THE IMPACT OF CHILDHOOD MEASURES OF GLYCEMIA AND INSULIN RESISTANCE FACTORS ON FOLLOW-UP GLYCEMIC MEASURES

by

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SIGNED: Carol Moffett
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With systems as the primary emphasis of my doctoral study, the concept of interacting elements giving rise to systems has been reinforced repeatedly. My work on this dissertation is a reflection of this process. The elements in this case are the many people who made my studies possible.

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DEDICATION

To my husband Rick, and my two daughters Jasmine and Jessica. Each in their own special ways have provided an immense about of joy and wonder in my life.
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ABSTRACT

The purpose of this research was to evaluate the impact of glycemic measures, and changes in identified risk factors (BMI, waist circumference, lipids, blood pressure) on follow-up glycemia, in Pima children at high risk for type two diabetes (type 2 DM).

I computed incidence and cumulative incidence of type 2 DM in Pima children 5-19 years of age between 1983 and 2004. Cox proportional hazards rates for development of type 2 DM were calculated by glycemic measure (HbA1C, 2\(^{0}\)PG, FPG) controlling for confounding factors (age, sex, BMI, blood pressure, and cholesterol). Diabetes was defined by the presence of at least one of four criteria: 1) 2\(^{0}\)PG of \(\geq 200\ \text{mg/dl}\), 2) FPG of \(\geq 126\ \text{mg/dl}\), 3) HbA1C \(> 8.0\%\), or 4) hypoglycemic treatment. Linear regression models were computed to identify the impact of changes in risk factors on changes in HbA1C. Only exams performed in non-diabetic children during childhood were included in the regression models.

Among 2658 non-diabetic children, 258 cases of diabetes occurred during mean 9.1 years of follow-up (1.5 - 21.7). The age-sex adjusted incident rate of diabetes was 19.0 cases per 1000 person-years, and cumulative incidence was 54% by age 40. Incidence rates increased with increasing baseline values of 2\(^{0}\)PG, and FPG, but not for HbA1C. For HbA1C the relationship was u-shaped with the lowest and highest quartiles having the highest DM rates. After adjustment for confounding risk factors using Cox proportional hazards analysis, the risk for diabetes increased 2-fold for every 10 mg/dl increase in FPG. Changes in waist circumference best predicted changes in HbA1C (\(R^2 = 0.48\), \(p<0.001\)). However, the ability of waist circumference to predict change is limited.
due to the powerful effect of regression to the mean, suggesting that these risk factors contribute very little to changes in HbA1C, at least in childhood.

Childhood levels of glycemia predict development of type 2 DM later in life. While changes in waist circumference are associated with only moderate changes in HbA1C, this does not refute the significant contribution of adiposity in childhood to the development of type 2 DM.
CHAPTER I - INTRODUCTION

Nurses are frequently instrumental in programs to screen, diagnose, and intervene in processes related to the development of type 2 diabetes mellitus (DM). The success of these programs depends critically on the availability of accurate and reliable methods of measuring glycemia and comorbid conditions. Current diagnostic measures of glycemia involve an eight-hour fasting period, repeated venipunctures, and (in some settings) ingestion of loading doses of glucose. These are difficult for adults to tolerate, but they are even less well tolerated by children. DM diagnostic processes that do not require fasting, venipunctures, and an unpalatable glucose load would be extremely helpful in the diagnostic process for children and also adults.

Type 2 DM results from an interplay of many factors that are more fully described in chapter two. Many of these factors could be considered predictors of disease progression. The usefulness of measures of predictive factors and measures of glycemia in children is the focus of this study. A secondary analysis of a longitudinal prospective diabetes study by National Institutes of Diabetes, and Digestive, and Kidney Diseases (NIDDK) conducted in the Gila River Indian Community since 1965. This extensive study has compiled data through an ongoing epidemiologic study that involves biannual exams on consenting Pima Indians from the Gila River Indian Community of Arizona. An analysis of these longitudinal data offers a unique opportunity to determine the predictive value of glycemic measurements made during childhood on the development of diabetes later in life. In addition, the relationship of changes in: BMI, lipids, blood pressure, and waist circumference (predictive factors) measured in childhood to changes in glycemia at the follow-up exam can be assessed.
Pima Indians are disproportionately affected by type 2 DM, with approximately 50% of adults over the age of 35 diagnosed with the disease (Knowler et al., 1978). Type 2 DM has also been found in Pima children as young as 4 years of age, though onset usually occurs during puberty (Fagot-Campagna et al., 2000). There are many factors that can at least partially explain the reason for the development of type 2 DM, and the NIDDK data analysis has contributed to much of what is known about how individuals develop the disease.

This chapter will provide an overview of type 2 DM and background information related to the disease in Pima Indians; in addition, it will also describe the purpose and significance of the research and the research questions to be answered.

Background

Worldwide there has been a tripling of the number of persons with diabetes since 1985 (Bloomgarden, 2004). As of 2005, at least 20.8 million people (7.0% of the population) have type 2 DM in the United States, and DM is the sixth leading cause of death (CDC, 2007). For Native Americans, the problem is even more acute. DM was virtually unknown in Native Americans at the beginning of the last century. In 1930, DM still was classified as “clinically nonexistent,” according to Indian Health Service sources. Today, 60-80% of Native Americans are likely to develop type 2 DM in their lifetime, compared with 20% of the US population as a whole (Fransceli, 2002). In one Arizona tribe, the Pima, a 1978 report indicated rates of 50% in tribal members over age 35, an incidence nineteen times higher than in a comparative white population (Knowler et al., 1978). For the Pima Indians, there is a significant impact of duration of diabetes on
death rates, with younger onset of DM implicated as the most common cause of death (Sievers et al., 1992).

According to the Diabetes Federation Consensus Conference (2003), type 2 DM in the young is an “evolving epidemic” driven by obesity and sedentary lifestyle. The prevalence of type 2 DM in adolescents is now equal to, or greater than, type 1 DM in many parts of the world (Weiss et al., 2005). Type 2 DM in children has been determined to be a health priority due to its implications for clinical and economic health care burden (Bloomgarden, 2004). Cardiovascular and kidney disease risk factors, including microangiopathy and proteinuria, are as common, or even more common, in children with type 2 DM as type 1 DM. Research related to children must identify the best methods of screening youth for type 2 DM, and developing knowledge about the progression of type 2 DM disease processes in children (Alberti et al., 2004).

“The available information on type 2 diabetes incidence and prevalence in childhood and adolescence is sparse compared with that for adults.” (Alberti et al., 2004, p. 1799). This is true for the U.S. population in general, as well as for Native American populations specifically. The burden of the disease in children is born disproportionately by girls and minority populations, most notably by indigenous peoples (Bloomgarden, 2004). The childhood diagnosis of the disease occurs most often during puberty.

Although population-based data are sparse for most groups, this is not true for the Pima Indians. An epidemiologic study of this population has been ongoing since 1965, performed by the NIDDK. For this population, measurements have been made as often as every two years from the age of 5 years on (wide variation in measurement intervals exist), making this an ideal population for answering questions about how type 2 DM
develops over time. Because type 2 DM is the only form of diabetes present in this population, the data are useful for the identification of factors in children that are predictive of the later onset of disease. These data also offer information that may be useful in determining which screening methods are most effective in children.

One study of the Pima Indians reported that adolescents from this population had a prevalence of type 2 DM 5-13 times that of the U.S. adolescent population (Fagot-Campagna et al., 2000). Another more recent Pima study reported a nearly 6 fold increased incidence of type 2 DM among children less than 15 years of age (Pavkov, in review). Type 2 DM in Pima children occurs predominantly in adolescence but has been found in a child as young as 4 years of age. In the Fagot-Campagna et al study, all of the diabetic children had at least one parent diagnosed with DM as well. For those children born to a mother with DM during pregnancy, the risk of disease was even greater.

Diabetic Screening

Type 2 DM

Type 2 DM is defined by the American Diabetes Association (ADA) as including:

1. Symptoms of diabetes and a casual plasma glucose of $\geq 200$ mg/dL (11.1 mmol/L).
2. A fasting plasma glucose (FPG) of $\geq 126$ mg/dL (7.0 mmol/L).
3. A 2-hour post-load glucose of $\geq 200$ mg/dL (11.1 mmol/L) during an oral glucose tolerance test (OGTT).

Impaired Glucose / Pre Diabetes

Impaired glucose (Pre-diabetes) is defined as either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (ADA, 2004). Impaired glucose or “pre-diabetes” can be diagnosed by one of two measurements of:
1. FPG between 100 and 125 mg/dL (5.6-6.9 mmol/L)
   - FPG values were decreased in 2003 from the prior cutoff point of 110-125 mg/dL.

2. Two-hour plasma glucose level of between 140 and 199mg/dL (7.8-11.1 mmol/L)

*Childhood Considerations*

The ADA criteria for diagnosing both type 2 DM and impaired glucose tolerances in childhood are the same as for adults. Most children with type 2 DM are:

1. Overweight or obese (85%)
2. Glycosuria without ketonuria (33% have ketonuria, and 5-25% have ketoacidosis)
3. May or may not have polyuria or polydipsia
4. No weight loss (usually)
5. Family history of diabetes (45-80% of cases)
6. Racial and ethnic disparities with over representation among:
   - Asian Americans
   - Hispanic Americans
   - African Americans
   - American Indian
7. Diagnosed after the age of 10 years, (usually middle to late puberty) (ADA, 2000).
8. IGT and type 2 DM diagnosis is best captured by the 2 hour OGTT (in 99.02% of cases) as opposed to the FPG measure (Gomez-Diaz et al., 2004, Sinha et al., 2004).
HbA1C: Glycemic Measure

HbA1C is not currently recognized as a screening measure for diagnosis of DM or IGT. However, several studies have reported the appropriateness of utilizing the HbA1C in combination with the FPG to improve the diagnosis of diabetes. These studies indicate that a HbA1C of >6.1% in combination with FPG elevations above 125 mg/dl or 140 mg/dl as the optimal method for capturing those who might otherwise only have DM diagnosed with a glucose tolerance test (Barr et al., 2002; Wang et al., 2002; Perry et al., 2001; and Simon et al., 1985). HbA1C has a life span of 120 days, and increases in proportion to the blood glucose level over the preceding 3 to 4 months (Peragallo-Dittko & Franz, 2003). This test is commonly used to monitor the clinical management of individuals who have already been diagnosed with DM, but has not been adopted for use in the diagnostic process, initially because of concerns over lack of laboratory standardization. However, the standardization of HbA1C results and methods among laboratories has been addressed (Rohlfing et al., 2000). Today HbA1C is acceptably standardized with excellent reliability, and has been shown to have a sensitivity of 66% and a specificity of 99% when compared with OGTT (Barr et al., 2002).

There are potential advantages to using HbA1C as a screening method and diagnostic measure of choice in children. This test does not require an 8-hour period of fasting and can be performed with finger-stick capillary blood collection techniques. The costs of performing two hour glucose tolerance tests in terms of time required of personnel and clients is more extensive, and the two hour test requires an 8 hour period of fasting prior to initiation of the testing (Rohlfing et al., 2000). The usefulness of HbA1C in the
diagnostic process for diabetes will be determined over time as more evidence is accumulated. This study will contribute to that body of evidence.

Purpose of the Research

The purpose of this research is to evaluate the usefulness of glycemic measures in children. As well as to examine the contribution of identified risk factors (BMI, waist circumference, lipids, blood pressure) to measures of glycemia (HbA1C, FPG, and 2o PG).

Research Questions

1. What effect does the level of:
   - HbA1C in childhood have on the development of an HBA1C of >8% later in life?
   - 2oPG in childhood have on the development of a two hour glucose of ≥200mg/dl later in life?
   - Fasting plasma glucose (FPG) in childhood has on the development of a FPG≥ 126 mg/dl later in life?

2. How do changes in BMI, lipids (Total Cholesterol, Triglycerides, High Density Lipoproteins), blood pressure and waist circumference correlate with changes in glycemic measure (HbA1C)?

Following a review of the literature, the cumulative incidence of elevated glycemic measures as noted over time in Pima Indians first examined in childhood (age ≥5 years to ≤19 years) were examined. Predictive factors were then explored to identify those risk factors that are most strongly associated with progression to disease. Once these risk
factors were identified, they were evaluated for their impact on changes in glucose measurement, especially HbA1C.

Significance of the Research

The Pima Indians, through their participation in a longitudinal epidemiologic study, have contributed much to our knowledge development related to type 2 DM. The data set associated with this study includes examinations of children as young as 5 years of age and follow-up exams as the children matured into adulthood. The data offer the possibility of further developing knowledge related to the usefulness of HbA1C in terms of its potential contribution to screening, diagnosis, and follow-up in multiple settings. Currently HbA1C can be obtained from a finger stick sample. A finger stick measurement that does not require a fasting state, is much more child friendly than the current screening requirements of fasting, and two-hour glucose tolerance testing.

The determination of the impact of changes in identified risk factors on measures of glycemia is useful knowledge for predicting the impact of alterations on these risk factors on the development of type 2 DM. Most of our scientific knowledge related to type 2 DM has been derived from data about adults. A focus on children offers the opportunity to develop strategies that could potentially alter the trajectory of disease progression. This information also becomes useful in cost benefit analysis. If the HbA1C is found to be a viable screening and ultimately diagnostic measurement then the cumbersome issues of fasting and two hour post load testing are eliminated. This is potentially more significant for children than for adults, though adults also would benefit from a shift in screening and diagnostic procedures.
Summary

Type 2 DM is a disease that is increasing rapidly in a global environment of obesity and sedentary lifestyles. There is a systemic impact, both from a clinical and economic perspective, on individuals and society. Children develop type 2 DM, in large part due to the same factors observed in adults that is, obesity and a sedentary lifestyle. Children who are exposed to diabetes in utero and or have a parent with diabetes experience increased risk for disease.

Type 2 DM is diagnosed according to strict criteria established by the ADA. It is primarily reliant on FPG and casual glucose levels that fall within specified parameters. A 2\textsuperscript{o}GTT is no longer recommended for diagnostic purposes in general clinical settings, although parameters for diagnosis with this measure are well established. The 2\textsuperscript{o}GTT has been demonstrated to be more useful than the FPG in the diagnosis of children with type 2 DM. Currently HbA1C is not recommended for use in the diagnostic or screening process. This secondary analysis of the prospective longitudinal study on Pima Indians will add to our knowledge of the usefulness of measures of glycemia in children. In addition, the research will identify and describe the impact of changes in metabolic/insulin resistance syndrome factors (BMI, waist circumference, lipids, and BP) on changes in glycemic measurements of HbA1C, FPG, and 2\textsuperscript{o}PG.
CHAPTER II: LITERATURE REVIEW AND THEORETICAL FRAMEWORK

The theoretical framework for this study emerged from a review of the extant literature. As described at the conclusion of the chapter, the theoretical framework details the development of type 2 Diabetes Mellitus (DM) and the impact of changes in risk factors for diabetes on glycemic measures (HbA1C, 2\textsuperscript{0} PG, FPG) at each stage. Closely intertwined with the diabetes risk factors are comorbid risk factors that are also associated with cardiovascular disease and end stage renal disease. These risk factors are often referred to as the metabolic syndrome, insulin resistance syndrome, or syndrome X. The interplay of these factors has implications for understanding the physiologic mechanisms in childhood that predispose to diabetes.

A preliminary, simple, 3-stage developmental model of type 2 DM was used to structure the literature review. Stage I is defined by obesity or increasing body mass index (BMI) and its contributing factors (genetics, uterine environment, inactivity, and over-nutrition). Stage II is defined by insulin resistance and its contributing factors (genetics, uterine environment, and inactivity), as well as central adiposity, hypertension, and elevated lipids and rising blood sugar. Stage III is defined by increasing blood sugar levels and beta cell exhaustion that eventually lead to the development of diabetes mellitus.

Stage I: Obesity/Increasing BMI

Severe obesity plays a prominent role in the pathogenesis of type 2 DM in children and adolescents (Rosenbloom et al., 1999). Importantly, obesity contributes to an increased risk of morbidity and mortality from cardiovascular disease, hypertension, dislipidemia, colorectal cancers, cholelithiasis, sleep apnea, joint problems and
osteoarthritis, polycystic ovary disease, fatty liver disease, and many others. The impact of obesity is heightened when it is associated with central adiposity and accompanied by hyperinsulinemia (Ferraro et al., 2003; Lobstein et al., 2004; Tremblay & Doucet, 2000; Zannolli et al., 1993). Life expectancy for those who are severely obese is reduced by 5 to 20 years (Olshansky et al., 2005).

