects [10, 15, 16, 17], only one study has analyzed proinsulin-glucose associations in nondiabetic subjects [15].

In addition, the possible role of obesity as an independent determinant of variation in proinsulin concentration has received only limited attention. In non-diabetic subjects, body mass index (BMI) and waist-hip ratio (WHR) have been reported to be positively correlated with proinsulin and, less consistently, inversely related to the PI/I ratio in a limited number of studies [15, 17, 24-28]. These papers presented the results of univariate analyses only, however, and the effect of confounding by other variables on these relationships is unknown. To our knowledge, the
relationship between proinsulin and percent body fat has not been explored, nor has the association with waist circumference alone, which has been shown to be superior to WHR as a surrogate measure of intra-abdominal fat [29].

A detailed examination of the associations of proinsulin concentration with adiposity and glucose tolerance would be of value in light of the emerging evidence for early beta cell dysfunction in the etiology of type 2 DM [2, 3]. We have previously documented high rates of obesity and glucose intolerance in a population-based study of Native Canadians in northern Ontario [30-32]. In the present paper, we employ data from this study to address the following specific research questions: (1) Are proinsulin levels elevated in subjects with type 2 DM or IGT relative to those with NGT? (2) What is the relationship between glucose and proinsulin concentrations within glucose tolerance categories? (3) Are measures of total and intra-abdominal adiposity independently related to proinsulin concentration?

SUBJECTS AND METHODS

The community of Sandy Lake, Ontario, is located roughly 2000 km northwest of Toronto in the Boreal Forest region of central Canada. Approximately 1600 people live in this isolated village, which is accessible only by air for most of the year. Historically, the inhabitants of this area lived in small nomadic groups and led a hunting and gathering subsistence typical of other North American subarctic populations. Their lives were physically active and their diet high in protein from wild meat and fish, with seasonal supplementation from berries and roots. The lifestyle of the people of this region has changed dramatically over the past several decades, with a marked decrease in physical activity and an alteration in diet to one characterized by excess consumption of saturated fat and processed foods [33]. This population is consequently undergoing an epidemiologic transition, with a rapid increase in morbidity related to chronic diseases, such as obesity and Type 2 DM [33-36].

Baseline Prevalence Survey

The methodology of the SLHDP prevalence study has been presented in detail in previous publications [30, 31]. Briefly, between July 1993 and December 1995, 728/1018 (72%) eligible residents of Sandy Lake aged 10-79 years participated in a population-based cross-sectional survey to determine the prevalence of Type DM and its associated risk factors. Signed informed consent was obtained from all participants, and the study was approved by the Sandy Lake First Nation Band
Council and University of Toronto Ethics Review Committee. The current study is based on data from 701 individuals for whom specimens were available for proinsulin determination.

Participants provided fasting blood samples for glucose, insulin, and proinsulin after an 8-12 hour overnight fast. A 75g oral glucose tolerance test (OGTT) was administered, and a second sample for glucose was drawn after 120 minutes. Individuals were excluded from the OGTT if they had physician diagnosed diabetes and were (a) currently receiving treatment with insulin or oral hypoglycemic agents, or (b) if they had a fasting blood glucose concentration exceeding 11.1 mmol/l. Women who were pregnant at the time of initial contact received their OGTT 3 months post-partum. Diabetes and IGT were diagnosed according to established criteria [37].

Insulin was measured using a radioimmunoassay (Pharmacia, Inc.) which has a lower detection limit of 22 pmol/l, and an interassay coefficient of variation of 7.2-8.8%. While this assay displays low cross-reactivity with C-peptide (<0.18%), the cross-reactivity with proinsulin is very high (100%), and thus reported values refer to concentrations of total immunoreactive insulin (IRI). Glucose concentration was determined using the glucose oxidase method. C-peptide level was measured using a radioimmunoassay (Diagnostic Products Corporation, Los Angeles) which has minimal detection limit of 43 pmol/l, and cross-reactivities of 0% with insulin and <13% with proinsulin.

Proinsulin concentration was determined using a human proinsulin radioimmunoassay (Linco Research Inc. [38]), which has a laboratory sensitivity of 3.5 pM, and a CV of 6.2-21.0%. This assay displays 46% cross-reactivity with des 31,32 proinsulin, the major form of circulating split proinsulin [39], and thus reported values refer to total proinsulin-like materials. Cross-reactivity with C-peptide, des 64, 65 proinsulin, and insulin is low (<0.1%). Proinsulin was measured in serum specimens that had been stored at -70°C for between 3-5 years at the Core Lab of the Banting and Best Diabetes Centre, University of Toronto. There is no information on the stability of proinsulin over time at this temperature.

Anthropometric measurements were performed with the volunteer wearing either light athletic clothing or undergarments and a hospital gown, and no shoes. Each measurement was performed twice, and the average was used in the analysis. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg using a hospital balance beam scale. Body mass index (BMI) was defined as weight/height² (Kg/m²). The waist was
measured to the nearest 0.5 cm at the point of narrowing between the umbilicus and xiphoid process; the hips were measured to the nearest 0.5 cm at the maximum extension of the buttocks. Waist hip ratio (WHR) was calculated as the ratio of these two circumferences. Percent body fat was estimated by bioelectrical impedance analysis (BIA) using the Tanita TBF-201 Body Fat Analyzer (Tanita Corp., Tokyo). High reproducibility of percent fat estimates using this machine in a sample from this population has been documented (intraclass correlation coefficient (ICC) = 0.99) [40], and the instrument has been validated by others against dual energy x-ray absorptiometry (DEXA) in both diabetic and non-diabetic populations [41-43].

**Prospective Study**

Subjects in the baseline survey who were found to be at high risk for subsequent diabetes, including those with impaired glucose tolerance (IGT) (n=74) [30] or normal glucose tolerance (NGT) with a 2 hour post challenge glucose level greater than or equal to 7.0 mmol/l (n=51), were invited to participate in a follow-up visit during the summer of 1998 to determine current glucose tolerance and risk factor status. Of the 125 individuals in this follow-up cohort, 3 (3 IGT, 0 NGT) had died, 11 (9 IGT, 2 NGT) were no longer living in the community, 2 (1 IGT, 1 NGT) were too sick to participate, and 14 (6 IGT, 8 NGT) refused to attend. Thus 95 (76%) members of this high-risk cohort participated in the follow-up examination. Non-participants did not differ significantly from participants in age, gender, anthropometric or metabolic variables (data not shown). Metabolic and anthropometric variables and glucose tolerance status (GTS) were determined using the same protocol employed during the 1993-1995 prevalence survey.

**Statistical Analyses**

The analytical convention in this body of literature is to “adjust” proinsulin level for insulin secretion, usually by employing the proinsulin-to-insulin ratio. This is problematic, for two reasons. First, peripheral insulin levels do not adequately represent insulin secretion given that “insulin undergoes a large and variable hepatic extraction as well as peripheral clearance that varies under different physiological circumstances” [44]. Second, Kronmal has pointed out that the use of ratio variables in correlation and regression can result in spurious results [45]. We have therefore opted to avoid the use of ratios and to conduct our analysis of proinsulin levels after adjustment for C-peptide, which is “co-secreted with insulin in an equimolar ratio, is not extracted by the liver, and has a constant peripheral clearance” [44].
All analyses were carried out using SAS version 6.12 [47]. The distributions of continuous variables were assessed for normality using the Shapiro and Wilk statistic and plots provided by SAS PROC UNIVARIATE, and the natural log transformation (log, sub) of skewed variables was used in subsequent multivariate analysis. Differences in proinsulin concentration by category (GTS and levels of anthropometry) were tested using analysis of covariance, adjusting for age, sex, C-peptide, percent body fat, and waist circumference.

Correlation and multiple linear regression techniques were employed to determine the independent contribution of anthropometric factors and glucose levels to variation in fasting proinsulin concentration within glucose tolerance categories. Scatterplots were examined to scan for departures from linearity. In lieu of BMI and WHR, we used percent body fat and waist circumference as measures of total and abdominal adiposity, respectively. As mentioned above, percent body fat measured by BIA has shown good criterion validity against DEXA [41-43], and waist circumference has been demonstrated to be superior to WHR as a measure of intra-abdominal fat [29]. The dependent variable in these models was \( \log_\text{e} \) fasting proinsulin. Both crude and adjusted (for sex, ln age and ln C-peptide) models are presented. We anticipated that gender-adiposity and waist circumference-percent body fat effect modifications were plausible, and we tested for these interactions by including cross-product terms in adjusted models. Stratum-specific results are presented when the p-value for the interaction term was <0.05, or when effects differed appreciably across strata. Model collinearity was assessed using the variance inflation factor (VIF) method described by Freund and Littell [46]. Based on these criteria, none of our models displayed evidence of marked collinearity.

In the analysis of the prospective data, the effect of both baseline levels and changes in \% body fat and waist circumference on both follow-up and change in proinsulin concentrations were evaluated using Spearman correlation analyses. Coefficients were adjusted for age, sex, C-peptide, and baseline and follow-up diabetes status. Models analyzing the effect of change in the independent variable (\% body fat or waist circumference) also included the baseline of that variable to adjust for the correlation between the initial and follow-up levels. Scatterplots were examined to screen for the presence of outliers.
RESULTS

Table 1 presents characteristics of participants in the SLHDP by glucose tolerance status (GTS). There was an increase in the mean of age, proinsulin, fasting and 2hr glucose, waist circumference and WHR with increasing GTS category. BMI as well as IRI and C-peptide concentrations were higher among subjects with IGT and DM compared to those among NGT.

Proinsulin Variation in Relation to Glucose Tolerance Status and Glucose Concentrations.

Figure 1 presents the frequency distribution of proinsulin concentration by GTS category. Each of the distributions was skewed, and there was notable overlap between the categories. After adjustment for age, sex, C-peptide, percent body fat and waist circumference, log$_{10}$ proinsulin level was significantly elevated in diabetic subjects (least square mean±standard error, 3.10±0.04 pmol/l) relative to both IGT (2.70±0.05 pmol/l) and NGT (2.39±0.02 pmol/l) subjects (both p<0.0001), and the concentration in those with IGT was significantly higher compared to subjects with NGT (p<0.0001). When normoglycaemic subjects were further stratified based on quartiles of fasting glucose concentration, proinsulin concentration increased continuously across these categories (Figure 2A). The trend was significant from both the lowest quartile of NGT (N1) through the highest NGT quartile (N4) ($p_{trend}$<0.0001), and from N1 through type 2 DM ($p_{trend}$<0.0001). Proinsulin concentration also increased with increasing quartile of 2 hour glucose concentration (Figure 2B), although the pattern was not as consistent as that for fasting glucose (N1 through N4, $p_{trend}$=0.08; N1 through type 2 DM, $p_{trend}$<0.0001).

We also examined the association between proinsulin and glucose within glucose tolerance categories using continuous variables. Unadjusted analysis indicated that fasting glucose concentration was positively related to proinsulin level, regardless of glucose tolerance status (Table 2). Pearson correlation coefficients indicated moderate but statistically significant associations ($r = 0.40$, $r = 0.30$, $r = 0.18$ for NGT, IGT and type 2 DM, respectively; all p-values <0.05). Two-hour glucose concentration was a significant variable only in those with normal glucose tolerance and diabetes. In multivariate models adjusting for age, sex, and insulin secretion (C-peptide), both fasting and 2-hour glucose levels were significant independent sources of variation in proinsulin level among subjects with NGT and diabetes (Table 2). While this was not the case in the IGT group, it is important to note that the positive fasting glucose coefficient in this group was within the confidence intervals for this variable among diabetic subjects, and that the p-value approached
statistical significance. This highlights the relatively limited statistical power in the IGT category. Partial correlation coefficients indicate that, after adjustment for other factors, fasting glucose concentration accounted for 4-11% of the variation in proinsulin concentration, while post-challenge glucose concentration accounted for 0-16%, with the large correlation among diabetic subjects referring to the subset of 72 subjects who were eligible for the OGTT (Table 2). Multivariate adjustment slightly reduced the magnitude if the associations among subjects with NGT, and slightly increased the magnitude among subjects with diabetes.

**Adiposity and Proinsulin Variation**

Table 3 presents results of crude and adjusted analyses examining the association between adiposity and proinsulin level. Waist circumference models are presented both pooled and stratified by gender given that initial analysis detected a significant interaction between gender and waist circumference (p=0.03). In subjects with NGT, crude models indicate significant associations with both % fat and waist circumference, with the latter explaining more of the variation in proinsulin level. After adjustment for age, sex and insulin secretion, % fat and waist circumference maintained their positive associations with proinsulin level; for waist circumference, this was the case for both pooled and gender-specific models (all p-values <0.0001). Percent body fat and waist circumference explained approximately 3% and 8%, respectively, of the variation in proinsulin concentration independent of other factors. Waist circumference was also positively related to proinsulin in diabetic subjects in both crude and adjusted models, although this was not the case for percent body fat. In models run separately by gender, waist circumference was a significant factor in female diabetic subjects only (males: partial r=0.06, p=0.69; females partial r=0.29, p=0.02). Adiposity variables did not appear to be associated with proinsulin level among individuals with IGT.

We further explored the relationship between adiposity and proinsulin concentration by adjusting the multivariate waist circumference models by % body fat (Table 4). The significant positive association with waist was maintained among subjects with NGT in both pooled and gender-specific models. Among diabetic subjects, there was a positive relationship between waist and proinsulin in the pooled model, although after stratification the effect appeared to be restricted to females. Figure 3 presents least square mean proinsulin concentrations by gender and quartiles of waist circumference. In both males and females with NGT, there is a clear trend of increasing proinsulin concentration with increasing levels of waist circumference after adjustment for age, insulin secretion, and percent body fat (males, \( p_{\text{trend}} < 0.0001 \); females, \( p_{\text{trend}} = 0.0002 \)).
Prospective Relationship between Adiposity and Proinsulin Concentration

The associations of baseline levels and change in adiposity with follow-up and change in proinsulin concentration are presented in table 5. After adjustment for age, sex, C-peptide concentration, and baseline and follow-up diabetes status, baseline waist circumference was a significant predictor of both follow-up and change in proinsulin concentration (partial r=0.27 and 0.24, respectively, both p<0.05). In gender specific analyses, both associations were stronger among women. Change in waist circumference predicted change in proinsulin concentration (partial r=0.20, p=0.08); again, the magnitude of association appeared to be stronger among women. Baseline % body fat also displayed a positive association with follow-up and change in proinsulin.

Subjects whose change in waist circumference was equal to or above the gender-specific median had a significantly larger change in proinsulin concentration compared to those whose waist circumference change was below the median (both sexes, 9.83 vs. -0.16 pmol/l, p=0.008 after adjustment for covariates) (Figure 4). The pattern was similar for both males and females, although the difference was statistically significant only among females.

DISCUSSION

In this paper we have documented significantly increased serum proinsulin concentrations in Native Canadian subjects with Type 2 DM and IGT compared to those with NGT. Further, we found that proinsulin concentrations increased across the spectrum of normal glucose tolerance, and that fasting and 2-hour glucose concentrations were associated with proinsulin concentration after adjustment for age, sex and insulin secretion in NGT and Type 2 DM. In addition, adiposity was positively and independently related to proinsulin concentration, and waist circumference appeared to be particularly detrimental to pancreatic beta-cell function after further adjustment for percent body fat. Finally, our prospective analyses demonstrated that baseline levels and change over time in waist circumference were significant predictors of follow-up and changes in proinsulin concentration.

Previous studies found that proinsulin concentrations and PI/I ratios were consistently elevated among individuals with Type 2 DM compared to those with NGT [6-19], and, in some cases, compared to those with IGT [10, 11, 13, 14]. While the findings of the present investigation
are not unique in this regard, this is the first study to examine proinsulin concentrations in aboriginal people from the Canadian subarctic, a population with very high rates of both pediatric and adult type 2 DM [34-36, 48, 49]. The literature regarding proinsulin concentrations in IGT is less consistent: Caucasian, Mexican-American and Japanese subjects with this metabolic disorder have elevated proinsulin concentrations and PI/I ratios relative to subjects with NGT [8, 12, 14, 16], while Pima Indian, Japanese-American and Finnish subjects with IGT do not [10, 15, 17]. In the present study, subjects with IGT had significantly higher proinsulin concentrations compared to those with NGT after adjustment for age, sex, insulin secretion and adiposity. Further, we found that proinsulin concentrations increased significantly across quartiles of normal glucose tolerance, an observation that extends the recent report of Meigs et al. [50], who documented similar trends in obesity, insulin levels and cardiovascular risk factors across the spectrum of NGT in the Framingham Offspring Study. These findings lend further support to the hypothesis that elevated proinsulin is a feature of phenotypes associated with risk for the development of Type 2 DM.

Both fasting and 2-hour glucose concentrations were positively and independently related to proinsulin level in subjects with NGT and diabetes, and the parameter for fasting glucose among those with IGT approached significance. This result among diabetic subjects has been reported previously [10], and is consistent with the hypothesis that glucose toxicity plays a role in the pathogenesis of beta cell decompensation [51]. Recent in vitro studies have documented increased proinsulin release by human β-cells after chronic exposure to glucose [52, 53, 54, 55, 56]. Subjects with NGT generally have lower glucose concentrations, however, and the result in this group is more difficult to explain. It is possible that elevated concentrations of proinsulin and glucose co-exist in individuals with a pre-diabetic phenotype and reflect environmentally- or genetically-determined beta-cell dysfunction. It has been reported, for example, that baseline concentrations of both fasting glucose and fasting proinsulin independently predict the development of diabetes [21, 23, 57]. In addition, recent investigations have demonstrated positive associations between hyperproinsulinaemia and more detailed clinical measures of beta cell dysfunction, including increased relative arginine-stimulated proinsulin secretion [58] and delayed insulin response during a hyperglycaemic clamp [59] in subjects with IGT, and reduced acute insulin response to arginine in diabetic subjects [60]. Alternatively, the toxicity of glucose to the beta cell may be in effect at lower glucose concentrations than previously thought, a possibility that deserves consideration given the accumulating evidence for early beta-cell dysfunction in diabetes pathogenesis [2].
Univariate results from previous papers have indicated that proinsulin concentrations are elevated in obese subjects [10, 11, 14, 18, 61, 62], and that proinsulin is positively correlated with BMI and WHR [15, 24, 25, 26, 27, 28]. The results of the present paper extend these findings by demonstrating independent associations with percent body fat and waist circumference in subjects with NGT and type 2 DM after adjustment for age, sex and insulin secretion. After further adjustment for total body adiposity, waist circumference was significantly related to proinsulin concentration in NGT (both sexes) and diabetes (women only), and, further, waist circumference was prospectively associated with changes in proinsulin concentration. These strong and generally consistent positive associations between waist circumference and proinsulin concentration suggest a distinctive, unfavorable role for intra-abdominal fat (IAF) in the natural history of beta-cell dysfunction. The possibility of a direct detrimental effect of intra-abdominal adipose tissue on the health of the beta cell, even prior to the development of diabetes, is supported by clinical studies demonstrating strong correlations between directly-measured IAF and insulin resistance [63], and by in vitro studies which have shown higher lypolytic activity of mesenteric and omental adipose tissue [64]. As is reviewed by Ferrannini [2], it is conceivable that this IAF-related insulin resistance might exacerbate existing beta cell dysfunction or injury, which may be present relatively early in diabetes pathogenesis. Recently, Kahn and co-workers [17] reported a significant crude association between directly-measured IAF and proinsulin in Japanese-American subjects.

Recent work by Unger’s group has suggested an alternative mechanism whereby excess adiposity might lead to increased proinsulin concentrations [65]. The adipocytes of obese individuals release high levels of free fatty acids (FFA) [65] which, in the short term, causes beta cell hyperplasia and hyperinsulinaemia, but with chronic exposure (and subsequent increase in FFA levels) leads to functional and morphologic changes in beta cells and consequent diabetes [65]. This notion has been supported with the documentation of substantial fat deposition in islets of obese rats, and the demonstration of FFA-induced loss of glucose-stimulated insulin secretion [66]. Increased FFA also induce nitric oxide synthase, and Shimabukuro et al. [67, 68] have shown that elevated FFA in rat beta cells cause increases in both nitric oxide levels and ceramide-mediated beta cell apoptosis (programmed cell death). The consequent reduction in the number of beta cells may result in elevated proinsulin concentration, in that the rate of secretion by remaining cells is increased, thereby decreasing the intracellular stores and forcing the release of incompletely processed materials [69, 70].
This interpretation is contrary to conclusions drawn from studies of the association between obesity and insulin resistance and PI/I. Reported relationships between measures of obesity and PI/I have been inconsistent [10, 11, 16, 26, 27], although two studies [10, 16] have reported significant inverse associations between BMI and PI/I in subjects with NGT. These results, taken together with data from clinical studies using more rigorous measures of insulin resistance, have been interpreted to suggest that non-diabetic beta cells are able to respond to the stress of insulin resistance (represented in the population-based studies by elevated adiposity) by increasing their secretion of true insulin without parallel or disproportionate increases in proinsulin secretion [19, 28]. While this hypothesis is clearly tenable, the results of studies using PI/I are not directly comparable to those from the present analysis, in that we elected not to use the PI/I. Rather, we used proinsulin concentration as our primary outcome variable, and adjusted our models for C-peptide, a reliable surrogate measure of insulin secretion. These decisions were based on statistical and physiological evidence available at the time the analysis was conducted [44, 45].

In conclusion, this paper lends further support to the observation of elevated concentrations of proinsulin among subjects with IGT and type 2 DM, and extends the phenomenon to Native Canadians from the central subarctic. We have further demonstrated independent positive associations between glucose concentrations and proinsulin in normoglycaemic and diabetic subjects, and have documented a possible detrimental role for intra-abdominal adiposity in beta cell function. These observations require confirmation in future studies. In particular, further analyses employing multivariate adjustment using C-peptide would be of interest.
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REFERENCES


69. Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ. Chronic exposure to free fatty acid reduces pancreatic beta-cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis transformation. J Clin Invest 1998;101:1094-1101.

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\(^1\) values for biochemical variables are fasting concentrations (mean ± standard deviation), unless otherwise indicated.

\(^2\) Sample sizes vary due to occasional missing values.

\(^3\) Total immunoreactive insulin.
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<td>Fasting glucose</td>
<td>1.3331 (0.4947)</td>
<td>0.30 (0.08-0.50)</td>
</tr>
<tr>
<td>2 hr glucose</td>
<td>0.6331 (0.6328)</td>
<td>0.12 (-0.11-0.34)</td>
</tr>
<tr>
<td>Type 2 DM (n=122)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.2450 (0.1230)</td>
<td>0.18 (0.00-0.35)</td>
</tr>
<tr>
<td>2 hr glucose&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.3534 (0.1747)</td>
<td>0.23 (0.00-0.44)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Natural log transformation of proinsulin used in analyses to improve normality.

<sup>2</sup>2-hour glucose only measured in 72 individuals in this category.

<sup>3</sup>Adjusted for sex, natural log (ln) of age, and C-peptide.
## TABLE 3. Crude and adjusted cross-sectional relationships between fasting proinsulin concentration and measures of obesity, by glucose tolerance status.

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Crude Analysis</th>
<th></th>
<th></th>
<th></th>
<th>Adjusted Analysis</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>r</td>
<td>(95% CI)</td>
<td>p-value</td>
<td>model R²</td>
<td>β (SE)</td>
<td>partial</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>NGT (n=505)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% body fat</td>
<td>0.0098 (0.0016)</td>
<td>0.26</td>
<td>(0.18-0.34)</td>
<td>&lt;0.0001</td>
<td>0.07</td>
<td>0.0074 (0.0020)</td>
<td>0.17</td>
<td>(0.08-0.25)</td>
</tr>
<tr>
<td>waist circumference</td>
<td>0.1345 (0.0015)</td>
<td>0.38</td>
<td>(0.30-0.45)</td>
<td>&lt;0.0001</td>
<td>0.14</td>
<td>0.0109 (0.0017)</td>
<td>0.28</td>
<td>(0.20-0.36)</td>
</tr>
<tr>
<td>males (n=232)</td>
<td>0.0174 (0.0021)</td>
<td>0.47</td>
<td>(0.36-0.57)</td>
<td>&lt;0.0001</td>
<td>0.22</td>
<td>0.0141 (0.0027)</td>
<td>0.32</td>
<td>(0.20-0.43)</td>
</tr>
<tr>
<td>females (n=273)</td>
<td>0.0100 (0.0020)</td>
<td>0.29</td>
<td>(0.18-0.40)</td>
<td>&lt;0.0001</td>
<td>0.08</td>
<td>0.0008 (0.0020)</td>
<td>0.24</td>
<td>(0.12-0.35)</td>
</tr>
<tr>
<td>IGT (n=74)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% body fat</td>
<td>0.0084 (0.0061)</td>
<td>0.16</td>
<td>(-0.07-0.38)</td>
<td>0.1769</td>
<td>0.03</td>
<td>-0.0043 (0.0075)</td>
<td>-0.07</td>
<td>(-0.31-0.18)</td>
</tr>
<tr>
<td>waist circumference</td>
<td>0.0054 (0.0055)</td>
<td>0.12</td>
<td>(-0.11-0.34)</td>
<td>0.3258</td>
<td>0.01</td>
<td>-0.0030 (0.0054)</td>
<td>-0.07</td>
<td>(-0.30-0.17)</td>
</tr>
<tr>
<td>males (n=16)</td>
<td>0.0116 (0.0136)</td>
<td>0.22</td>
<td>(-0.31-0.65)</td>
<td>0.4077</td>
<td>0.05</td>
<td>-0.0018 (0.0159)</td>
<td>-0.03</td>
<td>(-0.62-0.58)</td>
</tr>
<tr>
<td>females (n=58)</td>
<td>0.0048 (0.0061)</td>
<td>0.11</td>
<td>(-0.15-0.34)</td>
<td>0.4273</td>
<td>0.01</td>
<td>-0.0030 (0.0059)</td>
<td>-0.07</td>
<td>(-0.33-0.20)</td>
</tr>
<tr>
<td>Type 2 DM (n=122)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% body fat</td>
<td>0.0018 (0.0051)</td>
<td>0.03</td>
<td>(-0.15-0.21)</td>
<td>0.7203</td>
<td>0.00</td>
<td>0.0008 (0.0070)</td>
<td>0.01</td>
<td>(-0.18-0.20)</td>
</tr>
<tr>
<td>waist circumference</td>
<td>0.0177 (0.0047)</td>
<td>0.33</td>
<td>(0.15-0.48)</td>
<td>0.0003</td>
<td>0.11</td>
<td>0.0010 (0.0045)</td>
<td>0.20</td>
<td>(0.01-0.37)</td>
</tr>
<tr>
<td>males (n=45)</td>
<td>0.0157 (0.0084)</td>
<td>0.27</td>
<td>(-0.03-0.32)</td>
<td>0.0683</td>
<td>0.08</td>
<td>0.0036 (0.0090)</td>
<td>0.06</td>
<td>(-0.26-0.37)</td>
</tr>
<tr>
<td>females (n=72)</td>
<td>0.0177 (0.0058)</td>
<td>0.34</td>
<td>(0.12-0.53)</td>
<td>0.0032</td>
<td>0.12</td>
<td>0.0121 (0.0049)</td>
<td>0.29</td>
<td>(0.05-0.50)</td>
</tr>
</tbody>
</table>

1 Natural log transformation of proinsulin used in analyses to improve normality.
2 Adjusted for sex, natural log (ln) of age, and C-peptide.
TABLE 4. Cross-sectional relationships between fasting proinsulin concentration\(^1\) and waist circumference, after adjustment for age, insulin secretion and total body adiposity.

