

Inflammatory Cytokines and the Development for the Prediabetic Phenotypes of Impaired Insulin Secretion and Sensitivity in High-Risk Children

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A. Study Purpose and Rationale

The prevalence of type 2 diabetes mellitus (T2DM) among adolescents has increased 5-10 fold over the last decade and is now considered a “pediatric” disease, producing the same renal, neurological, and cardiovascular morbidities in children with T2DM as in Type 2 diabetic adults (1). The burden of pediatric diabetes falls disproportionately on African and Hispanic Americans, in whom between 25% and 50% of new-onset childhood diabetics are Type 2. (2) A better understanding of the clinical markers for pre-diabetic phenotypes will help in the identification of those at high risk for T2DM and would significantly reduce morbidity through earlier therapeutic intervention. Recently, elevated circulating concentrations of inflammatory markers [C-Reactive protein (CRP), interleukin-6 (IL-6) and plasminogen activator inhibitor-1 (PAI-1)] have been shown to be associated with an increased risk of developing type 2 DM in adults over 4-5 year followup periods, even when adjusted for other risk factors such as obesity, smoking, exercise, alcohol, and hormone replacement therapy (3-5). No such prospective studies have been performed in children, nor have there been studies of the associations of these inflammatory markers with pre-diabetic phenotypes in children. The purpose of this study is to investigate the association between inflammatory cytokines with the development of the prediabetic phenotypes of impaired insulin secretion and sensitivity in high-risk children. Such insights could help further establish the pathogenesis of T2DM in children as well as provide another means of risk stratification early intervention for the progression to T2DM.

B. Study Design and Statistical Analysis

This study will take place in the setting of an investigation already underway to examine the prevalence of pre-diabetic phenotypes, the response of these phenotypes to a supervised nutrition/health education/exercise program, and the progression of these phenotypes over 3 years in a predominantly Hispanic-American population at high risk for insulin resistance and T2DM. Our preliminary data show further evidence that the students at the school are at high risk for T2DM in that 50% of the students have a relative with T2DM and 25% of the students have a relative with the onset of disease before the age of 40. Further 37% of the students are overweight (BMI > 85th percentile for age and sex). Subjects will undergo measurements of diabetes risk factors (weight, height, abdominal circumference, cardiovascular risk profiles, measurement of insulin resistance and insulin secretory capacity before and after a three month period of intervention in the form of health education and three times weekly 40-45 minute aerobic exercise periods. Compliance with the intervention will be assessed one year after initial study completion by completion of behavioral questionnaires. Pre-diabetic phenotypes, including assessment of insulin sensitivity, (QUICKI), pancreatic islet cell function (AIR X QUICKI), anthropometry (height, weight, waist circumference, body composition), fitness, and diabetes-associated laboratory phenotypes (lipid profile, HgbA1C, and the inflammatory markers CRP, IL-6, and PAI-1) will be examined at 1 and 3 years after the completion of the initial studies. Values at 1 and 3 years will be compared to similar studies in a cohort of subjects who are classmates but did not participate in the program in middle school. *The primary outcome of this study protocol will be the correlation baseline inflammatory markers with insulin sensitivity and beta cell function at 3 years follow-up in the intervention group as well as the control group in the students who had normal insulin sensitivity (QUICKI) and secretion (AIR X QUICKI) at*

baseline. Based on preliminary data from a cohort of 8th grade students evaluated last year, we estimate that 85% of the subjects will demonstrate normal insulin sensitivity defined by QUICKI >0.3 and 77% will demonstrate unimpaired insulin secretion defined as AIR X QUICKI > 150 (> 1 S.D. below the mean for the two values