A worldwide problem, obesity has increased more than 75% since 1980 with more than one billion people now considered either overweight or obese. Of these, ten percent (10%) are school age children. Childhood obesity is significantly associated with severe obesity in adulthood (Lobstein et al., 2004). In the United States, sixty one percent (61%) of the population is considered to be overweight (BMI of 25-29.9) and 26% are obese (BMI of $\geq 30$) (Cummings & Schwartz, 2003). National Health and Nutrition Examination Survey (NHANES) data from the 2003-2004 surveys were evaluated to determine the prevalence of overweight among US children 2-19 years of age, in comparison with children of the same age in the 1971-1974 surveys. The percentage of overweight children increased to 13.9% for 2-5 year olds, to 18.8% for 6-11 year olds, and to 17.4% for 12-19 year olds. This is an increase of nearly two fold in twenty years (CDC, 2007). The number of overweight children in the U.S. continues to increase by about 0.5% annually (Ogden et al., 2002).

Overweight/Obesity Measurements

Determining rates of overweight and obesity in children can vary, depending on which measurements and references are used. Anthropometric measures of relative adiposity include weight, weight-for-height, BMI, skin-fold thickness, waist circumference, and waist-to-hip ratio. BMI, abdominal skin fold thickness, and waist
circumference measures correlate well with the gold standard of dual energy X-ray absorptiometry (DEXA). Studies indicate a correlation of 0.50-0.85 for BMI with DEXA, while waist circumference was found to correlate 0.83-0.84 with DEXA (Lobstein et al., 2004).

Variations in BMI among children are age and gender specific. In the first months of life, there is an initial rise in BMI, then a drop at around a year of age. Another increase in BMI begins around the sixth birthday, as age-dependent adipose tissue starts to form (Lobstein et al., 2004). Adipose cells are the principal sites of energy storage. By 15 weeks, fetal fat cells begin forming, with maximal proliferation in both the first year of life and in the period just prior to puberty. In children, adipose cells tend to be made up of a higher proportion of newer smaller cells, as compared with adult adiposity. As these small new cells age, they become larger and their volume expands (Lobstein et al., 2004).

Reference charts produced by the U.S. National Center for Health Statistics (NCHS) are clinically useful to determine how a child’s growth compares with nationally established norms. The NCHS recommends classifying children as overweight if they are at or above the 95th percentile and at risk for becoming overweight between the 85th and 95th percentile. It is currently not clear at what level of BMI adverse health outcomes can be predicted in children (Lobstein et al., 2004).

The US reference charts are not necessarily useful for international populations. Wang and Wang (2002) evaluated three references of international child and adolescent overweight and obesity:

1. The International Obesity Task Force (IOTF) reference chart is designed for use with children 2-18 years of age. Its tables were constructed using large data sets from six
developing and developed countries. These tables are age and sex specific with childhood BMIs identified that correspond with the adult cutoffs for overweight of 25 kg/m$^2$ and obesity of 30 kg/ m$^2$.

2. IN 1995, the World Health Organization (WHO) recommended using weight for height scores for children less than 10 years old. The weight for height score is plotted on a chart based on a standard reference population that gives a Z-score based on the difference between the observed value and the median reference value of a population. For children less than 10 years old, overweight is defined as weight for height Z-scores greater than two. A Z-score of two corresponds to the 97.7$^{th}$ percentile; a Z-score of 1.04 corresponds to the 85$^{th}$ percentile; and a Z-score of 0 is equivalent to the median or 50$^{th}$ percentile. In 1995, WHO recommended the use of BMI for children ≥9 years with references that are age-sex specific. Specifically, WHO recommended using weight for height scores at 85$^{th}$ percentile to define overweight, and a BMI of 85$^{th}$ percentile with triceps skin fold at the 90$^{th}$ percentile to define obesity in adolescents (10-19) (Lobstein et al, 2004. and Wang et al, 2002).

3. Must, Dallal and Deitz (MDD) is the reference generally used to define obesity or overweight in the U.S. for those 6-74 years of age. The MDD is based on BMIs (for age and sex), with the 85$^{th}$ percentile as the cutoff point for overweight and the 95$^{th}$ percentile as the cutoff point for obesity. The CDC (2000) revised their growth charts to include BMI and recommended that all children 2 years and older be plotted on this chart (Lobstein et al., 2004. and Wang et al., 2002). Dietz & Bellizzi (1999) identify children with a BMI between 25 and 29.9 BMI as grade 1 overweight and BMI 30 and greater as grade 2 overweight.
These three references were compared with measurements from developed and developing countries. The combined overweight and prevalence rates were similar for all references; but the WHO reference produced a slightly higher rate in children and a lower rate in adolescents than the IOTF, suggesting a need for caution when comparing rates based on different references. Differences also were noted in the growth patterns of children in developing countries and in populations of Asian-Pacific origin. This may, in part, be due to differences in maturation rates, as identified by mean age of menarche (12.6 years in US, 13.7 years in China, and 13.3 years in Russia). Growing evidence indicates that populations of Asian-Pacific origin experience greater obesity-related morbidity at the generally accepted cutoff points, indicating a need to lower BMI cutoffs for these groups to 23 for overweight, and to 25 for obesity.

Constructing appropriate reference indexes to assist in determining children and populations at risk related to variations in growth highlights the need for further knowledge development. This is supported by the following recommendations of the International Association for the Study of Obesity:

- “BMI (emphasis added) should be used as the main measure of overweight and obesity in childhood and adolescence for survey purposes.
- Research studies involving epidemiological or clinical data should ensure that BMI for age is expressed as a mean and standard deviation for each gender; and prevalence is estimated by using cut-off points from a clearly established reference. This will allow international comparison of data.
- Researchers should be encouraged to collect data on waist circumference in childhood and adolescence when performing epidemiological or clinical studies.
• Further research is needed to validate theBMIcon-for-age or waist-for-age cut off points associated with health risks in childhood and adolescence.

• Further research is needed into the effect of ethnicity on the interpretation of the definitions of ‘overweight’ and ‘obesity’.

• Further work is needed in establishing clinical definitions of ‘overweight’ and ‘obesity’ that are congruent with research definitions” (Lobstein, 2004, p.15.)

Overweight/Obesity of Pima Children

The average BMI in the Pima has increased 19% over the last 40 years (Pavkov et al., in review). Efforts to determine the anthropometric parameters of the population of Pima children utilized the CDC reference chart for comparative purposes. Values for children from birth through 21 months were calculated based on weight-for-length. For those children 2 through 20 years of age, BMI standards published by the CDC National Center for Health Statistics (NCHS) were used. Weight-for-length and BMI values were transformed into Z-scores to allow a determination of how each individual deviated from the reference values. Pima children (birth -20 years of age) were found to be significantly heavier (weight/height) at all ages after the first month of life when compared with data from NHANES II & I; although not when compared with data from NHANES III. This may be due to the upward trending of weight in the US population. The most dramatic increases occurred between 1 and 6 months of life and between 2 and 11 years of age. An analysis of Pima children 5-11 years of age during the decade from 1990-2000 provided strong evidence of an accumulation of adiposity in this group (Lindsay et al., 2002).

Adequately defining adiposity in individuals and populations requires the development and refinement of accurate measurements. Closer scrutiny of obesity has led
to our understanding of adiposity as the result of a complex system of interacting hormonal/chemical pathways influenced by genetics and the uterine environment, as well as behavioral components.

**Hormonal/Chemical Pathways**

Multiple regulatory systems operate to store and use energy. These systems signal appetite and satiety through chemical and hormonal messages sent via the sympathetic and parasympathetic nervous system as well as the endocrine system. Short term signaling arises from the gastrointestinal tract before and during food consumption (Lobstein et al., 2004). Insulin and leptin are hormones that circulate in proportion to body-fat content. They both enter the brain to interact with receptors and influence energy balance in a long acting manner. Leptin and insulin each play a role as they fluctuate in response to changes in body adiposity. A defect in leptin/insulin receptors or the development of leptin/insulin resistance is thought to contribute to most forms of obesity (Cummings & Schwartz, 2003).

Insulin and leptin are two of the hormones influenced by adipose tissue. Fat cells can be considered as a metabolic organ that secretes a large number of peptides and cytokines, prostaglandins, and steroid hormones (androgen and estrogen). These peptides influence fertility, sexual maturity, and cardiovascular and metabolic function. Obese children mature faster, with an earlier onset of puberty and accompanying spurt in stature. This early height spurt does not maintain the same velocity as in the non-obese, leading to a shorter stature in adulthood for the obese (Lobstein et al., 2004).
**Genetics**

Chemical and hormonal messaging and obesity are influenced by genetics. Obesity has a strong *heritability* component, perhaps as much as 50-90%. Heritability appears to be a more significant factor in early-onset (childhood) obesity than in adult onset obesity (Rosenbaum & Leibel, 1998). Genome scans in obesity studies are highly reproducible and, despite ethnic and environmental differences, the loci at chromosomes 2 and 10 are generally confirmed. Obesity is considered to be “oligogenic,” with expression modulated by “polygenic modifier genes” interacting with the environment in food choices, physical activity, and smoking (Froguel & Boutin, 2001).

A recently published study identified the gene, ENPP1 (ectonucleotide pyrophosphatase phosphodiesterase), also known as plasma cell glycoprotein. Variants in the gene ENPP1 expression are associated with a 50% increased risk of morbid obesity in adults and a 69% increased risk of childhood obesity (Meyre et al., 2005).

Efforts to identify the DNA marker for body fat in the Pima Indian population are ongoing. Norman et al. (1995) found a body fat linkage to tumor necrosis factor (TNF) ir24 located near 10kb from TNF-α. The role of TNF-α is increased in the adipose tissue of obese humans. Further studies have found evidence for a genetic influence on body fat at chromosomes 11q21-q22 and 3p24.2-p22 (Norman et al., 1997). Leptin is a hormone regulated by adipose tissue and is thought to correspond to the development of obesity. Walder et al. (1999) found chromosome 6p to be linked to plasma leptin concentrations.

A study examining the role of heritability, insulin, glucose, leptin and level of obesity in Pima children at 5 and 10 years of age found only a small role for heritability. The study was comprised of 138 children (65 boys, 73 girls). Height; weight (BMI from
NCHS growth charts, overweight ≥ 95th percentile, and risk for overweight ≥ 85th percentile; body composition (using 13O dilution spaces assuming water is 75% of fat free mass in girls and 74% in boys, and DEXA); parental obesity; and fasting plasma insulin, glucose, and leptin concentrations were entered into linear regression models. Of the variables tested, obesity at age 5 was the most significant predictor ($R^2 > 0.53$) of obesity at age 10. Other factors, including high maternal body mass index, elevated fasting plasma leptin concentrations, and low fasting plasma insulin concentrations, explained an additional 4% of the variance (Salbe et al., 2002).

**Uterine Environment**

Conflicting evidence exists regarding whether the uterine environment, independent of genetics, has an impact on the development of adiposity. Babies with very low and very high birth-weights are more likely to become obese (Lobstein et al., 2004). Pietilainen et al. (2002) found that twins who were larger at birth remained larger throughout life, thus implying an impact of the fetal environment on final body size. Two studies comparing birth weights of monozygotic and dichorionic twins concluded that the intra-uterine environment was critical to the development of height, but not to increased adiposity (Allison et al., 1995; Loos et al., 2001).

In contrast, there is strong evidence of the uterine environment’s contribution to adiposity in infants born to women with gestational diabetes. The gestational diabetes uterine environment seems to produce larger babies with greater fat mass and higher birth weights (Catalano et al., 2003; Gillman et al., 2003; Vohr & McGarvey, 1997). The fasting maternal glucose level has the greatest predictive strength, with higher maternal fasting glucose levels correlating with increased body fat in the infant (Catalano et al.,
This larger birth weight predisposes to overweight in adolescence (Gillman et al., 2003). The addition of excess caloric intake to an at-risk individual further increases the likelihood of obesity.

Over-Nutrition and Inactivity

WHO addressed the impact of a changing global environment on obesity:

“Changes in the world food economy have contributed to shifting dietary patterns, for example, increased consumption of energy-dense diets high in fat, particularly saturated fat, and low in unrefined carbohydrates. These patterns are combined with a decline in energy expenditure that is associated with a sedentary lifestyle motorized transport, labor-saving devices at home, the phasing out of physically demanding manual tasks in the workplace, and leisure time that is preponderantly devoted to physically undemanding pastimes” (2002, p.1-2).

Nutrient consumption and over consumption are the result of appetite and satiety chemical signaling, as noted earlier. Once weight has been gained, the body seems to defend the adiposity against large losses by triggering hunger and reducing satiety signals. In Western societies, adults gain .5-1.0 pounds per year during adulthood. The equivalent of adding 1 Ritz cracker per day (10-20 kcal/day) to the diet can produce this weight change (Cummings, & Schwarz, 2003; Franz et al., 2002). Specific nutrient intake has been evaluated for its impact, not only on obesity, but also on the development of diabetes. Dietary fat has been implicated in the development of diabetes and may have an adverse role in insulin sensitivity, though this has not been found in Pimas. All forms of dietary fat except n-3 fatty acids are considered problematic, though saturated fats are most consistently found to be adverse. In terms of diabetes, the impact of high fat diets is
primarily on energy balance. Diabetes is the result of weight gain associated with excess calorie consumption and adipose tissue accumulation (Franz et al., 2002).

Energy intake is the result of nutrient consumption, while energy expenditure is related to many aspects of cellular function, but also includes expenditure through physical activity. Physical inactivity has long been implicated in childhood obesity. This relationship has been documented in preschool children, and is also found in adolescents. Inactivity that involves excessive TV watching also encourages greater adiposity (Berkey et al., 2003; Davies et al., 1995; Dietz & Gortmaker, 1985). It is possible that obesity predisposes to inactivity, due to the metabolic demands of excess mass creating a cycle where inactivity contributes to further excess mass, which in turn limits activity (Norman et al., 2005; Rosenbaum & Leibel, 1998).

In summary, obesity is increasing throughout the world, due to a genetic predisposition and driven by the ease of access to calorie dense foods as well as the entrenchment of sedentary lifestyles. Childhood is also caught in the escalation of obesity, often with the uterine environment setting the stage. The physiologic alterations attributable to adipose tissue predispose to high risks for metabolic (DM), cardiovascular, orthopedic, and a multitude of other comorbid conditions that ultimately constrain the quality and quantity of life. Obesity will be represented in the conceptual model for this research as a precursor to insulin resistance.

Stage II: Insulin Resistance

Often there is a linkage between insulin secretion and insulin resistance. The early response to increasing glycemia is often hyperinsulinemia to offset the resistance at the cellular level to normal circulating insulin levels. This response changes with the onset of
diabetes, which is accompanied by an inadequate production of insulin, as well as declining insulin sensitivity.

Healthy skeletal muscle switches between lipid oxidation during fasting conditions and glucose metabolism and storage during insulin-stimulated conditions, with accompanying suppression of lipid oxidation. Insulin sensitivity refers to the ability of skeletal muscle cells to respond to the presence of insulin in the metabolism of glucose and suppression of lipid oxidation. Insulin resistance implies an inability (inflexibility) of the skeletal muscle cell to appropriately utilize available insulin to metabolize glucose and failure to suppress lipolysis (Kelley & Goodpaster, 2001). The interplay of the hormone insulin produced in the beta cells of the pancreas and the insulin receptors on the cell wall is complex. Insulin receptor mutations or modifications (down regulation) in the presence of obesity enhance insulin resistance. Pancreatic beta cells respond initially to insulin resistance through increased insulin production (hyperinsulinemia) in an attempt to offset the resistance at the cellular level. Pancreatic beta cells eventually are unable to meet the demands of hyperglycemia, resulting in a relative decline in insulin production (beta-cell exhaustion) (Kahn, 2005; Roith & Zick, 2001).