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Adjusted Analysis(^2)</th>
<th>(\beta) (SE)</th>
<th>partial (r)</th>
<th>(95% CI)</th>
<th>p-value</th>
<th>model R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT (n=505)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>waist circumference</td>
<td></td>
<td>0.0174 (0.0030)</td>
<td>0.25</td>
<td>(0.17-0.33)</td>
<td>&lt;0.0001</td>
<td>0.43</td>
</tr>
<tr>
<td>males (n=232)</td>
<td></td>
<td>0.0187 (0.0053)</td>
<td>0.23</td>
<td>(0.10-0.35)</td>
<td>0.0005</td>
<td>0.39</td>
</tr>
<tr>
<td>females (n=273)</td>
<td></td>
<td>0.0008 (0.0020)</td>
<td>0.17</td>
<td>(0.05-0.28)</td>
<td>0.0046</td>
<td>0.49</td>
</tr>
<tr>
<td>IGT (n=74)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>waist circumference</td>
<td></td>
<td>0.0035 (0.0081)</td>
<td>0.05</td>
<td>(-0.19-0.29)</td>
<td>0.6690</td>
<td>0.44</td>
</tr>
<tr>
<td>males (n=16)</td>
<td></td>
<td>-0.0102 (0.0216)</td>
<td>-0.15</td>
<td>(-0.69-0.49)</td>
<td>0.6477</td>
<td>0.29</td>
</tr>
<tr>
<td>females (n=58)</td>
<td></td>
<td>0.0074 (0.0090)</td>
<td>0.12</td>
<td>(-0.16-0.38)</td>
<td>0.4120</td>
<td>0.49</td>
</tr>
<tr>
<td>Type 2 DM (n=122)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>waist circumference</td>
<td></td>
<td>0.0138 (0.0057)</td>
<td>0.23</td>
<td>(0.05-0.40)</td>
<td>0.0172</td>
<td>0.32</td>
</tr>
<tr>
<td>males (n=45)</td>
<td></td>
<td>0.0006 (0.0122)</td>
<td>0.01</td>
<td>(-0.31-0.32)</td>
<td>0.9615</td>
<td>0.25</td>
</tr>
<tr>
<td>females (n=72)</td>
<td></td>
<td>0.0177 (0.0062)</td>
<td>0.33</td>
<td>(0.10-0.53)</td>
<td>0.0058</td>
<td>0.43</td>
</tr>
</tbody>
</table>

\(^1\)Natural log transformation of proinsulin used in analyses to improve normality.

\(^2\)Adjusted for sex, natural log (ln) of age and C-peptide, and percent body fat.
**TABLE 5. Prospective associations of baseline levels and change in anthropometric measurements with follow-up and change in fasting proinsulin concentration (total n = 90, male = 28, female = 62)\(^1\).**

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Proinsulin Concentration</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>partial Spearman r (95% CI) p-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>change</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>partial Spearman r (95% CI) p-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% body fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline(^2)</td>
<td>0.13 (-0.06-0.34) 0.2372</td>
<td>0.21 (-0.01-0.41) 0.0626</td>
<td></td>
</tr>
<tr>
<td>change(^3)</td>
<td>-</td>
<td>0.10 (-0.13-0.32) 0.3933</td>
<td></td>
</tr>
<tr>
<td>waist circumference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline(^2)</td>
<td>0.27 (0.06-0.46) 0.0135</td>
<td>0.24 (0.02-0.44) 0.0312</td>
<td></td>
</tr>
<tr>
<td>males</td>
<td>0.39 (-0.04-0.70) 0.0588</td>
<td>0.14 (-0.32-0.55) 0.5121</td>
<td></td>
</tr>
<tr>
<td>females</td>
<td>0.24 (-0.02-0.47) 0.0674</td>
<td>0.26 (-0.01-0.49) 0.0517</td>
<td></td>
</tr>
<tr>
<td>change(^4)</td>
<td>-</td>
<td>0.20 (-0.02-0.40) 0.0777</td>
<td></td>
</tr>
<tr>
<td>males</td>
<td>-</td>
<td>0.07 (-0.38-0.50) 0.7602</td>
<td></td>
</tr>
<tr>
<td>females</td>
<td>-</td>
<td>0.23 (-0.04-0.47) 0.0907</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Sample sizes vary slightly due to occasional missing values; Spearman correlation analyses; analyses with genders pooled also adjusted for sex.

\(^2\)Analyses adjusted for age, C-peptide concentration, baseline and follow-up diabetes status (and gender in pooled models).

\(^3\)Analyses adjusted for age, C-peptide concentration, baseline and follow-up diabetes status, and baseline % body fat (and gender in pooled models).

\(^4\)Analyses adjusted for age, C-peptide concentration, baseline and follow-up diabetes status, and baseline waist circumference (and gender in pooled models).
A: normal glucose tolerance (n=505, mean=12.0, SD=7.2, median=10);
se tolerance (n=74, mean=19.0, SD=12.4, median=16); C: type 2 diabetes mellitus
1.1, SD=15.7, median=25).
Table 2. Proinsulin concentrations (pmol/l) by categories of glucose tolerance status. Values are least-square means (with 95% CI) from analysis of covariance, adjusted for In age, sex, In C-peptide, and natural waist circumference. A: subjects with normal glucose tolerance categorized based on quartiles of fasting glucose concentration (N1: ≤5.0, N2: 5.1-5.3, N3: 5.4-5.6, N4: >5.6 mmol/l): N1 through DM2, p(trend)<0.0001; through N4, p(trend)<0.0001. B: subjects with normal glucose tolerance categorized based on quartiles of 2 hour glucose concentration (N1: ≤4.2, N2: 4.3-5.2, N3: 5.2-6.2, N4: >6.2): N1 through DM2, p(trend)<0.0001, N1 through N4, p(trend)=0.08.
Figure 3. Proinsulin concentrations (pmol/l) by gender and quartiles of waist circumference in subjects with normal glucose tolerance (males, n=232, females, n=273). Quartiles for males: 𝐾=78.9, 79.0-90.8, 90.9-101.6, >101.6 cm. Quartiles for females: 𝐾=76.0, 77.1-87.5, 87.6-97.0, >97.0 cm. Values are least-square means (with 95% CI) from analysis of covariance, adjusted for ln age, sex, ln C-peptide, and % body fat. Males (solid bars), p(trend)<0.0001; females (open bars), p(trend)=0.0002.
Figure 4. Relationship between change in waist circumference and change in proinsulin concentration. Data are least-square mean values (with 95% CI) for change in fasting proinsulin concentration, adjusted for age, change in C-peptide concentration, baseline and follow-up diabetes status, and baseline waist circumference using analysis of covariance (pooled models also adjusted for sex). Solid bars, change in waist circumference < the gender-specific median (males<4.63cm, females<3.25cm); open bars, change in waist circumference >/= the gender-specific median.
3.3 LINKING SECTION 1

The preceding paper documented significantly elevated proinsulin concentrations in subjects with diabetes and IGT, as well as a significant trend of increasing proinsulin concentrations across quartiles of glucose among those with NGT. In addition, adiposity was significantly associated with variation in proinsulin concentration in both cross-sectional and prospective analyses. These findings suggest that (1) proinsulin concentration is marker of beta cell dysfunction; (2) proinsulin concentrations begin to rise relatively early in the natural history of glucose intolerance; and (3) modifiable factors other than glucose are associated with variation in proinsulin concentration. Despite these significant independent associations with adiposity and glucose tolerance, however, a relatively large proportion of the variation in proinsulin concentration remains unexplained. This suggests that other modifiable factors may be playing an important role.

In this light, the association between parity and variation in proinsulin concentration was investigated. As is reviewed in the introduction to the next paper, previous studies reporting on the association between parity and both diabetes risk and insulin resistance have been highly inconsistent. Further, the association between parity and proinsulin concentration has not been studied to date. Understanding the association between parity and proinsulin is timely and appropriate for several reasons, in that it will help to (1) clarify the conflicting previous studies of parity and diabetes risk and insulin resistance, and (2) identify additional factors that are associated with early beta cell dysfunction.
PARITY AND ITS RELATIONSHIP WITH RISK OF GLUCOSE INTOLERANCE AND RELATED DISORDERS IN A NATIVE CANADIAN POPULATION EXPERIENCING AN EPIDEMIC OF TYPE 2 DIABETES MELLITUS.

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⁷Division of Endocrinology and Metabolism, Mt. Sinai Hospital and the University Health Network

Running title: Parity and risk of diabetes and related metabolic disorders

Key words:

Parity Diabetes, Type 2 Insulin resistance Proinsulin Leptin

Indians, North American Epidemiology

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ABSTRACT

The relationship between parity and risk of diabetes is controversial. In addition, little information is available regarding associations between parity and measures of insulin resistance and beta cell function. In the present paper, we report on the relationship between parity on risk of glucose intolerance and related metabolic disorders, including elevated insulin, leptin and proinsulin concentrations, using data from population-based study in a Native Canadian community currently experiencing an epidemic of type 2 diabetes. Female participants (n=383, age 12-79) provided fasting blood samples for determination of glucose, insulin, C-peptide, proinsulin and leptin concentrations. A 75g oral glucose tolerance test was administered, and diabetes and impaired glucose tolerance were diagnosed according to WHO criteria. Waist circumference was measured, and % body fat was determined using bioelectrical impedance analysis. Information regarding occurrence of live births and previously diagnosed diabetes was obtained from interviewer-administered questionnaires, and current use of oral contraceptives (OC) was determined from medical records. Logistic regression analysis indicated that parity was associated with a significantly reduced risk of diabetes among women age 30 and older (nulliparous vs. ≥ 1 birth, OR=0.31, 95% CI 0.11-0.88, p<0.05) after adjustment for age, waist circumference and OC use. A similar non-significant reduction in risk was also apparent among younger women. Despite their lower age-adjusted adiposity (p<0.05 for both waist and % body fat), nondiabetic nulliparous women had significantly lower insulin sensitivity and significantly elevated concentrations of fasting insulin, C-peptide, proinsulin and leptin relative to those reporting 3-4 live births (all p<0.05) in analyses adjusted for age and waist circumference. Adjustment for OC use did not appreciably alter these findings. Our results regarding diabetes risk and insulin concentrations are consistent with those from studies conducted among Pima Indians, and indicate that nulliparity is associated with marked insulin resistance, beta cell dysfunction and hyperleptinaemia. Further, these data suggest the presence of a relatively prevalent, diabetes-prone phenotype within the nulliparous sub-cohort of this population.
INTRODUCTION

The relationship between parity and risk of diabetes has been examined in a large number of published studies [1]. As pointed out by others [1, 2], most reported associations between parity and diabetes have not been adjusted for age or body adiposity, both of which are likely to be important confounding factors. In the nine studies that have presented results adjusted for age and adiposity [1-9], the findings have been highly inconsistent: three reported a positive relationship with diabetes risk [3-5], five found no effect [1, 2, 6, 7, 9], and one demonstrated a protective association with parity [8]. The role of childbearing in the pathogenesis of diabetes thus remains controversial.

Discordant results have also been reported regarding the effect of parity on insulin concentrations. After adjustment for obesity, Kritz-Silverstein and coworkers found that the number of pregnancies had a significant positive relationship with fasting insulin concentrations as well as an inverse relationship with insulin sensitivity, after adjustment for waist-hip ratio and other covariates [10]. Reports from the Pima and San Luis Valley diabetes studies, however, documented inverse associations between parity and insulin concentrations [7, 8]. A similar inverse relationship of parity on C-peptide concentration has been reported, although this has been examined in only one study [7]. Recently, Cowan et al. found that parity was not related to variation in fasting insulin concentrations among American Indian women participating in the Strong Heart Study [9]. The findings regarding parity and insulin concentrations have been variably interpreted to suggest that insulin resistance would predict an increased risk of diabetes among both parous women [5, 10], and, conversely, nulliparous women [8], should either association ultimately be established.

While the precise etiology of insulin resistance is unknown, it is believed to result from a complex interaction of genetic and environmental factors [11]. For many years beta cell decompensation was thought to follow insulin resistance in the natural history of glucose intolerance [12]. Evidence is accumulating, however, to support the notion that beta cell dysfunction occurs earlier in the pathogenesis of the disease, and further, that it interacts with insulin resistance in a complex feedback mechanism in non-diabetic individuals [13, 14]. Although this hypothesis is difficult to test because direct assessment of beta cell function is not possible [13], circulating proinsulin concentrations have been suggested to provide a surrogate estimate of beta cell dysfunction [15]. While proinsulin concentrations among normoglycaemic subjects are generally low compared to subjects with diabetes, there is substantial variation in the distribution of proinsulin among non-diabetic subjects [16], with higher concentrations being predictive of the development
of diabetes in prospective studies [17-20]. In light of the inconsistent results of previous studies of childbearing and insulin concentrations, as well as the substantial cross-reactivity of conventional insulin assays with proinsulin-like material, it would be of interest to examine the effect of parity on proinsulin concentration in non-diabetic women.

The discovery of leptin has also had implications for understanding the relationship between parity and metabolic disorders associated with glucose intolerance [21]. Leptin is secreted by adipocytes and acts as a barometer of adipose tissue mass. In humans it is strongly correlated with BMI, percent body fat and insulin concentrations [22-25]. Paradoxically, in two recent studies leptin concentrations were lower among subjects with diabetes compared to controls [22, 26]. Recent evidence from studies of both mouse models and human subjects suggests leptin may play an important role in specific aspects of reproduction [27-29]. This study has therefore also examined the relationship between parity and leptin.

In the present paper, we report the analysis of the relationship between parity and the risk of glucose intolerance and related metabolic disorders, including elevated leptin and proinsulin concentrations, using data from the Sandy Lake Health and Diabetes Project (SLHDP). The SLHDP is an ongoing population-based epidemiologic study of diabetes and associated risk factors, which has been conducted in partnership with Sandy Lake First Nation, an isolated Native Canadian community in northern Ontario [22, 30, 31]. Rates of type 2 diabetes mellitus (type 2 DM) and impaired glucose tolerance (IGT) among women in this community are very high, (age-adjusted prevalence rates: IGT, 19.8%; type 2 DM, 28.0%) and the age of onset of glucose intolerance is relatively young [31]. Further, families in Sandy Lake are large, and women tend to initiate childbearing at an early age. The elucidation of a diabetogenic role for either parity or nulliparity would thus be of substantial public health importance for this community. The specific objectives of the present study were (1) to evaluate the effect of parity on risk of type 2 DM and IGT, and (2) to assess the relationship between parity and variation in concentrations of insulin, proinsulin, C-peptide and leptin.
SUBJECTS AND METHODS

The methodology of the SLHDP prevalence study has been presented in detail previously [22, 30, 31]. Briefly, between July 1993 and December 1995, 728/1018 (72%) eligible residents of Sandy Lake aged 10-79 participated in a population-based cross-sectional survey to determine the prevalence of Type 2 DM and its associated risk factors. Signed informed consent was obtained from all participants, and the study was approved by the Sandy Lake First Nation Band Council and University of Toronto Ethics Review Committee. The analyses in this paper are based on data from 383 Sandy Lake females age 12-79 years, for whom information was available on glucose tolerance status and for whom serum specimens were available for determination of insulin, proinsulin and C-peptide and leptin.

Participants provided fasting blood samples for glucose, insulin, proinsulin and leptin after an 8-12 hour overnight fast. A 75g oral glucose tolerance test (OGTT) was administered, and a second sample for glucose was drawn after 120 minutes. Individuals were excluded from the OGTT if they had physician-diagnosed diabetes and were (a) currently receiving treatment with insulin or oral hypoglycemic agents, or (b) if they had a fasting blood glucose concentration exceeding 11.1 mmol/L. Women who were pregnant at the time of initial contact received their OGTT at least 3 months post-partum. Diabetes and IGT were diagnosed according to World Health Organization criteria [32].

Insulin was measured using a radioimmunoassay technique (Pharmacia, Inc) which has a lower detection limit of 22 pmol/l, and an interassay coefficient of variation (CV) of 7.2-8.8%. This assay displays a very high degree of cross-reactivity with proinsulin (100%), and thus the reported values refer to concentrations of total immunoreactive insulin (IRI) [33]. Glucose concentration was measured using the glucose oxidase method [30]. C-peptide level was measured using a radioimmunoassay (Diagnostic Products Corporation, Los Angeles) which has minimal detection limit of 43 pmol/l, and cross-reactivities of 0% with insulin and <13% with proinsulin. Proinsulin was determined using a human proinsulin radioimmuno assay which has a laboratory sensitivity of 3.5 pM, and a CV of 6.2-21.0% (Linco Research Inc., St. Louis, MO). This assay displays 46% cross-reactivity with des 31,32 proinsulin, the major form of circulating split proinsulin, and thus reported values refer to total proinsulin-like materials [34]. Cross-reactivity of this assay with des 64, 65 proinsulin, insulin and C-peptide is very low (<0.1%). Proinsulin was measured in serum
specimens that had been stored at -70°C for between 3-5 years at the Core Lab of the Banting and Best Diabetes Centre, University of Toronto. Serum leptin was measured using a radioimmunoassay prepared using recombinant human leptin as the standard. It has a minimum detectable concentration of 0.5 μg/l and the limit of linearity is 100 μg/l (Linco Research, St. Louis, MO) [37]. Insulin resistance and beta cell function were estimated from fasting glucose and insulin concentrations using the homeostasis model assessment (HOMA IR and HOMA β-cell, respectively) of Matthews and colleagues [38]. These indices have been validated against gold standard measures of insulin resistance and beta cell function [38].

Anthropometric measurements were performed with the volunteer wearing either undergarments and a hospital gown or light athletic clothing, and no shoes. Each measurement was performed twice, and the average was used in the analysis. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg using a hospital balance beam scale. Body mass index (BMI) was defined as weight/height$^2$ (kg/m$^2$). The waist was measured to the nearest 0.5 cm at the point of narrowing between the umbilicus and xiphoid process (as viewed from behind); the hips were measured to the nearest 0.5 cm at the maximum extension of the buttocks. Waist hip ratio (WHR) was calculated as the ratio of these two circumferences. Percent body fat (% fat) was estimated by bioelectrical impedance analysis (BIA) using the Tanita TBF-201 Body Fat Analyzer (Tanita Corp., Tokyo). High reproducibility of percent fat estimates using this machine (intraclass correlation coefficient (ICC) = 0.99) [39] in a sample from this population has been documented, and the instrument has been validated by others against dual energy x-ray absorptiometry (DEXA) in subjects with and without diabetes [40-42].

Information on number of live births and current age of each child was obtained using standardized questionnaires administered by trained interviewers [30]. Information regarding abortions, miscarriages and still births was not collected, and thus “parity” in the present study refers to live full-term births of one or more infant(s). Women with diabetes were also asked about the duration of their diabetes and current method of treatment (diet, oral hypoglycaemic agents, or insulin). Information regarding current use of oral contraceptives (OC) was obtained, with consent, from medical records maintained at the community clinic.

Statistical Analyses
All analyses were carried out using SAS version 6.12 [43]. For women with previously diagnosed diabetes, live births that occurred after the onset of the disease were excluded from the calculation of number of live births to ensure that risk estimates related to exposures that occurred prior to the onset of diabetes. The distributions of continuous variables were assessed for normality, and the natural log transformation of skewed variables was used in subsequent multivariate analyses. The association between parity and risk of diabetes was assessed using multiple logistic regression. We evaluated the risk associated with parity using 3 different approaches: parity modeled (1) as a continuous variable (risk per 1 additional live birth); (2) as a dichotomous variable (nulliparous vs. parous); (3) as a 4-level categorical variable with nulliparity as the reference category (vs. 1-2 births, 3-4 births, and 5 or more births). Crude odds ratios were examined, as well as odds ratios adjusted for ln age, waist circumference, and use of oral contraceptives. Analysis of covariance was employed to calculate adjusted mean levels of anthropometric (% fat and waist circumference) and metabolic (glucose, insulin, HOMA IR, C-peptide, proinsulin and leptin) variables by parity category. P-values for pairwise comparisons between individual least-square means of interest were calculated both with and without adjustment for multiple comparisons. The Tukey-Kramer method was employed for pairwise multiple comparison adjustment because it is considered more powerful for this application than the Bonferroni, Sidak or Scheffe approaches, and has performed well in Monte Carlo studies [43]. Natural logarithms of the dependent variables were used in these analyses, and the results were back-transformed for presentation in the figures, with 95% confidence intervals.

We anticipated that age might modify the association of parity with risk of both diabetes and related metabolic abnormalities, and we tested for these effect modifications by examining results of analyses conducted within age strata. In addition, age-parity interaction terms were included in models and the magnitude and significance of these variables were evaluated. Stratum-specific results are presented when the p-value for the interaction term was <0.05, or when effects differed appreciably across strata.

The analytical convention in the literature is to “adjust” proinsulin concentration for insulin secretion, usually by employing the proinsulin-to-insulin ratio. This is problematic, for two reasons. First, peripheral insulin concentrations do not adequately represent insulin secretion given that “insulin undergoes a large and variable hepatic extraction as well as peripheral clearance that varies under different physiological circumstances” [44]. Second, Kronmal has pointed out that the use of ratio variables in correlation and regression can result in spurious findings [45]. We have therefore opted to avoid the use of ratios and to analyze effect of proinsulin on levels of CVD risk factors...
after adjustment for C-peptide, which is "co-secreted with insulin in an equimolar ratio, is not extracted by the liver, and has a constant peripheral clearance" [44].

RESULTS

Anthropometric and metabolic characteristics of participants in the current analysis are presented in Table 1. The average age was approximately 30 years, and the average parity was 2.4 births. The prevalence of glucose intolerance was high, with 14.2% of women having IGT and 18.1% having newly or previously diagnosed diabetes.

Table 2 presents characteristics of SLHDP participants by 4-level parity category. Age, waist circumference, and fasting and 2-hour glucose and leptin concentrations increased across parity categories, while HOMA β-cell function and insulin concentrations decreased with increasing parity. HOMA IR and proinsulin concentration displayed U-shaped patterns, with higher concentrations among both nulliparous women and those with 5 or more live births. Diabetes prevalence increased steadily across parity categories, ranging from 13.2% among nulliparous women to 38.9% among women reporting 5 or more births. IGT was also positively related to parity, although women with 3-4 births had the highest prevalence of this condition. After adjustment for age, trends in metabolic and anthropometric characteristics across parity categories were less clear (Table 2). However, nulliparous women were distinctive in that they were more insulin resistant, had higher concentrations of fasting and 2hr glucose and proinsulin, as well as lower beta cell function, total and regional adiposity and leptin concentration compared to parous women (Table 2).

Results of multiple logistic regression analyses of parity and risk of diabetes are presented in Table 3. Interaction testing suggested that the effect of parity on diabetes risk was different between young (age 19-29) and older (age 30+) women, and thus results are presented separately for these age strata. When analyzed as a continuous variable, the number of births was associated with a slight, non-significant reduction in risk among older women after adjustment for age (odds ratio (OR) = 0.94, 95% confidence interval (CI) 0.83-1.07 per live birth). The magnitude of this risk estimate did not change after adjustment for waist circumference and OC use. No such reduction in risk was apparent among younger women. Compared to nulliparous older women, those who had experienced at least one live birth were at a statistically significant 3.6-fold reduced risk of diabetes after adjustment for age (OR=0.28, 95% CI 0.19-0.76). The protective effect of parity among older women was not altered appreciably after further adjustment for waist circumference and OC use.
Among younger women, there was some suggestion of reduced risk associated with parity in the fully adjusted logistic regression model, although this estimate was not statistically significant (OR=0.58, 95% CI 0.13-2.68). Analysis of parity as a 4-level categorical variable indicated that older women who reported having 3 or 4 live births were at especially reduced risk of diabetes after adjustment for age (OR=0.21, 95% CI 0.07-0.62). This risk estimate was largely unchanged after further adjustment for waist circumference and OC use. Risk of diabetes was also reduced among those reporting both 1-2 and 5 or more births compared to nulliparous older women. Among younger women, there was some suggestion of a U-shaped relationship, with reduced risk in the intermediate categories of parity, and an elevated risk among women reporting 5 or more births compared to nulliparous women. None of these estimates was significant at the 5% level. Logistic regression analysis of risk of IGT compared to NGT revealed that parity did not appear to be related to this condition after adjustment for age, waist circumference and OC use (data not shown).