Projected Testing Schedule							
Subject Group	Site	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Experimental Subjects	MS 54 pre-intervention	40	40	40	40	0	200
	MS 54 post-intervention	40	40	40	40	0	200
	HS 9 th grade	32	32	32	32	32	160
	HS 11 th grade	0	0	24	24	24	72
	Total	122	122	136	136	56	552
Control Subjects	HS 9 th grade	10	10	10	10	10	50
	HS 11 th grade	0	0	10	10	10	30
	Total	0	10	20	20	20	80

a. Statistical Analysis

Statistical analysis will be performed using the Statistica statistical software package. The following criteria will be examined:

b. Initial Screening

- Range of inflammatory marker levels and their association with pre-diabetic phenotypes (impaired insulin sensitivity and/or secretion), and other laboratory parameters (Hgb A1C, lipids), fitness, anthropometric (waist circumference, body composition, and historical data (family history of type 2 diabetes).

c. Testing following 3 Months of Health/Nutrition Education and Exercise

- Range of inflammatory marker levels and their association with changes in prediabetic phenotypes (impaired Insulin sensitivity and/or secretion), other laboratory parameters (Hgb A1C, lipids), fitness, anthropometric (waist circumference, body composition, and historical data (family history of type 2 diabetes).

d. Follow-up Testing

- Range of inflammatory marker levels and their association with changes in prediabetic phenotypes (impaired Insulin sensitivity and/or secretion), other laboratory parameters (Hgb A1C, lipids), fitness, anthropometric (waist circumference, body composition, and historical data (family history of type 2 diabetes) data in 9th and 11th grade.
- Comparison of inflammatory marker levels and the prevalence of pre-diabetic phenotypes (Impaired insulin sensitivity and/or secretion) other laboratory parameters (Hgb A1C, lipids), fitness, anthropometric (waist circumference, body composition, and historical data (family history of type 2 diabetes) data in 9th and 11th grade between subjects participating in the health intervention and the control subjects who did not.

e. Primary outcome statistical analysis:

i. Power

The primary outcome of this study will be the correlation of baseline inflammatory markers and insulin sensitivity and secretion at 3 years follow-up for the intervention group and 2 years follow-up for the control group for the subjects with normal insulin sensitivity and secretion at baseline. For the experimental group, with 61 eligible subjects projected to complete the study, a correlation coefficient of 0.35 will be detectable testing for 80% power at $p=0.05$. For the control group, with 23 subjects projected to complete the study, a correlation coefficient of 0.56 will be detectable testing for 80% power at $p=0.05$.

α , or Type I error, Is defined as rejecting null hypothesis when it is actually true. For this study, α is defined by the statement that the inflammatory markers are associated with the subsequent development of insulin resistance and or sensitivity, when they really are not

β , or Type II error is defined as accepting null hypothesis when it is actually false. For this study, β is defined by the statement that the inflammatory markers are not associated with the subsequent development of Insulin resistance and/or sensitivity when they really are.

ii. Logistic multivariate analysis

Conditional logistic regression adjusting for age, sex, BMI, family history in a first degree relative, insulin resistance and secretion status at baseline will be performed for all three inflammatory markers. Odds ratios indicating a 1 SD increase of the variables of interest will be calculated.

C. Study Procedures

a. Medical Family History

A complete medical and family history will be obtained at enrollment and amended at subsequent study periods including questions regarding family history of diabetes, diabetes-related phenotypes and activity level. Medical and family history will be reviewed with parents in person or by telephone.

b. Anthropometry

Anthropometry will be obtained in all subjects at all study periods. All students will undergo measurements of height, weight, waist circumference, and body composition by electrical impedance immediately before each IVGGT.