In children with insulin resistance, impaired glucose tolerance (IGT) can occur in the presence of relatively well-preserved beta cell function. Insulin resistance occurs to some extent normally with puberty. Obesity further compounds the demands for insulin associated with the insulin resistance of puberty. This seems to explain the surge of pubertal and post pubertal diagnosis of diabetes especially among girls (Rosenbloom et al., 1999; Sinha et al., 2002).
Quantification of Insulin Resistance

Determining insulin resistance requires specific measurement techniques. Sinha et al. (2002) assessed childhood and adolescent beta cell function by calculating an insulinogenic index using a ratio of the increment of plasma insulin level to the plasma glucose level during the first 30 minutes after the oral glucose load. \((I_{30\text{min}} - I_{0\text{min}}/G_{30\text{min}} - G_{0\text{min}})\). This index correlates with the early insulin response measured with a hyperglycemic clamp. The hyperglycemic clamp technique involves the administration of a hyperglycemia-inducing bolus of dextrose (25%) followed by dextrose infusion to maintain hyperglycemia (200 mg/dl, 12.5 mmol/liter), as a method to measure insulin action and to estimate insulin sensitivity, noninsulin-dependent glucose uptake, glucose effectiveness, \(\beta\)-cell secretory capacity, and hepatic insulin clearance. Insulin resistance can also be calculated using the homeostatic model (HOMO), as the product of fasting plasma insulin (in microunits per milliliter) and fasting plasma glucose (in millimoles per liter) divided by 22.5. Another insulin resistance estimate is the quantitative insulin sensitivity check index (QUICKI) using the formula \(1/\log(I_0) + \log(G_0)\), where \(I_0\) is fasting insulin, and \(G_0\) is the fasting glucose. The HOMO model and the QUICKI both correlate well with the euglycemic-hyperinsulinemic clamp \((r = -0.71, p < 0.001)\) (Katz et al., 2000; Keskin et al., 2005). The euglycemic–hyperinsulinemic clamp is considered the gold standard for measurement of insulin resistance. This test is conducted following an overnight fasting period. A continuous intravenous infusion of regular insulin is given at a rate to maintain plasma insulin with the intent of suppressing hepatic glucose production. At the same time, a glucose infusion is adjusted to maintain euglycemia.
Higher levels of glucose requirements are indicative of insulin sensitivity while lower levels indicate insulin resistance.

Another technique used to determine insulin resistance and beta cell function is through measurement of proinsulin levels. Proinsulin is a precursor to insulin production. Measurements of proinsulin to insulin ratios are sometimes used to determine which individuals might be experiencing a failure of beta cell functioning. The proinsulin/insulin ratio has implications for use in predicting progression to diabetes in adults. However, Sinha et al. (2002) did not find evidence of usefulness for proinsulin measurements in children.

*Factors Contributing to Insulin Resistance*

*Genetics.* All aspects of insulin resistance, including progressive beta cell failure, are influenced by genetic contributions. Pankow et al. (2004) found that children of parents with insulin resistance were more likely to be insulin resistant. This was evident even when adjustments for adiposity were made, suggesting a role for genetics as well as for environmental factors. Further evidence of a genetic link is found in the variations in insulin sensitivity noted in racial and ethnic groups. African Americans, Hispanic, and Pima children have lower insulin sensitivity (greater insulin resistance) than non-Hispanic white children. However, African American children compensate with a higher acute insulin response to glucose, at least in part due to a reduction in liver extraction of insulin, whereas Hispanic children compensate through a greater insulin secretion (Goran et al., 2002). Hanson et al. (2001) analyzed the heritability of diminished insulin sensitivity and deficient insulin secretion in Pima Indians. These two factors appear to be
related to genetics--perhaps through the diabetes-susceptibility gene on chromosome 1q, which appears to affect insulin secretion.

Meyre et al. (2005) identified a gene ENPP1 (ectonucleotide pyrophosphatase phosphodiesterase, also known as plasma cell glycoprotein 1, which blocks insulin-induced changes in insulin receptor conformation. Variants in the gene promote obesity, which encourages an excessive accumulation of fat, which in turn boosts insulin resistance. Children with this gene (haplotype) had higher fasting glycemia and were more than three times as likely to have glucose intolerance and type 2 DM, while the haplotype more than doubled risk of type 2 DM among parents. This study provides a link between obesity and insulin resistance at the molecular level.

Inactivity. A genetic predisposition to insulin resistance is compounded by a sedentary lifestyle. Throughout the world, there is an increasing reliance on motorized transportation and entertainment activities that encourage little to no large muscle movement. In general, everyday survival is no longer reliant on physical movement; as a result, one of the pathways for energy disposal is under utilized. When muscle is active, a complex system of enzymes, as well as increased blood supply to the muscle, facilitates glucose uptake, independent of insulin. When an adequate supply of insulin is present, physical activity works in synergy with insulin to enhance glucose uptake both during and following exercise. For individuals predisposed to insulin resistance, the working muscle provides a mechanism to manage rising blood glucose levels (Sigal et al., 2004).

Uterine-environment. Individuals can be predisposed to insulin resistance at very early life stages through exposure to certain uterine environments. Results of a Danish study of twins revealed a higher prevalence of type 2 DM and IGT among adults who had
a low birth weight or lower weight at 1 year (Poulsen et al., 1997). This suggests a role for intrauterine malnutrition in the disease process.

Maternal diabetes during pregnancy is linked to insulin resistance and eventual diabetes in offspring. Cho et al. (2000) noted that the uterine environment contributes to abnormalities in weight and glucose tolerance in the offspring of diabetic mothers with abnormal metabolism. For the Pima Indians, risks for type 2DM begin in the uterine environment of diabetic pregnancies because of a lower acute insulin response to glucose (Bogardus and Tataranni, 2002; Dabelea et al., 2000). The diabetic uterine environment also predicts elevations in systolic blood pressure and HbA1C during childhood (Bunt et al., 2005). The mechanisms that contribute to the influence of the uterine environment on insulin resistance presumably involve an increase in glucose, amino acids and free fatty acids transmission from the mother to the developing fetus in women with poor metabolic control. These factors, in turn, provoke fetal hyperinsulinemia and alterations in fetal metabolism leading to macrosomia. “Excessive insulin secretion in utero, as assessed by amniotic fluid insulin concentration, is a predictor of both obesity and IGT in adolescence” (Silverman et al., 1998, p. 142).

Insulin. Insulin concentrations in utero contribute to insulin resistance in the developing fetus. As adiposity is acquired, fat cells further enhance the insulin resistance process through complex chemical signaling involving leptin and insulin. Eventually insulin fails to regulate plasma glucose levels due to problems at the cellular receptor level and/or diminution of insulin supply over time. Initially, insulin resistance produces hyperinsulinemia. In some cases, hyperinsulinemic responses arise from an abnormally
low early insulin response followed by excessively high compensatory insulin production by the pancreatic beta cells (Guzzaloni et al., 2002).

**Metabolic Syndrome**

Insulin resistance is integral to concepts related to the metabolic syndrome and the insulin resistance syndrome. The metabolic syndrome is defined as a combination of the factors of hypertension, dyslipidemia, insulin resistance, hyperinsulinemia, glucose intolerance, and obesity (particularly central obesity). This combination of factors is thought to promote the development of diabetes and cardiovascular disease. Both WHO and the National Cholesterol Education Program’s Adult Treatment Panel (NCEP) or (ATP) have established criteria for the diagnosis of metabolic syndrome (Table 1).

**TABLE 1. Definitions of Metabolic Syndrome** (Bloomgarden, 2004)

<table>
<thead>
<tr>
<th>Organization</th>
<th>Waist circumference or waist/hip</th>
<th>Lipids</th>
<th>Blood Pressure</th>
<th>Urinary Albumin</th>
<th>Glucose</th>
</tr>
</thead>
</table>
| WHO                   | Recommended glucose parameter plus 2 others | Waist/hip ratio 
>0.90 in men 
>0.85 in women | Serum triglycerides 
>150 mg/dl or HDL < 35 mg/dl in men and <39 mg/dl in women | >140/90 mm/Hg. | Excretion rate of 
>20 µg/min. | **Must have** 
DM, IFG, IGT, or HOMA insulin resistance |
| NCEP ATP III          | Requires at least 3 of the parameters | >102 cm in men 
>88 cm in women | Serum triglycerides 
>150 mg/dl and HDL cholesterol <40 mg/dl in men and <50 mg/dl in women | >130/85 mm/Hg | Serum glucose 
>110 mg/dl |
When metabolic syndrome in Pima Indian adults (those 20 years of age and older) was evaluated using both NCEP and WHO criteria; the WHO criteria predicted diabetes with more specificity and sensitivity. This difference is thought to occur because the WHO criteria require hyperglycemia or insulin resistance in addition to other metabolic abnormalities, whereas the NCEP criteria treat the components more equally and do not require hyperglycemia or insulin resistance to diagnose the metabolic syndrome.

An analysis was conducted on data collected from Pima Indians over 20 years of age with an initial non-diabetic visit and at least one subsequent visit between Feb.1993 and May 1998. The sample included 890 participants (549 women, and 341 men with mean age of 33.3 ± 10.3 years). Sixteen percent, or 144, of the 890 subjects developed diabetes at a median of 4.1-years after the initial visit. A factor analysis revealed that the variables best predicting the onset of diabetes were insulinemia and obesity. Dyslipidemia, in the form of high triglycerides and low HDL, was a significant but more modest predictor of diabetes, while high blood pressure was weakly associated. A combination of insulinemia, body size, and lipids had a significant predictive value as compared with blood pressure (Hanson et al., 2002).

Another study of children (Weiss et al., 2004) used criteria modified from NCEP and WHO for diagnosis of metabolic syndrome. Modification was required because body proportions change during puberty and may vary among different races and ethnicities; also, differences in waist-to-hip ratios are difficult to interpret in children.

The factors evaluated in this study were:

1. Obesity, defined as BMI z score of 2.0-2.5, and Severely Obese, defined as BMI z score greater than 2.5
2. Systolic or diastolic blood pressure value that exceeded the 95th percentile for age and sex.

3. Abnormalities in fasting triglycerides and HDL cholesterol adjusted for age, sex, and race or ethnic group (>95th percentile for triglycerides; <5th percentile for HDL).

4. IGT defined as a glucose level greater than 140 mg/dl (7.8 mmol/liter) but <200 mg/dl (11.1 mmol/liter) at two hours.

Children were classified as having metabolic syndrome if they met three or more of the above criteria. Degree of insulin resistance was determined using a homeostasis model assessment (HOMA) model in which scores ranged from 0-15 with higher scores indicating greater insulin resistance. The study found that the metabolic syndrome was prevalent in obese children and adolescents and that its prevalence increased in a direct relationship to the degree of obesity. This study did not determine, however, which factors predict the later development of diabetes or cardiovascular disease (Weiss et al., 2004).

Metabolic syndrome variables found to predict type 2 DM in a study of Pima children include the two-hour plasma glucose, fasting serum insulin, relative weight, and a parent with diabetes (McCance et al., 1994). The study evaluated 1258 Pima children aged 5-19 years with a mean follow-up of 8.4 years. In this group, 1120 children had at least one parent with type 2 DM; and 101 (9.0%) of these children were diagnosed with type 2 DM at follow-up. For the 138 children who had no diabetic parents, only 1 was diagnosed with type 2 DM at follow-up.
Insulin Resistance Syndrome

The metabolic syndrome as defined by NCEP Adult Treatment Panel (ATP) III is focused primarily on coronary heart disease (CHD) and does not recommend routine measurement of insulin sensitivity or of inflammatory markers. The insulin resistance syndrome (IRS) has only recently re-emerged as a diagnostic category in the literature; it is sometimes used interchangeably with metabolic syndrome and Syndrome X. The metabolic syndrome includes non-metabolic components such as central adiposity and hypertension and, as a result, some consider the term to be a misnomer (Davidson, 2003). IRS excludes individuals with DM and is best diagnosed with the 2-hour post glucose challenge measurement for insulin resistance. IRS is thought to be a continuum of risk that is dependent on the number and severity of components. Early identification of IRS allows for focused treatment, aggressive lifestyle intervention, and the possibility of identifying family members at risk. Risk factors and criteria (described below) are identified in Table 2. Both the American Association of Clinical Endocrinologists (AACE) and the European Group for the Study of Insulin Resistance (EGIR) have established parameters (Table 2) for the syndrome.

The AACE definition of IRS is based on the presence of at least two of three criteria (lipid, BP, Glucose) and one of seven factors (waist circumference, race/ethnicity, lifestyle, BMI, or age, diagnosis of certain diseases, family history of certain diseases, history of gestational DM or IGT/IFG.). EGIR definition of IRS is based on simpler criteria: a diagnosis of hyperinsulinemia and at least two other abnormal factors (fasting plasma glucose, blood pressure, lipids, and waist circumference).
TABLE 2. *Insulin Resistance Syndrome*

<table>
<thead>
<tr>
<th>Organization</th>
<th>Lipids</th>
<th>Blood Pressure</th>
<th>Glucose</th>
<th>Waist circumference</th>
<th>Additional Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGIR IRS</strong></td>
<td><strong>Criteria</strong></td>
<td><strong>Criteria</strong></td>
<td><strong>Criteria</strong></td>
<td><strong>Criteria</strong></td>
<td><strong>Criteria</strong></td>
</tr>
<tr>
<td><em>Fasting Hyperinsulinemia And Must have at least 2</em></td>
<td>Triglycerides &gt;2.0 mmol/l</td>
<td>≥140/90 mmHg</td>
<td>FPG ≥6.1 mmol/l</td>
<td>Men ≥94 cm.</td>
<td>*Polycystic ovarian syndrome (PCOS), *<em>non-alcoholic fatty liver disease (NAFLD), (Bloomgarten, 2004)</em></td>
</tr>
<tr>
<td></td>
<td>HDL &lt;1.0 mmol/l</td>
<td>Or</td>
<td>Treated for high BP</td>
<td>Women ≥80 cm.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Or</td>
<td>Treated for dyslipidemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Factor</strong></td>
<td><strong>Factor</strong></td>
<td></td>
<td></td>
<td><strong>Factor</strong></td>
</tr>
<tr>
<td></td>
<td>Men &gt;40 inches</td>
<td>Women &gt;35 inches</td>
<td></td>
<td></td>
<td>Non-Caucasian ethnicity</td>
</tr>
<tr>
<td><strong>AACE IRS</strong></td>
<td><strong>Criteria</strong></td>
<td><strong>Criteria</strong></td>
<td><strong>Criteria</strong></td>
<td><strong>Factor</strong></td>
<td></td>
</tr>
<tr>
<td><em>Must have at least 2 criteria And at least 1 factor</em></td>
<td>Triglycerides &gt;150 mg/dl</td>
<td>&gt;130/85 mmHg.</td>
<td>FPG 110-125 mg/dl</td>
<td>Non-Caucasian ethnicity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>Or</td>
<td>2-h post load 140-200 mg/dl</td>
<td>Sedentary lifestyle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• men &lt;40 mg/dl</td>
<td>Or</td>
<td>BMI &gt;125.0 kg/m²</td>
<td>Age &gt;40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• women &lt;50 mg/dl</td>
<td></td>
<td></td>
<td>Diagnosis of: CVD, hypertension, *PCOS, **NAFLD, or acanthosis nigricans.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Family history of: DM, hypertension or CVD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>History of: Gestational DM or IGT/IFG</td>
<td></td>
</tr>
</tbody>
</table>

Factors identified as contributing to the metabolic syndrome (obesity, dyslipidemia, hyperglycemia/hyperinsulinemia, and hypertension) have been studied separately, as well as in combination, as summarized in the following sections.
Lipids

Body mass index is intertwined with the other variables of the metabolic syndrome in most studies. Gostynski et al. (2004) evaluated the relationship of total cholesterol and body mass index by gender and age in adults, using data from the WHO Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project, for 1979 through 1989. Data were collected from several countries and communities and primarily sampled 35-64 year olds, though some centers included 5-34 year olds as well. The results indicated that the prevalence of hypercholesterolemia (PHC) increased more rapidly in females than males; although women younger than 50 years had lower PHC than males, and after the age of 50 years had higher PHC than males. For males, PHC rose most dramatically until age 39 years, but then stabilized from age 40 years on. The prevalence of obesity, showed the same trends, with the prevalence in women increasing more steeply with age in comparison with men. The interaction between age and cholesterol was positive, as was the relationship of BMI with cholesterol. When age-sex analysis was performed, the strongest BMI related increase in PHC was noted in males age 25-39 years and females 25-34 years. There was no significant relationship of BMI with PHC in women 50-64 years and only a weak relationship in men of the same age.