Figure 1 presents mean levels of fasting insulin, HOMA IR, C-peptide, proinsulin and HOMA β-cell adjusted for age, waist circumference and diabetes status (proinsulin concentration also adjusted for C-peptide) among non-diabetic women. Fasting insulin and proinsulin concentrations and HOMA IR displayed U-shaped relationships with parity, with lower levels of these variables among women in the intermediate categories of parity. Nulliparous women were significantly more insulin resistant and had significantly higher concentrations of fasting insulin and C-peptide compared to those reporting 3-4 live births (Figure 1 A-C: all \( p < 0.05 \)). Further, proinsulin concentration among nulliparous women was significantly elevated compared to women with 1-2 or 3-4 live births (Figure 1 D: both \( p < 0.01 \)), and nulliparous women had significantly higher HOMA β-cell function compared to women in the two highest categories of parity (Figure 1 E: both \( p < 0.05 \)). Additional adjustment of these mean levels by OC use did not change the results (data not shown). P-values calculated using the Tukey-Kramer method of multiple comparison adjustment (reported in the legend of the figure) were slightly larger than unadjusted p-values. These relationships were also examined by comparing nulliparous women to parous women using dichotomous variables. Nulliparous women had significantly higher adjusted levels of fasting insulin, HOMA β-cell (both \( p < 0.05 \)), and fasting proinsulin (\( p < 0.001 \)) compared to parous women.

Figure 2 demonstrates that both waist circumference and percent body fat were lower among non-diabetic nulliparous compared to non-diabetic parous women after adjustment for age and diabetes status (Figure 2 A, C). Despite this relationship, however, leptin concentrations among
nulliparous women were significantly higher than among women who reported having at least one live birth after adjustment for age, diabetes status and either waist circumference or percent body fat (Figure 2 B, D). The difference was particularly apparent after adjustment for percent body fat, with leptin concentrations being significantly lower in all categories of parity (Figure 2 D, all p<0.05). Additional adjustment of these mean levels by OC use did not change the results (data not shown). P-values calculated using the Tukey-Kramer method of multiple comparison adjustment (reported in the legend of the figure) were larger than unadjusted p-values, although many remained highly statistically significant. In dichotomous comparisons, nulliparous women had significantly lower adjusted levels of both waist circumference (p<0.01) and percent body fat (p<0.0001), but higher adjusted levels of leptin (p<0.001).

DISCUSSION

In this paper we have demonstrated that nulliparity is associated with an increased risk of diabetes among women age 30 years and older in a Native Canadian population after adjustment for age, waist circumference and OC use. In addition, non-diabetic nulliparous women had lower insulin sensitivity, higher beta cell function, and higher concentrations of fasting insulin, proinsulin and leptin compared to those in the intermediate categories of parity (1-4 live births) after adjustment for covariates. Taken together our results suggest that both insulin resistance and beta cell dysfunction are elevated in nulliparous compared to parous women.

As mentioned above, previously-reported associations between parity and diabetes risk have been highly inconsistent, even in the body of studies that have appropriately adjusted for confounding factors [1-9]. While most covariate-adjusted studies have reported no significant relationship [1, 2, 6, 7, 9], three have documented elevated glucose concentrations or increased diabetes risk [3-5]. Conversely, our findings indicate a significant reduction in diabetes risk with parity, and are in line with those from Charles et al.'s study of Pima Indian women, a population similar to that from Sandy Lake with respect to both Native North American ethnicity and high diabetes risk [8]. The prevalence of glucose intolerance was high among nulliparous compared to parous women in univariate results from another study of indigenous North Americans [9], although multivariate analysis did not indicate a significant relationship. This may have been due, however, to the fact that parity was parameterized only as a continuous variable, which may not have allowed for the detection of increased risk associated with nulliparity (vs. one or more births). Other
explanations for discrepant results in the literature are not readily apparent, although it is possible that the relationship between parity and diabetes risk is variable across ethnic groups who differ by genetic background and may be subject to distinctive environmental attributes.

Both the fasting insulin concentration and the HOMA insulin resistance index have shown reasonably high criterion validity against gold standard measures of insulin resistance in non-diabetic individuals, and can thus be considered acceptable surrogate indicators of insulin resistance for large epidemiologic projects [38, 46]. In the present study, we found inverse relationships between parity and both fasting insulin concentrations and the HOMA insulin resistance index, with significantly higher levels among nulliparous women compared to those with 3-4 births. This finding is consistent with Charles et al. [8], who reported that nulliparous women had higher insulin levels than those who had borne children. Insulin concentrations were also inversely associated with parity among participants in the San Luis Valley Diabetes Study [7], although recent results from the Strong Heart Study do not confirm these findings [9].

Previous validation exercises have demonstrated that the HOMA β-cell function index correlates moderately to strongly with gold standard measures of insulin secretion [38]. Among non-diabetic women in the present study, HOMA β-cell function decreased in a stepwise manner through categories of parity, with the highest level among nulliparous women. Taken together with our findings regarding insulin resistance, this pattern suggests that the pancreatic beta cells of women the higher categories of parity (3 or more births) are required to secrete less insulin compared to nulliparous women, whose beta cells have a larger degree of insulin resistance to overcome.

The interpretation of our findings regarding C-peptide concentration is less clear. C-peptide is thought to be a reliable indicator of beta cell secretion, in that it is co-secreted with insulin in equimolar amounts but does not undergo the same variable hepatic extraction [44]. The low concentration of this molecule in women reporting 3-4 births is consistent with the concept that individuals in this category have higher insulin sensitivity and thus their beta cells are required to secrete less insulin compared to nulliparous women. The elevated C-peptide concentration associated with 5 or more births suggests that insulin secretion in these women may also be enhanced in the face of insulin resistance. Although a positive relationship between parity and insulin resistance has been reported elsewhere, this notion is not supported by other findings of the
present investigation. In the only other epidemiological study of parity and C-peptide concentrations, Alderman et al. found that the sum of 1 and 2 hour OGTT stimulated C-peptide concentrations decreased with increasing parity [7].

While it is recognized that both insulin resistance and beta cell dysfunction are important elements in the pathogenesis of diabetes, the sequence of these events remains controversial [13, 14]. Recent literature syntheses have drawn attention to the likelihood that these conditions not only coexist in the pre-diabetic state, but that they exacerbate each other through multiple feedback mechanisms [13, 14]. Elevated proinsulin concentration has been proposed as a surrogate indicator of beta cell dysfunction [15]. Proinsulin concentrations are elevated among subjects with IGT, Type 2 DM and gestational diabetes mellitus (GDM) [16, 48-50], and higher concentrations have been shown to predict the development of diabetes in prospective studies [17-20]. Further, previous studies have reported statistically significant correlations between proinsulin concentration and more detailed measures of beta cell function [51-53]. In the present study, non-diabetic nulliparous women had proinsulin concentrations which were significantly higher than those at intermediate levels of parity, suggesting that the nulliparity was associated with a relatively higher degree of beta cell dysfunction. To our knowledge, this is the first study to investigate the relationship between parity and proinsulin concentrations in a population-based study, and these findings extend the evidence supporting a possible role of insulin resistance and beta cell dysfunction in nulliparity.

In interpreting the elevated insulin concentrations and diabetes risk among nulliparous Pima women participating in their study, Charles et al. suggested that hormonal changes associated with insulin resistance could be related to increased risk of conditions causing infertility, including polycystic ovary syndrome (PCOS) [8]. This hypothesis is supported by the work of Dunai and coworkers, who documented lower rates of insulin-mediated glucose disposal in women with PCOS relative to both controls and women with Type 2 DM [54]. In addition, women with PCOS have been shown to be at elevated risk for a number of insulin resistance-related conditions, including Type 2 DM, GDM, hypertension, cardiovascular disease and dyslipidaemia [55]. Finally, three recent studies have presented evidence of beta cell dysfunction in women with PCOS and related conditions. O'Meara et al. found that the incremental secretory response to meals was reduced among women with functional ovarian hyperandrogenism [56]; Ehrmann and colleagues reported insulin secretory defects subjects with PCOS who had a family history of diabetes [57]; and Dunai and Finegood demonstrated a significantly decreased disposition index among women with PCOS [58].
Homozygous recessive ob mice, which produce no leptin, have multiple metabolic abnormalities, including infertility [27]. In 1996 Chehab et al. reported that administration of human recombinant leptin to these mice corrected their infertility [27], a finding which led to the suggestion that leptin might have a role in reproduction, and raised the possibility that women with PCOS might be leptin resistant [29]. While results to date have been highly inconsistent [28, 59-64], the question has yet to be addressed in a population-based sample. Our finding of significantly elevated leptin concentrations among nulliparous women could be interpreted to support the hypothesis of leptin resistance among women with reproductive abnormalities, including PCOS. We have previously reported significant independent associations between leptin concentration and percent body fat, waist circumference and fasting insulin in our study population [22]. In a recent study by Gonzalez et al., tumor necrosis factor alpha (TNF-α) concentrations were elevated in women with PCOS [65]. We found that TNF-α was significantly related to fasting insulin, HOMA IR and leptin in a random sample of women from the current study population [66]. Unfortunately, we do not have any information on the underlying determinants of nulliparity in this population, including the prevalence of PCOS or other forms of infertility. Population surveys have indicated, however, that PCOS is highly prevalent among pre-menopausal women [55] and is associated with obesity and insulin resistance. Our data indicating significantly elevated insulin resistance, beta cell dysfunction and leptin concentration in nulliparous women suggests that PCOS or related reproductive disorders might be contributing to nulliparity in this population.

One potential limitation of the present study involves the lack of information on abortions, miscarriages and stillbirths. However, in a previous study that analyzed risk of diabetes associated with both number of pregnancies and number of live births, the patterns of association were similar between the two exposure measures [5]. We also did not have information regarding the presence of a medical history of GDM. In a previous study among the Ojibway and Oji-cree of northwestern Ontario, a previous diagnosis of GDM was associated with a significant increased risk of subsequent GDM [67]. Thus it seems highly unlikely that previous GDM explains the decreased diabetes risk among parous women in the present study. Finally, infertility is known to be one consequence of infection with sexually transmitted diseases, the prevalence of which is known to be high in this population [68]. We did not have any data on medical history of STD infections or STD-associated infertility, although it appears unlikely that these traits would confound the associations examined in this paper.
In conclusion, we have demonstrated that parity is associated with a reduced risk of type 2 DM, and that nulliparous women were less insulin sensitive and had higher concentrations of insulin, C-peptide, proinsulin and leptin compared to women with 1-4 births. These results suggest that nulliparous women in this population should be considered to be at increased risk of developing type 2 diabetes and related metabolic disorders.
ACKNOWLEDGEMENTS

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REFERENCES


64. El Orabi H, Ghalia AA, Khalifa A, Mahfouz H, El Shalkani A, Shoieb N. Serum leptin as an additional possible pathogenic factor in polycystic ovary syndrome.


TABLE 1. Anthropometric, metabolic and reproductive characteristics of female participants in the Sandy Lake Health and Diabetes Project (n=383)\(^1\).

<table>
<thead>
<tr>
<th>Variable</th>
<th>mean</th>
<th>(SD)</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.97</td>
<td>(15.64)</td>
<td>12.00-79.96</td>
</tr>
<tr>
<td>Number of live births</td>
<td>2.57</td>
<td>(2.79)</td>
<td>0-14</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>6.54</td>
<td>(3.32)</td>
<td>3.7-25.5</td>
</tr>
<tr>
<td>2-hour glucose (mmol/l)</td>
<td>7.20</td>
<td>(4.12)</td>
<td>2.6-32.1</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>140.39</td>
<td>(119.97)</td>
<td>25-1592</td>
</tr>
<tr>
<td>HOMA insulin resistance index (IR)</td>
<td>6.00</td>
<td>(7.49)</td>
<td>0.77-104.17</td>
</tr>
<tr>
<td>HOMA beta-cell % (β-cell)</td>
<td>177.64</td>
<td>(127.50)</td>
<td>4.66-1041.67</td>
</tr>
<tr>
<td>Fasting C-peptide (pmol/l)</td>
<td>721.91</td>
<td>(450.76)</td>
<td>90-4050</td>
</tr>
<tr>
<td>Fasting proinsulin (pmol/l)</td>
<td>15.50</td>
<td>(11.17)</td>
<td>3-94</td>
</tr>
<tr>
<td>Fasting leptin (ng/ml)</td>
<td>21.41</td>
<td>(13.09)</td>
<td>2.7-83.8</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>42.72</td>
<td>(10.31)</td>
<td>8.1-61.2</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>91.90</td>
<td>(13.35)</td>
<td>61.0-142.5</td>
</tr>
</tbody>
</table>

Diabetes (%) 18.1
Impaired glucose tolerance (%) 14.2
Current use of oral contraceptives (%) 2.0

\(^1\)sample sizes vary slightly for specific variables due to occasional missing values
TABLE 2. Anthropometric and metabolic characteristics of female participants in the Sandy Lake Health and Diabetes Project, stratified by parity category.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nulliparous (n=129)</th>
<th>1-2 births (n=83)</th>
<th>3-4 births (n=99)</th>
<th>5+ births (n=72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cr.</td>
<td>mean (SD)</td>
<td>(95% CI)</td>
<td>cr.</td>
</tr>
<tr>
<td>Age</td>
<td>cr. 2</td>
<td>20.53 (11.77)</td>
<td>27.19 (10.18)</td>
<td>33.98 (9.49)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>ad. 2</td>
<td>6.42 (6.01-6.86)</td>
<td>6.10 (5.69-6.53)</td>
<td>5.73 (5.37-6.11)</td>
</tr>
<tr>
<td>2h glucose (mmol/l)</td>
<td>cr. 2</td>
<td>6.41 (4.06)</td>
<td>6.81 (3.87)</td>
<td>7.35 (3.33)</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>ad. 2</td>
<td>150.30 (174.21)</td>
<td>142.66 (96.79)</td>
<td>132.26 (70.71)</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>cr. 2</td>
<td>6.07 (10.14)</td>
<td>6.05 (6.01)</td>
<td>5.51 (5.03)</td>
</tr>
<tr>
<td>HOMA β-Cell</td>
<td>ad. 2</td>
<td>126.98 (109.1-147.7)</td>
<td>144.17 (123.3-168.7)</td>
<td>152.93 (131.9-177.2)</td>
</tr>
<tr>
<td>C-peptide (pmol/l)</td>
<td>cr. 2</td>
<td>684.68 (530.24)</td>
<td>728.66 (416.21)</td>
<td>679.80 (385.88)</td>
</tr>
<tr>
<td>Proinsulin (pmol/l)</td>
<td>ad. 2</td>
<td>599.44 (531.8-675.7)</td>
<td>648.07 (572.5-733.7)</td>
<td>561.16 (498.9-631.2)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>cr. 2</td>
<td>15.97 (12.36)</td>
<td>15.82 (12.95)</td>
<td>13.58 (8.32)</td>
</tr>
<tr>
<td>% body fat (%)</td>
<td>ad. 2</td>
<td>14.51 (12.87-16.37)</td>
<td>13.08 (11.56-14.08)</td>
<td>11.09 (9.87-12.46)</td>
</tr>
<tr>
<td>Variable</td>
<td>Value</td>
<td>95% CI</td>
<td>P-value</td>
<td>Value</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------------</td>
<td>--------------</td>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>13.2</td>
<td>(8.00-19.89)</td>
<td></td>
<td>15.7</td>
</tr>
<tr>
<td>IGT (%)</td>
<td>6.2</td>
<td>(3.86-8.77)</td>
<td></td>
<td>12.1</td>
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<tr>
<td>Current use of oral contraceptives (%)</td>
<td>0.0</td>
<td>(0.00-0.00)</td>
<td></td>
<td>3.6</td>
</tr>
</tbody>
</table>

-all values fasting unless otherwise indicated
-cr. = crude; ad. = adjusted for age using analysis of covariance; least square means calculated from natural log tx for all variables (except % body fat and waist circumference) and back transformations presented in table
-sample sizes vary for certain variables due to occasional missing values
-crude prevalence
### TABLE 3. Association between parity and risk of diabetes, Sandy Lake Health and Diabetes Project.

<table>
<thead>
<tr>
<th>Model</th>
<th>Adjusted for age</th>
<th>Adjusted for age waist circumference and oc use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Age 12-29 (n=215)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Births&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.10</td>
<td>0.74-1.62</td>
</tr>
<tr>
<td>Nulliparous vs. parous&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.95</td>
<td>0.23-3.96</td>
</tr>
<tr>
<td>Categories</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 births</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>1-2 births</td>
<td>0.97</td>
<td>0.21-4.43</td>
</tr>
<tr>
<td>3-4 births</td>
<td>0.71</td>
<td>0.11-4.67</td>
</tr>
<tr>
<td>5+ births</td>
<td>2.35</td>
<td>0.26-21.67</td>
</tr>
<tr>
<td><strong>Age 30+ (n=168)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Births&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.94</td>
<td>0.83-1.06</td>
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<td>0 births</td>
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</tr>
<tr>
<td>1-2 births</td>
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<td>0.11-1.27</td>
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<tr>
<td>3-4 births</td>
<td>0.21</td>
<td>0.07-0.62</td>
</tr>
<tr>
<td>5+ births</td>
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<td>0.11-1.01</td>
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<tr>
<td><strong>All ages (n=383)</strong></td>
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<tr>
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<tr>
<td>5+ births</td>
<td>0.51</td>
<td>0.19-1.32</td>
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</tbody>
</table>

<sup>1</sup>logistic regression analysis
<sup>2</sup>continuous variable: increase in risk per additional live birth
<sup>3</sup>codes: 0=nulliparous, 1=parous
Figure 1. Mean (95% CI) levels of metabolic variables in non-diabetic women by parity category, after adjustment for age, waist circumference and glucose tolerance status (NGT vs. IGT) by analysis of covariance, Sandy Lake Health and Diabetes Project.

A: fasting insulin; B: HOMA insulin resistance (IR); C: fasting C-peptide; D: fasting proinsulin (also adjusted for C-peptide); E: HOMA beta cell function (also adjusted for HOMA IR).

* p<0.05 vs. nulliparous; #, p<0.01 vs. nulliparous;
\( ^\wedge \), p<0.05 versus 1-2 births; #, p<0.05 vs. 3-4 births.

Tukey-Kramer multiple comparison adjustment: a, p=0.08; b, p=0.19; c, p=0.15; d, p=0.13; e, f, p=0.02; g, p=0.07; h, p=0.05; i, p=0.17.
Figure 2. Mean (95% CI) levels of anthropometric variables and leptin concentration in non-diabetic women by parity category, determined by analysis of covariance, Sandy Lake Health and Diabetes Project. A: waist circumference adjusted for age and diabetes status (NGT vs. IGT); B: fasting leptin concentration adjusted for age, waist circumference and diabetes status; C: percent body fat adjusted for age and diabetes status; D: fasting leptin adjusted for age, waist circumference and diabetes status. *, p<0.05 versus nulliparous; @, p<0.01 vs. nulliparous. Tukey-Kramer multiple comparison adjustment: a, p=0.08; b, p=0.05; c, p=0.16; d, p=0.001; e, p=0.006; f, p=0.01; g, p=0.007; h, p=0.15.
3.5 LINKING SECTION 2

Results of the previous paper indicated that nulliparous women had significantly increased risk of diabetes, as well as a greater degree of insulin resistance and higher circulating levels of proinsulin. Taken together with the findings of paper 1, these results further support the notions that (1) proinsulin concentration, an indicator of beta cell dysfunction, is elevated early in the natural history of glucose intolerance; (2) a number of factors are associated with variation in proinsulin concentration, including glucose tolerance, adiposity and parity; and (3) despite having a more favourable anthropometric profile, nulliparous women are more insulin resistant and have higher concentrations of insulin, proinsulin, and leptin compared to their parous counterparts, a finding which suggests that nulliparity may be associated with a pre-diabetic phenotype in this population.

Insulin resistance is a well-documented characteristic of the early phases of the natural history of glucose intolerance. This metabolic trait is known to cluster with other risk factors for diabetes and heart disease, and this collection of features has been termed Syndrome X, or the insulin resistance syndrome. Dyslipidaemia has been highlighted as a central feature of the insulin resistance syndrome, although the exact mechanism determining elevated lipids in insulin resistance remains unclear. This is due in part to the use in many earlier studies of non-specific insulin assays that cross-react to a large degree with proinsulin. The coexistence of both dyslipidemia and hyperproinsulinemia in subjects with diabetes and IGT, along with the documentation of high cardiovascular morbidity in the human proinsulin arm of a clinical trial, generated the hypothesis that proinsulin may be directly involved in the pathogenesis of cardiovascular disease. Although a limited number of studies have reported independent cross-sectional associations between proinsulin and cardiovascular disease risk factors, this relationship has yet to be examined among Native Canadians, a population with both high diabetes risk and increasing cardiovascular morbidity. In addition, the prospective relationship between proinsulin concentration and cardiovascular risk factors has not been explored. The goal of the following paper was to address these gaps in the scientific literature.
CROSS-SECTIONAL AND PROSPECTIVE ASSOCIATIONS BETWEEN PROINSULIN AND CARDIOVASCULAR RISK FACTORS IN A NATIVE CANADIAN COMMUNITY EXPERIENCING RAPID CULTURAL TRANSITION

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Running title: Proinsulin and cardiovascular risk factors

Key words:

Proinsulin  Lipids  Blood pressure  Indians, North American  Epidemiology

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ABSTRACT

The causative role of proinsulin in cardiovascular disease is controversial. Two important research questions have yet to be addressed in this body of literature. First, are proinsulin concentrations associated with CVD risk factors among North American indigenous populations? Second, what are the prospective relationships between proinsulin and CVD risk factors in non-diabetic subjects who are at risk of developing diabetes? We examined these specific issues using cross-sectional and longitudinal data from a Native Canadian community experiencing an epidemic of type 2 diabetes mellitus.

Between 1993 and 1995, 72% of eligible members of an isolated Native Canadian community participated in a population-based baseline survey to determine the prevalence and risk factors for type 2 DM. Samples for glucose, C-peptide, proinsulin, lipids and lipoproteins were drawn after an overnight fast, and a 75g oral glucose tolerance test was administered with a second sample for glucose drawn after 120 minutes. Blood pressure and waist circumference were measured, and percent (%) body fat determined by bioelectrical impedance analysis. In the present study, subjects with normal glucose tolerance (NGT, n=505) and impaired glucose tolerance (IGT, n=74) were included in cross-sectional analyses. In 1998, 95 individuals who at baseline had IGT or NGT with an elevated 2-hour glucose concentration (≥ 7.0 mmol/l) participated in a follow-up evaluation using the protocol employed at baseline. Cross-sectional and prospective associations between proinsulin and cardiovascular risk factors were assessed using correlation and multiple linear regression.

After adjustment for age, sex, C-peptide, and waist circumference, fasting proinsulin concentration was significantly associated with concurrently measured lipid concentrations in subjects with NGT (triglyceride: partial r=0.20, p<0.0001; total cholesterol, partial r=0.12, p<0.01; LDL-cholesterol, partial r=0.10, p<0.05; HDL-cholesterol, partial r=-0.14, p<0.01). Among subjects with IGT, fasting proinsulin was inversely correlated with HDL-cholesterol (partial r=-0.22, p=0.0656). Proinsulin concentration and blood pressure were not cross-sectionally associated in either glucose tolerance category. After adjustment for age, sex, change in C-peptide, change in waist circumference, diabetes status, and the baseline level of the dependent variable, change in fasting proinsulin was prospectively related to changes over 4 years in triglyceride (partial r=0.24, p<0.05), total (partial r=0.32, p<0.01), and LDL-cholesterol (partial r=0.23, p<0.05). A post-hoc analysis of these data motivated by recent literature regarding the lipotoxicity hypothesis of diabetes.
[Unger RH, Diabetes 1995;44:863-70] suggested the possibility that elevated baseline triglyceride and decreased baseline HDL-cholesterol concentrations were associated with increases over time in proinsulin concentration.

These results confirm previously reported cross-sectional associations between proinsulin and lipid concentrations, and suggest the possibility that dyslipidaemia may play a role in beta-cell decompensation.
INTRODUCTION

Ischaemic heart disease and stroke together account for half of the total mortality among individuals with Type 2 diabetes mellitus (Type 2 DM) [1]. Further, the risk of a first myocardial infarction (MI) among subjects with diabetes approximates that for re-infarction among non-diabetic patients with previous MI [2]. This highly unfavorable cardiovascular profile is foreshadowed by a period during which both diabetic and pre-diabetic individuals are dyslipidaemic and hypertensive [3-5]. It has been suggested that these abnormalities might be a consequence of the extended period of exposure to insulin resistance-related hyperinsulinaemia [6, 7]. While this hypothesis has been supported by the findings of both in vivo and in vitro studies [8-14], the issue remains controversial [15].

The assessment of the relationship between insulin and cardiovascular risk factors is complicated by the fact that conventional insulin radioimmunoassays display a high degree of cross-reactivity with proinsulin and its split products [16]. Given that proinsulin concentrations are disproportionately elevated in subjects with diabetes [17-30] and (in some studies) impaired glucose tolerance (IGT) [19, 21, 22, 23, 25-30], it is conceivable that this prohormone may have particularly detrimental metabolic effects in the pathogenesis of cardiovascular disease (CVD). Support for this theory emerged during the 2-year interim analysis of a clinical trial comparing human proinsulin and human insulin [31]. Six myocardial infarctions (including 2 deaths) occurred in the human proinsulin group, and none in the comparison group.

Cross-sectional relationships between proinsulin and intermediate quantitative traits associated with CVD have been examined in a limited number of studies [32-36]. Among both diabetic and non-diabetic subjects, proinsulin has exhibited moderate but significant independent associations with blood pressure (BP) and concentrations of total cholesterol, triglyceride, LDL- and HDL-cholesterol [32-36]. In addition, weak but significant associations between proinsulin concentrations and both intima-media (carotid artery) wall thickness (IMT) [37] and LDL particle size [38-40] have been reported.