c. Rapid IVGGT

The rapid IVGGT will be performed in all subjects at all study periods. The IVGGT is an established endocrine test that has been used widely in children from infancy through adulthood but the standard IVGGT takes 3 hours and examines different phases of insulin release. We are interested only in pancreatic Insulin secretory capacity which is reflected in phase I (early, insulin released at 3 and 5 minutes following an intravenous glucose load) and so will use a substantially abbreviated version of this test. Insulin sensitivity will be measured using the quantitative insulin sensitivity check index (QUICKI). Insulin secretory capacity will be defined as acute insulin secretory response (AIR) and calculated as the incremental rise in circulating insulin concentrations following an intravenous glucose load. Students will be seen in the schools' health centers for initial and follow-up studies between 9 AM and 10 AM before breakfast. A 19 gauge butterfly needle will be placed in an antecubital or dorsal hand vein and blood will be drawn for fasting glucose, insulin, proinsulin, C-reactive protein (CRP), interleukin-6 (IL-6), plasminogen activator inhibitor- 1 (PAI-1), Hgb A1C, cholesterol, HDL, LDL, and triglyceride levels. Students will then receive a standard intravenous glucose load (0.5 gm/kg, maximum 25 gm) over 3-4 minutes and blood will be drawn through the same line for insulin at 1, 3, and 5 minutes after glucose administration. The total amount of blood drawn is less than 40 cc. The students will then be fed breakfast by the investigators and returned to regular classes.

The QUICKI is well correlated with insulin sensitivity measured by oral and intravenous glucose tolerance testing in adults and children and for following changes in insulin sensitivity over time (6). It is defined as $QUICKI = 1/[\log(I_0) + \log(G_0)]$ where I_0 is the fasting insulin and G_0 is the fasting glucose. Though clear pediatric and adult standards for the QUICKI are not available, values <0.31 are greater than 1 S.D. below the mean in adolescents in our preliminary data.

Acute insulin response (AIR, mean incremental rise in plasma insulin at 3 and 5 minutes following an intravenous glucose load), correlates with first phase Insulin release measured by IVGGT (6) and low AIR is predictive of progression to Impaired glucose tolerance or frank T2DM in Pima Indians (7).

Because of the significant negative correlation of QUICKI and AIR, insulin secretory capacity cannot be assessed by AIR alone and insulin secretory capacity will be defined as $AIR \times QUICKI$ (8). Though clear standards of QUICKI, AIR, and $AIR \times QUICKI$ values across ethnic groups have not been

identified, values greater than 1 S.D. below the mean based on our initial data will be considered as suggestive of impaired Beta-cell secretory capacity. In addition, we will measure anti-islet cell, anti-insulin, and anti-GAD antibodies in all subjects with pre-diabetic phenotypes to ascertain that they are not developing Type I diabetes. Since one or more of these antibodies may be present in as many as 30% of T2 diabetics, children with auto-antibodies will not be excluded from statistical analysis but may be analyzed as a separate group.

d. Laboratory Assay

All assays are performed by the core laboratory at the The New York Presbyterian Medical Center. Lipid profiles (cholesterol, triglycerides, HDL, LDL) will be measured colorimetrically. Glucose will be measured by colorimetric assay using the glucose hexokinase method (Glucose/HK, Boehringer Mannheim, Werk Penzberg, Federal Republic of Germany). Plasma insulin will be measured by solid phase ¹²⁵I-radioimmune assay (Coat-a-count, DPC, Los Angeles, CA). Hemoglobin A1C will be measured colorimetrically. CRP and PAI-1 will be determined by ELISA. IL-6 will be assayed by RIA. Anti-insulin antibodies will be measured by RIA. Anti-islet cell antibodies will be measured by chemiluminescence. Anti-GAD antibodies will be measured using a monoclonal antibody assay.

D. Study Drugs

No drugs will be investigated in this study.

E. Medical Device

No Medical devices will be investigated in this study.

F. Study Questionnaires

No questionnaires will be used in this study.