Atherosclerosis begins in childhood. Nationally, among adolescents (12-17 years of age) and adults, total cholesterol levels have fallen by an average 7 mg/dL between the 1960s and the early 1990s. Females, however, have higher mean total cholesterol and LDL-C levels than males; and black females have experienced the smallest decrease. The NHANES III (1988-1994) examined 7,499 children 4-19 years of age for serum total

The decline in lipids over time is not noted in Pima adolescents, however. In 1970, Pima children had lower cholesterol measurements than the general population. More recent data indicate that their values for cholesterol will soon be equivalent to the general population. A study of 4993 Pima children aged 10-17 years from 1970-2001 found that mean total cholesterol concentrations increased significantly in boys, but not in girls. For boys, the 1970-1975 mean total cholesterol measured 148 mg/dl and rose to 158 mg/dl in 2000-01 (p<0.001). For girls, the change was from 150 mg/dl in 1970-75 to 152 mg/dl (p=0.214). For adolescent boys, there was also an increase from 23% (1993-1994) to 32% (2001) in the proportion of LDL-C concentration above 110 mg/dl. The BMI for boys increased from 23.0 to 24.5 kg/m² (1970-1974) and from 27.6 to 28.3 kg/m² (2000-2001) (Williams et al., unpublished).

**Blood pressure**

Elevations in blood pressure are associated with cardiovascular disease in a manner similar to dyslipidemia. There is evidence that both dyslipidemia and hypertension are associated with increases in BMI. This finding was demonstrated in an African (Nigerian) population that is becoming westernized. When the BMI reached a threshold of greater than 21.5 kg/m², it correlated with increases in blood pressure (Bunker et al., 1995). Usually cardiovascular events occur in adults who are in the fifth decade of life. Childhood increases in blood pressure predict adult hypertension, which in turn leads to cardiovascular disease.
The effect of increasing obesity on blood pressure was explained in children 8-17 years in NHANES. In the 1999-2000 data, both systolic and diastolic blood pressure levels increased with age; and this also was true of all race and ethnicity classifications. When adjustments were made for differences in age, race, and sex, the mean systolic blood pressure was 1.4 mm Hg (95%CI, 0.6-2.2 mmHg; $P<.001$) and the mean diastolic pressure was 3.3 mm Hg (95% CI, 2.1-4.5 mm Hg; $P<.001$) higher in 1999-2000 than in 1988-1994. The increasing prevalence of overweight and obesity among all segments of the population is thought to contribute to these increases (Muntner et al., 2004).

To summarize, insulin resistance is identified as a precursor to the development of diabetes mellitus. Obesity is implicated in the development of insulin resistance along with genetics, inactivity, and a hostile uterine environment. Insulin resistance occurs when an inadequate supply of insulin and/or cellular receptor defects interfere with appropriate glucose uptake and disposal. Obesity, hyperlipidemia, hypertension, and rising blood glucose are components of the insulin resistance syndrome, a risk profile for diabetes mellitus and cardiovascular disease. Cardiovascular problems are usually comorbid with diabetes and complicate the disease process for those who progress on to develop diabetes.
Stage III: Increasing Blood Sugar and Diabetes Mellitus

As insulin resistance progresses plasma glucose levels rise, the pancreatic beta cells eventually become exhausted and rapidly decline in function, resulting in the onset of diabetes.

Impaired Glucose/Pre-Diabetes

Impaired Glucose tolerance (IGT) and impaired fasting glucose (IFG) are found in approximately 6.9% of the U.S. population. About a third of individuals with IGT (140-199 mg/dL) or IFG (110-125 mg/dL) progress to type 2 DM if no intervention occurs (Barr et al., 2002). A study (Edelstein et al., 1997) of six populations found that incidence rates of type 2 DM increased with rising FPG and IGT levels; however, the rates rose in a much more constant manner with increasing 2-hour post load levels. This study also noted that baseline BMI was a significant predictor of IGT progression to type 2 DM.

Abnormal Plasma Glucose in Children and Adolescents

The ADA criteria for diagnosing both type 2 DM and glucose intolerance in childhood are the same as in adults. Most (85%) children with type 2 DM are overweight or obese, have glycosuria without ketonuria, may or may not have polyuria or polydipsia, and usually have no weight loss. Approximately 33% of children, however, have ketonuria and 5-25% will have ketoacidosis at the time of diagnosis with type 2 DM. A family history of diabetes (45-80%) usually exists; and racial and ethnic disparities are evident in the over representation among Americans of Asian, Hispanic, African, and American Indian decent. Children are usually diagnosed after the age of 10 years, i.e., in middle to late puberty (ADA, 2000).
The diagnosis of IGT and type 2 DM is best captured by the 2 hour OGTT in children and adolescents as opposed to the FPG measure (Conwell et al., 2004; Sinha et al., 2002; Perry et al., 2001). According to Sinha et al. (2004), children and adolescents with IGT were almost entirely captured by abnormalities in the 2-hour post-load glucose level, while the FPG levels remained normal (<110 mg/dL) in the IGT group and in the IFG range (110-125 mg/dL) for those with diabetes. However, this study was conducted prior to the new standard for IFG of 100-125 mg/dL DM. A group of obese and overweight children 4-17 years of age were evaluated using the new IFG parameters to determine the prevalence of IFG and IGT in a group from Mexico City (Gomez-Diaz et al., 2004). The study found that the 2003 criteria were an improvement in terms of their ability to capture many more of the children who also had an elevated 2-hour post load glucose response. The sensitivity of IFG to detect IGT improved from 26.6% to 36.7%. However, only 40.8% of children with IFG also had IGT; and the test failed to predict 61.1% of cases with IGT. This study seemed to confirm the need to continue using the OGTT to diagnose impaired glucose and diabetes in children and adolescents, even with the revised 2003 IFG category.

*Glycosolated Hemoglobin*

Type 2 DM and impaired glucose are diagnosed solely based on elevations in plasma glucose concentration. Glycemia is a continuously distributed measure. The threshold that determines who has type 2 DM has been based on the risk for microvascular complications (retinopathy) (Barr et al., 2002; Knowler et al, 1990). Elevations in plasma glucose occur in the presence of other processes. The most directly related measure of average glucose concentration is hemoglobin glycosylation (HbA1C).
A link has been established between HbA1C levels and advanced glycosylation end product formation in human red blood cells. As stated earlier in this chapter, HbA1C is the most abundant minor hemoglobin component in the red blood cell and has a life span of 120 days. HbA1C increases in proportion to the blood glucose level over the preceding 3 to 4 months (Peragallo-Dittko & Franz, 2003). Excessive glycosylation of proteins is determined to be a major cause of microvascular and neuropathic diabetic related complications. Links have also established a continuous relationship between elevations of HbA1C and cardiovascular disease as well as total mortality, even at moderate levels of HbA1C (5-6.9%) in individuals who were not diagnosed with diabetes (Khaw et al., 2004).

HbA1C is a commonly accepted measure of the effectiveness of DM treatment, but is not used for diagnosis of DM. The test has become acceptably standardized with excellent reliability and has a sensitivity of 66% and a specificity of 99% when compared with OGTT. HbA1C is considered to be more reliable than OGTT (Barr et al., 2002). HbA1C values are closely associated with plasma glucose levels over a four-month period. The measurement is a weighted average, with 50% of the value explained by the most recent month, 25% of the value by the preceding month, and 25% accounted for plasma glucose concentration 3 and 4 months prior to the test. HbA1C is the gold standard for glycemic control and correlates best with mean plasma glucose. Both fasting and post-prandial glucose excursions contribute to HbA1C. Postprandial glucose elevations often precede elevations in fasting plasma glucose in the development of diabetes. Postprandial glucose levels in approximately a third of individuals can rise above the 200 mg/dl level with HbA1C levels of <7% (Schrot, 2004). There is evidence
that the post-prandial glucose contributes more to lower HbA1C (<8.4%) values, while fasting glucose is more contributory to higher HbA1C levels (>8.4 %) (Monnier et al., 2003). One caveat to interpreting HbA1C values relates to elevations in HbA1C values in the presence of iron deficiency anemia and subsequent lowering of HbA1C values with iron treatment (Coban et al., 2004).

Davidson and coworkers (1999) evaluated the diagnostic criteria for both type 2 DM and FPG based on old and new diagnostic criteria, using the HbA1C to add credibility to the diagnosis. The old diagnostic criteria for DM were based on a FPG of ≥140 mg/dl instead of the current standard of ≥126 mg/dl or higher; there was no change in the two hour or random plasma glucose measurement of ≥200mg/dl. It should be noted that normal and impaired fasting glucose criteria have been revised downward in the years since this study. Davidson and colleagues (1999) evaluated two large data sets, NHANES III and Meta-Analysis Research Group (MRG), for HbA1C related to the four criteria shown in Table 3. The HbA1C values for normal intermediate and elevated were determined by establishing upper limits of normal, upper limits of normal plus 1%, and greater than upper limit of normal plus 1% for each data set.
TABLE 3. Comparison of HbA1C values from NHANES III & MRG Data Sets For Normal, IGT, FPG & DM Categories

<table>
<thead>
<tr>
<th>Diagnostic Parameter</th>
<th>NHANES III</th>
<th></th>
<th>MARG</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HbA1C %</td>
<td></td>
<td>HbA1C %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤6.1</td>
<td>6.2-7.0</td>
<td>≥7.1</td>
<td>≤6.3</td>
</tr>
<tr>
<td>Norm FPG (≤110mg/dL)</td>
<td>97.3%</td>
<td>2.7%</td>
<td>0.1%</td>
<td>96.2%</td>
</tr>
<tr>
<td>IFG (110-125mg/dL)</td>
<td>86.7%</td>
<td>13.1%</td>
<td>0.2%</td>
<td>81.4%</td>
</tr>
<tr>
<td>DM new guidelines FPG (126-139mg/dL)</td>
<td>60.9%</td>
<td>35.8%</td>
<td>3.4%</td>
<td>59.6%</td>
</tr>
<tr>
<td>DM old guidelines FPG (≥140 mg/dL)</td>
<td>18.6%</td>
<td>32.5%</td>
<td>48.9%</td>
<td>16.7%</td>
</tr>
</tbody>
</table>

Based on this information the authors proposed that the diagnosis of diabetes be made using the old standard i.e., FPG of ≥140 mg/dL. They recommended further that if the new standard of FPG of >126 mg/dL is used, the diagnosis, DM, should be reserved for those who also have a significant level of glycoslylation (elevated HbA1C).

*Beta Cell (β-cell) Exhaustion*

Alterations in β-cell function in type 2 DM are gaining more attention. Pancreatic β-cell problems are evident in reduced response to glucose changes in “pulsatile and oscillatory insulin secretion” and changes in the ease of converting proinsulin to insulin (Kahn, 2005). Type 1 DM has been considered a separate entity that is driven by an immune attack on the beta cells. β-cell dysfunction in type 2 DM has been relatively neglected until recently.
The Accelerator Hypothesis re-evaluates the classifications of type 1 and type 2 diabetes and suggests that they are one entity. This unifying theory “at its simplest can be reduced to the unfavorable interplay of two phenomena: disturbed β-cell apoptosis and insulin resistance. Apoptosis occurs throughout life at a variable rate but is intrinsically higher in those who are susceptible to diabetes” (Wilkin, 2001, p. 919). In type 2 DM, the loss of beta cells is driven primarily by weight gain and physical inactivity. The β-cells are metabolically and immunogenically upregulated when stressed by rising blood sugar (glucotoxicity), which “accelerates” β-cell loss in a slow progressive manner. Insulin resistance is the result of progressively increasing body weight and is considered the reason for increasing numbers of children developing type 2 DM. The β-cells initially attempt to overcome the rising glucose due to insulin resistance through hyperplasia and increased insulin secretion. The mounting insulin resistance eventually becomes too great for the overworked beta cells, and accelerated beta-cell apoptosis occurs. Apoptosis, or the physiologic process of cell turnover (death), is capable of inducing an immune response from primed cytotoxic T cells and induced autoimmunity. In type 1 DM, weight increase, insulin resistance, and metabolic upregulation occur in the same manner. However, in type 1 DM, an aggressive immune response genotype against the upregulated β-cells maximally “accelerates” the loss of beta cells.

According to the ‘Accelerator Hypothesis’, the link between weight gain, insulin resistance, and beta cell apoptosis is related to an immune response by tumor necrosis factor-α (TNF-α) that is secreted in large amounts by visceral fat. TNF-α, a cytokine increases insulin resistance at the insulin receptor and accelerates beta cell apoptosis. In addition, visceral fat produces fatty acids, which escalate beta-cell apoptosis. Wilkin
(2001) concludes that the best mechanism for preventing both type 1 and type 2 DM is to control weight gain and, with it, insulin resistance.

Type 2 Diabetes Mellitus

Diabetes occurs as the result of a progressive loss of beta cells in the pancreas. Insulin resistance is thought to be the factor that leads to the beta cell loss in type 2 DM. Type 2 DM is defined by the American Diabetes Association (ADA) as including:

1. “Symptoms of diabetes and a casual plasma glucose of \( \geq 200 \text{ mg/dL} \) (11.1 mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polydypsia, polyuria, and unexplained weight loss.

2. A fasting plasma glucose (FPG) of \( \geq 126 \text{ mg/dL} \) (7.0 mmol/L). Fasting is defined as no energy intake for at least 8 hours.

3. A 2-hour post load glucose of \( \geq 200 \text{ mg/dL} \) (11.1 mmol/L) during an oral glucose tolerance test (OGTT). The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day. The third OGTT is not recommended for routine clinical use.” (2005, p. S41).

Pima Indian children 10-14 years old have a prevalence of type 2 DM of 22.3 per 1000; while 15-19 year olds have a prevalence of 50.9 per 1000, as compared with 4.1 per 1000 prevalence for the U.S. (NHANES III). The mean age at diagnosis of type 2 DM was 12-16 years of age, though the youngest Pima child was 4 years at the time of diagnosis. All of these children had at least one parent with type 2 DM. The disease is
most often diagnosed during puberty, with more females than males presenting with the
diagnosis. Children who had a mother with diabetes during pregnancy were more at risk
than children whose mothers developed diabetes after the pregnancy. Low birth weight is
also associated with the diagnosis diabetes in Pima children, although not in other
populations (Fagot-Campagna et al, 2000).

Conceptual Model

The conceptual model for the study (Fig. 1) presents factors considered to
contribute to the development of type 2 DM. The literature suggests that excess fat
expressed as increasing BMI combined with insulin resistance is the impetus for the
eventual expression of DM. Contributing to the increasing BMI and insulin resistance are
genetics and uterine environment factors over which the individual has no control.
Inactivity and over-nutrition are lifestyle factors that are modifiable and provide an
opportunity for intervention.

The lower part of the model describes the links between measures associated with
insulin resistance and factors described in the model. It is the usefulness and relative
predictability of these measures that are the focus of this study, which will evaluate how
factors associated with increasing BMI and insulin resistance in childhood impact
measurements of glycemia. This conceptual model implies that changes in BMI, lipids,
blood pressure, waist circumference and previous glycemia measures promote changes in
glycemia at follow-up. The degree to which each of these factors predicts changes, and
the specificity and accuracy of available measures for each factor will be explored in this
research.
Simplified Model of Progression to DM

- Genetics
- Uterine Environment
- Inactivity

Over-nutrition

BMI → Insulin Resistance → Blood Sugar → Beta-cell exhaustion → DM

Stage I

Stage II

Stage III

Relationship of Predictor Factors to Changes in Glycemia

Childhood & Interim Exams

- BMI
- Lipids
- BP
- Waist Circum.
- HbA1C
- 2°GTT
- FPG

Last Follow-up

- HbA1C
- 2°GTT
- FPG

FIGURE 1: Conceptual Framework
Summary

The conceptual model for this study was determined through a review of the literature. The literature supports, with overwhelming evidence, the dire consequences of obesity—not only in terms of its relationship to the development of type 2 DM, but also its impact on virtually all systems of the body. The aspect of obesity that is applicable to this study primarily relates to those factors associated with insulin resistance and type 2 DM, with an emphasis on their presentation in childhood. This conceptual model describes in simplified terms the manner in which type 2 DM develops. The associated model presents the conceptualization of the impact of changes in risk factors on glycemic measures (HbA1C, 2^0 GTT, FPG). These diabetes risk factors are identified as factors in the metabolic/insulin resistance syndromes and are also associated with cardiovascular disease and end stage renal disease. The chapter presented the dynamics of these risk factors in terms of their usual measurements and as they relate to disease. The literature also suggests the importance of identifying the physiologic mechanisms in childhood that predispose to disease.
CHAPTER III: METHODS AND DESIGN

This chapter describes the research methods and the database used to conduct the study. The study focused on the usefulness of measures of glycemia in children. The ongoing Pima longitudinal epidemiologic study offered an opportunity to determine the predictive value of these childhood measurements on the development of diabetes. The incidence rate as well as the cumulative incidence of diabetes and of elevated HbA1C was computed. In addition, the effect of BMI, lipids, blood pressure, and waist circumference on changes in glycemia was examined. Determining the predictive value of measures of glycemia during childhood has implications for diagnosis, clinical practice, and interventional studies related to children.