Despite these positive associations with CVD risk factors, inconsistent evidence has emerged from 4 studies of the role of proinsulin in documented cardiovascular disease. Katz and colleagues [41] found no independent relationship between coronary artery disease and proinsulin concentration after adjustment for BMI, and Yudkin et al. [42] reported that proinsulin was not a
significant risk factor for prevalent or incident coronary heart disease (diagnosed by electrocardiogram) after adjustment for age, sex and BMI. Contrary to these studies, however, Bavenholm et al. [43] documented higher adjusted proinsulin concentrations in young men presenting with a first MI compared to controls, as well as significant independent associations between proinsulin concentration and global coronary atherosclerosis score. Further, Lindahl and coworkers [44] recently reported the results of a nested case-control study which indicated that subjects in the highest quartile of proinsulin concentration had a 3-fold increased risk of acute MI compared to those in the lowest quartile after adjustment for confounding factors. Thus, the role of proinsulin concentrations in atherogenesis remains controversial.

Two important research questions have yet to be addressed in this body of literature. First, are proinsulin concentrations associated with CVD risk factors among North American aboriginal people, a population with increasing rates of both type 2 DM [45] and coronary heart disease [46]? Second, what are the prospective relationships between proinsulin and CVD risk factors in non-diabetic subjects who are at risk of developing diabetes? We examined these specific issues using data from the cross-sectional and longitudinal components of the Sandy Lake Health and Diabetes Project (SLHDP) [47, 48]. The residents of Sandy Lake are Native Canadians from the isolated subarctic region of northern Ontario, a population that has traditionally experienced relatively low rates of cardiovascular mortality [49]. Westernization is occurring rapidly in this region, however, and has resulted in an epidemiological transition characterized by dramatic increases in the prevalence of obesity and type 2 DM [50, 51]. While rates of dyslipidaemia and hypertension are remarkably low among normoglycaemic individuals in this population, the situation is reversed in the presence of glucose intolerance [52]. Indeed, despite the relatively recent emergence of epidemic diabetes in this area [53], it is becoming apparent that this disease is already having a detrimental effect on the cardiovascular health of its residents [54].

SUBJECTS AND METHODS


The methodology of the SLHDP prevalence study has been presented in detail in previous publications [47, 48, 55]. Briefly, between July 1993 and December 1995, 728/1018 (72%) residents of Sandy Lake aged 10-79 participated in a population-based cross-sectional survey to determine the prevalence of Type DM and its associated risk factors. Signed informed consent was obtained from all participants, and the study was approved by the Sandy Lake First Nation Band Council and
University of Toronto Ethics Review Committee. The cross-sectional component of the current study is based on data from the 579 individuals identified as having normal glucose tolerance (NGT) or IGT in this survey and for whom specimens were available for proinsulin determination.

Participants provided fasting blood samples for glucose, C-peptide, proinsulin and lipids after an 8-12 hour overnight fast. A 75g oral glucose tolerance test (OGTT) was administered, and a second sample for glucose was drawn after 120 minutes. Individuals were excluded from the OGTT if they had physician diagnosed diabetes and were (a) currently receiving treatment with insulin or oral hypoglycemic agents, or (b) if they had a fasting blood glucose concentration exceeding 11.1 mmol/l. Women who were pregnant at the time of initial contact received their OGTT 3 months post-partum. Diabetes and IGT were diagnosed according to World Health Organization criteria [56].

C-peptide concentration was measured using a radioimmunoassay (Diagnostic Products Corporation, Los Angeles) which has minimal detection limit of 43 pmol/l, and cross-reactivities of 0% with insulin and <13% with proinsulin. Proinsulin was determined using a human proinsulin radioimmunoassay which has a laboratory sensitivity of 3.5 pM, and a CV of 6.2-21.0% (Linco Research Inc., St. Louis, MO). This assay displays 46% cross-reactivity with des 31,32 proinsulin, the major form of circulating split proinsulin, and thus reported values refer to total proinsulin-like materials [57]. Cross-reactivity of this assay with des 64, 65 proinsulin, insulin and C-peptide is very low (<0.1%). Proinsulin was measured in serum specimens that had been stored at -70°C for between 3-5 years at the Core Lab of the Banting and Best Diabetes Centre, University of Toronto. Glucose concentration was determined using the glucose oxidase method. Cholesterol, triglyceride, and HDL-cholesterol concentrations were determined using methods described in the Lipid Research Clinics manual of operations [59]. LDL-cholesterol concentration was calculated using the Friedwald formula [60].

Anthropometric measurements were performed with the participant wearing either undergarments and a hospital gown or light athletic clothing, and no shoes. Each measurement was performed twice, and the average was used in the analysis. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg using a hospital balance beam scale. Body mass index (BMI) was defined as weight/height² (Kg/m²). The waist was measured to the nearest 0.5 cm at the point of narrowing between the umbilicus and xiphoid process (as viewed from behind); the hips were measured to the nearest 0.5 cm at the maximum
extension of the buttocks. Waist hip ratio (WHR) was calculated as the ratio of these two circumferences. Percent body fat (% fat) was estimated by bioelectrical impedance analysis (BIA) using the Tanita TBF-201 Body Fat Analyzer (Tanita Corp., Tokyo). High reproducibility of percent fat estimates using this machine in a sample from this population has been documented (intraclass correlation coefficient (ICC) = 0.99) [61], and the instrument has been validated by others against dual energy x-ray absorptiometry (DEXA) in both diabetic and non-diabetic populations [62-64].

BP was measured in the right arm with the participant seated and the arm bared. Systolic BP was recorded to the nearest 2 mm HG at the appearance of the first Korotkoff sound (phase I), and diastolic BP was recorded to the nearest 2 mm Hg at the appearance of the fifth Korotkoff sound (phase V). Two measurements were performed using a hand held aneroid sphygmomanometer, and the average of the two was used in the analysis.

Follow-Up Survey, 1998

During the summer of 1998, Sandy Lake residents who were found, at the time of the baseline survey, to have impaired glucose tolerance (IGT) (n=74) or normal glucose tolerance (NGT) with a 2 hour post challenge glucose concentration greater than or equal to 7.0 mmol/l (n=51) were invited to participate in a follow-up visit to determine current glucose tolerance status. Of the 125 individuals in the follow-up cohort, 3 (3 IGT, 0 NGT) had died, 11 (9 IGT, 2 NGT) were no longer living in the community, 2 (1 IGT, 1 NGT) were too sick to participate, and 14 (6 IGT, 8 NGT) refused to attend. Thus 95 (76%) members of this high-risk cohort participated in the follow-up examination. Concentrations of lipid and metabolic variables and anthropometric measurements were assessed using field and laboratory methods and equipment identical to those used during the baseline survey. The median follow-up period was 4 years. Signed informed consent was obtained from all subjects, and the follow-up protocol was approved by the Sandy Lake First Nation Band Council and the University of Toronto Ethics Review Committee.

Statistical Analyses

The analytical convention in this body of literature is to “adjust” proinsulin concentration for insulin secretion, usually by employing the proinsulin-to-insulin ratio. This is problematic, for two reasons. First, peripheral insulin concentrations do not adequately represent insulin secretion given that “insulin undergoes a large and variable hepatic extraction as well as peripheral clearance that varies under different physiological circumstances” [65]. Second, Kronmal has pointed out that the
use of ratio variables in correlation and regression can result in spurious findings [66]. We have therefore opted to avoid the use of ratios and to analyze effect of proinsulin on concentrations of CVD risk factors after adjustment for C-peptide, which is “co-secreted with insulin in an equimolar ratio, is not extracted by the liver, and has a constant peripheral clearance” [65].

The association between proinsulin concentrations and CVD risk factors was investigated using concurrent measures collected during the 1993-1995 baseline prevalence survey, described below as the “cross-sectional study”. The prospective associations of baseline concentrations and change in proinsulin concentration with change in CVD risk factors over 4 years were investigated using data from both the baseline prevalence (1993-1995) and follow-up (1998) surveys; these are described below as the “prospective study”. All analyses were carried out using SAS version 6.12 [67].

**Cross-Sectional Study**

The distributions of continuous variables were assessed for normality, and the natural log transformations of skewed variables were used in subsequent analyses. Crude and adjusted associations between concurrent measures of proinsulin and cardiovascular risk factors (lipids and blood pressure) were assessed using correlation coefficients and multiple linear regression within categories of glucose tolerance (NGT or IGT). For each dependent variable, three models were constructed which included the following independent variables: (i) proinsulin alone; (ii) proinsulin, age, sex, and C-peptide, and (iii) proinsulin, age, sex, C-peptide and waist circumference. Waist circumference was used in lieu of WHR as a measure of abdominal adiposity, given that the former is superior as an estimate of intra-abdominal fat mass [68]. Additionally, the problem of ratio variables is avoided [66]. We tested for statistical interactions between sex and proinsulin by adding a (sex x proinsulin) term to each of the final models, as well as by examining stratum-specific results. As none of the interaction parameter estimates was significant at the 5% level (all p>0.51) and the results of stratum specific models were not markedly different, we present models with the sexes pooled. Model collinearity was assessed using the variance inflation factor (VIF) method described by Freund and Littell [69]. Based on these criteria, none of models displayed evidence of marked collinearity. Analysis of covariance was employed to estimate mean concentrations of lipids and lipoproteins for each quartile of proinsulin concentration, after adjustment for age, sex, C-peptide concentration, and waist circumference. P-values for pairwise comparisons between individual least-square means of interest were calculated both with and without adjustment for multiple comparisons. The Tukey-Kramer method was employed for pairwise multiple comparison
adjustment because it is considered more powerful for this application than the Bonferroni, Sidak or Scheffe approaches, and has performed well in Monte Carlo studies [67]. Natural logarithms of the dependent variables were used in these analyses, and the results were back-transformed for presentation in the figures, with 95% confidence intervals.

**Prospective Study**

Changes in metabolic variables and lipids were calculated as the follow-up level (1998) minus the baseline level (1993-1995). The relationship was assessed between baseline concentration and change over the follow-up period in proinsulin concentration and both follow-up and change in cardiovascular risk variables. Due to skewness in the distribution of both predictor and outcome variables, Spearman correlation coefficients were employed to assess these relationships, with and without adjustment for age, sex, C-peptide concentration, waist circumference, baseline and follow-up diabetes status, and baseline levels of the dependent variable.

**RESULTS**

**Cross-Sectional Study**

Characteristics of subjects included in the cross-sectional study are presented in table 1. Individuals with IGT were older and had higher waist circumference and both systolic and diastolic blood pressure compared to subjects with NGT. In addition, subjects with IGT had higher concentrations of proinsulin, C-peptide and lipids (except HDL-cholesterol) compared to those with NGT.

Table 2 presents results of correlation and multiple linear regression analyses examining crude and adjusted cross-sectional relationships between fasting proinsulin concentration and CVD risk factor levels in subjects with NGT. In unadjusted analyses, proinsulin was significantly and positively correlated with total cholesterol, triglyceride, and LDL-cholesterol and significantly negatively correlated with HDL cholesterol. These relationships were attenuated slightly but remained significant after controlling for age, gender, C-peptide and waist circumference (triglyceride: partial r=0.20, p<0.0001; total cholesterol, partial r=0.12, p=0.0095; LDL-cholesterol, partial r=0.10, p=0.0208; HDL-cholesterol, partial r=-0.14, p=0.0028). In the fully adjusted model, proinsulin concentration explained approximately 4% of the variation in triglyceride concentration. Proinsulin concentrations accounted for smaller amounts of independent variation in total, LDL-, and HDL cholesterol (1-2%). Proinsulin displayed a positive crude association with blood pressure
among subjects with NGT, although coefficients were not significantly associated with variation BP after adjustment for waist circumference and other covariates.

To further explore the independent associations between proinsulin and lipid concentrations, we calculated mean lipid concentrations by quartiles of proinsulin concentration, after adjustment for age, sex, C-peptide and waist circumference (Figure 1, A-D). Total cholesterol and triglyceride concentrations increased in a stepwise manner across quartiles of proinsulin concentration (p\text{trend}=0.0034 and p\text{trend}<0.0001, respectively), and for both outcome variables subjects in the highest quartile had significantly elevated concentrations relative to those in the baseline quartile (Figure 1, A & B). Similar patterns were evident for LDL- and HDL-cholesterol (inverse association), although the trends were less strongly significant (Figure 1, C & D; p\text{trend}=0.0122 and p\text{trend}=0.0241).

Proinsulin concentration was generally not associated with other CVD risk factors in those with IGT. Only HDL-cholesterol concentration was associated with proinsulin concentration (Table 3; partial r=-0.22, p=0.0656), with the magnitudes of regression and partial correlation coefficients similar to or stronger than those from analyses among individuals with NGT. The lack of statistical significance at the 5% level of the association between HDL-cholesterol and proinsulin concentrations is attributable to the much smaller number of subjects in this sub-cohort.

**Prospective Study**

Table 4 presents mean values of baseline and change variables for subjects who participated in the follow-up survey. Spearman correlation coefficients between baseline and change in fasting proinsulin concentration and change in cardiovascular risk factors are presented in table 5. Baseline proinsulin concentration was not significantly associated with change in cardiovascular risk factors, although paradoxically, baseline proinsulin concentration displayed a significant inverse association with follow-up cholesterol concentration. However, after adjustment for age, sex, change in C-peptide concentration, baseline and follow-up diabetes status, and baseline level of the dependent variable, change in proinsulin concentration was associated with changes in concentrations of cholesterol (r=0.31, p<0.01) and triglyceride (r=0.25, p<0.05). Examination of the distributions for changes in concentrations of total cholesterol and triglyceride indicated the presence of one individual with large changes in these values. Partial correlation coefficients were attenuated slightly when this subject was eliminated from the analysis (total cholesterol, partial r = 0.27, p<0.05; triglyceride, partial r=0.18, p=0.11).
DISCUSSION

In the present study we have reported significant cross-sectional associations between fasting proinsulin and lipid concentrations in subjects with NGT after adjustment for covariates including age, sex, insulin secretion and intra-abdominal obesity. In subjects with IGT, an association was found with HDL-cholesterol only. To our knowledge, these relationships have not previously been described in North American indigenous people, a population undergoing rapid epidemiologic transition. Further, for the first time we have documented prospective relationships between change in proinsulin concentration and change in CVD risk factors. Change in proinsulin concentration over a 4-year period was positively associated with change in triglyceride as well as total and LDL-cholesterol after adjustment for age, sex, change in insulin secretion, change in waist circumference, glucose tolerance status and baseline level of the dependent variable.

Our findings are consistent with the results of previous research examining cross-sectional relationships between proinsulin concentration and cardiovascular risk factors in non-diabetic subjects. In the three studies that presented results adjusted for age, sex and adiposity [33-35], triglyceride concentration was consistently related to proinsulin concentration. Independent associations were reported for LDL and HDL cholesterol in two of the three papers [34, 35]. Our paper further contributes to this body of literature by presenting results of a prospective analysis, and by adjusting both cross-sectional and prospective models by C-peptide, a measure of insulin secretion that is superior to insulin, as discussed above.

Confirmation of the hypothesis of an independent predictive role for proinsulin in the development of dyslipidaemia will require the demonstration of a prospective association between baseline proinsulin concentration and change over time in lipid concentrations after adjustment for confounding factors. We were unable to demonstrate such an association in the small prospective study reported here. Rather, we found that change over the follow-up period in proinsulin concentration was significantly associated with changes in lipid concentrations, indicating that proinsulin and lipid concentrations appear to increase concurrently. Although this finding suggests that an unmeasured common factor might be responsible for deterioration in both parameters, the possibility of an independent pathogenic role for proinsulin cannot be eliminated.

The physiological mechanism responsible for the association between proinsulin concentration and lipids or lipoproteins has yet to be determined. As mentioned, it is possible that a
common factor is responsible for both increased proinsulin and increased lipid concentrations. We have previously described positive relationships between waist circumference and both lipid [52] and proinsulin concentrations [70] in this population, and thus it is conceivable that intra-abdominal fat (IAF) is playing such a role. Haffner et al. have proposed such a mechanism for IAF in explaining increased levels of proinsulin, plasminogen activator inhibitor-1 (PAI-1), and intima-media wall thickness [37]. In our study, however, the associations between proinsulin and CVD risk factors remained significant after adjustment for waist circumference (a reliable indicator of intra-abdominal fat) in both cross-sectional and prospective analyses. This finding suggests that the pathogenic role for proinsulin might be at least partially independent of intra-abdominal fat.

However, there is currently only limited information supporting the notion of a direct pathogenic role for proinsulin in atherogenesis or dyslipidemia, especially given its low relative concentration in non-diabetic subjects and its limited metabolic activity [34]. The metabolic clearance rate of proinsulin is substantially lower than that of insulin, however, possibly resulting in prolonged exposure of susceptible tissues to proinsulin molecules [43]. Bavenholm and coworkers have speculated that proinsulin might be directly implicated in atherogenesis and thrombogenesis based on in vitro data suggesting that insulin propeptides stimulate the “synthesis and secretion of fibrinolytic proteins by hepatocytes and endothelial cells” [43, 71, 72]. In recent research, significant associations between proinsulin concentration and LDL particle size have been documented [38-40], and it has been suggested that this relationship may operate through the hypertriglyceridaemia that characterizes states of beta cell dysfunction [40]. Triglyceride is an important determinant of LDL particle size [40], and previous research demonstrated that impaired insulin secretion was associated with higher nonesterified fatty acid concentrations in men, which in turn was related to elevated triglyceride concentrations [73].

Alternatively, it is possible that the pathogenic mechanism may operate in the opposite direction, with higher baseline circulating lipid concentrations leading to subsequent beta cell dysfunction and elevations in proinsulin concentrations. In the ZDF rat, short term exposure to high levels of circulating free fatty acids (FFA) causes beta-cell hyperplasia and hyperinsulinaemia, but chronic exposure and subsequent increases in FFA levels lead to functional and morphologic changes in beta cells and consequent diabetes [74]. Substantial fat deposition in islets of obese ZDF rats has been documented, as well as the demonstration of FFA-induced loss of glucose-stimulated insulin secretion in these animals [75]. Increased FFA also induce nitric oxide synthase, and Shimabukuro et al. [76, 77] have shown that elevated FFA in rat beta cells cause increases in both
nitric oxide levels and ceramide-mediated beta cell apoptosis (programmed cell death). The consequent reduction in the number of beta cells may result in elevated proinsulin concentration, in that the rate of secretion by remaining cells is increased, thereby decreasing the intracellular stores and forcing the release of incompletely processed materials [78, 79]. In light of this evidence, we evaluated the prospective association between baseline lipid concentrations on follow-up and change in proinsulin concentration in a post-hoc analysis (Table 6). Baseline triglyceride concentration displayed a positive and significant association with change over the follow-up period in proinsulin concentration, whereas baseline HDL-cholesterol concentration showed a significant inverse association with change in proinsulin concentration. These findings are consistent with the lipotoxicity hypothesis of beta-cell dysfunction, although they require confirmation in future studies.

In summary, this study has demonstrated significant independent cross-sectional and prospective relationships between fasting proinsulin concentrations and CVD risk factors. We have previously reported that variation in lipids, lipoproteins, and blood pressure in this population is related to age, obesity, cigarette smoking, glucose intolerance and inherited factors, including variation in the APOC3, AGT, FABP2, and HNF-1α genes [48, 80-84]. It appears, then, that complex genetic and environmental mechanisms are responsible for the regulation of lipid concentrations and blood pressure in Native Canadians. In addition, these data raise the possibility that lipotoxicity to the beta-cell may help to explain previous cross-sectional associations between lipid and proinsulin concentrations.
REFERENCES


70. Hanley AJG, McKewon-Eyssen G, Harris SB, Hegele RA, Wolaver TMS, Kwan J, Connelly PW, Zinman B. Proinsulin obesity and glucose tolerance status in a Native Canadian community with high rates of glucose intolerance (manuscript submitted for publication).


78. Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ. Chronic exposure to free fatty acid reduces pancreatic beta-cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. J Clin Invest 1998;101:1094-1101.


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<table>
<thead>
<tr>
<th>Variable</th>
<th>NGT n=505; (232 male, 273 female)</th>
<th>IGT n=74 (16 male, 58 female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at screening (years)</td>
<td>Mean 25.59 ± 12.92</td>
<td>Mean 39.10 ± 17.68</td>
</tr>
<tr>
<td>Proinsulin (pmol/l)</td>
<td>Mean 12.04 ± 7.15</td>
<td>Mean 19.00 ± 12.37</td>
</tr>
<tr>
<td>C-peptide (pmol/ml)</td>
<td>Mean 0.60 ± 0.37</td>
<td>Mean 0.84 ± 0.44</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>Mean 4.29 ± 0.88</td>
<td>Mean 4.77 ± 0.71</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>Mean 1.26 ± 0.61</td>
<td>Mean 1.68 ± 0.78</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>Mean 2.46 ± 0.75</td>
<td>Mean 2.78 ± 0.59</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>Mean 1.26 ± 0.27</td>
<td>Mean 1.23 ± 0.29</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>Mean 88.56 ± 13.60</td>
<td>Mean 97.62 ± 10.76</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>Mean 113.72 ± 13.71</td>
<td>Mean 124.25 ± 17.55</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>Mean 64.50 ± 11.32</td>
<td>Mean 69.47 ± 12.67</td>
</tr>
</tbody>
</table>

1 all metabolic variables are fasting concentration
## TABLE 2. Crude and adjusted cross-sectional relationships between fasting proinsulin concentration and cardiovascular risk factors in subjects with normal glucose tolerance (n=503).

<table>
<thead>
<tr>
<th>Dependent Variable¹</th>
<th>Adjustment²</th>
<th>Statistics for Proinsulin Variable</th>
<th>Full model R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>t</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
<td>0.0657</td>
<td>3.54</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>0.0682</td>
<td>3.43</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>0.0536</td>
<td>2.61</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-</td>
<td>0.3835</td>
<td>10.10</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>0.2646</td>
<td>6.01</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>0.1958</td>
<td>4.41</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>-</td>
<td>0.0982</td>
<td>3.60</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>0.1097</td>
<td>3.69</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>0.0701</td>
<td>2.32</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-</td>
<td>-0.1455</td>
<td>-7.70</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>-0.1135</td>
<td>-4.78</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>-0.0711</td>
<td>-3.01</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>-</td>
<td>0.0325</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>0.0345</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>0.0148</td>
<td>1.26</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>-</td>
<td>0.0456</td>
<td>2.86</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>0.0303</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>0.0026</td>
<td>0.14</td>
</tr>
</tbody>
</table>

¹natural log transformations used in analyses correct skewness.
²natural log transformations of age and C-peptide used in analysis correct skewness.
³95% confidence intervals for partial correlation coefficient.
<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Adjustment</th>
<th>Statistics for Proinsulin Variable</th>
<th>Full model R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>t</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
<td>-0.0119</td>
<td>-0.34</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>-0.0200</td>
<td>-0.57</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>-0.1973</td>
<td>-0.55</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-</td>
<td>0.1378</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>0.0332</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>0.0384</td>
<td>0.36</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>-</td>
<td>0.0279</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>0.0271</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>0.0260</td>
<td>0.45</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-</td>
<td>-0.1606</td>
<td>-3.20</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>-0.1149</td>
<td>-1.89</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>-0.1152</td>
<td>-1.87</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>-</td>
<td>-0.0107</td>
<td>-0.34</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>0.0149</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>0.0203</td>
<td>0.59</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>-</td>
<td>-0.0243</td>
<td>-0.55</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide</td>
<td>-0.0460</td>
<td>-0.86</td>
</tr>
<tr>
<td></td>
<td>Age, sex, C-peptide, waist</td>
<td>-0.0457</td>
<td>-0.85</td>
</tr>
</tbody>
</table>

1 natural log transformations used in analyses correct skewness.
2 natural log transformations of age and C-peptide used in analysis correct skewness.
3 95% confidence intervals for partial correlation coefficient.
TABLE 4. Characteristics of subjects with normal and impaired glucose tolerance from the follow-up phase of the Sandy Lake Health and Diabetes Project (males=29, females=66)\(^1\).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline values</th>
<th>Change over follow-up period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
</tr>
<tr>
<td>NGT/IGT/DM (n)</td>
<td>41/54/0</td>
<td>54/17/24</td>
</tr>
<tr>
<td>Age at baseline (years)</td>
<td>35.10 16.97</td>
<td>-  -</td>
</tr>
<tr>
<td>Proinsulin (pmol/l)</td>
<td>17.06 11.60</td>
<td>3.66 18.40</td>
</tr>
<tr>
<td>C-peptide (pmol/ml)</td>
<td>0.83 0.43</td>
<td>0.30 0.49</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.70 0.85</td>
<td>0.09 1.38</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.61 0.75</td>
<td>0.29 2.20</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.78 0.68</td>
<td>-0.08 0.54</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.19 0.26</td>
<td>0.02 0.20</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.35 11.37</td>
<td>3.16 5.78</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>121.00 15.48</td>
<td>4.68 13.10</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>68.74 11.08</td>
<td>4.74 11.51</td>
</tr>
</tbody>
</table>

\(^1\)Sample sizes vary slightly for some variables due to occasional missing values.
TABLE 5. Prospective associations of baseline levels and change in proinsulin concentration with changes in lipid concentrations and blood pressure.

<table>
<thead>
<tr>
<th>Cardiovascular Risk Factor Concentration Level</th>
<th>Proinsulin Concentration</th>
<th>( \text{Baseline} ) partial r (95% CI) p-value</th>
<th>( \text{Change over 4 years} ) partial r (95% CI) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol follow-up(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>change(^3) over 4 years</td>
<td>-0.24 (-0.44 - -0.02)</td>
<td>0.0311</td>
<td>-</td>
</tr>
<tr>
<td>Triglyceride follow-up(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>change(^3) over 4 years</td>
<td>-0.16 (-0.37 - 0.06)</td>
<td>0.1462</td>
<td>-</td>
</tr>
<tr>
<td>LDL-C follow-up(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>change(^3) over 4 years</td>
<td>-0.09 (-0.31 - 0.14)</td>
<td>0.4231</td>
<td>-</td>
</tr>
<tr>
<td>HDL-C follow-up(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>change(^3) over 4 years</td>
<td>-0.05 (-0.27 - 0.17)</td>
<td>0.6602</td>
<td>-</td>
</tr>
<tr>
<td>Systolic BP follow-up(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>change(^3) over 4 years</td>
<td>-0.15 (-0.36 - 0.07)</td>
<td>0.1684</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic BP follow-up(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>change(^3) over 4 years</td>
<td>0.07 (-0.15 - 0.29)</td>
<td>0.5341</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^1\)Spearman correlation analysis; sample sizes vary slightly due to occasional missing values.
\(^2\)Analyses adjusted for age sex, c-peptide concentration, change in waist circumference, baseline and follow-up diabetes status, and baseline level of the dependent variable.
\(^3\)Analyses adjusted for age, sex, change in C-peptide concentration, change in waist circumference, baseline and follow-up diabetes status, and baseline level of the dependent variable.
TABLE 6. Results of post-hoc analysis of examining prospective associations of baseline levels and change in lipid concentrations with changes in proinsulin concentrations.