G. Study Subjects

a. Inclusion/Exclusion Criteria

- Baseline Insulin sensitivity and secretion of QUICKI >0.3 and QUICKI X AIR >150.
- No evidence of type 1 DM as defined by a lack of detectable anti-insulin, anti-GAD, or anti-islet cell antibodies (this is not required, since as many as 30% of type 2 DM patients have been reported to be positive for at least one of these autoantibodies while less than 8% are positive for two or more, of them). These antibodies will be measured in all subjects, and those in whom the presence of two or more autoantibody types is detected will be excluded.
- No evidence of a chronic inflammatory condition by medical history
- Subjects with an acute illness at IVGGT times will be tested at least 2 weeks after resolution of symptoms.

Minors will be the subjects of this study. Informed consent and assent will be obtained before the study begins. They will be free to drop out of the study at any time. Individuals identified as being either insulin resistant or having impaired Beta-cell function will be referred to the Pediatric Diabetes Clinic at the Naomi Berrie Diabetes Center at Columbia University for further evaluation once the study is completed. Individuals identified as being diabetic will be referred immediately to the Pediatric Diabetes Clinical and the Naomi Berrie Diabetes Center. The results of all studies will be fully discussed with students and their families, and if desired, copies of all tests will be available to the primary care physicians caring, for the participants.

The Population represented in the El Camino class and high school whose Students will participate is 85% Hispanic or Latino, 12 % Black, and 3% multiethnic, thus reflecting ethnic groups at high risk for T2DM.

H. Recruitment of Subjects

All students in the 8th grade class at MS 54 and will be invited to participate in the intervention study. All students in the 9th grade class at the high schools involved will be invited to participate as controls. Subjects will be excluded only if they are pregnant, have an acute illness during blood drawing, or if they are known to be insulin-dependant diabetics. Written informed consent (offered in English and Spanish) will be obtained from all subjects and their families. Once consent is obtained students and their families will be asked to complete a questionnaire regarding their family and personal medical histories. The investigators will make themselves directly available to students and parents to answer any questions regarding this study. Students will in no way be coerced to participate.

I. Confidentiality of Study Data

All data and specimens obtained for this study will be used solely for the experimental protocol described. Data will be uniquely coded and will be stored in a secure location accessible only to the investigators. The results of these studies will be kept in strict confidence but will be discussed with students and their families.

J. Potential Conflict of Interest

The investigators in this study will not benefit financially in any way other than the results of the investigation.

K. Location of Study

The study will be conducted in the school setting. The nurses' office will be the site of all phlebotomy procedures. The school principal's signature will be obtained for permission prior to starting the study.

L. Potential Risks

We do not believe that any aspect of this study constitutes a significant hazard to a carefully selected subject population. The only anticipated risks are bruising and discomfort at venipuncture sites during the rapid IVGTT. Rapid administration of glucose should not produce any side effects and subjects are fed breakfast immediately following the test to insure against the unlikely possibility of subsequent rebound hypoglycemia. Physicians or nurse practitioners perform all blood testing. Discomfort is minimized by application of a local anesthetic cream (Elamax) to potential venipuncture sites 15-30 minutes before blood drawing or glucose administration. Subjects are free to terminate testing at any time and at the first sign of venous irritation or of I.V. infiltration, testing will be stopped.

M. Potential Benefits

Subjects benefit immediately from the assessment of their diabetes risk. We will recommend referral of children who are identified as being at a high risk for the development of type 2 DM based on these tests to the pediatric endocrinology and diabetes clinics at Columbia Presbyterian Medical Center and St. Luke's/ Roosevelt Hospital Medical Center. We will also furnish copies of laboratory results to the subjects' primary care physicians if so directed by the families. These referrals will be made via

discussion with the families of the participants by one of the pediatric endocrinologists associated with the study.

N. Alternative Therapies

This study does not involve an experimental therapy.

O. Compensation to subjects

Subjects will not be compensated.

P. Costs to Subjects

Subjects will not incur cost as a result of participating in the study.

Q. Minors as Research Subjects

Approval from the Department of Pediatrics Committee on Human Investigation will be obtained.

R. Radiation or Radioactive Substances

This study does not involve any radiation or radioactive substances.

S. Works Cited

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