Research Questions

1. What effect does the level of:

   - HbA1C in childhood have on the development of an HBA1C of >8% later in life?
   - Two hour plasma glucose (2^0PG) in childhood have on the development of a 2^0PG of >200mg/dl later in life?
   - Fasting plasma glucose (FPG) in childhood have on the development of a FPG≥126 mg/dl later in life?

2. How do changes in BMI, lipids (Total Cholesterol, Triglycerides, and High Density Lipoproteins), blood pressure and waist circumference predict changes in glycemic measures (HbA1C, Fasting Glucose, and 2^0PG)?
Methods

Design

This research is a secondary data analysis of a primary longitudinal prospective diabetes study NIH/NIDDK has conducted in the Gila River Indian Community since 1965. The secondary data analysis will be limited to data collected prior to 12/31/04.

Data Source and Collection

The National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) has conducted a longitudinal study of Diabetes in the Gila River Indian Community since 1965. Community members who are primarily Pima are invited to participate in research examinations every two years from 5 years of age on. Participants who have been fully informed consent to the examination. Minors have parental/guardian consent and also assent to the procedures. All protocols have been reviewed and approved by the NIDDK institutional review board (IRB). These examinations are done at a clinic in Sacaton, Arizona. Demographic data, anthropometric measures, venous blood samples, urine samples, EKG, dxa scan, and dilated eye exam are performed by trained personnel employed by NIDDK.

Measures and Procedures

Blood (venous plasma) samples are obtained after an overnight fast, and stand in EDTA. Plasma is then separated after centrifugation at ~700g for 15 minutes at 10° Centigrade. Glucose is measured while the subject is fasting (12 hour) and two-hours after a 75 g carbohydrate load. The diagnosis of diabetes is made based on the WHO (1985) criteria. HbA1C was measured from November 1989 to the present time. Assays from November 1989 to December 2000 used the Bio Rad high-performance
chromatography and immunoassay methods (HPLC) while those from January 2000 to present utilize the TOSO assay (National Glycohemoglobin Standardization Program standards). Fasting lipids (serum), inclusive of total cholesterol, triglycerides, high-density lipoproteins (HDL) and calculated low-density lipoproteins (LDL), were measured since June 1, 1993. Prior to that time total cholesterol alone was measured. Height and weight are measured using a fixed stadiometer and calibrated scales. From this, a BMI (kg/m$^2$) is calculated. Waist circumference is measured to the nearest centimeter at the narrowest point or at the umbilicus. Blood pressure is measured with a mercury sphygmomanometer and a large adult cuff to the nearest 2 mmHg with subjects resting in the supine position. The diastolic reading is based on the fourth Korotkoff sound. Height, weight, and BMI in children are compared with standards published by the Centers for Disease Control and Prevention, National Center for Health Statistics. The extent to which each individual deviates from the reference value is calculated according to a normalized score (Lindsay, 2004).

Sample

The population used for this analysis included all subjects with at least one examination as a child (5-19 years of age) and a minimum of one follow-up examination at which HbA1c and lipid fractions were measured. Accordingly, only examinations occurring after June 1, 1983 were included. The sample size was 2658.
Data Management

Data collected from the examinations are entered and stored in a secure computer system housed at NIDDK. Security on this system requires password access. Passwords are provided on a limited basis to certain NIDDK employees. As an NIDDK employee, I worked through the established security system of the Institute. NIDDK continuously monitors all individuals working on NIDDK computers or through remote access VPN accounts. For the purposes of this study, the data set with identifiers was used in the analysis phase, while all reports contain only de-identified information that has been aggregated.

Human Subjects

Protocols are in place to assure human subjects protection. These protocols are reviewed annually or more frequently if modifications are made. These reviews are accomplished through the NIDDK institutional review board process (IRB). All personnel who have participant contact have been trained in appropriate procedures. Informed consent is provided by the individual or if a minor by the parent/guardian with an assent from the minor. Participants are informed of their study results. Referral mechanisms are in place for appropriate follow-up if participants should need such services. Approval for this study also was obtained from the Investigational Review Board (IRB) at the University of Arizona.
Data Analysis

Question One

To answer Question 1 required computing the incidence rate, the cumulative incidence, and hazards rate ratio—using Cox proportional hazards modeling of elevated glycemia (HbA1C, 2°PG, FPG) in individuals measured first as children between the ages of 5-19.

Incidences and Cumulative Incidence. Incidence and cumulative incidence rates are useful in quantifying the frequency of disease in a population. When a disease (DM) is identified it is termed an outcome event. Time is an important element in the calculation with incidence time determined by the amount time that elapses from no disease to onset of disease, no disease, or death. Every member of a population accumulates a specific amount of time for the risk period; the sum of these times for all members is the total person-time at risk. The incidence rate can then be calculated with the number of new cases of DM divided by the person-time.

\[
\text{Incidence rate} = \frac{\text{Number of cases of a Disease onsets}}{\Sigma \text{ time spent in population persons}}
\]

The cumulative incidence, or frequency of development of elevated glycemia, will be reported in 5-year age increments. Cumulative incidence is a probabilistic model that estimates the average risk for members of a group who are at risk but disease free at the first evaluation. Risk is a conditional probability with a time reference “where:

\[
R(t_0, t) = \hat{C}I(t_0, t) = I/N_0;
\]

\[
R(t_0, t) = \Delta t \text{ risk of disease (for an individual)};
\]

\[
CI = \text{average } \Delta t \text{ risk for members of the cohort};
\]

\[
\Delta t = t - t_0 \text{ - duration of follow-up};
\]
I = number of incident cases of disease;

\( N'_0 \) = number of persons at risk at the start of the follow-up period, i.e. at time \( t_0 \)

(Note: The hat over a parameter indicates an estimator of that parameter” (Kleinbaum D, 1982 as presented in Epidemiology Notebook, p. 110).

A simplified equation is:

Confidence Interval = \( \frac{\text{Number of new cases of a disease during a given time period}}{\text{Total population at risk (Fromm, R.,1996)}} \)

Constraints to the use of this method are related to the loss of participants from observation due to: 1) death from any cause, 2) changes or events that remove the participant from risk, 3) loss to follow-up, or 4) an extended intake period.

**FIGURE 2: Example: Simple Cumulative Method of Risk Estimation**

Key:  
\( \bullet \) = New occurrence of disease  
\( O \) = Death  
\( - \) = Living with disease X
=Living without disease X (but at risk)

Assumptions:

- All events occur at the midpoints of each year interval
- Disease X is incurable in this study death is relevant only if it removes people as potential cases.

Estimated 20 year risk of disease X = \( R_{(0,20)} = \frac{3}{5} = 0.60 = 60\% \)

(Note: The denominator for this estimate is 5 (not 7) because subjects 3 and 6 were not at risk at the start of the follow-up. In addition, the early deaths of subjects 2 and 5 create a problem in knowing whether these individuals would have developed the disease had they lived long enough (Kleinbaum D, 1982).

*Cox Proportional Hazards.* Cox proportional hazards analysis was used to evaluate the effect of glycemic measures (HbA1C, 2⁰PG, FPG) in childhood on the development of type 2 DM later in life while controlling for the potentially confounding effects of age, sex, BMI, cholesterol, and MAP. The Cox model computes the hazard rate of developing type 2 DM relative to an exposure variable, in this case glycemia, while controlling for the confounding effects of covariates. A hazard rate ratio for a one-unit change in exposure level is the effect measure of interest. In a valid proportional hazards model, this hazard ratio is constant over time, an assumption that can be tested statistically.
The proportional hazards assumption assumes that effect parameters multiply hazard: for example, if a low HbA1C halves your hazard at time 0, it also halves your hazard at time 1, or at time 0.5, or at time t for any value of t. The effect parameters estimated by the proportional hazards model are reported as hazard ratios.

To ensure validity of the models, each glycemic measure model was evaluated to determine if it satisfied the proportionality assumption. In addition, interaction terms between covariates were examined to determine if one covariate influenced another.

A time dependent analysis was conducted, which uses values of covariates from each research examination. If values changed at an examination the new values were included for the appropriate time periods.

**Question Two**

The analysis done to answer Question 2 used multiple linear regression models. We used multiple linear regressions to evaluate the effect of changes in measurements of metabolic factors on changes in HbA1C. A best-fit model was sought to identify the metabolic factors that best predicted a change in HbA1C. The best fit included those variables, which alone or in combination, achieved the highest R² value.

Changes in each of the variables were identified by computing the difference between measures at each follow-up exam in childhood divided by differences in age at each follow-up exam, yielding a slope for the variable of interest. For example, if the current HbA1C was 5.1% and the previous HbA1C was 4.9% and current age was 13.5 years and the previous age was 11.5 years the slope of HbA1C was 0.1%/year. In equation format:
The analysis evaluated changes in variables in three ways. The first, a short time span group, involved restricting the analysis to subjects with intervals between examinations of 1.5 to 2.5 years. The second, an unrestricted time span group, allowed any span of time between exams as long as the exams were successive and occurred during childhood (5-19 years of age). A third approach, age and gender categorization sorted the exams into age groups (5-19, 5-9, 10-14, 15-19), and gender (male, female). This categorization was performed for both time span groups.

The analysis of the short time span group and age and gender categorization were done to diminish the impact of maturational changes on the variables and to examine how variables might change in an intervention project of short duration. This shortened time frame and the limitations of age and gender categorizations, however, reduces the sample size, and amplifies slope estimate errors. The unrestricted time span group allowed for analyses of the strength of the models in the context of a larger sample size in an expanded time span between exams.

Linear regression models were run using multiple combinations of the independent variables of interest (BMI, lipids, MAP, and waist circumference) in relationship to the dependent variable (HbA1C). Validity of the linear regression models was assessed with residual plot analysis.
Summary

The usefulness of measures of glycemia in children is the focus of this study. An analysis of the Pima longitudinal epidemiologic study offers an opportunity to determine the predictive value of these measurements made during childhood on the development of diabetes later in life (incidence, cumulative incidence, Cox proportional hazards). In addition, the relationship of changes (increase or decrease) in: BMI, lipids, blood pressure, waist circumference, and glycemia (predictive factors) measured in childhood and in subsequent exams on changes in glycemia at the final exam will be assessed using multiple linear regression models. Determining the value of these measures of predictive factors during childhood has implications for diagnosis, clinical practice, and interventional studies related to children.
CHAPTER IV: RESULTS

This chapter describes the results of the analyses done to examine the usefulness of glycemic measures in children. Data from the ongoing Pima epidemiologic study were used to determine the predictive value of glycemic measurements made during childhood on the development of diabetes later in life. The predictive value of measures of glycemia made during childhood has implications for diagnosis, clinical practice, and interventional studies related to diabetes prevention in children. In addition, changes in metabolic factors associated with the metabolic syndrome, including body mass index (BMI), waist circumference, blood pressure and cholesterol, were evaluated to determine how they predict changes in glycemia. Some of these metabolic factors are predictive of diabetes and contribute to the development of diabetic complications.

Description of the Sample

A retrospective analysis was conducted using the Pima Indian data set, which is maintained by the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK). A subset of the larger NIDDK data set was created to examine the effect of childhood glycemia (HbA1C, 2oPG, FPG) on development of diabetes in later life. The effects of changes in BMI, waist circumference, blood pressure (BP) and lipids on changes in glycemia were also examined. Inclusion in the subset was premised on:

1) An initial exam as a non-diabetic child, 5-19 years of age, with at least 50% American Indian heritage and at least one follow-up exam.

2) The presence of three glycemic measures (HbA1 or HbA1C, 2oPG, FPG) at all exams.
3) Childhood measures of waist circumference, height, weight, BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP), mean arterial pressure (MAP) \((2 \times DBP + SBP)/3\), serum cholesterol, high density lipoproteins (HDL), and triglycerides.

**Participants**

There were 2,658 individuals (1,227 boys, 1,431 girls) who met the inclusion criteria. Subjects were divided into three groups on the basis of age at their initial childhood exam; 929 (35%) were in the 5-9 year age group, 1,210 (46%) were in the 10-14 year age group, and 519 (19%) were in the 15-19 year old age group. These 5-year age group categories permitted examination of effects in children at similar levels of maturation (primary school age, early adolescence and late adolescence). Tanner staging, a method of defining pubertal development, of children is not done as part of the research examination, and pubertal changes were identified only in girls by the onset of menarche.

**TABLE 4. Age and Gender Characteristics of Study Population at Baseline**

<table>
<thead>
<tr>
<th>Initial Exam</th>
<th>N and Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td>Age Group (yrs) / Gender</td>
<td></td>
</tr>
<tr>
<td>5-9</td>
<td>929 (35%)</td>
</tr>
<tr>
<td>Male</td>
<td>451 (17%)</td>
</tr>
<tr>
<td>Female</td>
<td>478 (18%)</td>
</tr>
<tr>
<td>10-14</td>
<td>1210 (46%)</td>
</tr>
<tr>
<td>Male</td>
<td>564 (21%)</td>
</tr>
<tr>
<td>Variable</td>
<td>Female</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>646 (24%)</td>
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</table>

**TABLE 5. Baseline Characteristics of Study Population**

<table>
<thead>
<tr>
<th>Variable (N)</th>
<th>Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP mmHg (2653)</strong></td>
<td><strong>105 (60-300)</strong></td>
</tr>
<tr>
<td>• 5-9 age group (930)</td>
<td>• 96 (60-300)</td>
</tr>
<tr>
<td>• 10-14 age group (1208)</td>
<td>• 108 (60-300)</td>
</tr>
<tr>
<td>• 15-19 age group (515)</td>
<td>• 112 (72-170)</td>
</tr>
<tr>
<td><strong>DBP mmHg (2645)</strong></td>
<td><strong>58 (20-104)</strong></td>
</tr>
<tr>
<td>• 5-9 age group (928)</td>
<td>• 54 (20-95)</td>
</tr>
<tr>
<td>• 10-14 age group (1203)</td>
<td>• 59 (20-92)</td>
</tr>
<tr>
<td>• 15-19 age group (514)</td>
<td>• 63 (20-104)</td>
</tr>
<tr>
<td><strong>MAP mmHg (2645)</strong></td>
<td><strong>74 (33-115)</strong></td>
</tr>
<tr>
<td>• 5-9 age group (928)</td>
<td>• 68 (33-110)</td>
</tr>
<tr>
<td>• 10-14 age group (1203)</td>
<td>• 75 (38-111)</td>
</tr>
<tr>
<td>• 15-19 age group (514)</td>
<td>• 80 (47-115)</td>
</tr>
<tr>
<td><strong>FPG mg/dl (2658)</strong></td>
<td><strong>89 (60-120)</strong></td>
</tr>
<tr>
<td>Test</td>
<td>5-9 age group (932)</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>2º PG mg/dl (2658)</td>
<td>86 (60-110)</td>
</tr>
<tr>
<td>*HbA1C % (2658)</td>
<td>4.8 (2.1-7.9)</td>
</tr>
<tr>
<td>Cholesterol mg/dl (2658)</td>
<td>149 (62-275)</td>
</tr>
<tr>
<td>Triglycerides mg/dl (1072)</td>
<td>84 (8-512)</td>
</tr>
<tr>
<td>HDL mg/dl (1099)</td>
<td>45 (21-102)</td>
</tr>
</tbody>
</table>
- 15-19 age group (128)