<table>
<thead>
<tr>
<th>Baseline Lipid Concentration</th>
<th>Proinsulin Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follow-up</td>
</tr>
<tr>
<td></td>
<td>partial r (95% CI) p-value</td>
</tr>
<tr>
<td>Cholesterol&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-0.01 (-0.23 - 0.21) 0.9607</td>
</tr>
<tr>
<td>Triglyceride&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.18 (-0.04 - 0.38) 0.1130</td>
</tr>
<tr>
<td>LDL-C&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.02 (-0.20 - 0.24) 0.8484</td>
</tr>
<tr>
<td>HDL-C&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-0.20 (-0.40 - 0.02) 0.0732</td>
</tr>
</tbody>
</table>

<sup>1</sup>Sample sizes vary slightly due to occasional missing values.
<sup>2</sup>Analyses adjusted for age sex, c-peptide concentration, change in waist circumference, baseline and follow-up diabetes status, and baseline level of the dependent variable.
Figure 1. Mean lipid concentrations (in pmol/l, with 95% confidence intervals) in subjects with normal glucose tolerance, by quartile of proinsulin concentration, adjusted for age, sex and waist circumference by analysis of covariance. Quartile ranges: Q1, 3-7 pmol/l; Q2, 8-10 pmol/l; Q3, 11-13 pmol/l; Q4, > 13 pmol/l.
3.7 LINKING SECTION 3

The results of the preceding paper demonstrate significant cross-sectional associations between proinsulin and lipids, as well as, for the first time, evidence that they change together. The findings of the thesis thus far have suggested that beta cell dysfunction, as indicated by elevated proinsulin concentration, is apparent in the earlier stages of glucose intolerance, is associated with modifiable factors such as total and regional adiposity, and may help to explain the elevated cardiovascular risk of subjects with IGT and type 2 DM. Further, the prospective findings regarding abdominal obesity and lipid concentrations raise the possibility that a lipotoxic process may at least in part be responsible for beta cell dysfunction.

The high prevalence rates of both type 2 DM and IGT in Sandy Lake have been reported previously. IGT is a significant phase in the natural history of diabetes in that it indicates increased risk for progression to diabetes, and thus represents an important opportunity for intervention. Although metabolic factors associated with risk of progression from IGT to diabetes have been evaluated in several populations, little is known of these relationships among Native Canadians. In light of this paucity of knowledge and of the findings of the thesis thus far regarding proinsulin concentration, it would be of value to examine the importance of proinsulin concentration and other metabolic factors in risk of progression to diabetes among high-risk subjects, including those with IGT. The specific objectives of the following paper, then, are to assess the association between the risk of hyperglycaemic progression and baseline values and change over 4 years of follow-up in measures of percent body fat, waist circumference, fasting insulin, insulin resistance and proinsulin.
A PROSPECTIVE EVALUATION OF INSULIN RESISTANCE AND BETA CELL FUNCTION IN A NATIVE CANADIAN POPULATION WITH HIGH RATES OF GLUCOSE INTOLERANCE

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²Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, Ontario
³Centre for Studies in Family Medicine, University of Western Ontario, London, Ontario
⁴Robarts Research Institute, London, Ontario
⁵Department of Nutritional Sciences, University of Toronto, Toronto, Ontario
⁶Banting and Best Diabetes Centre, University of Toronto, Toronto, Ontario
⁷Division of Endocrinology and Metabolism, Mt. Sinai Hospital and the University Health Network, Toronto, Ontario

Running title: Insulin resistance, beta cell function and risk of glucose intolerance.

Key words:

Diabetes, Type 2  Insulin resistance  Proinsulin  Obesity

Indians, North American  Epidemiology  Impaired glucose tolerance

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Phone: (416) 586-8747; fax (416) 586-8785; email: zinman@mshri.on.ca
ABSTRACT

Both insulin resistance and beta-cell dysfunction are involved in the natural history of type 2 diabetes, although the sequence of these events remains controversial. The objectives of the present study were to assess the association between the risk of hyperglycaemic progression and baseline values and change over time in measures of adiposity, insulin resistance, and proinsulin, a surrogate measure of beta cell dysfunction. Between 1993 and 1995, 72% of eligible members of an isolated Native Canadian community participated in a population-based survey to determine the prevalence and risk factors for type 2 diabetes. In 1998, 95 individuals who at baseline had impaired glucose tolerance (IGT) or normal glucose tolerance (NGT) with an elevated 2-hour glucose concentration (≥ 7.0 mmol/l) participated in a follow-up evaluation using the protocol employed at baseline. Samples for glucose, insulin, C-peptide, and proinsulin were drawn after an overnight fast, and waist circumference and percent (%) body fat were measured. Insulin resistance and beta cell function were estimated using the homeostasis model assessment (HOMA IR and HOMA β-cell). A 75g oral glucose tolerance test was administered with a second sample for glucose drawn after 120 minutes; diabetes and IGT were diagnosed using WHO criteria. Prospective associations between glycaemic progression and independent variables were assessed using correlation and multiple logistic regression analyses. The rate of progression to diabetes was 25.3% after four years of follow-up, and was higher among subjects with IGT compared to those with NGT (adjusted odds ratio (OR) = 3.36, 95% confidence interval (CI) 1.05-10.71). After adjustment for age, sex, change in waist circumference and baseline glucose tolerance status (GTS), significantly increased risk of diabetes development was associated with changes in proinsulin concentration (2.20, 95% CI 1.21-4.00 per 15 pmol/l), and HOMA IR (1.67, 95% CI 1.10-2.55 per 2 units). Models which included measures of both change in insulin resistance and change in beta cell function indicated the independent contribution of these disorders in diabetes risk. Baseline proinsulin concentration did not predict the subsequent development of diabetes, although this may have been related to the fact that baseline proinsulin concentrations were already relatively high compared to other study populations. These data suggest that both insulin resistance and beta cell dysfunction are important predictors of diabetes in high-risk individuals.
INTRODUCTION

The natural history of type 2 diabetes mellitus (type 2 DM) is thought to involve both insulin resistance and beta cell dysfunction [1]. Controversy exists, however, regarding the sequence of these events in the deterioration of glucose tolerance [2]. Some reviewers have suggested that obesity-associated insulin resistance occurs early in the disease course and is largely responsible for the development of impaired glucose tolerance (IGT), while beta cell decompensation takes place subsequently and induces the IGT-diabetes transition [3]. Others have highlighted studies indicating that beta cell dysfunction is present early in the natural history of diabetes, and further, that the existence of this condition will exacerbate insulin resistance in non-diabetic individuals [1, 4].

Prospective studies of non-diabetic subjects at high-risk for the development of diabetes, including individuals with impaired glucose tolerance (IGT), are beneficial in helping to clarify the importance of these factors in the intermediate stages of diabetes natural history. While the results of these studies to date have generally been in agreement regarding the predictive value of fasting and 2-hour glucose concentrations [5-11], findings concerning anthropometric variables and insulin concentrations have been less consistent. In the majority of papers that employed WHO diagnostic criteria for IGT, body mass index (BMI) was related to progression in univariate analysis [5-15], although, in the seven positive studies that further evaluated the association using multivariate analysis [5-9, 11, 15], BMI was a significant independent predictor in only two [11, 15]. To our knowledge, risk associated with percent body fat, a possibly superior measure of total body adiposity [16-18], has not been evaluated as a risk factor among high-risk subjects. In addition, the role of abdominal obesity has received only limited attention in this literature. Significant univariate relationships have been reported for both waist circumference and waist-hip ratio (WHR) [10], whereas only two projects assessed the independent association between upper body obesity and diabetes development [6, 7]. The independent contribution of waist circumference alone, which has been shown to be superior to WHR as a measure of intra-abdominal adipose tissue [19], has not been evaluated as a diabetes risk factor in these high-risk groups, nor has change in anthropometry over time.

Elevated fasting insulin concentration, a valid surrogate measure of insulin resistance in non-diabetic individuals [20], has also been documented as a risk factor for progression to diabetes among subjects with IGT. Significant univariate relationships were reported in the Pima [6] and San Luis Valley Diabetes Studies [8], as well as in two Scandinavian populations [9, 15], although only in
the North American groups were the associations significant in multivariate analysis. Interpretation of these fasting insulin results is complicated, however, by the fact that conventional insulin radioimmunoassays cross-react with proinsulin-like materials [21]. Proinsulin, which is elevated in subjects with type 2 DM and gestational DM [22, 23], is the physiological precursor to insulin [24], and raised concentrations are considered to be an indicator of beta cell dysfunction [25]. Specific measurement of this prohormone could facilitate further elucidation of the role of beta cell dysfunction in hyperglycaemic progression. A number of recent studies have found that elevated proinsulin concentration predicts the development of diabetes in subjects with IGT [26, 27] and NGT [28-32]. Change in proinsulin concentration over time, however, has not been examined.

In the present study, we explored the associations between insulin resistance and beta cell dysfunction and risk of glucose tolerance deterioration in a group of subjects at high-risk for the development of diabetes. The study was conducted in a Native Canadian population with high rates of diabetes and IGT [33, 34]. The specific objectives of the present paper were to assess the association between the risk of hyperglycaemic progression and baseline values and change over the follow-up period in measures of percent body fat, waist circumference, fasting insulin, homeostasis model indices of insulin resistance and beta cell function (HOMA IR and HOMA \( \beta \)-cell), C-peptide and proinsulin.

SUBJECTS AND METHODS

The methodology of the Sandy Lake Health and Diabetes Project (SLHDP) has been presented in detail in previous publications [33, 34]. Briefly, between July 1993 and December 1995, 728/1018 (72%) eligible residents of Sandy Lake aged 10-79 volunteered to participate in a population-based cross-sectional survey to determine the prevalence of Type 2 DM and its associated risk factors. Signed informed consent was obtained from all participants, and the study was approved by the Sandy Lake First Nation Band Council and the University of Toronto Ethics Review Committee. Subjects in this baseline survey who were found to be at high-risk for subsequent diabetes, including those with impaired glucose tolerance (IGT) (n=74) [34] or normal glucose tolerance (NGT) with a 2 hour post challenge glucose concentration greater than or equal to 7.0 mmol/l (n=51), were invited to participate in a follow-up visit during the summer of 1998 to determine current glucose tolerance and risk factor status. Of the 125 individuals in the follow-up cohort, 3 (3 IGT, 0 NGT) had died, 11 (9 IGT, 2 NGT) were no longer living in the community, 2
(1 IGT, 1 NGT) were too infirm to participate, and 14 (6 IGT, 8 NGT) refused to attend. Thus 95 (76%) members of this high-risk cohort participated in the follow-up examination. Non-participants did not differ significantly from participants in age, gender, anthropometric or metabolic variables (data not shown).

**Baseline Prevalence Survey Procedures, 1993-1995**

Volunteers provided fasting blood samples for glucose, insulin, proinsulin and lipids after an 8-12 hour overnight fast. A 75g oral glucose tolerance test (OGTT) was administered, and a second sample for glucose was drawn after 120 minutes. Individuals were excluded from the OGTT if they had physician diagnosed diabetes and were (a) currently receiving treatment with insulin or oral hypoglycemic agents, or (b) if they had a fasting blood glucose concentration exceeding 11.1 mmol/l. Women who were pregnant at the time of initial contact received their OGTT 3 months post-partum. Diabetes and IGT were diagnosed according to World Health Organization criteria [35].

Insulin was measured using a radioimmunoassay technique (Pharmacia, Inc) which has a lower detection limit of 22 pmol/l, and an interassay coefficient of variation (CV) of 7.2-8.8%. This assay displays a very high degree of cross-reactivity with proinsulin (100%), and thus reported values for “insulin” refer to concentrations of total immunoreactive insulin (IRI) [36]. Glucose concentration was determined using the glucose oxidase method. We employed fasting insulin and glucose concentrations to estimate insulin resistance (HOMA IR) and beta cell function (HOMA β-cell) using the homeostasis model assessment calculation of Matthews et al. [37]. These indices have previously been validated against gold standard measures of insulin resistance and beta cell function [37]. C-peptide concentration was measured using a radioimmunoassay (Diagnostic Products Corporation, Los Angeles) which has minimal detection limit of 43 pmol/l, and cross-reactivities of 0% with insulin and <13% with proinsulin. Proinsulin was determined using a human proinsulin radioimmunoassay technique, which has a laboratory sensitivity of 3.5 pM, and a CV of 6.2-21.0% (Linco Research Inc [38]). This assay displays 46% cross-reactivity with des 31,32 proinsulin, the major form of circulating split proinsulin [39, 40], and thus reported values refer to total proinsulin-like materials. Cross-reactivity of this assay with des 64, 65 proinsulin, insulin and C-peptide is very low (<0.1%). Proinsulin was measured in serum specimens that had been stored at -70°C for between 3-5 years at the Core Lab of the Banting and Best Diabetes Centre, University of Toronto.
Anthropometric measurements were performed with the volunteer wearing either undergarments and a hospital gown or light athletic clothing, and no shoes. Each measurement was performed twice, and the average was used in the analysis. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg using a hospital balance beam scale. Body mass index (BMI) was defined as weight/height\(^2\) (Kg/m\(^2\)). The waist was measured to the nearest 0.5 cm at the point of narrowing between the umbilicus and xiphoid process (as viewed from behind); the hips were measured to the nearest 0.5 cm at the maximum extension of the buttocks. Waist hip ratio (WHR) was calculated as the ratio of these two circumferences. Percent body fat was estimated by bioelectrical impedance analysis (BIA) using the Tanita TBF-201 Body Fat Analyzer (Tanita Corp., Tokyo). High reproducibility of percent fat estimates using this machine (intraclass correlation coefficient (ICC) = 0.99) \[41\] in a sample from this population has been documented, and the instrument has been validated by others against dual energy x-ray absorptiometry (DEXA) \[42-44\] in diabetic and non-diabetic subjects.

Follow-Up Survey Procedures, 1998

Field and laboratory methods and equipment employed during the follow-up survey were identical to those used during the baseline survey. Signed informed consent was obtained from all subjects, and the follow-up protocol was approved by the Sandy Lake First Nation Band Council and the University of Toronto Ethics Review Committee.

Statistical Analyses

All analyses were carried out using SAS version 6.12 \[45\]. The distributions of continuous variables were assessed for normality, and the natural log transformations of non-normal variables were used to correct skewness. Differences in mean values between groups were compared using t-tests or the non-parametric Wilcoxon rank sum test, and differences in proportions using chi-square tests. Risk factors for progression to diabetes were evaluated using logistic regression analyses. The dependent variable in these models was development of diabetes (yes/no). Separate models for each independent variable of interest were constructed; these variables included presence of IGT at baseline (yes/no), baseline values of fasting and 2-hour glucose, and baseline and change over the follow-up period in concentrations of fasting insulin, HOMA IR, HOMA \(\beta\)-cell, fasting C-peptide and fasting proinsulin, as well as baseline and change over the follow up period in percent body fat and waist circumference. These independent variables were modeled as continuous variables, and
the odds ratio was estimated to describe the increase in risk per 1 SD change for baseline variables, and for the following differences for change variables: Δ fasting insulin, 50 pmol/l; Δ HOMA IR, 2 units; Δ HOMA β-cell, 50%, Δ fasting C-peptide, 200 pmol/l, Δ fasting proinsulin, 15 pmol/l, Δ % fat, 10%; Δ waist, 10 cm. Age, sex, waist circumference, and baseline GTS were included as potential confounding factors.

We also employed Spearman correlation analyses to evaluate deterioration or improvement of glucose concentrations as continuous outcome variables. Independent variables in these models were similar to those outlined above for the logistic regression analyses, and included baseline fasting and 2-hour glucose, baseline values and change over the follow-up period in concentrations of fasting insulin, HOMA IR, HOMA β-cell, fasting C-peptide and fasting proinsulin, as well as baseline and change over the follow up period in percent body fat and waist circumference. Scatterplots were examined prior to correlation analyses to ensure linearity of the relationships.

The analytical convention in this body of literature is to ‘adjust’ proinsulin concentration for insulin secretion, usually by employing the proinsulin-to-insulin ratio (P/I ratio). This is problematic, for two reasons. First, peripheral insulin concentrations do not adequately represent insulin secretion given that “insulin undergoes a large and variable hepatic extraction as well as peripheral clearance that varies under different physiological circumstances” [46]. Second, Kronmal has pointed out that the use of ratio variables in correlation and regression can result in spurious results [47]. We have therefore opted to avoid the use of the P/I ratio and to conduct our analysis of proinsulin concentrations after adjustment for C-peptide, which is “co-secreted with insulin in an equimolar ratio, is not extracted by the liver, and has a constant peripheral clearance” [46].

RESULTS

Baseline characteristics of study participants are presented in Table 1. Subjects with IGT at baseline were older and had higher concentrations of 2-hour glucose and proinsulin compared to those with NGT at baseline. In addition, subjects with IGT had higher concentrations of fasting glucose, were more insulin resistant and had higher beta cell activity compared to those with NGT.
Twenty-four subjects (25.3%) developed type 2 DM after four years of follow-up, corresponding to a progression rate of 6.3% per year. Duration of follow-up was not significantly related to risk of diabetes development (data not shown). Subjects with IGT had a higher rate of deterioration to diabetes compared to NGT subjects (34.55% vs. 12.50%, p=0.015, Table 2). Subjects who developed type 2 DM had significantly higher 2-hour glucose concentration and waist circumference at baseline (both p<0.05). Those who developed diabetes were more insulin resistant and had higher beta cell function and concentrations of insulin, C-peptide, proinsulin, and higher % body fat and compared to those who did not progress to diabetes, although these differences did not reach statistical significance due to the small number of individuals who progressed to diabetes (Table 2). Increases over the follow-up period in HOMA IR, fasting proinsulin and percent body fat were significantly larger in subjects who progressed to diabetes compared to those who did not (all p<0.01, Table 2).

We used logistic regression analysis to estimate the risk of progression to diabetes associated with baseline GTS (Table 3). Subjects with IGT at baseline were at more than 3-fold higher risk of diabetes at follow-up compared to those with NGT. This risk estimate was essentially unchanged after adjustment for age, sex, and waist circumference (odds ratio (OR) 3.36, 95% confidence interval (CI) 1.05-10.71) (Table 3).

Logistic regression was further employed to evaluate risk of progression to diabetes associated with baseline and change over time in metabolic and anthropometric variables (Table 4). After adjustment for age and sex, baseline levels of 2-hour glucose and waist circumference were each associated with a 1.8-fold (per 1 SD) increased risk of progression to diabetes. The magnitude of the risk estimate for 2-hour glucose concentration was reduced slightly with further adjustment for baseline GTS and waist circumference. Change over time in HOMA IR, proinsulin concentration and percent body fat were also significant risk factors for progression to diabetes. After adjustment for age, sex, baseline GTS, and changes in waist circumference and insulin secretion (fasting C-peptide concentration), change in fasting proinsulin held a greater than 2-fold risk of deterioration to diabetes (OR=2.20, 95% CI 1.21-4.00 per 15 pmol/l change). In addition, increased risk of diabetes was associated with an increase in HOMA IR (adjusted OR=1.67, 95% CI 1.10-2.55 per 2 unit change) as well as a decrease in HOMA β-cell (adjusted OR=0.76, 95% CI 0.60-0.98) after adjustment for age, sex, baseline GTS and change in waist circumference. The independent prospective roles of changes in beta cell function and insulin resistance were also
assessed in two separate multivariate logistic models (Table 5). In each model, both an increase in the insulin resistance parameter (HOMA IR or fasting insulin) and an increase in the parameter for beta cell dysfunction (increase in proinsulin or decrease in HOMA β-cell) over the follow-up period were significantly associated with risk of diabetes development, although the parameter for change in fasting insulin was not significant when modeled with change in proinsulin.

Deterioration or improvement in glucose tolerance was also assessed using follow-up and change in glucose concentrations as continuous outcome variables (Table 6). Baseline fasting insulin and C-peptide concentrations as well as HOMA IR and waist circumference were positively associated with fasting glucose concentrations at follow-up (all p-values <0.05) in age- and sex-adjusted models. After adjustment for age, sex, baseline GTS, initial glucose concentration and waist circumference, both baseline HOMA IR and baseline fasting C-peptide showed moderate associations with follow-up and change in fasting glucose concentrations. Changes over the follow-up period in fasting insulin, HOMA IR, HOMA β-cell, fasting proinsulin and waist circumference were associated with change in fasting glucose concentrations (age and sex adjusted, all p-values <0.001). Change over time in 2 hour glucose concentration, on the other hand, was associated with changes in HOMA IR, proinsulin, waist circumference and % body fat (age and sex adjusted, all p-values <0.01). The direction and magnitude of these associations were not appreciably altered in the fully adjusted models (age, sex, baseline GTS, initial glucose concentration and change in waist circumference).

DISCUSSION

In this paper, we have reported that subjects with IGT were at an increased risk of progression to diabetes relative to subjects with NGT who had an elevated 2-hour glucose concentration. In logistic regression analysis, risk of deterioration to diabetes was associated with baseline 2-hour glucose concentration and waist circumference, as well as changes in HOMA IR, HOMA β-cell and proinsulin. When modeled together, changes in both insulin resistance and beta cell function were independent contributors to risk of diabetes development. When glucose tolerance was analyzed as a continuous outcome variable, baseline insulin, C-peptide and waist circumference, as well as changes over time in insulin, HOMA IR, HOMA β-cell, proinsulin, and adiposity were positively associated with follow-up concentrations and change in glucose.
Among subjects with IGT, the rate of progression to diabetes was 8.6%/year, versus 3.1% per year among those in the NGT group. Rates of progression in previous studies of IGT cohorts have ranged from as low as 2.0%/year [48] to over 10%/year [7, 49, 50]. Despite these high rates of progression, the usefulness of IGT as an epidemiological or clinical category has been questioned [51, 52]. Critics draw particular attention to the low short-term reproducibility of a single diagnosis of this condition [52]. Notwithstanding these criticisms, a number of studies have reported that IGT is a significant independent risk factor for the development of diabetes [6, 53]. In the present study, IGT was a risk factor for progression even when the comparison group was comprised of subjects with NGT who had high post-challenge glucose concentrations. This result confirms that IGT is an important predictor of diabetes in this population.

Results of our logistic regression analyses indicated that baseline proinsulin concentration was not significantly associated with the development of diabetes in the full prospective cohort. This was an unexpected finding in light of the results of a number of previous investigations which have reported that baseline proinsulin was an independent predictor of deterioration to diabetes in subjects with IGT and NGT [26-32]. It is possible that this discordant finding might be explained by differences in the natural history of diabetes between different populations. Proinsulin concentrations among non-converters in the present study were high compared to concentrations in non-converters in two studies from this literature that employed the same proinsulin radioimmunoassay (Bowsher and colleagues [38]) [27, 30]. It is conceivable, then, that proinsulin concentrations begin to increase at an earlier stage of diabetes pathogenesis in Native Canadian subjects.

Our results indicating strong associations between change in proinsulin and change in glucose concentrations extend previous findings regarding the role of proinsulin in the natural history of diabetes [26-32]. In particular, this result highlights the close link between increasing proinsulin concentration and deterioration of glucose tolerance among high-risk non-diabetic subjects. There are two possible mechanistic explanations for this concomitant increase. First, increasing proinsulin concentrations could have been the result of increasing glucose concentrations. While a number of recent papers have reported increased release of proinsulin by beta cells after chronic glucose exposure [54, 55], the glucose concentrations employed in those studies were substantially higher (≥20 mmol/L) than those normally seen in subjects with NGT or IGT. In another study, proinsulin secretion was not increased in normal subjects after an 8-hour hyperglycaemic clamp [56]. Second, increasing glucose concentrations could have been the result of
declining beta cell function, which is reflected by increasing proinsulin concentrations. Several lines of evidence suggest that this is the more likely explanation [24, 25]. Proinsulin is increased in non-diabetic siblings of subjects with Type 1 DM [57], as well as in subjects who have undergone hemipancreatectomy [58]. In addition, recent investigations have demonstrated increased relative arginine-stimulated proinsulin secretion in women with IGT [59], as well as associations between hyperproinsulinaemia and more detailed clinical measures of beta cell dysfunction, including delayed insulin response during a hyperglycaemic clamp [60] in IGT, and reduced acute insulin response to arginine in diabetic subjects [61].

Our study showed that baseline levels and changes in HOMA IR, fasting insulin, percent body fat and waist circumference were positively associated with changes in glucose concentrations, which suggests that insulin resistance is also an important factor in hyperglycaemic progression in this high-risk subgroup. Both HOMA IR and fasting insulin demonstrate acceptable criterion validity against gold standard measures of insulin resistance in non-diabetic subjects [16, 37]. In addition, waist circumference has been shown to be a valid surrogate measure of intra-abdominal adipose tissue [62], which is known to be particularly detrimental to insulin sensitivity [63, 64]. As mentioned, results of previous prospective studies of subjects with IGT have been inconsistent regarding the importance of fasting insulin and obesity. Discordant results regarding the role of obesity may be partly related to the use of BMI as an index of adiposity in the majority of these studies. The relationship between BMI and gold standard measures of body fat has been shown to be dependent on age, sex, ethnicity and body build, and thus BMI might not be a valid measure of total body adiposity [16-18]. In addition, BMI may not adequately capture excess central or abdominal adiposity. In studies that have measured body fat distribution, significant univariate relationships have been reported for both waist circumference and waist-hip ratio [10], whereas only one project assessed the independent contribution of upper body obesity using measures of skinfold thickness [8]. It is also possible that ethnic differences in the relative importance of insulin resistance explain the inconsistency in the results of these studies [65].

Multivariate modeling indicated that both increases in insulin resistance and decreases in beta cell function (the latter measured as either increased proinsulin concentration or decreased HOMA B-cell) contributed independently to the risk of diabetes development. The co-existence of these disorders in the natural history of diabetes has been highlighted in recent literature syntheses [1, 2, 4]. Ferrannini [1] has suggested that the primacy of either insulin resistance or beta cell dysfunction in the etiology of diabetes is unlikely. Rather, it appears that these two disorders
exacerbate each other through tightly connected multiple feedback mechanisms. Nagi and coworkers have reported that both insulin resistance and reduced early insulin response predicted progression to diabetes among Pima subjects with IGT [66]. The Pimas are similar to the population of Sandy Lake in both Native North American ancestry and very high population prevalence of glucose tolerance abnormalities. In addition, Haffner and colleagues have previously documented the independent contributions of HOMA IR and HOMA β-cell in diabetes risk, and have highlighted the importance of accounting for insulin resistance when assessing insulin secretion using the HOMA β-cell index [67, 68]. In particular, our finding that changes over time in both insulin resistance and beta cell function are important in the development of diabetes is consistent with the recent work of Weyer and colleagues [69], who found that deterioration in glucose tolerance among Pima subjects was associated with longitudinal declines in both insulin-stimulated glucose disposal and acute insulin response.

One potential criticism of this study is the relatively small sample size, and hence the low statistical power for detecting small effects. Our study was population-based, however, and the eligible subjects we identified to address the research questions of interest represented the universe of individuals with IGT and high-risk NGT in this particular population [33]. Seventy-six percent of eligible subjects participated, and baseline variables in these individuals were not significantly different from values in non-participants, suggesting that volunteer bias is unlikely to effect the generalizability of the findings.

In conclusion, we found that measures of both insulin resistance and beta cell function were important factors in determining hyperglycaemic progression in high-risk non-diabetic subjects. This finding supports the notion that the natural history of diabetes is the result of a complex interaction between insulin resistance and beta cell dysfunction.
ACKNOWLEDGEMENTS

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REFERENCES


Release of incompletely processed proinsulin is the cause of disproportionate proinsulinaemia of NIDDM. Diabetes 1997;46:1725-32.


High risk of progression to NIDDM in South African Indians with impaired glucose tolerance. Diabetes 1993;42:556-63.


Impaired glucose tolerance: is it a risk factor for diabetes or a diagnostic ragg? BMJ 1990;301:397-402.


Prolonged exposure of human beta cells to high glucose increases their release of proinsulin during acute stimulation with glucose or arginine. J Clin Endocrinol Metab 1999;84:1386-90.


Hyperproinsulinemia is associated with increased beta cell demand after hemipectectomy in humans. J Clin Invest 1996;97:455-60.

Relative hyperproinsulinemia as a sign of islet dysfunction in women with impaired glucose tolerance. J Clin Endocrinol Metab 1999;84:2068-74.


Table 1. Baseline characteristics of follow-up study participants, both pooled and stratified by baseline glucose tolerance status.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Subjects (n=95)</th>
<th>Subjects by Baseline Glucose Tolerance Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NGT (n=40)</td>
</tr>
<tr>
<td>Gender (% male / % female)</td>
<td>29.50/70.50</td>
<td>37.50 / 62.50</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.10 (16.97)</td>
<td>29.21 (14.47)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.73 (0.55)</td>
<td>5.60 (0.41)</td>
</tr>
<tr>
<td>2-hour glucose (mmol/l)</td>
<td>8.28 (1.06)</td>
<td>7.34 (0.23)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>161.55 (97.35)</td>
<td>140.86 (55.00)</td>
</tr>
<tr>
<td>HOMA IR (units)</td>
<td>5.78 (3.71)</td>
<td>4.91 (2.04)</td>
</tr>
<tr>
<td>HOMA β-cell (%)</td>
<td>208.23 (123.94)</td>
<td>188.89 (73.13)</td>
</tr>
<tr>
<td>Fasting C-peptide (pmol/l)</td>
<td>827.61 (428.36)</td>
<td>772.16 (365.03)</td>
</tr>
<tr>
<td>Fasting proinsulin (pmol/l)</td>
<td>17.06 (11.60)</td>
<td>13.71 (6.41)</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>41.54 (10.55)</td>
<td>39.99 (11.66)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.35 (11.37)</td>
<td>94.37 (13.19)</td>
</tr>
</tbody>
</table>

Values are means (±SD) or proportions.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Maintained NGT/IGT (n=71)</th>
<th>Developed Type 2 DM (n=24)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline GTS (% NGT / % IGT)</td>
<td>87.50 / 65.45</td>
<td>12.50 / 34.55</td>
<td>0.015</td>
</tr>
<tr>
<td>Gender (% male / % female)</td>
<td>78.57 / 73.13</td>
<td>21.43 / 26.87</td>
<td>0.578</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.87 (18.27)</td>
<td>38.73 (11.91)</td>
<td>0.141</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.69 (0.47)</td>
<td>5.84 (0.75)</td>
<td>0.446</td>
</tr>
<tr>
<td>2-hour glucose (mmol/l)</td>
<td>8.10 (1.02)</td>
<td>8.81 (1.03)</td>
<td>0.004</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>158.12 (96.39)</td>
<td>171.87 (101.67)</td>
<td>0.388</td>
</tr>
<tr>
<td>HOMA IR (units)</td>
<td>5.65 (3.74)</td>
<td>6.17 (3.69)</td>
<td>0.321</td>
</tr>
<tr>
<td>HOMA β-cell (%)</td>
<td>200.21 (104.09)</td>
<td>232.29 (171.00)</td>
<td>0.648</td>
</tr>
<tr>
<td>Fasting C-peptide (pmol/l)</td>
<td>800.43 (372.32)</td>
<td>909.13 (566.79)</td>
<td>0.235</td>
</tr>
<tr>
<td>Fasting proinsulin (pmol/l)</td>
<td>16.59 (11.84)</td>
<td>18.42 (11.01)</td>
<td>0.417</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>41.05 (11.08)</td>
<td>43.06 (8.80)</td>
<td>0.439</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>94.94 (12.13)</td>
<td>100.45 (7.57)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

**Change variables**

- Δ Fasting glucose | -0.18 (0.66) | 2.26 (3.55) | <0.001
- Δ 2-hour glucose | -1.45 (1.81) | 5.27 (4.07) | <0.001
- Δ Fasting insulin | -36.26 (95.80) | -14.82 (107.55) | 0.129
- Δ HOMA IR | -1.39 (3.79) | 1.19 (3.16) | 0.005
- Δ HOMA β-cell | -43.86 (112.48) | -7.50 (137.25) | 0.218
- Δ Fasting C-peptide | 305.13 (416.07) | 281.59 (662.77) | 0.877
- Δ Fasting proinsulin | -0.06 (17.61) | 14.35 (16.65) | <0.001
- Δ Percent body fat | 3.30 (8.61) | 9.00 (8.69) | 0.009
- Δ Waist circumference | 2.91 (6.03) | 3.89 (5.01) | 0.471

Values are mean (±SD) or proportion

1Test performed on log-transformed variable

2Wilcoxon rank sum test
TABLE 3. Baseline glucose tolerance status and risk of progression to diabetes\textsuperscript{1}.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Crude Analysis</th>
<th>Analysis adjusted for age and sex</th>
<th>Analysis adjusted for age, sex, and waist circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR\textsuperscript{2}</td>
<td>95% CI</td>
<td>p-value</td>
</tr>
<tr>
<td>Baseline GTS (NGT=0, IGT=1)\textsuperscript{3}</td>
<td>3.69</td>
<td>1.24-10.98</td>
<td>0.019</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Outcome variable: development of diabetes (no=0, yes=1)

\textsuperscript{2}Abbreviations: OR, odds ratio; CI, confidence interval; GTS, glucose tolerance status; NGT, normal glucose tolerance; IGT, impaired glucose tolerance.
TABLE 4. Baseline and change over time in anthropometric and metabolic characteristics and risk of progression to diabetes. 

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Crude Analysis</th>
<th>Analysis adjusted for age and sex</th>
<th>Analysis adjusted for age, sex, waist circumference, and baseline GTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>p-value</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>1.26 0.85-1.86</td>
<td>0.252</td>
<td>1.20 0.81-1.80</td>
</tr>
<tr>
<td>2-hour glucose</td>
<td>1.86 1.19-2.93</td>
<td>0.007</td>
<td>1.80 1.14-2.86</td>
</tr>
<tr>
<td>Log, fasting insulin</td>
<td>1.23 0.77-1.99</td>
<td>0.385</td>
<td>1.36 0.79-2.32</td>
</tr>
<tr>
<td>Log, HOMA IR</td>
<td>1.28 0.79-2.06</td>
<td>0.319</td>
<td>1.37 0.81-2.34</td>
</tr>
<tr>
<td>Log, HOMA β-cell</td>
<td>1.13 0.73-1.74</td>
<td>0.593</td>
<td>1.24 0.76-2.03</td>
</tr>
<tr>
<td>Log, fasting C-peptide</td>
<td>1.33 0.83-2.12</td>
<td>0.235</td>
<td>1.34 0.82-2.17</td>
</tr>
<tr>
<td>Log, fasting proinsulin</td>
<td>1.21 0.77-1.90</td>
<td>0.414</td>
<td>1.22 0.77-1.94</td>
</tr>
<tr>
<td>Log, fasting proinsulin</td>
<td>1.09 0.63-1.88</td>
<td>0.759</td>
<td>1.11 0.64-1.94</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>1.23 0.73-2.09</td>
<td>0.435</td>
<td>1.33 0.58-3.04</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>2.86 1.58-5.20</td>
<td>0.044</td>
<td>1.61 0.95-3.43</td>
</tr>
<tr>
<td>Δ fasting insulin</td>
<td>1.14 0.85-1.52</td>
<td>0.380</td>
<td>1.07 0.79-1.45</td>
</tr>
<tr>
<td>Δ HOMA IR</td>
<td>1.71 1.17-2.50</td>
<td>0.006</td>
<td>1.67 1.13-2.47</td>
</tr>
<tr>
<td>Δ HOMA β-cell</td>
<td>1.16 0.91-1.48</td>
<td>0.219</td>
<td>1.12 0.86-1.45</td>
</tr>
<tr>
<td>Δ Fasting C-peptide</td>
<td>0.98 0.80-1.19</td>
<td>0.843</td>
<td>0.93 0.76-1.14</td>
</tr>
<tr>
<td>Δ Fasting proinsulin</td>
<td>2.20 1.27-3.80</td>
<td>0.005</td>
<td>2.09 1.22-3.59</td>
</tr>
<tr>
<td>Δ Fasting proinsulin</td>
<td>2.85 1.25-4.42</td>
<td>0.008</td>
<td>2.21 1.20-4.06</td>
</tr>
<tr>
<td>Δ Percent body fat</td>
<td>2.47 1.22-4.99</td>
<td>0.012</td>
<td>2.65 1.22-5.73</td>
</tr>
<tr>
<td>Δ Waist circumference</td>
<td>1.35 0.60-3.04</td>
<td>0.467</td>
<td>1.46 0.59-3.62</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; CI, confidence interval; GTS, glucose tolerance status.
Outcomes variable: development of diabetes (no=0, yes=1).

In baseline models, "waist circumference" = baseline waist circumference; in change models, "waist circumference" = change in waist circumference.

Odds ratios refer to an increase in the level of the independent variable of 1 SD based on the distribution in subjects who did not progress to diabetes.

Odds ratios refer to an increase in the level of the independent variable of: Δ fasting insulin, 50 pmol/l; Δ HOMA IR, 2 units; Δ HOMA β-cell, 50%, Δ fasting C-peptide, 200 pmol/l, Δ fasting proinsulin, 15 pmol/l, Δ % fat, 10%; Δ waist, 10 cm.

OR=0.96, 95% CI 0.48-1.93, p=0.912 after further adjusted for HOMA IR; * also adjusted for C-peptide; † also adjusted for change in C-peptide.
TABLE 5. Independent contribution of insulin resistance and beta cell function in risk of diabetes development

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ HOMA IR</td>
<td>6.23</td>
<td>1.89-20.61</td>
<td>0.003</td>
</tr>
<tr>
<td>Δ HOMA β-cell</td>
<td>0.36</td>
<td>0.16-0.83</td>
<td>0.016</td>
</tr>
<tr>
<td>Model 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ HOMA IR</td>
<td>1.73</td>
<td>0.99-3.02</td>
<td>0.052</td>
</tr>
<tr>
<td>Δ fasting proinsulin</td>
<td>1.68</td>
<td>0.95-2.98</td>
<td>0.079</td>
</tr>
<tr>
<td>Model 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ fasting insulin</td>
<td>3.72</td>
<td>1.56-8.88</td>
<td>0.003</td>
</tr>
<tr>
<td>Δ HOMA β-cell</td>
<td>0.30</td>
<td>0.15-0.61</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ fasting insulin</td>
<td>1.12</td>
<td>0.68-1.84</td>
<td>0.652</td>
</tr>
<tr>
<td>Δ fasting proinsulin</td>
<td>2.14</td>
<td>1.18-3.87</td>
<td>0.012</td>
</tr>
</tbody>
</table>

<sup>a</sup>Abbreviations: OR, odds ratio; CI, confidence interval.

<sup>b</sup>Odds ratios refer to an increase in the level of the independent variable of: Δ HOMA IR, 2 units; Δ HOMA β-cell, 50%; Δ fasting proinsulin, 15 pmol/l; Δ fasting insulin, 50 pmol/l.

<sup>c</sup>Outcome variable: development of diabetes (no=0, yes=1)

<sup>d</sup>Adjusted for age, sex, change in waist circumference, and baseline GTS.

<sup>e</sup>Also adjusted for change in C-peptide.
TABLE 6. Relationship of baseline and change over time in metabolic and anthropometric variables with follow-up and change over time in fasting and 2-hour glucose concentrations.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted for age and sex</th>
<th>Adjusted for age, sex, baseline glucose tolerance status, waist circumference, and initial glucose concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting glucose</td>
<td>2-hour glucose</td>
</tr>
<tr>
<td></td>
<td>follow-up</td>
<td>change</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>0.23*</td>
<td>-0.03</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.27*</td>
<td>-0.10</td>
</tr>
<tr>
<td>HOMA β-cell</td>
<td>0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>Fasting C-peptide (pmol/ml)</td>
<td>0.22*</td>
<td>0.02</td>
</tr>
<tr>
<td>Fasting proinsulin (pmol/l)</td>
<td>0.12</td>
<td>-0.09</td>
</tr>
<tr>
<td>Fasting proinsulin (pmol/l)</td>
<td>0.02</td>
<td>-0.11</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>0.16</td>
<td>0.09</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.26*</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Change

| ΔFasting insulin (pmol/l)       | 0.13      | 0.32†  | 0.08     | 0.10   | 0.24*     | 0.20|| 0.00     | 0.01     |
| ΔHOMA IR                       | 0.37§     | 0.61§  | 0.26*    | 0.27*  | 0.52§     | 0.53§  | 0.17     | 0.19||     |
| ΔHOMA β-cell                   | 0.14      | 0.28†  | 0.06     | 0.09   | 0.27*     | 0.23*  | 0.00     | 0.03     |
| ΔFasting C-peptide (pmol/ml)   | 0.10      | 0.16   | 0.03     | 0.07   | 0.14      | 0.08   | 0.01     | 0.01     |
| ΔFasting proinsulin (pmol/l)   | 0.45§     | 0.51§  | 0.36†    | 0.43§  | 0.51§     | 0.48§  | 0.32†    | 0.34†    |
| ΔFasting proinsulin (pmol/l)   | 0.42§     | 0.47§  | 0.33†    | 0.42§  | 0.48§     | 0.47§  | 0.31†    | 0.34†    |
| ΔPercent body fat (%)          | 0.06      | 0.12   | 0.06     | 0.32†  | -         |      | -        | -        |
| ΔWaist circumference (cm)      | 0.15      | 0.36‡  | 0.15     | 0.34†  | -         |      | -        | -        |

1 analyses using Spearman correlation coefficients.
2 fasting or 2 hour glucose
3 in baseline models, "waist circumference" = baseline waist circumference; in change models, "waist circumference" = change in waist circumference.
4 also adjusted for C-peptide
5 p<0.10; † p<0.05; ‡ p<0.01; § p<0.001
The previous paper documented the importance of changes in both insulin resistance and beta cell function in risk of diabetes development among subjects at high-risk for diabetes at baseline. While a number of previous papers have examined metabolic factors and risk of progression to diabetes among high risk subjects, very little information is available regarding lifestyle factors and diabetes risk in cohorts with high-risk phenotypes. Results from prospective studies in the United States and Europe have suggested the importance of lifestyle factors in determining risk of diabetes among general population cohorts. Limited information is available, however, regarding the association between lifestyle factors and diabetes development among subjects at high risk of diabetes, including those with IGT. In addition, the association between lifestyle factors and risk of diabetes among Native Canadians, a population that is experiencing both epidemic diabetes and rapid cultural transition, has received only limited attention.

In light of these gaps in the literature, the following paper presents the results of an investigation of the role of lifestyle factors, including smoking, alcohol consumption, and dietary intake, and risk of progression to diabetes among subjects who at baseline had either IGT or NGT with elevated post-challenge glucose concentrations.
LIFESTYLE FACTORS AND RISK OF DETERIORATION IN GLUCOSE TOLERANCE IN A POPULATION-BASED FOLLOW-UP STUDY OF HIGH-RISK NATIVE CANADIAN SUBJECTS.

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Running title: Lifestyle factors and glucose tolerance deterioration.

Key words:

Diabetes, Type 2 Impaired glucose tolerance Smoking Alcohol

Diet Physical Activity Indians, North American Epidemiology

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ABSTRACT

Only limited information is available regarding the association between modifiable lifestyle factors and risk of glycaemic progression among subjects in the intermediate stages of diabetes natural history. In the present paper, we report on an evaluation of the prospective relationship between risk of deterioration to type 2 diabetes mellitus (type 2 DM) and lifestyle practices, including smoking, alcohol consumption, and dietary intake. We followed subjects at high-risk for diabetes, defined as those who, during a 1993-95 baseline prevalence and risk factor survey, had either impaired glucose tolerance (IGT) or normal glucose tolerance (NGT) with an elevated 2-hour glucose concentration (≥ 7.0 mmol/l). In 1998, 76% of eligible subjects (n=95) attended a follow-up evaluation (median follow-up period was 4 years). In both the baseline and follow-up examinations, a 75g oral glucose tolerance test was administered, and Type 2 DM and IGT were diagnosed using WHO criteria. Results of logistic regression analysis indicated that, compared to never and former smokers, current smokers at baseline were at increased risk for diabetes development after adjustment for confounding factors (OR=3.50, 95% confidence interval (CI), 1.19-10.29). In addition, dietary calcium intake was associated with a significantly decreased adjusted diabetes risk (OR=0.54, 95% CI 0.31-0.95 per 1 SD difference). Increased alcohol consumption was a significant risk factor for diabetes after adjustment for age and sex (OR=1.66, 95% CI 1.06-2.60 per 1 SD difference in weighted consumption frequency scale), although the magnitude of the estimate was reduced slightly in fully adjusted models. Analyses were also suggestive of increased risk of diabetes with frequent lard consumption, as well as decreased risk of diabetes with infrequent fish consumption, although these odds ratios were not statistically significant. The results of this study indicate that risk of Type 2 DM is associated with modifiable lifestyle factors, even among subjects in the intermediate stages of disease natural history.
INTRODUCTION

The etiology of type 2 diabetes mellitus (Type 2 DM) is complex, and is thought to involve an interaction of genetic, environmental and lifestyle factors [1]. Regarding the latter, both total body and central adiposity have been prospectively associated with risk of diabetes development in a number of populations [1-4]. In addition, several recent cohort studies have demonstrated the protective role of physical activity in the epidemiology of this condition [5-8]. However, the importance of other modifiable lifestyle factors, including diet [9-13], cigarette smoking [7, 14-16], and alcohol consumption [7, 15, 17-20] is much less clear.

Prospective evaluations of non-diabetic subjects at high risk for the development of diabetes, including individuals with impaired glucose tolerance (IGT), are beneficial in helping to clarify the importance of lifestyle factors in the intermediate stages of diabetes natural history. To date, however, most follow-up studies of individuals with IGT have focused on the roles of anthropometric and metabolic factors and risk of deterioration to diabetes [21-31]. To our knowledge, in only a few papers have lifestyle factors and risk of progression been examined [24, 25, 29, 31]. Marshall and colleagues reported that increased baseline total fat intake was an independent risk factor for the development of Type 2 DM [24], and Eriksson et al. found that several measures of physical fitness level were lower in subjects with baseline IGT who eventually developed diabetes [25]. While a lack of association with smoking has been reported in 3 studies [21, 25, 31], the exposure was treated as a simple dichotomous variable, and thus these studies would not have been able to detect risk associated with higher amounts of smoking.

Over the past several decades, many Native communities in Canada have experienced rapid Westernization, which has brought about largely detrimental changes in diet composition and levels of regular physical activity [32]. As a consequence, Native Canadians are experiencing an epidemiological transition, with increases in mortality and morbidity attributable to chronic diseases [33]. We have recently documented high rates of obesity and type 2 DM in an isolated First Nation in northern Ontario [34]. In the present paper, we report on an evaluation of the prospective relationship between risk of diabetes development and lifestyle practices, including smoking, alcohol consumption, and dietary intake, among high-risk subjects in this community. We defined high-risk subjects as those who, at baseline, had either IGT or normal glucose tolerance (NGT) with an elevated 2-hour glucose concentration (≥ 7.0 mmol/L).
SUBJECTS AND METHODS

The methodology of the Sandy Lake Health and Diabetes Project (SLHDP) has been presented in detail in previous publications [34-37]. Between July 1993 and December 1995, 728/1018 (72%) eligible residents of Sandy Lake aged 10-79 participated in a cross-sectional survey to determine the prevalence of Type DM and its associated risk factors. Signed informed consent was obtained from all participants, and the study was approved by the Sandy Lake First Nation Band Council and University of Toronto Ethics Review Committee. Participants in this baseline survey who were found to be at high risk for subsequent diabetes, including those with impaired glucose tolerance (IGT) (n=74) or normal glucose tolerance (NGT) with a 2 hour post challenge glucose level greater than or equal to 7.0 mmol/l (n=51), were invited to participate in a follow-up visit during the summer of 1998 to determine current glucose tolerance and risk factor status. Of the 125 individuals in this follow-up cohort, 3 (all IGT) had died, 11 (9 IGT, 2 NGT) were no longer living in the community, 2 (1 IGT, 1 NGT) were too infirm to participate, and 14 (6 IGT, 8 NGT) refused to attend. Thus 95 (76%) members of this high-risk cohort participated in the follow-up examination. Non-participants did not differ significantly from participants in age, gender, anthropometry, metabolic or lifestyle variables (data not shown).

Baseline Prevalence Survey Procedures, 1993-1995

Participants provided fasting blood samples for glucose after an 8-12 hour overnight fast. A 75g oral glucose tolerance test (OGTT) was administered, and a second sample for glucose was drawn after 120 minutes. Individuals were excluded from the OGTT if they had physician diagnosed diabetes and were (a) currently receiving treatment with insulin or oral hypoglycemic agents, or (b) if they had a fasting blood glucose concentration exceeding 11.1 mmol/l. Women who were pregnant at the time of initial contact received their OGTT 3 months post-partum. Diabetes and IGT were diagnosed according to World Health Organization criteria [38]. Glucose concentration was determined using the glucose oxidase method.

Anthropometric measurements were performed with the participant wearing either undergarments and a hospital gown or light athletic clothing, and no shoes. Each measurement was performed twice, and the average was used in the analysis. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg using a hospital balance beam scale. Body mass index (BMI) was defined as weight/height^2 (Kg/m^2). The waist was
measured to the nearest 0.5 cm at the point of narrowing between the umbilicus and xiphoid process (as viewed from behind); the hips were measured to the nearest 0.5 cm at the maximum extension of the buttocks. Waist hip ratio (WHR) was calculated as the ratio of these two circumferences.

Lifestyle practices were assessed using standardized, interviewer-administered questionnaires [35, 39]. Smoking history was determined, including time since cessation among former smokers, as well as duration in years among ever smokers and usual number of cigarettes per day among current smokers. Pack-years were calculated for current smokers as \(\text{years of smoking} \times \frac{\text{number of cigarettes/day}}{20}\). Frequency of alcohol consumption (4-levels) as well as usual amounts of beer, wine and liquor consumed during each drinking occasion were determined. A drinking intensity scale was calculated as \(\text{frequency of drinking} \times \text{usual amount per occasion}\). Nutrient intake was assessed by 24-hour dietary recall, with volumes and portion sizes were estimated using measuring cups and spoons, and 2- and 3-dimensional food models. The coding and preliminary analyses of these data have been reported elsewhere [40, 41]. In the present analysis, we evaluated possible associations with total energy intake and with macronutrients including protein, carbohydrate, starch, simple sugar, fibre, total fat, saturated fat, and poly- and monounsaturated fat. We also investigated the risk associated with micronutrients including calcium, vitamin A, vitamin C, niacin, and folate. Three-month food consumption patterns, including common wild and store-bought foods and seasonal variation in intake, were determined using a 34-item semi-quantitative food frequency questionnaire [42]. In the present analysis, we evaluated risk of diabetes associated with frequency of consumption of lard/butter, fish, fruits and vegetables given the biological plausibility of a role for these foods in diabetes etiology.