**Waist Circumference cm (1581)**

- 5-9 age group (694)
- 10-14 age group (705)
- 15-19 age group (182)

**Body Mass Index kg/m² (2656)**

- Male (1227)
  - 5-9 age group (932)
    - Male (452)
    - Female (480)
  - 10-14 age group (1210)
    - Male (565)
    - Female (645)
  - 15-19 age group (514)
    - Male (209)
    - Female (304)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male Frequency</th>
<th>Female Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9</td>
<td>452</td>
<td>480</td>
</tr>
<tr>
<td>10-14</td>
<td>565</td>
<td>645</td>
</tr>
<tr>
<td>15-19</td>
<td>209</td>
<td>304</td>
</tr>
</tbody>
</table>

**Waist Circumference cm (1581)**

- 5-9 age group (694)
- 10-14 age group (705)
- 15-19 age group (182)

**Body Mass Index kg/m² (2656)**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male Frequency</th>
<th>Female Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9</td>
<td>452</td>
<td>480</td>
</tr>
<tr>
<td>10-14</td>
<td>565</td>
<td>645</td>
</tr>
<tr>
<td>15-19</td>
<td>209</td>
<td>304</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male Frequency</th>
<th>Female Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9</td>
<td>452</td>
<td>480</td>
</tr>
<tr>
<td>10-14</td>
<td>565</td>
<td>645</td>
</tr>
<tr>
<td>15-19</td>
<td>209</td>
<td>304</td>
</tr>
</tbody>
</table>

**Body Mass Index kg/m² (2656)**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male Frequency</th>
<th>Female Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9</td>
<td>452</td>
<td>480</td>
</tr>
<tr>
<td>10-14</td>
<td>565</td>
<td>645</td>
</tr>
<tr>
<td>15-19</td>
<td>209</td>
<td>304</td>
</tr>
</tbody>
</table>

- 43 (21-93)

**76 (27-142)**

- 66 (27-117)
  *(excluded 1 child age 8 with measure=0)*
- 81 (50-137)
- 94 (64-142)

23.9 (13.2 -59.4)

- 23.1 (13.6-55.3)
- 24.5 (13.2-59.4)
- 20.3 (13.2-55.3)
- 20.2 (13.6-55.3)
- 20.4 (13.2-40.4)
- 24.6 (13.7-51.4)
- 23.7 (13.8-49.0)
- 25.3 (13.7-51.4)
- 28.5 (16-59)
- 27.2 (17.3-48.6)
- 29.3 (16.0-59.3)

*HbA1 in 1213 individuals was converted to HbA1C using the formula (0.99 X HbA1)-1.535=HBa1C*
Research Question One

1. What effect does the level of:

   - HbA1C in childhood have on the development of an HbA1C >8% later in life?
   - 2⁰PG in childhood have on the development of a 2⁰PG ≥200mg/dl later in life?
   - FPG in childhood have on the development of a FPG≥ 126 later in life?

To answer this three-part question, I computed the incidence and cumulative incidence of diabetes according to baseline levels of these glycemic measures. Results were expressed by quartiles of baseline glycemia. Cox Proportional Hazards modeling was used to examine the effect of glycemia on diabetes after controlling for the potential confounding effects of age, sex, BMI, MAP, and cholesterol. All children were free of diabetes when first evaluated. The follow-up exams could occur in either childhood or adulthood based on when the individual was examined in the biennial clinic. Diabetes was defined by the presence of at least one of four criteria: 1) 2⁰PG of ≥200 mg/dl. 2) FPG of ≥126 mg/dl., 3) HbA1C > 8.0%, or 4) hypoglycemic treatment.

There were 258 cases of type 2 DM, of which 62 (24%) were diagnosed in childhood. Of these childhood cases, 23 (38%) were diagnosed with a 2-hour postprandial glucose (2⁰PG) only, 35 (57%) with both a FPG and 2⁰PG elevation, 3 (5%) with a FPG only, and 1 (2%) with HbA1C of 8.8% (FPG of 101 mg/dl and 20PG of 161 mg/dl). For adults the 2⁰PG alone identified 62 (31%), the FPG and 2⁰PG 111 (57%), the FPG alone 23 (12%), and 1 (0.05%) with HbA1C of 10.2% (FPG of 100 mg/dl, and 2⁰PG of 153 mg/dl) (Fig.3).
Incidence

Incidence was calculated as the number of new cases of diabetes that developed in study participants during the study period (1983-2004) per 1000 person-years. For example, if 100 children entered the study and two of these children developed type 2 DM at the end of 10 years follow-up, the incidence rate would be two per 1000 person-years assuming no deaths or other loss to follow-up during the study period.

Person-time accumulated from the baseline examination until the diagnosis of DM, death, or the last exam on or before December 31, 2004, whichever came first. The criteria for diagnosis of type 2 DM were based on measures of glycemia obtained in the NIH biennial clinic using the ADA criteria of FPG $\geq 126$ mg/dl or 2°PG of $\geq 200$ mg/dl, a history of receiving treatment for type 2 DM, or an HbA1C $\geq 8\%$. Follow-up did not continue after 40 years of age, due to the limited person-time of follow-up after that age.
Incidence rates were standardized to the 2000 US census population, and age-sex adjusted diabetes rates were computed. Standardization allows for interpreting the results according to the age distribution of the U.S. population. Among 2658 non-diabetic children, 258 cases of diabetes occurred during an average of 9.1 (range=1.5 - 21.7) years of follow-up. Incidence rates were calculated based on 24,075.2 person-years of follow-up (Table 6). The overall age and sex-adjusted rate was 19.0 cases per 1000 person years with an incidence rate of 15.1 for males and 22.8 for females per 1000 person years. The incidence rate increased steadily with age in males and females, with slightly higher rates in the females in each age category except for the 30-34 year olds.

**Cumulative Incidence**

*Cumulative incidence* provides an estimate of the probability or risk of developing type 2 DM by the end of a specified time interval. For this study, cumulative incidence was defined as the proportion of subjects within an age group who developed diabetes by the time they reached the oldest age in that group. Cumulative incidence was calculated from the incidence rate, assuming stability of incidence over the time interval of interest. The cumulative incidence of diabetes was 54% by age 40 years (Table 6 and Figure 3).

**TABLE 6. Age-Sex Specific Incidence and Cumulative Incidence of Diabetes**

<table>
<thead>
<tr>
<th>Age Group (yrs)</th>
<th>Cases</th>
<th>Person Years</th>
<th>Incidence</th>
<th>Cumulative Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>% With type 2 DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>802.7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Age Group</td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
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<td></td>
</tr>
<tr>
<td>10-14</td>
<td>8</td>
<td>13</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3007.1</td>
<td>3471.9</td>
<td>6479.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>4.7</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.6</td>
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<tr>
<td>15-19</td>
<td>17</td>
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<td>7005.7</td>
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<td>4.5</td>
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<td>13.6</td>
<td>16.0</td>
<td>13.6</td>
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<tr>
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<td>10.7</td>
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<td>25-29</td>
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<td>72</td>
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<td>1079.3</td>
<td>1572.9</td>
<td>2652.2</td>
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<tr>
<td></td>
<td>27.0</td>
<td>33.6</td>
<td>27.2</td>
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<td>22.1</td>
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<td>30-34</td>
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<td>44</td>
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<tr>
<td></td>
<td>436.2</td>
<td>689.2</td>
<td>1125.5</td>
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<tr>
<td></td>
<td>60.2</td>
<td>39.1</td>
<td>39.1</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
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</tr>
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<td>------</td>
<td>--------</td>
<td>-------</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>11</td>
<td>13</td>
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</tr>
<tr>
<td></td>
<td>72.7</td>
<td>122</td>
<td>193.8</td>
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</tr>
<tr>
<td></td>
<td>66.2</td>
<td>109.7</td>
<td>67.1</td>
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</tr>
<tr>
<td>Adjusted Sex</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td>15.1</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td>22.8</td>
<td></td>
</tr>
<tr>
<td>Age-Sex</td>
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</tr>
<tr>
<td>Adjusted</td>
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<td></td>
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</tr>
<tr>
<td>Total</td>
<td>258</td>
<td>24075.2</td>
<td>24075.2</td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 4. Cumulative Incidence of DM**
Glycemic Measure Quartiles

Glycemic measures were divided into quartiles to examine the effect of various levels of glycemia on the incidence of type 2 DM. The age-sex adjusted incidence of type 2 DM was calculated in each quartile, and confidence intervals were computed for the age-sex adjusted rates. Trends in 2⁰PG and FPG were examined by the Mantel-Hanzel extension test; the statistical significance of the trends is noted in Figures 3 and 4. The Mantel-Hanzel extension test computes a chi-square with one degree of freedom to determine whether there is a statistically significant monotonic trend, an increase or decrease in disease frequency with increasing levels of exposure. This test was not appropriate for HbA1C because the relationship was not monotonic, but instead was u-shaped (Fig. 4). DM incidence rate ratios (IRR) were computed across quartiles with the first quartile serving as the referent. IRR is the ratio of two incidence rates. In this case, the incidence rate for a specific quartile, divided by the incidence rate of first quartile, gives a relative measure of the effect of a higher quartile of glycemia. This allowed comparison of each of the later quartiles with the first quartile.

HbA1C Incidence by Quartile. The incidence of diabetes derived from baseline HbA1C quartiles is presented in Figure 4. There is a u-shaped pattern for the incidence of DM by quartiles with a lower incidence of DM in the second and third quartile lower than in the first and fourth, as noted below:

- IRR 0.6 (95% CI=0.2-1.5) times as high in quartile 2 as in quartile 1
- IRR 0.7 (95% CI=0.3, 1.6) times as high in quartile 3 as in quartile 1
- IRR 1.6 (95% CI=0.8, 3.1) times as high in quartile 4 as in quartile 1
FIGURE 5. Age and Sex Adjusted Incidence (95% CI) of Type 2 DM Based on Baseline HbA1C Quartile

2<sup>o</sup>PG Incidence by Quartile. The incidence of diabetes derived from quartiles of baseline 2<sup>o</sup>PG is presented in Figure 5. Diabetes incidence for this measure increased monotonically, with the greatest increases in the second and fourth quartiles.

- IRR 3.8 (95% CI=1.6, 9.1) times as high in quartile 2 as in quartile 1
- IRR 3.8 (95% CI=1.7, 8.4) times as high in quartile 3 as in quartile 1
- IRR 6.6 (95% CI=3.6, 12.2) times as high in quartile 4 as in quartile 1
FIGURE 6. Age-Sex Adjusted Incidence (95% CI) of Type 2 DM Based on Baseline 2⁰PG Quartile

**FPG Incidence by Quartile.** The incidence of DM derived from quartiles of baseline FPG is presented in Figure 6. The pattern of DM incidence was monotonic for this measure as well.

- IRR 2.3 (95% CI=0.9, 5.9) times as high in quartile 2 as in quartile 1
- IRR 2.4 (95% CI=0.9, 6.4) times as high in quartile 3 as in quartile 1
- IRR 5.5 (95% CI=2.5, 11.9) times as high in quartile 4 as in quartile 1
Cox Proportional Hazards

*Cox proportional hazards analysis* was used to evaluate the effect of glycemic measures (HbA1C, 2⁰PG, FPG) in childhood on the development of type 2 DM later in life while controlling for the potentially confounding effects of age, sex, BMI, cholesterol, and MAP. The Cox model computes the hazard rate of developing type 2 DM relative to an exposure variable, in this case glycemia, while controlling for the confounding effects of covariates. A hazard rate ratio for a one-unit change in exposure level is the effect measure of interest. In a valid proportional hazards model, this hazard ratio is constant over time, an assumption that can be tested statistically. The *proportional hazards assumption* is that effect parameters multiply hazard: for example, if a low HbA1C halves your hazard at time 0, it also halves your hazard at time 1, or at
time 0.5, or at time $t$ for any value of $t$. The effect parameter(s) estimated by the proportional hazards model are reported as hazard ratios.

To ensure validity of the models, each glycemic measure model was evaluated to determine if it satisfied the proportionality assumption. The proportionality assumption was not violated for HbA1C or FPG models, but it was violated for the 2°PG, so models of 2°PG were not computed. In addition, interaction terms between covariates were examined to determine if one covariate influenced another. Since these product terms did not improve the regression models, they were not included in the final models.

I conducted a time dependent analysis, which uses values of covariates from each research examination. If values changed at an examination the new values were included for the appropriate time periods. The time dependent model was calculated for HbA1C and FPG and the results are presented below.

Because of the non-linear nature of the relationship between HbA1C and diabetes in the stratified analysis (Figure 4), I conducted two separate proportional hazards analyses to examine more closely the nature of this relationship. For one analysis, a quadratic term was added to the model because the effect of this variable was u-shaped (quadratic). In another analysis, the baseline HbA1C values were divided into quartiles as defined previously (Figure 4) for the stratified analysis.

In the proportional hazards model that included the quadratic term, there was a significant positive relationship between HbA1C and diabetes (Table 7). The quadratic term was highly significant, confirming the non-linear relationship noted previously in the statistical analysis.
TABLE 7. Effect of HbA1C and Squared HbA1C on Type 2 DM in a Time Dependent
Proportional Hazards Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta$</th>
<th>Hazards Rate</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.9</td>
<td>0.71-1.22</td>
<td></td>
</tr>
<tr>
<td>Age (10 year intervals)</td>
<td>1.5</td>
<td>1.05-2.04</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (50 mg/dl)</td>
<td>1.0</td>
<td>0.79-1.17</td>
<td></td>
</tr>
<tr>
<td>BMI (5 kg/m$^2$)</td>
<td>1.2</td>
<td>1.16-1.33</td>
<td></td>
</tr>
<tr>
<td>MAP (10 mmHg)</td>
<td>1.0</td>
<td>0.91-1.18</td>
<td></td>
</tr>
<tr>
<td>HbA1C (1%)</td>
<td>-3.45 (p&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1C squared</td>
<td>0.41 (p&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$HbA1C (1%) *$</td>
<td></td>
<td>$\chi^2$=52.5514 $p&lt;.0001$</td>
<td></td>
</tr>
<tr>
<td>$HbA1C squared *$</td>
<td></td>
<td>$\chi^2$=52.5514 $p&lt;.0001$</td>
<td></td>
</tr>
</tbody>
</table>

*A likelihood ratio test was used to determine the significance of the effect of HbA1C. The quadratic term for HbA1c is statistically significant (p<.0001), confirming the u-shaped relationship between HbA1C found in the earlier stratified analysis (Figure 2).

The proportional hazards model that included quartiles of baseline HbA1c is not as powerful as the quadratic model because glycemia is not analyzed as a continuous variable, but this approach can be used to estimate the effect of HbA1C on the incidence of type 2 DM. This analysis indicates that a glucose value in the second quartile range imparts, on average, a 1.2-fold (95% CI 0.8-1.7) higher risk of diabetes than in the first quartile. The incidence in the third quartile is 1.3 times (95% CI 0.9-1.9) as high, and in fourth quartile is 2.7 times (95% CI 1.9-3.9) as high as in the first quartile (Table 8).
### TABLE 8. Effect of Quartiles of HbA1C on Type 2 DM in a Proportional Hazards Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazards Rate Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1.0</td>
<td>0.8-1.3</td>
</tr>
<tr>
<td>Age (10 year intervals)</td>
<td>1.5</td>
<td>1.1-2.0</td>
</tr>
<tr>
<td>Cholesterol (50 mg/dl)</td>
<td>1.0</td>
<td>0.8-1.2</td>
</tr>
<tr>
<td>BMI (5 kg/m²)</td>
<td>1.2</td>
<td>1.2-1.3</td>
</tr>
<tr>
<td>MAP (10 mmHg)</td>
<td>1.0</td>
<td>0.9-1.2</td>
</tr>
<tr>
<td>HbA1C quartile 2 (1%)</td>
<td>1.2</td>
<td>0.8-1.7</td>
</tr>
<tr>
<td>HbA1C quartile 3 (1%)</td>
<td>1.3</td>
<td>0.9-1.9</td>
</tr>
<tr>
<td>HbA1C quartile 4 (1%)</td>
<td>2.7</td>
<td>1.9-3.9</td>
</tr>
</tbody>
</table>

Table 9 presents the predictive value of FPG on the development of type 2 DM from a time dependent Cox model analysis. Each 10mg/dl increase of FPG nearly doubled the incidence of type 2 DM after controlling for the effects of age, sex, cholesterol, BMI, and MAP.