*Follow-Up Survey*

In 1998, glucose tolerance and risk factor status among participants in the follow-up survey (n=95, as described above) were determined. Field and laboratory methods and equipment employed during the follow-up survey were identical to those used during the baseline survey. Signed informed consent was obtained from all subjects, and the follow-up protocol was approved by the Sandy Lake First Nation Band Council and the University of Toronto Ethics Review Committee.
**Statistical Analyses**

All analyses were carried out using SAS version 6.12 [43]. The distributions of continuous variables were assessed for normality, and the natural log transformations of non-normal variables were used in the analysis when the transformation improved the normality of the distribution. Differences in mean values between groups were compared using t-tests. Risk factors for progression to diabetes were evaluated using logistic regression analyses. The dependent variable in these models was development of diabetes (yes/no). Separate models for each independent variable of interest were constructed, including baseline cigarette smoking, alcohol consumption, nutrient intake from the 24-hour recall and patterns of food consumption from the food frequency questionnaire. When independent factors were modeled as continuous variables, the odds ratio (OR) refers to an increase in risk per 1 SD change among subjects who did not progress to diabetes. Consumption of fruits, vegetables, and fruits and vegetables combined were modeled as additive scales of reported consumption frequency of individual foods from the food frequency questionnaire (see Table 1 for food items). Both crude and adjusted analyses were conducted. Multivariate odds ratios were adjusted for potential confounding factors, including age, sex, baseline diabetes status, change in waist circumference, smoking, alcohol consumption, and energy intake. Finally, we included duration of follow-up as a continuous variable in all fully adjusted models. This variable had no effect on the magnitude or significance of the ORs (data not shown), and it was thus not included in the models presented in the tables.

**RESULTS**

Over a median follow-up period of 4.2 years (range 3.0-5.2 years), 24 subjects developed diabetes (baseline NGT: 5/40, baseline IGT 19/55). Duration of follow-up was not significantly related to risk of diabetes development (data not shown).

Table 1 presents baseline characteristics of study participants based on their follow-up diabetes status. Those who progressed to diabetes were slightly older and more likely to have had IGT at baseline (12.5 versus 34.6%, p=0.015). The proportions of current smokers (70.8% versus 45.7%, p=0.06) and heavy drinkers (12.5% versus 1.43%, p=0.08) were higher among progressors, as was the proportion of subjects reporting butter or lard consumption at least once per day (83.3%
versus 64.8%, p=0.09). In addition, subjects progressing to diabetes reported lower consumption of calcium (384.2mg versus 727.9mg, p=0.02) and vitamin C (41.4mg versus 58.9mg, p=0.09).

Logistic regression analysis indicated that cigarette smoking was associated with risk of progression to diabetes (Table 2). After adjustment for age and sex, current smokers at baseline were significantly more likely to have progressed to diabetes relative to never and former smokers. The magnitude of this association increased with further adjustment for baseline diabetes status, change in waist circumference, reported alcohol consumption and energy intake (OR=3.35, 95% confidence interval (CI), 1.14-9.84). When the factor years of smoking was analyzed as a continuous variable, each additional year of exposure incurred a 1.8-fold increased risk of diabetes progression in the fully-adjusted model (OR=1.82, 95% CI, 1.06-3.13 per log year). There was also a dose-response relationship across increasing categories of smoking.

Alcohol consumption measured using the drinking intensity scale was associated with a significant age- and sex-adjusted diabetes risk (OR 1.66, 95% CI 1.06-2.60 per 1 SD difference). In addition, subjects who reported drinking on more that fifty occasions over the past year were at significantly increased risk of progression to diabetes compared to non-drinkers (Table 3, OR=15.89, 95% CI, 1.41-178.79). The magnitude and significance of these associations were attenuated, however, after further adjustment for baseline diabetes status, change in waist circumference, smoking and energy intake. Subjects in the highest category of the drinking intensity scale had a three-fold age- and sex-adjusted higher risk of progression to diabetes. The magnitude of this estimate was essentially the same in the full multivariate model (OR=3.01, 95% CI, 0.73-12.42).

Table 4 presents results of the analysis of nutrient intake by 24-hour dietary recall. After adjustment for age and sex, high calcium intake was associated with a significantly decreased risk of diabetes development (OR=0.54, 95% CI 0.31-0.95 per 1 SD difference). The magnitude of this association was unchanged after further adjustment for baseline diabetes status, change in waist circumference, current smoking, alcohol consumption and energy intake, although the confidence intervals became larger. Results of logistic regression analysis of data from the food frequency questionnaire were suggestive of increased risk of diabetes with frequent lard consumption (≥ once/day versus < once/day), as well as with infrequent fish consumption.
DISCUSSION

The rapidly increasing burden of type 2 DM in developing countries, migrant groups and populations experiencing rapid lifestyle transition strongly suggests that modifiable factors are exerting an important effect in the etiology of this disease [1]. In the present study, we found prospective associations between diabetes risk and a number of lifestyle factors, including cigarette smoking, alcohol consumption, and dietary calcium intake.

Although there is inconsistency in the literature regarding the role of cigarette smoking in the etiology of glucose intolerance [7, 14-16], our finding of a significant relationship between smoking and diabetes risk is in line with three recent population-based prospective studies [14-16]. In addition, we found evidence for a dose-response effect, with a significant increase in risk for each additional year of smoking, as well as an elevated risk among the most exposed in terms of pack-years. Rimm and coworkers have also reported dose-response relationships between smoking and diabetes risk in two U.S. population-based cohort studies [14-15]. Three previous studies of subjects with IGT found no significant effect of smoking on risk of diabetes development [21, 25, 31], although these investigations may have been limited in that the exposure was treated as a simple dichotomous variable. Smoking may increase the risk for diabetes through a number of mechanisms. Several studies have suggested that smoking induces insulin resistance [44-46], although others have not supported this finding [47-49], and it has been suggested that the opposite is true [50]. It is also possible, as pointed out by Rimm et al. [14], that the chemicals in tobacco smoke may damage insulin producing beta cells through the action of free radicals [51]. Our finding of an increased risk of diabetes associated with smoking in this well-defined high-risk non-diabetic group suggests that smoking may exert a harmful effect during the intermediate phases of the natural history of the disease. This hypothesis will require confirmation in future projects.

We found that high exposure to alcohol was associated with increased risk of diabetes, although this association was attenuated somewhat after adjustment for other risk factors, including smoking. The results of previous studies of alcohol consumption and diabetes development have been mixed, with the suggestion of both detrimental [17, 20] and protective [7, 15] relationships, and in some cases, no effect [19]. While moderate alcohol consumption has been shown to improve
insulin sensitivity [52], a recent rodent model study has demonstrated that elevated plasma levels of two diols associated with alcoholism can reduce whole body glucose uptake [53]. It is conceivable, then, that frequent and/or heavy alcohol consumption is harmful to glucose metabolism.

Low dietary calcium intake, measured by 24-hour recall, was significantly associated with progression to diabetes in the present study. Colditz et al. [11] have also reported a significant inverse relationship between diabetes risk and dietary calcium, and Boucher et al. [54] reported that vitamin D deficiency was related to glucose intolerance and impaired insulin secretion. Calcium is required by the endopeptidases involved in proinsulin cleavage [55, 56], and it is possible that low dietary calcium is involved in defective insulin secretion. Alternatively, low calcium intake may be a marker of another (unidentified) dietary risk factor for diabetes.

In a previous study, Marshall et al. found that increased baseline total fat intake, assessed by 24-hour recall, was an independent risk factor for the development of Type 2 DM among subjects with IGT [24]. In contrast, our results indicated a modest, non-significant protective association between both total and saturated fat intake during the previous 24-hour period and risk of diabetes. One possible explanation for this divergent finding is that a single 24-hour may not adequately capture usual dietary fat intake in this population. It is of interest, however, that lard and butter intake assessed by a 3-month food frequency questionnaire was associated with a non-significant 2-fold increased risk of diabetes. Additionally, we found a non-significant reduced risk associated with frequent fish consumption, a result which has been reported in 3 previous studies [57-59]. It is possible, then, that the role of fat diabetes pathogenesis is dependent on chain length and degree of saturation.

Our study has three potential limitations which should be considered when interpreting the findings. First, the sample size is relatively small, and thus the study has limited statistical power to detect small effects. This characteristic can be defended from a public health perspective, however, in that the detection of large effects at this time is reasonable in light of the magnitude of the current diabetes epidemic in the study community. In addition, in regular meetings with the study investigators, the community leadership has identified subjects with IGT as a priority for detailed follow-up and future evaluation. In fact, the cohort in the present study represents 76% of the universe of individuals with IGT or high-risk NGT, and thus a large sample size was not available. Second, the dietary measurement instruments used in this study are susceptible to non-differential misclassification error. This type of measurement error will, in most cases, bias effect estimates
towards the null value, and thus our risk estimates associated with lifestyle factors are likely to be conservative. It has been recently suggested that non-differential misclassification of exposure will, in some situations, bias effect estimates away from the null [60-62]. However, it appears that the likelihood of this effect is especially increased with the categorization of continuous variables [63, 64], as well as in the intermediate categories of polychotomous exposure variables [65, 66]. These circumstances are unlikely to have affected the results of the present study due to the fact that our polychotomous variables do not display aberrant patterns of association, and we avoided the categorization of continuous variables wherever possible. Finally, we did not collect information about drinking cessation, and it is possible that there were a number of ex-drinkers in the non-drinking sub-group. Clinical experience in this population suggests that ex-drinkers are usually characterized by high alcohol exposure prior to cessation, and therefore we suspect that their presence in the reference group would result in bias of effect estimates towards the null.

In conclusion, this study has identified significant prospective associations between diabetes risk and a number of lifestyle factors, including cigarette smoking, alcohol consumption, and dietary calcium intake. We have previously reported that subjects with IGT in this study were at increased risk of progression to diabetes compared to those with high-risk NGT [67]. The present findings indicate that risk for Type 2 DM may be modifiable through lifestyle changes, even among subjects in the intermediate stages of the disease process.
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REFERENCES


67. Hanley AJG, McKewon-Eysen G, Harris SB, Wolever TMS, Hegele RA, Gittelsohn J, Zinman B. Deterioration in glucose tolerance is related to baseline levels and change insulin resistance, proinsulin concentration and obesity: results from a population-based follow-up study of high-risk subjects (submitted manuscript).
TABLE 1. Baseline lifestyle variables in study participants, by follow-up glucose tolerance status.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Maintained Glucose tolerance status after 4 years of follow-up</th>
<th>Developed Glucose tolerance status after 4 years of follow-up</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGT/IGT (n=71)</td>
<td>Type 2 DM (n=24)</td>
<td></td>
</tr>
<tr>
<td>Baseline GTS (% NGT / % IGT)</td>
<td>87.50 / 65.45</td>
<td>12.50 / 34.55</td>
<td>0.015</td>
</tr>
<tr>
<td>Gender (% male / % female)</td>
<td>78.57 / 73.13</td>
<td>21.43 / 26.87</td>
<td>0.578</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.87 (18.27)</td>
<td>38.73 (11.91)</td>
<td>0.141</td>
</tr>
<tr>
<td><strong>Baseline Smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% ever smokers</td>
<td>68.57</td>
<td>87.50</td>
<td>0.123</td>
</tr>
<tr>
<td>% current smokers</td>
<td>45.71</td>
<td>70.80</td>
<td>0.060</td>
</tr>
<tr>
<td># years of smoking(^4)</td>
<td>13.50 (12.10)</td>
<td>15.95 (11.38)</td>
<td>0.433</td>
</tr>
<tr>
<td># cigarettes / day(^5)</td>
<td>9.25 (8.94)</td>
<td>10.06 (7.95)</td>
<td>0.746</td>
</tr>
<tr>
<td><strong>Baseline Alcohol Consumption</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% non-drinkers</td>
<td>61.4</td>
<td>45.8</td>
<td>0.274</td>
</tr>
<tr>
<td>% frequent drinkers(^1)</td>
<td>1.43</td>
<td>12.50</td>
<td>0.083</td>
</tr>
<tr>
<td># drinks / occasion(^2)</td>
<td>12.33 (11.71)</td>
<td>16.38 (13.96)</td>
<td>0.342</td>
</tr>
<tr>
<td>drinking intensity scale(^3)</td>
<td>196.46 (1223.11)</td>
<td>340.63 (823.90)</td>
<td>0.097</td>
</tr>
</tbody>
</table>

1 self-reported drinking more than 50-times per year
2 drinkers only (non-progressors, n=27; progressors, n=13)
3 midpoint of frequency category x usual amount consumed per occasion (non-drinkers=0)
4 ever smokers only (non-progressors, n=48; progressors, n=21)
5 current smokers only (non-progressors, n=32; progressors, n=17)
TABLE 1 (continued). Baseline lifestyle variables in study participants, by follow-up glucose tolerance status.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Maintained NGT/GT (n=71)</th>
<th>Developed Type 2 DM (n=24)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2232.84 (1113.23)</td>
<td>1991.83 (1326.73)</td>
<td>0.200*</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>16.03 (6.77)</td>
<td>16.31 (8.33)</td>
<td>0.417*</td>
</tr>
<tr>
<td>Total Fat (%)</td>
<td>36.45 (10.00)</td>
<td>33.35 (9.96)</td>
<td>0.191</td>
</tr>
<tr>
<td>Saturated Fat (%)</td>
<td>13.21 (4.22)</td>
<td>12.11 (4.38)</td>
<td>0.279</td>
</tr>
<tr>
<td>Polyunsaturated Fat (%)</td>
<td>4.85 (2.07)</td>
<td>4.56 (1.92)</td>
<td>0.626</td>
</tr>
<tr>
<td>Monounsaturated Fat (%)</td>
<td>13.27 (4.49)</td>
<td>12.55 (4.64)</td>
<td>0.507</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>45.73 (11.71)</td>
<td>48.47 (9.83)</td>
<td>0.307</td>
</tr>
<tr>
<td>Starch (%)</td>
<td>25.01 (9.22)</td>
<td>27.15 (11.28)</td>
<td>0.359</td>
</tr>
<tr>
<td>Sugar (%)</td>
<td>20.55 (12.05)</td>
<td>21.03 (12.72)</td>
<td>0.866</td>
</tr>
<tr>
<td>Fibre (g/1000kcal)</td>
<td>4.97 (2.46)</td>
<td>6.36 (4.48)</td>
<td>0.146*</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>727.93 (927.75)</td>
<td>384.21 (262.54)</td>
<td>0.016*</td>
</tr>
<tr>
<td>Vitamin A (RE)</td>
<td>692.00 (2183.15)</td>
<td>265.38 (299.23)</td>
<td>0.109*</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>58.90 (56.32)</td>
<td>41.43 (36.17)</td>
<td>0.085</td>
</tr>
<tr>
<td>Niacin (NE)</td>
<td>39.20 (18.79)</td>
<td>38.31 (29.73)</td>
<td>0.317*</td>
</tr>
<tr>
<td>Folate (mg)</td>
<td>170.74 (82.28)</td>
<td>159.38 (79.50)</td>
<td>0.557</td>
</tr>
</tbody>
</table>

Baseline Intake of Specific Foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Maintained</th>
<th>Developed</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butter and Lard (% reporting ≥ once/day)</td>
<td>64.8</td>
<td>83.3</td>
<td>0.088</td>
</tr>
<tr>
<td>Fish (% reporting ≥ 1-2x/week)</td>
<td>36.6</td>
<td>25.0</td>
<td>0.298</td>
</tr>
<tr>
<td>Fruit Scale (additive scale: wild berries, fresh &amp; canned fruit)</td>
<td>5.15 (2.32)</td>
<td>5.33 (2.43)</td>
<td>0.748</td>
</tr>
<tr>
<td>Vegetable Scale (additive scale: peas, corn, carrots, other vegetables)</td>
<td>6.65 (3.66)</td>
<td>8.00 (3.48)</td>
<td>0.119</td>
</tr>
<tr>
<td>Combined Fruit and Vegetable Scale (additive scale: fruit + vegetables scales)</td>
<td>11.84 (5.01)</td>
<td>13.33 (5.10)</td>
<td>0.214</td>
</tr>
</tbody>
</table>

* from 24 hour recall
* from 3-month food frequency questionnaire
* t-test performed on log-transformed variable
<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Crude Analysis</th>
<th>Analysis adjusted for age and sex</th>
<th>Analysis adjusted for age, sex, Δ waist circumference, baseline diabetes status, alcohol consumption, and energy intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>p-value</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>Never (0) versus ever (1) smokers</td>
<td>3.21 0.87-11.90 0.081</td>
<td>2.82 0.74-10.81 0.130</td>
<td>2.85 0.69-11.08 0.148</td>
</tr>
<tr>
<td>Never/former (0) versus current (1) smokers</td>
<td>2.67 1.01-7.05 0.048</td>
<td>2.71 1.01-7.26 0.048</td>
<td>3.35 1.14-9.84 0.028</td>
</tr>
<tr>
<td>Years of smoking (continuous variable: OR per log 1 year increase(^1))</td>
<td>1.61 1.09-2.39 0.016</td>
<td>1.67 1.04-2.70 0.036</td>
<td>1.82 1.06-3.13 0.029</td>
</tr>
<tr>
<td>Categorical:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smokers</td>
<td>1.00 - -</td>
<td>1.00 - -</td>
<td>1.00 - -</td>
</tr>
<tr>
<td>Former smokers</td>
<td>2.13 0.45-10.12 0.324</td>
<td>1.72 0.34-8.83 0.515</td>
<td>1.40 0.25-7.82 0.703</td>
</tr>
<tr>
<td>Current smokers - light(^2)</td>
<td>2.71 0.59-12.39 0.200</td>
<td>2.49 0.54-11.53 0.244</td>
<td>2.64 0.51-13.60 0.247</td>
</tr>
<tr>
<td>Current smokers - heavy(^3)</td>
<td>5.90 1.37-25.35 0.017</td>
<td>5.25 1.18-23.37 0.029</td>
<td>5.91 1.15-30.28 0.033</td>
</tr>
</tbody>
</table>

Outcome variable: development of diabetes (no=0, yes=1)
\(^1\)natural log of 1 year increase
\(^2\)pack-years ≤ 3
\(^3\)pack-years > 3
TABLE 3. Baseline alcohol consumption and risk of progression to diabetes.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Crude Analysis</th>
<th>Analysis adjusted for age and sex</th>
<th>Analysis adjusted for age, sex, Δ waist circumference, baseline diabetes status, smoking, and energy intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR  95% CI</td>
<td>p-value</td>
<td>OR  95% CI</td>
</tr>
<tr>
<td>Non-drinkers (0) versus (1) drinkers</td>
<td>1.88 0.74-4.80 0.186</td>
<td>2.22 0.83-5.94 0.112</td>
<td>1.81 0.62-5.27 0.280</td>
</tr>
<tr>
<td>Drinking intensity¹ (continuous variable)²</td>
<td>1.48 0.98-2.25 0.064</td>
<td>1.66 1.06-2.60 0.028</td>
<td>1.22 0.98-1.53 0.080</td>
</tr>
<tr>
<td>Categorical (frequency):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-drinkers</td>
<td>1.00 - -</td>
<td>1.00 - -</td>
<td>1.00 - -</td>
</tr>
<tr>
<td>1-10x / year</td>
<td>1.68 0.59-4.83 0.334</td>
<td>2.04 0.67-6.16 0.208</td>
<td>1.50 0.46-4.94 0.505</td>
</tr>
<tr>
<td>Up to 50x / year</td>
<td>1.14 0.21-6.29 0.878</td>
<td>1.29 0.23-7.35 0.776</td>
<td>1.59 0.24-10.73 0.634</td>
</tr>
<tr>
<td>More than 50x / year</td>
<td>12.00 1.14-126.79 0.039</td>
<td>15.89 1.41-178.79 0.025</td>
<td>10.05 0.62-163.32 0.105</td>
</tr>
<tr>
<td>Categorical (drinking intensity):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-drinkers</td>
<td>1.00 - -</td>
<td>1.00 - -</td>
<td>1.00 - -</td>
</tr>
<tr>
<td>Light drinkers</td>
<td>1.60 0.50-5.08 0.425</td>
<td>1.78 0.54-5.81 0.324</td>
<td>1.23 0.33-4.53 0.761</td>
</tr>
<tr>
<td>Heavy drinkers</td>
<td>2.33 0.74-7.31 0.146</td>
<td>3.08 0.89-10.61 0.075</td>
<td>3.01 0.73-12.42 0.127</td>
</tr>
</tbody>
</table>

Outcome variable: development of diabetes (no=0, yes=1)

¹OR per 1 SD difference in non-progressors
²natural log transformation used in analysis
# TABLE 4. Baseline nutrient intake and risk of progression to diabetes.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Crude Analysis</th>
<th>Analysis adjusted for age and sex</th>
<th>Analysis adjusted for age, sex, Δ waist circumference, baseline diabetes status, smoking, and alcohol consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>p-value</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>Energy (kcal)'</td>
<td>0.72 0.47-1.11</td>
<td>0.141</td>
<td>0.77 0.49-1.19</td>
</tr>
<tr>
<td>Protein (%)'</td>
<td>1.01 0.64-1.61</td>
<td>0.963</td>
<td>1.01 0.62-1.64</td>
</tr>
<tr>
<td>Total Fat (%)</td>
<td>0.73 0.45-1.17</td>
<td>0.192</td>
<td>0.67 0.41-1.10</td>
</tr>
<tr>
<td>Saturated Fat (%)</td>
<td>0.77 0.47-1.24</td>
<td>0.277</td>
<td>0.72 0.44-1.18</td>
</tr>
<tr>
<td>Polyunsaturated Fat (%)'</td>
<td>0.89 0.55-1.43</td>
<td>0.622</td>
<td>0.86 0.53-1.41</td>
</tr>
<tr>
<td>Monounsaturated Fat (%)</td>
<td>0.85 0.53-1.37</td>
<td>0.503</td>
<td>0.80 0.49-1.29</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>1.29 0.79-2.11</td>
<td>0.305</td>
<td>1.40 0.84-2.33</td>
</tr>
<tr>
<td>Starch (%)</td>
<td>1.24 0.79-1.93</td>
<td>0.355</td>
<td>1.22 0.77-1.91</td>
</tr>
<tr>
<td>Sugar (%)</td>
<td>1.04 0.66-1.64</td>
<td>0.865</td>
<td>1.12 0.70-1.81</td>
</tr>
<tr>
<td>Fibre (g/1000kcal)'</td>
<td>1.48 0.87-2.53</td>
<td>0.148</td>
<td>1.47 0.85-2.54</td>
</tr>
<tr>
<td>Calcium (mg)'</td>
<td>0.52 0.30-0.90</td>
<td>0.020</td>
<td>0.54 0.31-0.95</td>
</tr>
<tr>
<td>Vitamin A (RE)'</td>
<td>0.71 0.43-1.19</td>
<td>0.192</td>
<td>0.72 0.43-1.21</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>0.66 0.36-1.19</td>
<td>0.163</td>
<td>0.69 0.38-1.26</td>
</tr>
<tr>
<td>Niacin (NE)</td>
<td>0.78 0.52-1.17</td>
<td>0.229</td>
<td>0.82 0.54-1.25</td>
</tr>
<tr>
<td>Folate (mg)</td>
<td>0.84 0.46-1.52</td>
<td>0.533</td>
<td>0.87 0.47-1.64</td>
</tr>
</tbody>
</table>

Outcome variable: development of diabetes (no=0, yes=1)

Odds ratios refer to an increase in the level of the independent variable of 1 SD based on the distribution in subjects who did not progress to diabetes.

' natural log transformed variable used in analysis
TABLE 5. Baseline pattern of food consumption\(^1\) and risk of progression to diabetes.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Crude Analysis</th>
<th>Analysis adjusted for age and sex</th>
<th>Analysis adjusted for age, sex, Δ waist circumference, baseline diabetes status, smoking, alcohol consumption and energy intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI  p-value</td>
<td>OR 95% CI  p-value</td>
<td>OR 95% CI  p-value</td>
</tr>
<tr>
<td>Butter and Lard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1x/day (0) vs. ≥1x/day (1)</td>
<td>2.72 0.84-8.83 0.097</td>
<td>2.55 0.76-8.48 0.128</td>
<td>2.00 0.54-7.40 0.300</td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never or rarely</td>
<td>1.00 -</td>
<td>1.00 -</td>
<td>1.00 -</td>
</tr>
<tr>
<td>1-3x/month</td>
<td>1.26 0.41-3.83 0.688</td>
<td>1.07 0.33-3.40 0.913</td>
<td>1.08 0.28-4.15 0.907</td>
</tr>
<tr>
<td>≥1x/week</td>
<td>0.66 0.19-2.31 0.509</td>
<td>0.53 0.14-1.97 0.340</td>
<td>0.44 0.10-1.86 0.265</td>
</tr>
<tr>
<td>Fruit Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-unit difference</td>
<td>1.07 0.72-1.59 0.745</td>
<td>1.03 0.68-1.54 0.903</td>
<td>1.07 0.67-1.69 0.786</td>
</tr>
<tr>
<td>Vegetable Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-unit difference</td>
<td>1.50 0.90-2.53 0.123</td>
<td>1.51 0.89-2.58 0.127</td>
<td>1.53 0.82-2.86 0.177</td>
</tr>
<tr>
<td>Combined Fruit &amp; Vegetable Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-unit difference</td>
<td>1.34 0.85-2.12 0.215</td>
<td>1.32 0.82-2.10 0.251</td>
<td>1.35 0.78-2.34 0.280</td>
</tr>
</tbody>
</table>

Outcome variable: development of diabetes (no=0, yes=1)
*Test performed on log transformed variable
\(^1\)From 3-month food frequency questionnaire
Chapter 4

Discussion & Conclusions

4.1. Summary, Implications and Future Directions

The following is a summary of the findings of this dissertation, organized according to the original objectives of the studies and including a brief discussion of the implications of each result. Some of the usual features of the Discussion section of academic documents, including comparisons with previous research findings and the consideration of possible pathophysiological mechanisms are included in the Discussion sections of the individual papers presented in Chapter 3, and this information will not be repeated here. In addition, a brief consideration of possible future research is offered.

Objective I (a): To determine whether fasting proinsulin concentrations are elevated among individuals with glucose tolerance abnormalities, including Type 2 DM and IGT, relative to those with NGT.

Results: After adjustment for insulin secretion and other covariates, proinsulin concentration was significantly elevated in subjects with both diabetes and IGT compared to those with NGT. In addition, proinsulin concentrations increased significantly across quartiles of fasting glucose among subjects with NGT.

Implications: These findings confirm that patterns of glucose intolerance-associated hyperproinsulinaemia are present in a Native Canadian population with high rates of obesity and diabetes, and support the notion that elevated proinsulin concentrations are a surrogate marker for beta cell dysfunction. High proinsulin concentrations among those with less advanced states of hyperglycaemia suggest that beta cell function may begin to decline early in the evolution of diabetes in this population.
Future Directions: It would be of interest to examine the criterion validity of proinsulin concentration against more detailed measures of beta cell dysfunction in a sample of subjects from this population. This validation would provide further evidence that elevated proinsulin concentration is a surrogate indicator of beta cell dysfunction among Native Canadians from the eastern subarctic.

Objective I (b): To investigate the relationship between proinsulin level and concurrent measures of anthropometry, specifically percent body fat and waist circumference.

Results: After adjustment for age, sex and insulin secretion, proinsulin concentrations were significantly associated with percent body fat and, in particular, waist circumference.

Implications: This finding suggests that abdominal adiposity is detrimental to beta cell function. Although this interpretation is contrary to that accompanying some previous reports, recent research in rodent models has demonstrated that obesity is associated with excess fat deposition in pancreatic islets and subsequent beta cell dysfunction.

Future Directions: Confirmation of the association between proinsulin concentration and total and abdominal obesity using more detailed methods of anthropometric assessment would be of interest. Hydrodensitometry (underwater weighting) and dual energy x-ray absorptiometry (DEXA) are considered gold standard measures of total body adiposity, whereas intra-abdominal fat area can be determined using magnetic resonance imaging [Jensen 1992].

Objective I (c): To determine whether baseline levels and change over time in percent body fat and waist circumference are associated with follow-up and change in proinsulin concentrations.

Results: Both baseline and change in waist circumference were associated with follow-up concentrations and change in proinsulin after adjustment for covariates including insulin secretion.

Implications: This finding indicates a prospective relationship between abdominal obesity and declining beta cell function, and, together with the cross-sectional findings from objective I (b), is
suggestive of a detrimental role for intra-abdominal adipose tissue in the health of the pancreatic beta cell.

**Future Directions:** Confirmation of these prospective associations in future studies is required. In addition, the prospective relationship between directly measured intra-abdominal fat and proinsulin concentration would be of value.

**Objective I (d):** To evaluate the association of parity with proinsulin and risk of glucose intolerance.

**Results:** There was a significant inverse association between parity and risk of diabetes in older women, and the suggestion of a similar relationship among younger women. In addition, nulliparity was associated with insulin resistance and increased concentrations of insulin, proinsulin and leptin.

**Implications:** These results suggest either that nulliparity is associated with a diabetes-prone phenotype, or that parity reduces the risk of diabetes and related conditions. In addition, the findings are of interest given that concordant results have been reported among the Pima Indians of Arizona, a population that is similar in both indigenous North American ancestry and high diabetes risk.

**Future Directions:** A number of issues related to this finding require clarification and/or additional research: (i) The relationship between parity and risk of diabetes in other populations indigenous to North America should be evaluated. (ii) Studies should be conducted which employ detailed questionnaires for the measurement of reproductive history. These instruments should be designed specifically to evaluate the relationship between aspects of reproduction and diabetes risk. Information should include documentation of abortion, miscarriage, still birth, live birth, duration of pregnancy, menstrual patterns, self-selected nulliparity, and adverse reproductive histories (including PCOS and related disorders). (iii) There is a need for more detailed examination of leptin concentrations, proinsulin concentrations and beta cell function in various states of nulliparity. (iv) The prevalence of PCOS among Native Canadian women should be determined.
Objective I (e): To assess the relationship between proinsulin concentration and concurrent measures of the following cardiovascular disease (CVD) risk factors: cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) levels, and systolic and diastolic blood pressure.

Results: In subjects with NGT, proinsulin was significantly positively associated with concentrations of triglyceride as well as total and LDL cholesterol, and was negatively associated with HDL cholesterol. In subjects with IGT, proinsulin was significantly negatively associated with HDL cholesterol.

Implications: These findings suggest that elevated proinsulin concentrations may be related to the adverse cardiovascular profile of subjects with both IGT as well as NGT who have elevated glucose concentrations. This finding is consistent with observations reported in other populations.

Future Directions: Further research is necessary to uncover the pathophysiological mechanisms behind the consistent association between proinsulin and lipid concentrations.

Objective I (f): To determine whether baseline levels and change over time in proinsulin concentration are associated with follow-up and change in the above-listed CVD risk factors.

Results: Change in proinsulin concentration was significantly associated with change in lipid concentrations, although baseline proinsulin concentration did not predict change in lipids. In a post-hoc analysis, elevated baseline concentrations of triglyceride and reduced baseline concentrations of HDL-C were significantly associated with changes in proinsulin concentrations.

Implications: These findings confirm that beta cell function and lipid concentrations decline concomitantly. The results of the post-hoc analysis could be interpreted to support the lipotoxicity hypothesis of diabetes, which suggests that long-term exposure to elevated circulating concentrations of free fatty acids (FFA) is directly related to functional and morphologic changes to the beta cells.
Future Directions: To date this is the first study to have evaluated the prospective associations between proinsulin and lipid concentrations. Additional prospective studies of this relationship are required.

Objective II (a): To determine whether the presence of baseline IGT as well as baseline levels and change over time in proinsulin, insulin resistance, and adiposity are associated with the development of diabetes and changes in glucose concentrations;

Results: After adjustment for covariates, subjects with IGT at baseline were at significantly elevated risk of progression to diabetes compared to those with NGT. In addition, changes over time in proinsulin concentration and insulin resistance were associated with risk of deterioration to diabetes. In adjusted multivariate models, measures of both change in insulin resistance and change in beta cell dysfunction were independently associated with diabetes risk. Baseline proinsulin concentration was not associated with risk of progression to diabetes, although this prohormone was present in high concentrations at baseline in all cohort members, suggesting that early beta cell dysfunction is a characteristic of non-diabetic subjects at high risk for diabetes.

Implications: These results demonstrate that both insulin resistance and beta cell dysfunction are associated with risk of progression to diabetes. In addition, the relatively high proinsulin concentrations among those with IGT suggest that beta cell dysfunction appears at a relatively early stage of disease evolution in this population. Finally, despite criticisms of its clinical and research utility, a diagnosis of IGT indicated significantly increased risk of diabetes even when compared to subjects with NGT who had elevated post-challenge glucose concentrations.

Future Directions: Additional studies of the prospective relationships of beta cell function and insulin resistance with risk of diabetes in Native Canadians would be of value.

Objective II (a): To assess the importance of smoking, alcohol consumption, dietary intakes of fat and fiber, physical activity, and fitness level in determining risk of development of diabetes and changes in glucose concentrations.
Results: Cigarette smoking was significantly related to risk of progression to diabetes. In addition, low calcium consumption was associated with reduced risk of diabetes. Finally, there was a suggestion of a positive association between high alcohol intake and risk of diabetes.

Implications: These results indicate that previous observations of diabetes risk associated with cigarette smoking can be extended to Native Canadians, a population with high prevalence of smoking, but low consumption on a per-smoker basis. Further, there is the suggestion that other modifiable lifestyle factors, including calcium and alcohol consumption, may be associated with risk of diabetes among high-risk subjects in this population.

Future Directions: Additional studies of the association between lifestyle factors and diabetes risk in Native Canadians are required.

4.2. Synthesis

The results of this study suggest that beta cell dysfunction, represented here by elevated proinsulin concentration, is (1) apparent relatively early in the pathogenesis of diabetes, (2) is associated with several metabolic, anthropometric and lifestyle factors, and (3) may be related to subsequent risk of glucose intolerance. While the presence of beta cell dysfunction among subjects with diabetes is well known, these findings lend support to the less well established notion that this condition exists earlier in the pathogenesis of type 2 diabetes, and further, that it is associated with modifiable risk factors. Thus, the evidence from these studies is apparently not consistent with the 2-stage model of diabetes natural history, which emphasizes early insulin resistance and a later emergence of beta cell dysfunction (see Chapter 1, Figure 1). Rather, the findings seem to be in line with more recently proposed models which highlight an earlier appearance of beta-cell dysfunction and an interaction of this state with insulin resistance (see Chapter 1, Figure 2).

A number of the findings regarding associated anthropometric, metabolic and reproductive factors also provide new insight into the pathobiology of beta cell dysfunction. This study has presented the most detailed analyses to date regarding the association of anthropometry and proinsulin concentration. In particular, both the cross-sectional and prospective findings suggest that abdominal obesity contributes significantly to the early appearance of beta cell dysfunction. Further, the elevated proinsulin concentrations among nulliparous women in this study extend the
findings of Charles and colleagues [1994], who reported excess diabetes and insulin resistance in nulliparous Pima Indian women. Taken together, data from these two studies suggest the presence of a relatively prevalent, highly diabetes-prone phenotype among the nulliparous sub-cohorts of certain Native North American populations with high rates of glucose intolerance. Finally, all previous reported associations of proinsulin and lipid and lipoprotein associations had been cross-sectional, and thus the cause-effect relationship was unclear. Although the findings of the prospective component of this study were somewhat unexpected, a reasonable biological mechanism can be proposed to account for the association of dyslipidemia and subsequent decline in beta cell function (see below).

A number of previous studies (reviewed in Chapter 1) have demonstrated that elevated baseline proinsulin concentration is associated with risk of progression to diabetes, suggesting that proinsulin might have clinical utility in identifying subjects at high risk for subsequent diabetes. The results of the prospective component of the present study did not confirm these findings, possibly due to the fact that baseline proinsulin concentrations in this high-risk cohort were notably higher than in cohorts in other studies. Thus it appears that proinsulin may not be clinically useful in subjects in the study population who are already at high risk for diabetes on the basis of 2-hour post-challenge glucose levels. It is possible that proinsulin may have clinical or diagnostic utility among individuals in this population that are more representative of the spectrum of normal glucose tolerance.

While a number of possible mechanisms could be cited to explain these findings, the possible role of lipotoxicity [Unger 1995] is particularly intriguing. Briefly, the lipotoxicity hypothesis maintains that short term exposure to high levels of circulating FFA causes beta-cell hyperplasia and hyperinsulinaemia, but chronic exposure and subsequent increases in FFA levels lead to functional and morphologic changes in beta cells and consequent diabetes [Unger 1995]. Substantial fat deposition in islets of obese ZDF rats has been documented, as well as the demonstration of FFA-induced loss of glucose-stimulated insulin secretion in these animals [Lee et al. 1994]. Increased FFA also induce nitric oxide synthase, and Shimabukuro et al. [1997, 1998] have shown that elevated FFA in rat beta cells cause increases in both nitric oxide levels and ceramide-mediated beta cell apoptosis (programmed cell death). The consequent reduction in the number of beta cells may result in elevated proinsulin concentration, in that the rate of secretion by remaining cells is increased, thereby decreasing the intracellular stores and forcing the release of incompletely processed materials [Bollheimer et al. 1998, Furukawa et al. 1999].
Although FFA were not directly measured in the present study, a body of indirect evidence supports the lipotoxicity hypothesis as a possible pathogenic mechanism. First, the majority of the risk factors associated cross-sectionally and prospectively with variation in proinsulin concentration in the present study are also associated with elevated concentrations of FFA (Figure 8). These factors include (1) abdominal obesity [Unger 1995], which may act independently or as part of a pathway which includes lifestyle factors; (2) lifestyle factors including cigarette smoking [Freeman et al. 1998] and excess intake of saturated fat; and (3) elevated triglyceride and low HDL-C concentrations [Lewis 1997]. In addition, elevated lipid and FFA concentrations have been documented in women with PCOS [Holte et al 1994, Robinson et al. 1996], a condition contributing to infertility that may be associated with lifestyle risk factors such as diet and physical activity [Huber-Buchholz et al. 1999]. While it is conceivable that PCOS explains a substantial proportion of nulliparity in the study population, this aspect of the hypothesis will remain speculative until the true prevalence of PCOS in the study population is determined. Second, although the lipotoxicity hypothesis has been demonstrated primarily in ZDF rats [Unger 1995], the development of diabetes in this animal model closely resembles that among the human subjects in the present study (i.e. classical obesity-associated type 2 diabetes with lipid abnormalities among subjects with both diabetes and IGT).

Figure 9 presents a schematic of the possible role of lipotoxicity in the pathogenesis of beta cell dysfunction in the context of findings from the present study. This model indicates that nulliparity is associated with both insulin resistance and beta cell dysfunction, which in turn are likely interrelated through multiple feed-back mechanisms [Ferrannini 1998]. The physiologic features of nulliparity that lead to these abnormalities remain to be elucidated, although as mentioned, the possible role of PCOS cannot be eliminated. In addition, abdominal adiposity and dyslipidemia are associated with beta cell dysfunction cross-sectionally, and they predict further deterioration in beta cell function, which in turn (and in combination with an increase in insulin resistance) is related to the development of diabetes. As indicated in the schematic, it is possible that the elevated concentrations of FFA that characterize abdominal obesity and elevated lipid concentrations are playing a pathogenic role in these relationships. Finally, cigarette smoking, excessive alcohol consumption and possibly high saturated and low omega-3 fatty acid intake contribute to diabetes risk among high-risk subjects through increases in FFA, beta cell toxicity, insulin resistance or other as yet unidentified mechanism.
Elevated circulating levels of FFA may also be contributing to diabetes pathogenesis via an independent detrimental effect on insulin sensitivity in muscle and liver. Under the Randle, or glucose-fatty acid cycle, it is proposed that in conditions of increased availability of FFA, both glucose oxidation and glucose uptake are decreased in muscle. The latter phenomenon appears to be especially pronounced with chronic FFA elevations [Boden 1996, 1997]. It has been suggested that the preferential utilization of FFA may have a survival advantage, in that glucose is spared for use in critical tissues, such as those in the central nervous system [Boden 1997]. In addition, high FFA concentrations increase hepatic glucose production and decrease extraction of insulin from the blood by the liver [Yki-Jarvinen and Williams 1991].

It is unlikely that these findings are the result of diabetes-induced differences in the peripheral catabolism of proinsulin. Kahn and Halban [1997] have demonstrated a close correlation between basal and 3-minute arginine-stimulated proinsulin concentrations in a sample of subjects with NGT and NIDDM (r=0.88, p<0.0001). Stimulated concentrations measured during this time period are reliable because variations in peptide clearance rates will have “less impact on their relative levels in the circulation” [Kahn and Halban 1997].
FIGURE 8. Possible risk-factor pathways involved in elevated free fatty acid concentrations.

- Abdominal obesity
- Smoking
  - ↑ Saturated fat intake
  - ↓ n-3 fat intake
- PCOS (?)
- ↑ Free Fatty Acids
  - ↑ Triglyceride, ↓ HDL-C
FIGURE 9. Possible role of lipotoxicity in the pathogenesis of beta cell dysfunction in the present study.

NGT → IGT → DM

Metabolic & reproductive conditions associated with nulliparity affect insulin resistance-beta cell dysfunction loop:
- PCOS
- genetic factors

Metabolic conditions associated with elevated circulating concentrations of free fatty acids affect insulin resistance-beta cell dysfunction loop:
- abdominal adiposity
- high triglyceride / low HDL-C
- PCOS (?)

Lifestyle factors associated with elevated circulating concentrations of free fatty acids and/or toxicity to beta cells exacerbate existing beta-cell dysfunction:
- cigarette smoking
- excessive alcohol consumption
- high saturated/low n-3 fat intake
4.3. Strengths and Limitations

4.3.1. Use of Cross-Sectional Data

In the present study, both cross-sectional and prospective data were used to evaluate associations between exposures and outcomes. Kelsey and coworkers [1986, pg. 187] have highlighted a number of strengths of cross-sectional studies, including:

(1) Appropriateness for studies of diseases of slow onset and long duration for which medical care is not sought until the condition has reached a fairly advanced stage. Type 2 DM clearly falls into this category given its insidious pathogenesis and long pre-clinical phase [Rewers and Hamman 1995]. It has been estimated that as many as 50% of cases in a general population may be undiagnosed at any one time [Jarrett 1989, Harris et al. 1998]. In addition, although the mortality rate of subjects with diabetes is substantially higher than among the non-diabetic population [Geiss et al. 1995], long term survival after diagnosis is fairly good compared to many other chronic conditions. Further, elevated proinsulin concentration, the other major outcome variable of interest in the present study, is not routinely evaluated outside research settings nor is it a recognized clinical condition. In this light, classic applications of both the case-control and cohort designs would have been challenging for the present study given the relative difficulty in defining incident cases of both diabetes and elevated proinsulin concentration.

(2) External generalizability. Because cross-sectional studies are usually conducted within entire general populations or samples thereof, they tend not to be limited by biases related to necessity, accessibility or willingness to seek medical care. As mentioned, a high proportion (73%) of eligible members of the community participated in the baseline survey.

(3) Cost efficiency. Given the relatively short time period of data collection, management and entry, costs associated with cross-sectional studies are often lower than for case-control and cohort studies of long duration.

In addition to these strengths, a number of criticisms of the use of cross-sectional data to report etiological associations have been presented [Kelsey et al. 1986, pg. 187; Rothman and Greenland 1998, pg. 75], including:
(1) **Length-biased sampling: the under-representation of cases with short duration of illness.**
As mentioned above, both diabetes and elevated proinsulin concentration are conditions of long duration, and thus this limitation is unlikely to apply to the present study.

(2) **Unclear cause-effect relationships given concurrent measures of exposure and outcome.**
This is a potentially serious concern, given the importance of temporality as a criterion for causation in the epidemiological paradigm. However, there are a number of design features of the present study that are likely to have alleviated or attenuated this limitation.

(a) For several variables, exposure history was collected for time periods that are likely to be more etiologically relevant than at the cross-section [Rothman and Greenland 1998, pg. 75]. These include parity (entire live birth history, with post-diagnosis births excluded in analysis), smoking (entire life history), alcohol (usual consumption over the past year), and food frequency questionnaire (past 3 months).

(b) If past exposure either corresponds closely with current exposure or is likely to be recalled inaccurately, concurrent measures may be reasonably used as proxies for relevant past exposure [Rothman and Greenland 1998, pg. 75]. Current measures of adiposity, for example, may be reasonable estimates of past adiposity given that body weight tracks over time [Guo and Chumlea 1999].

(c) Finally, a number of the cross-sectional associations reported in this thesis were supported in results from the smaller prospective study. These include the adiposity→proinsulin, proinsulin→glucose, and proinsulin→lipid associations.

4.3.2. **Low Statistical Power of Prospective Study**

The prospective portion of this study was characterized by relatively low statistical power, and thus was unable detect small effects. This was unavoidable given the characteristics and size of the baseline cohort, as well as the research priorities of the community partners. The 125 members of the baseline cohort who were identified for re-examination represented the universe of subjects in
this population with IGT or “high risk” NGT, defined as those with post-challenge glucose levels ≥ 7.0 mmol/l. The 95 subjects (76%) who participated did not differ from non-participants in terms of baseline anthropometric, metabolic or lifestyle variables, thus these follow-up analyses are unlikely to suffer from volunteer bias. In addition, the community partners, including both political leadership and grass-roots organizations, had identified subjects with IGT as a priority for detailed follow-up and future study.

The low statistical power of these prospective analyses resulted in a number of effect estimates that were not statistically significant at the conventional 5% level. As discussed below (Section 4.2.3), however, conventional epidemiologic wisdom has highlighted the value of estimation over strict significance testing.

Finally, in light of the magnitude of the current diabetes epidemic in the study community, the ability to detect only large effects at this time might be defended from a public health perspective, in that the most important risk factors can be identified and addressed first.

4.3.3. Statistical Estimation versus Significance Testing

This issue is discussed in detail by Rothman and Greenland [1998, pgs. 183-199], who argue for the use of statistical estimation over statistical testing as the preferred method of presentation of epidemiological findings. They suggest that reporting a point estimate along with confidence intervals is much more informative than simply presenting a p-value, in that three pieces of information are simultaneously communicated: the magnitude of the association of interest, the random variability of that point estimate, and the consistency between the data and the hypothesis of interest. To illustrate the last feature, consider an odds ratio of 2.5 with 95% confidence intervals of 2.0 to 3.0. Given that the confidence interval excludes the null value 1.0, the null hypothesis of no effect can be rejected at the 5% level. The use of statistical estimation, then, is consistent with the view that epidemiology is a measurement exercise rather than a decision-making exercise, and thus requires more detailed statistical methods than “the simple dichotomy produced by hypothesis testing” [Rothman and Greenland 1998, pg. 189], including estimates of the variability of ones measurement.
4.3.4. Misclassification of Lifestyle Exposure Measurement and Variable Skewness

It is possible that the measurement of certain lifestyle exposures in the present study, including diet, was characterized by varying degrees of misclassification error. However, this misclassification is likely to be non-differential (i.e. similar between those with and without the outcomes under study), and thus effect estimates would have been biased towards the null [Armstrong et al. 1992, pg. 59]. It has been recently suggested that non-differential misclassification of exposure will, in some situations, bias effect estimates away from the null [Dosemeci et al. 1990, Flegal et al. 1991, Brenner 1993]. However, it appears that the likelihood of this effect is especially increased with the categorization of continuous variables [Brenner and Blettner 1993, Brenner and Loomis 1994], as well as in the intermediate categories of polychotomous exposure variables [Birkett 1992, Correa-Villasenor et al. 1995]. These circumstances are unlikely to have affected the results of the present study due to the fact that our polychotomous variables do not display aberrant patterns of association, and we avoided the categorization of continuous variables wherever possible.

The food frequency instrument used in the present study was non-quantitative and contained only 34 items, and was thus much simpler than those that have been previously used in population-based epidemiologic studies [Willett 1990, pg. 69]. In addition, the validity and reproducibility of this questionnaire were not determined. The decision not to use a more detailed food frequency questionnaire was made by the study investigators based on the large number of other measurements that were being conducted during the single visit to the SLHDP project house. It is encouraging to note, however, that despite the likely exposure misclassification of this instrument, significant relationships between diabetes risk and patterns of dietary intake determined from this instrument have been documented in this population [Gittelsohn et al. 1998]. This suggests that, despite its simplicity, the questionnaire is able to differentiate endpoint subgroups in the population in terms of dietary intake.

As discussed elsewhere, the 24-hour food frequency questionnaire provides a 1-day snapshot of an individual's diet and therefore may not accurately document usual dietary intake [Willett 1990, pg. 59]. The limitation is of less concern, however, when there is little day to day variation in the diet of study subjects [Willett 1990, pg. 59]. The relatively limited range of both wild and store-bought foods currently available in Sandy Lake suggests that the 24-hour recall may have higher reliability compared to non-reserve populations, although non-differential misclassification remains a likely problem.
Finally, the Shapiro and Wilk statistic is very sensitive for the detection of departures from normality, and thus there was the possibility that, for certain continuous variables, the natural log transformation was used where it was not essential to do so. However, this likely led to underestimates of association and would not be expected to produce falsely positive associations.

4.4. Conclusions and Recommendations

(1) Beta cell dysfunction, estimated here using concentrations of circulating proinsulin, is a clear feature of not only diabetes but also pre-diabetic states including IGT and NGT with an elevated glucose concentration. Further studies to identify factors associated with early beta cell injury are warranted.

(2) Adiposity, especially abdominal adiposity, plays an important role in early beta cell pathology. Existing public health education programmes targeting the maintenance of healthy body weights should be continued, and new strategies for special populations, including Native Canadians, should be initiated.

(3) Conditions associated with nulliparity may indicate high-risk for diabetes in this population, and thus the glucose tolerance status of women with PCOS or related reproductive disorders and should be investigated.

(4) Concentrations of proinsulin and lipids are cross-sectionally associated and appear to deteriorate together. Given the substantial cardiovascular disease burden of individuals with diabetes and IGT, determining the temporal sequence and pathogenic mechanisms of this association should be a priority of future research activities.

(5) Risk of diabetes development appears to be modifiable through lifestyle change even among subjects at high risk for diabetes. The development and maintenance of primary and secondary prevention programmes focusing on lifestyle modification in First Nations groups should be supported. In addition to diet, physical activity and weight maintenance, smoking cessation programmes tailored to First Nations should be developed given the high prevalence of this risk factor in this population and its apparent relationship with diabetes risk.
4.4. References


Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 1997;46:3-10.

Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ. Chronic exposure to free fatty acid reduces pancreatic beta-cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. J Clin Invest 1998;101:1094-1101.


Lewis GF. Fatty acid regulation of very low density lipoprotein production. Curr Opin Lipidol 1997;8:146-53.


Appendix 1

Contributions of the Author to the Design and Implementation of the Sandy Lake Health and Diabetes Project, and to the Studies Presented in this Thesis

In accordance with the recommendations of the committee that examined the protocol for this thesis, the following information outlines the specific contributions of the author to the design and implementation of the surveys from which data were obtained for use in the present studies.

Baseline Survey, 1993-1995

The author was not involved in the initial conceptualization of the project nor in the writing of the original grants, all of which took place prior to the hiring of the author as Project Coordinator in February of 1993. Upon arrival in Sandy Lake, however, the author made substantial contributions to the design, pilot testing, and modification of the questionnaires and other aspects of the protocol, including the anthropometric measurements and fitness testing. In addition, the author led a number of instructional sessions during the training and certification of the Community Surveyors. Further, the author coordinated and supervised the field work, data collection and initial analysis of information from the baseline survey. This work included blood sample collection and processing, and administration of the oral glucose tolerance tests.

Follow-up Survey, 1998

As the follow-up survey was a central element of the author's thesis protocol, he had the primary responsibility for the conceptualization, design and implementation of the study. In addition, the author coordinated the field work, data collection and analysis for this phase of the data collection.
Motivation for Measurement of Proinsulin Concentration, 1998

The motivation for the measurement of circulating proinsulin concentrations from saved specimens arose from discussions between the author and Dr. Zinman regarding the feasibility and usefulness of this exercise. While the author did not directly perform the measurements, he spent several sessions observing these procedures at the Banting and Best Diabetes Centre Core Lab, University of Toronto. In addition, the author has met several times with Mr. Jeremy Kwan, Manager of the Core Lab, to discuss technical aspects of the radioimmunoassay kits that were used to measure concentrations of proinsulin, insulin, C-peptide and leptin.