### TABLE 9. Effect of FPG on Type 2 DM in a Time Dependent Proportional Hazards Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazards Rate Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.8</td>
<td>0.6-1.1</td>
</tr>
<tr>
<td>Age (10 year intervals)</td>
<td>1.3</td>
<td>0.9-1.9</td>
</tr>
<tr>
<td>Cholesterol (50 mg/dl)</td>
<td>1.0</td>
<td>0.8-1.2</td>
</tr>
<tr>
<td>BMI (5 kg/m²)</td>
<td>1.2</td>
<td>1.1-1.3</td>
</tr>
<tr>
<td>MAP(10 mmHg)</td>
<td>1.0</td>
<td>0.9-1.2</td>
</tr>
<tr>
<td>FPG (10 mg/dl)</td>
<td>1.9</td>
<td>1.7-2.2</td>
</tr>
</tbody>
</table>
Research Question Two

2. How do changes in BMI, lipids (Total Cholesterol, Triglycerides, and HDL), blood pressure (MAP) and waist circumference predict changes in glycemic measures (HbA1C, FPG, and 2\textsuperscript{nd} PG)?

To answer question two, changes in BMI, lipids, MAP, and waist circumference were examined in relationship to changes in HbA1C alone. Changes in FPG and 2\textsuperscript{nd} PG are being examined by others in NIDDK and are not included in this study. Determining the usefulness of metabolic measures in relationship to HbA1C has meaning for intervention projects with children at risk for diabetes. If BMI, waist circumference and MAP changes (all non-invasive anthropometric measures) are associated with changes in HbA1C, a predictor of diabetes, these variables can each be measured with relative ease in a school based study without having to collect blood. The HbA1C collection in the school is also done with the relative ease of a finger stick. Measurements of risk factors that require an 8 hour period of fasting (Lipids, FPG) along with a 75 gram glucose load and an additional measurement 2 hours later (2\textsuperscript{nd} PG) are more demanding strategies and are potentially more difficult to apply in a school-based populations.

*Question Two Participants*

As mentioned earlier, the study population was drawn from the NIDDK database using measurements taken in children at exams between 1983 and 2004. 1983 was the first year that HbA1 was measured. Measures of lipids other than cholesterol were first made in 1993 and waist circumference was added to the examination in 1988. Of the 2,658 individuals identified in the baseline population to address question one, a subset was used for the present analysis. To be eligible for this analysis, subjects had to have
participated in two or more examinations in childhood that contained the measurements of interest. In addition:

1. Each child could contribute multiple data points based on the numbers of exams during childhood. For example if a child was examined at ages 5, 7, 9, 11, 13, 15, 17, & 19 years, that child would contribute 7 measures of change for each variable measured.

2. Examinations after the onset of type 2 DM were excluded from this analysis.

3. Examinations where the measure of interest was missing were also excluded from this analysis.

### TABLE 10. Baseline Characteristics of Question 2 Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Short Time Span (N=678)</th>
<th>Unrestricted Time Span (N=1010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>13.5 (6.7-19.9)</td>
<td>13.6 (6.7-19.9)</td>
</tr>
<tr>
<td>Previous Age</td>
<td>11.5 (5.1-18.2)</td>
<td>11.6 (5.1-18.2)</td>
</tr>
<tr>
<td>HbA1C %</td>
<td>5.1 (3.9-7.2)</td>
<td>4.8 (2.8-7.2)</td>
</tr>
<tr>
<td>Previous HbA1C%</td>
<td>4.9 (2.5-6.1)</td>
<td>4.8 (2.5-6.4)</td>
</tr>
<tr>
<td>HbA1C slope</td>
<td>0.08 (-0.53-+1.14)</td>
<td>0.03 (-0.91-+1.14)</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>27.7 (14.5-55.4)</td>
<td>26.9 (14.5-55.4)</td>
</tr>
<tr>
<td>Previous BMI kg/m²</td>
<td>24.8 (13.9-50.8)</td>
<td>24.1 (13.9-50.8)</td>
</tr>
<tr>
<td>BMI slope kg/m²</td>
<td>1.44 (-6.9-+6.8)</td>
<td>1.37 (-6.9-+9.7)</td>
</tr>
<tr>
<td>Waist Circ. cm</td>
<td>87.3 (50-146)</td>
<td>85.9 (50-146)</td>
</tr>
</tbody>
</table>
Regression Models

I used multiple linear regressions to evaluate the effect of changes in measurements of metabolic factors on changes in HbA1C. A best-fit model was sought to identify the metabolic factors that best predicted a change in HbA1C. The best fit included those variables, which alone or in combination, achieved the highest $R^2$ value.

Many linear regression models were run using multiple combinations of the independent variables of interest (BMI, lipids, MAP, and waist circumference) in relationship to the dependent variable (HbA1C). Validity of the linear regression models was assessed with residual plot analysis. When measures of the HDL and triglycerides (lipids) were included in the analysis, the numbers of exams available was diminished, since these tests were only recently introduced as part of the research examinations.

Changes in BMI and waist circumference were most strongly associated with changes in HbA1C. In the final models, 678 individuals were included in the short time span group and 1010 in the unrestricted times span group. More limited data sets were created for the step-wise multiple linear regression models that included HDL and triglycerides.
All variables were evaluated using both the slope measures and the previous values for each variable. Thus, the independent measures used for analysis were BMI slope, previous BMI, total cholesterol slope, previous cholesterol, triglyceride slope, previous triglyceride, HDL slope, previous HDL, MAP slope, previous MAP, waist circumference slope, and previous waist circumference along with previous HbA1C. The inclusion of previous HbA1C as an independent variable was done to account for regression to the mean, an artifact present when analyzing change between 2 points. The dependent variable of interest was HbA1C slope.

Analyses were done using investigator selection of variables, as well as step-wise multi-linear regression. The step-wise regression models suggested that the variables associated with the greatest $R^2$ values were BMI and waist circumference. BMI was measured throughout the study period 1983-2004, waist circumference from 1988 through 2004, and triglycerides and HDL from 1993 through 2004. When larger data sets were created by eliminating the HDL and triglyceride measures, changes in waist circumference was consistently associated with changes in HbA1C within most subgroups ($R^2=0.48$, $p<0.0001$). Changes in BMI were also associated with changes in HbA1C in a manner similar to waist circumference using the data set 1988-2004 ($R^2=0.48$, $p<0.0001$). However BMI was less significant when the data set was expanded to 1983-2004 ($R^2=0.30$, $p<0.0001$). Reasons for this are unclear. As a result, waist circumference, rather than BMI, is presented in the best-fit model, although it is important to note that BMI and waist circumference are strongly co-related.

The model that best predicted changes in HbA1C included slope of waist circumference, previous waist circumference, and previous HbA1C. The $R^2$ for this
model was 0.48 for the short time span (1.5-2.5 years between exams) group and was 0.50 for the unrestricted time span group. The Short Time-Span Group alone is presented due to the minimal differences between this group and the Unrestricted Time-Span Group. Age was included in the final model as a potential confounding variable and was found to have no impact on the prediction of changes in HbA1C.

In all models, the contribution of the previous HbA1C value to the prediction was large. The partial $R^2$ (pR$^2$) for previous HbA1C in the all-inclusive 5-19 age group was 0.43 ($p>0.0001$), indicating that waist circumference slope and previous waist circumference contributed only a pR$^2$ of 0.05 to the predicted change in HbA1C. A discussion of the implications of this finding will be presented in Chapter 5. This model was statistically significant for all age and gender sub-groups, with the exception of the youngest boys (5-9 year olds) (Table 11, 12, 13, 14). This linear regression model is represented graphically in Figure 7, with residual plots (a visual display of the plot dispersion around the best-fit line) used to assess the validity of the regression model represented in Figure 8.

TABLE 11. Short Time Span Best-Fit Model for Childhood Measures Associated with Changes in HbA1C

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>5-19 age</th>
<th>M 5-19</th>
<th>F 5-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.51**</td>
<td>1.51**</td>
<td>1.50**</td>
</tr>
<tr>
<td>Prev HbA1C</td>
<td>-0.34**</td>
<td>-0.33**</td>
<td>-0.34**</td>
</tr>
<tr>
<td>Waist Slope</td>
<td>0.01**</td>
<td>0.007**</td>
<td>0.013**</td>
</tr>
<tr>
<td>Prev Waist</td>
<td>0.002**</td>
<td>0.002**</td>
<td>0.002**</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.48</td>
<td>0.47</td>
<td>0.50</td>
</tr>
</tbody>
</table>
**TABLE 12. Short Time Span Best-Fit Model for Childhood Measures Associated with Changes in HbA1C 5-9 Year Old Age Group Male and Female**

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>5-9</th>
<th>M 5-9</th>
<th>F 5-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.56</td>
<td>1.63**</td>
<td>1.4**</td>
</tr>
<tr>
<td>Prev HbA1C</td>
<td>-0.35**</td>
<td>-0.37**</td>
<td>-0.32**</td>
</tr>
<tr>
<td>Waist Slope</td>
<td>0.007*</td>
<td>NS</td>
<td>0.04**</td>
</tr>
<tr>
<td>Prev Waist</td>
<td>0.003**</td>
<td>0.03**</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R²</th>
<th>0.50</th>
<th>NA</th>
<th>0.41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prev HbA1C pR² §</td>
<td>0.46*</td>
<td>0.54*</td>
<td>0.35*</td>
</tr>
</tbody>
</table>

*N 1008 445 561

*p<.05, **p<.01 §Partial R²
### TABLE 13. *Short Time Span Best Fit Model for Childhood Measures Associated with Changes in HbA1C 10-14 Year Old Age Group Male and Female*

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>10-14</th>
<th>M 10-14</th>
<th>F 10-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.54</td>
<td>1.36</td>
<td>1.46</td>
</tr>
<tr>
<td>Prev HbA1C</td>
<td>-0.33**</td>
<td>-0.29**</td>
<td>-0.32**</td>
</tr>
<tr>
<td>Waist Slope</td>
<td>0.01**</td>
<td>0.007**</td>
<td>0.011**</td>
</tr>
<tr>
<td>Prev Waist</td>
<td>0.002**</td>
<td>0.0009**</td>
<td>0.002**</td>
</tr>
<tr>
<td>Model R²</td>
<td>0.50</td>
<td>0.51</td>
<td>0.49</td>
</tr>
<tr>
<td>pR² Prev HbA1C</td>
<td>0.46**</td>
<td>0.44**</td>
<td>0.44**</td>
</tr>
<tr>
<td>N</td>
<td>541</td>
<td>241</td>
<td>299</td>
</tr>
</tbody>
</table>

*p<.05, **p<.01 §Partial R²

### TABLE 14. *Short Time Span Best Fit Model for Childhood Measures Associated with Changes in HbA1C 15-19 Year Old Age Group Male and Female*

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>15-19</th>
<th>M 15-19</th>
<th>F 15-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.39**</td>
<td>0.90**</td>
<td>1.6**</td>
</tr>
<tr>
<td>Prev HbA1C</td>
<td>-0.34**</td>
<td>-0.24**</td>
<td>-0.39**</td>
</tr>
<tr>
<td>Waist Slope</td>
<td>0.015**</td>
<td>0.11*</td>
<td>0.02**</td>
</tr>
<tr>
<td>Prev waist</td>
<td>0.003**</td>
<td>0.003*</td>
<td>0.02*</td>
</tr>
<tr>
<td>R²</td>
<td>0.47</td>
<td>0.26</td>
<td>0.58</td>
</tr>
<tr>
<td>pR² Prev HbA1C</td>
<td>0.25**</td>
<td>0.08**</td>
<td>0.33**</td>
</tr>
<tr>
<td>N</td>
<td>195</td>
<td>70</td>
<td>124</td>
</tr>
</tbody>
</table>

*p<.05, **p<.01 §Partial R²
FIGURE 8. *Best-Fit Model: Slope of HbA1C as predicted by slope of Waist Circumference & Previous Waist & Previous HbA1C for all 2-point plots less than 2.5 years between measurements*
Summary

Two research questions were addressed in this chapter. Both questions relied on childhood measures of glycemia and metabolic factors derived from the NIDDK epidemiologic study conducted in the Pima Indians of the Gila River Indian Community from 1983 through 2004. All children were free of type 2 DM at the time of their initial examination and each had measures of HbA1C, 2\(^0\)PG, and FPG at all exams. Measures of the other factors were variable, with BMI, total cholesterol, and MAP most frequently
measured, whereas waist circumference was measured only from 1988 on, and triglycerides and HDL lipids from 1993 on.

The results of the analyses for Question 1 showed a steady increase in the incidence rate of type 2 DM with age and a cumulative incidence of 54% by age 40. The overall age-sex-adjusted incidence rate was 19.0 cases per 1000 person-years. Females bore a greater disease burden with 22.8 cases per 1000 person-years, whereas the rate was 15.1 cases per 1000 person-years in males. When incidence rates were divided into baseline quartiles by glycemic measure, the predicted pattern of increasing rates with increasing baseline values was noted for $2^0\text{PG}$, and FPG, but not for HbA1C.

HbA1C presented a u-shaped relationship with the incidence of type 2 DM based on baseline HbA1C quartiles. The lowest and highest quartiles had the highest incidence rates of type 2 DM. When examined by Cox proportional hazards analysis, the u-shaped relationship between HbA1C and type 2 DM was again noted. Successive quartiles had hazard rate ratios of 1.2 to 2.7 times that of the first quartile.

The Cox model was appropriate for analyzing the relationship between FPG and diabetes but not $2^0\text{PG}$ and diabetes. For $2^0\text{PG}$, the assumption of proportionality necessary for the Cox analysis was violated. With FPG, the model indicated that for every 10 mg/dl increase in FPG there was a 2-fold increase in the risk for developing type 2 DM.

Question 2 was addressed using multiple linear regression techniques that tested the association between changes in measures of metabolic factors and changes in measures of HbA1C. Waist circumference was the best predictor of changes in HbA1C, although BMI was almost as good. The waist circumference model that controlled for
BMI, MAP, waist circumference, and lipids (total cholesterol, HDL, triglycerides) achieved an $R^2$ value of approximately 0.50. The ability of waist circumference to predict change however is limited due to a strong tendency for the overall effect to be accounted for by regression to the mean, as noted by the partial $R^2$ values of the previous HbA1C included in the model, suggesting that these metabolic factors contribute very little to changes in HbA1C, at least in childhood.
CHAPTER V: DISCUSSION AND IMPLICATIONS

This chapter provides a contextual basis for understanding the findings of the study of childhood glycemia and the metabolic syndrome risk factors that may influence changes in glycemia. Findings identified in the study are presented and addressed in relationship to the literature. In addition, the strengths, limitations and implications of these findings are addressed.

Childhood levels of glycemia (HbA1C, FPG, & 2PG) predict development of type 2 DM later in life. In addition, changes in waist circumference and BMI, two components of the metabolic syndrome, are most strongly associated with changes in HbA1C. These two risk factors, however, contribute only moderately to the overall change in HbA1C. In general, changes in the metabolic syndrome risk factors including BMI, MAP, waist circumference, and lipids are poor predictors of glycemic change in childhood, as measured by HbA1C. These findings imply that glycemic measures (HbA1C, FPG, & 2PG), rather than these surrogate markers of glycemic changes, are necessary to adequately assess the effect of diabetes prevention in children.

Incidence and Cumulative Incidence of Type 2 DM

Over half of Pima Indians will develop diabetes by the age of 40 years, and the incidence is higher in women than in men. Recent evidence (Pavkov et al.; in review) indicates the incidence rates have remained stable over the past 40 years, with the exception of the children. In Pima Indians under 15 years old, the incidence of diabetes has increased by nearly six fold during this period and the increase in diabetes is associated with a substantial increase in the frequency of obesity in youth. The increasing
incidence of diabetes in children gives added emphasis to the need for intervention in the
disease process at younger ages, ideally beginning prior to conception.

Glycemic Measures

*HbA1C*

HbA1C was of primary interest in this study for assessing the risk of diabetes, due to the ease with which it can be measured and its unique ability to assess average glycemia over an extended period. The person does not need to fast, receive a glucose load, or undergo venipuncture; an adequate sample can be obtained with a capillary finger stick. This measure assesses glucose control for a period of approximately 120 days prior to the draw, although the value is weighted to the previous month. The ADA does not use HbA1C as a diagnostic criterion for diabetes, although it may be a useful screening tool for identifying people at risk for diabetes in epidemiologic studies.

The incidence of diabetes increased in this study with increasing glycemia in childhood, when glycemia is assessed by FPG or 2°PG. On the other hand, the relationship between HbA1C and diabetes incidence in this study was u-shaped, and this relationship remained after controlling for potentially confounding variables in a Cox proportional hazards model. Such a relationship has not been reported previously and I can offer no reason for this observation.

Among Pima children in the current study, the mean HbA1C was between the 20\textsuperscript{th} and 40\textsuperscript{th} percentile of HbA1C reference values in the US as published by Saddine et al. (2002). Each age group of children were plotted using the HbA1C reference chart using the mean HbA1C for each age group; for 5-9 year olds the mean HbA1C (4.8\%) fell at the 40\textsuperscript{th} percentile; for 10-14 year olds the mean HbA1C (4.8\%) fell at the 30\textsuperscript{th} percentile, and for 15-19 year olds the mean HbA1C (4.6\%) fell at the 20\textsuperscript{th} percentile.
African American and Mexican American children are two groups at high risk for type 2 DM. NHANES noted that these two groups maintained a significantly higher HbA1C percentage than did non-Hispanic Whites (Eldeirawi et al., 2003). For Pima children, a group at high risk for type 2 DM, to have mean HbA1C values below the 50th percentile when compared to the general US population is unexpected and suggests ethnic differences in HbA1C.

Sixty-two children in this study developed type 2 DM in childhood. Of these, 29 (47%) had an HbA1C above 6.03% and 1 had an HbA1c below 4.07% (1%) at the time of diagnosis. The remaining 36 (52%) children had HbA1C within the normal range 4.07-6.03%. One child with an HbA1C of 8.8% had a FPG of 101 mg/dl and 2ºPG of 161 mg/dl. By the criteria used in this study, the child was considered to have type 2 DM. In adult Pima Indians, an elevated HbA1c (>6.03%) is highly specific (91%) and sensitive (85%) for detecting DM, but a normal HbA1C (4.07-6.03%) does not exclude the diagnosis (Little et al., 1988). McCance et al (1994) noted that an HbA1C of 7.85% is an appropriate DM diagnostic level in adult Pima Indians and found that it was equal to FPG and 2ºPG for predicting microvascular complications, including retinopathy and nephropathy. Using an HbA1C of 7.85%, as the diagnostic criterion would have identified only 12 of the 62 children diagnosed with type 2 DM in the current study. Schrot (2004) reported that a third of individuals could have postprandial glucose excursions above normal while maintaining HbA1C of less than 7%. This observation has meaning in the current study, where the majority of HbA1C values were in the normal range despite diabetes level 2ºPG, and for childhood DM diagnosis that appears to be heavily dependent on the glucose challenge for provocation of an elevated blood
sugar. The complexities of glycemia in childhood suggest that HbA1C gives meaning to the disease process identifying whether the disease process involves primarily postprandial excursions or a more continuous hyperglycemia.

$2^0_{PG}$ and $FPG$

$2^0_{PG}$ and $FPG$ diagnostic criteria for DM are well established. However, the time intensive measurement of $2^0_{PG}$ has fallen out of favor and been replaced with the random glucose measurement in general practice guidelines (ADA, 2000). For children, however, several authors consider the $2^0_{PG}$ to be the best measure for detecting type 2 DM and impaired glucose regulation (Conwell et al., 2004; Sinha et al., 2002; Perry et al., 2001;).

In the current study, higher childhood $2^0_{PG}$ at baseline predicted a higher incidence of type 2 DM. Sixty-two children were diagnosed with type 2 DM during childhood. The $2^0_{PG}$ was the only glycemic measure to reach diagnostic threshold in 38% of the children, while 56% were diagnosed with both the FPG and $2^0_{PG}$. Only three children (5%) were diagnosed by FPG alone.

The current study confirms the usefulness of both $2^0_{PG}$ and FPG for childhood screening and research related to diabetes as identified in the WHO and ADA criteria. While FPG contributes very little to diagnosing type 2 DM in children it is useful for identifying children who may have increased risk for the disease. The $2^0_{PG}$ is indispensable to diagnosing diabetes in children.

Metabolic Risk Factors

$BMI$ and Waist Circumference

Changes in BMI and waist circumference were associated with changes in HbA1C ($p>0.001$) in linear regression analysis. However, their contribution to the change
in HbA1C was moderate. Most of the change in HbA1C values was attributable to regression to the mean. For this reason changes in both BMI and waist circumference are of very little value in predicting changes in glycemia in non-diabetic children as measured by HbA1C.

Despite the limited utility of changes in measures of obesity (BMI and waist circumference) in the current study for predicting changes in HbA1C, both of these measures are associated with the development of type 2 DM. Hanson et al (2002) found obesity and insulinemia to strongly predict type 2 DM in Pimas older than 20 years of age. Franks (in review for publication) reported that BMI, waist circumference, and 2⁰PG were the strongest individual predictors of type 2 DM in children 5-19 year olds. The strength of adiposity as a predictor of diabetes in these two Pima studies indicates the value of these risk factors, though changes in adiposity are of lesser value for predicting change in glycemia in non-diabetic children.

The average child in this study was overweight, with a BMI at the 95th percentile using parameters of overweight identified in Lobstein et al., 2004. Lindsay et al. (2002) noted that Pima children were consistently heavier than the US population and gained weight most rapidly between birth and six months of age and again between 2 and 11 years of age in a comparative analysis with NHANES. In the current study, mean BMI was determined for gender in each age group (5-9, 10-14, and 15-19) and plotted using growth charts from both the US National Center for Health Statistics (NCHS) and the International Obesity Task Force (IOTF). The average Pima child was at about the 95th percentile using NCHS, growth charts. These children reached an overweight category at
or above the 98th percentile using the IOTF growth charts with the oldest girls (15-19 years) plotted at the 99.6th percentile.

Change in waist circumference was the strongest predictor of change in HbA1C in all childhood age and gender categories ($p > .001$). The Pima children in the current study had waist circumferences consistently above the 75th percentile for age and gender when compared with the US population. Change in BMI was also a predictor, but not as strong as change in waist circumference in all age groups and gender categories. Franks et al. (in review) noted waist circumference to be a strong predictor of type 2 DM in Pima children 5-10 years of age, waist circumference, but a weaker predictor in the older children. The two studies differed in terms of: 1) outcome measure, with change in HbA1C (current) as opposed to type 2 DM (Franks et al, in review), 2) change in waist circumference (current) as opposed to baseline waist circumference (Franks et al., in review).

Waist circumference is a measure of abdominal fat (central adiposity). Abdominal fat is related to the development of type 2 DM and cardiac disease and is a criterion in both the Metabolic Syndrome and Insulin Resistance Syndrome literature (Bloomgarten, 2004). Li et al. (2007) identified a recent trend of disproportionate increase in waist circumference as compared with BMI in US children. Fat cells secrete large numbers of peptides and cytokines, prostaglandins and steroid hormones (androgen and estrogen) (Lobstein et al, 2004). Insulin receptor modifications (down regulation), in the presence of obesity, leads to insulin resistance, an initial vigorous hyperinsulinemic response (in children), and eventually an increase in glycemic levels (Kahn, 2005; Sinha et al., 2002, Roith & Zick, 2001). This helps explain the link between adiposity and the progression to type 2 DM.


**Lipids**

In the current study, changes in lipids were not associated with changes in HbA1C except in subgroups of the study population. This finding was true of total cholesterol, measured throughout the study, as well as HDL and triglycerides, measured from 1994 through 2004. The subgroups where changes in lipids did have significance were 5-9 year old girls with triglycerides (p<0.01) and HDL (p<0.05), 10-14 year old girls with total cholesterol (p<0.05), and 15-19 year old boys with triglycerides (p<0.01). Franks et al. (in review for publication) noted that lipids were weak predictors of type 2 DM in Pima children, although HDL was a better predictor than triglycerides.

Williams (unpublished) noted increases in total cholesterol to be positively associated with increases in BMI in Pima children 10-17 years of age. His study spanned the years 1970-2001, and both mean cholesterol and BMI increased throughout this time period. As noted earlier, BMI is also associated with the development of type 2 DM. Adiposity and lipidemia are implicated in cardiovascular disease, which complicates type 2 DM.

**Blood Pressure/MAP**

Changes in blood pressure were not associated with changes in HbA1C in linear regression models. The study by Franks et al. (in review), found that systolic and diastolic blood pressures were the weakest predictors of type 2 DM of all the metabolic risk factors. These findings imply that blood pressure is not a useful predictor of glycemia, as well as of type 2 DM. However, blood pressure does have an impact on complications of type 2 DM (cardiomyopathy, and nephropathy). When DM develops,
the ensuing hypertension and hyperglycemia contribute to the development and progression of micro- and macro-vascular disease.

Blood pressure is positively associated with BMI. A study using NHANES data noted a trend of increasing blood pressure in children 8-17 years of age when two time frames were compared (1988-1994 with 1999-2000). One explanation for this observation was the simultaneous increase of BMI in the population over the same time periods (Muntner et al 2004). BMI seems to be the common denominator between glycemia, blood pressure, and lipids. However, while lipids and blood pressure are not directly associated with glycemia or the development of type 2 DM, they are both associated with diabetic complications.

Conceptual Framework

A conceptual framework was identified in chapter two to provide a visual model of the manner in which type 2 DM develops. This model also hypothesized that changes in measurements of childhood risk factors (discussed above) would predict changes in the glycemic measures. The entire model was not tested. Changes in glycemic measures were not used to predict changes in follow-up glycemic measures. In addition, the model was restricted to non-diabetic childhood exams with HbA1C as the only outcome measure. I have depicted the model with “+” sign next to the risk factors that were significant and a “-” sign next to those that weren’t significant (FIGURE 10).
Simplified Model of Progression to DM

- Genetics
- Uterine Environment
- Inactivity

Over-nutrition

\[ \text{BMI} \] \[ \uparrow \] \text{Insulin Resistance} \[ \uparrow \] \text{Blood Sugar} \[ \text{Beta-cell exhaustion} \] \[ \rightarrow \] \text{DM}

**Stage I**
**Stage II**
**Stage III**

**Tested**

**Relationship of Predictor Factors to Changes in Glycemia**

**Childhood & Interim Exams**

- BMI +
- Lipids -
- BP -
- Waist Circum. +
- HbA1C
- 2⁰GTT
- FPG

**Childhood 1.5-2year Follow-up between exams**

- HbA1C +
- 2⁰GTT
- FPG

Not Tested

FIGURE 10. Conceptual Framework as Tested
Strengths, Limitations and Implications

The strength of this study is its longitudinal design and the large population-based sample of children. Metabolic syndrome risk factors were part of the dataset, although lipids (HDL and triglycerides) were only measured in the latter half of the study period so their effects could not be adequately assessed. Despite this limitation, it was possible to identify the manner in which changes in metabolic risk factors contributed to change in the glycemic measure of HbA1C in children who did not have type 2 DM.

Another potential limitation is related to the population of children who participate in the NIDDK biennial exams (selection bias). These children are not actively recruited; instead they may have been brought to the clinic by concerned parents or referred for testing as the result of a school-screening program or by a physician. This referral pattern could skew the childhood population to one at greater risk of type 2 DM than the risk inherent in the broader community.

The overall implications of this study relate to evidence that FPG and 2⁰PG are important glycemic measures for the identification of children at risk for progression to type 2 DM. The 2⁰PG measure is the best measure for identifying type 2 DM in children, whereas FPG is relatively ineffective for diagnostic purposes. HbA1C was of interest as a measure in childhood due to its non-linear u-shaped relationship to incidence of type 2DM, its variability in relationship to FPG and 2⁰PG, and its association with the metabolic risk factors. Interpreting the meaning of this measure as it relates to type 2 DM diagnosis calls into question how it is possible for a child to reach diabetic levels of glycemia with 2⁰PG while maintaining a normal or below normal HbA1C. One interpretation could be that the HbA1C is not useful as a determinant of progression to
disease in children. HbA1C does add a broadened perspective by contributing an average level of glycemia for a three-month period of time as opposed to the snap shot perspective of FPG and 2⁰PG. McCance et al. (1994) identified an HbA1C threshold of 7.85% to be associated with microvascular complications in adults, suggesting that HbA1C in adults was useful for diagnosis of diabetes. Establishing such a threshold in children is problematic. Complications in children usually present 10-15 years after disease onset. Whether HbA1C should be incorporated into the diagnostic process is currently unclear. The criteria for diagnosis of type 2 DM were established in adults, and have been applied to children. Whether these criteria are adequate for children is uncertain. The effect of the glycemic measures and metabolic risk factors on the development of diabetic complications after the onset of diabetes in children is beyond the scope of this paper.

Another implication of the study relates to the poor performance of changes in metabolic syndrome risk factors as surrogate measures to identify changes in glycemia (HbA1C). While these factors did not predict change in HbA1C, adiposity is strongly associated with the development of type 2 DM. For this reason, intervention studies need to encourage diet and exercise as a strategy to prevent type 2 DM. However, to measure the effect of these studies on the development of diabetes, glycemic measures (FPG, 2⁰PG, and HbA1C) are needed.

This study has implications for nurses and clinicians working with children at high risk for type 2 DM whether in the clinical setting or in an interventional study. Knowledge of the role and function of each glycemic measure as it relates to children is important for diagnostic, therapeutic, and prevention strategies related to type 2 DM.
HbA1C provides a broadened dimension when combined with values based on FPG and 2\textsuperscript{nd}PG, and is warranted when assessing children at risk for diabetes. Measures of FPG and 2\textsuperscript{nd}PG are point-in time-measurements while HbA1C assess the average level of glycemia of 3 months. An elevation in any of the glycemic measures indicates a child at greater risk than others for diabetes, a child in need of intervention (lifestyle) and monitoring. Despite the relatively moderate role of changes in metabolic risk factors and HbA1C noted in this study, there are many indications that efforts to reduce adiposity through diet and exercise are effective at preventing type 2 DM (Schlessinger et al., 2005). Nurses interested in the prevention of type 2 DM can intervene at the individual level as well as at the community level by participating in strategies that support lifestyle changes, advocating for appropriate foods and physical exercise in schools, and through community efforts to structure neighborhood environments to encourage walking and biking.

Summary

Childhood levels of glycemia predict development of type 2 DM later in life. While changes in waist circumference are associated with only moderate changes in HbA1C, this does not refute the significant contribution of adiposity in childhood to the development of type 2 DM. The implication for nursing is that lifestyle changes must be addressed to prevent and diminish the threat of diabetes in childhood and all segments of the population.
APPENDIX A:

UNIVERSITY OF ARIZONA HUMAN SUBJECTS REVIEW
16 November 2005

Carol Moffett, Ph.D. candidate
Advisor: Judith Effken, Ph.D.
College of Nursing
P.O. Box 210203

RE: THE IMPACT OF CHILDHOOD MEASURES OF GLYCEMIC AND INSULIN RESISTANCE FACTORS ON FOLLOW-UP GLYCEMIC MEASURES

Dear Ms. Moffett:

We received documents concerning your research proposal as cited above. This project involves the analysis of de-identified lab data collected from 1500 Pima Indian Community children ages 5-19 at the time of collection related to understanding the factors related to HbA1c and other measures of glycemia in children. These data are being provided to you by the NIH/NIDDK for analysis. The procedures to be followed in this study pose no risk to subjects providing the data. Regulations issued by the U.S. Department of Health and Human Services [45 CFR Part 46.110(b)(2)] exempt this type of research from review by our Institutional Review Board.

Exempt status is granted with the understanding that no further changes or additions will be made either to the procedures followed or to the consenting instrument used (copies of which we have on file) without the review and approval of the Human Subjects Committee and your College or Departmental Review Committee. Any research related physical or psychological harm to any subject must also be reported to each committee.

Thank you for informing us of your work. If you have any questions concerning the above, please contact this office.

Sincerely yours,

Rebecca W. Dahl, RN, Ph.D.
Director
Human Subjects Protection Program

RWD:pm

cc: Departmental/College Review Committee
REFERENCES


