

GLUCOSE INTOLERANCE (ALL CENTERS)

Specific Aims/Hypotheses

The objectives of this study are: 1) to determine glucose tolerance status including non-insulin dependent diabetes mellitus (NIDDM) and impaired glucose tolerance (IGT), for half of the Year 10 CARDIA participants; and 2) to examine the associations of cardiovascular disease (CVD) risk factors with glucose tolerance status. The main goal is to clarify whether in younger adults, glucose intolerance (NIDDM and IGT)/hyperglycemia is associated with CVD risk factors independent of insulin, and whether certain lifestyle factors and changes in lifestyle factors are associated with glucose intolerance/hyperglycemia. Specifically, the proposed study will explore the following questions:

1. Do young adults with glucose intolerance have higher insulin (fasting and post-load), blood pressure (BP), total plasma cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), uric acid, fibrinogen and other hemostatic factors and lower high density lipoprotein cholesterol (HDL-C) than young adults with normal glucose tolerance? Are the differences in these CVD risk factors between glucose groups independent of body mass index (BMI) and insulin?
2. Are insulin levels (fasting and post-load) positively associated with BP, TC, TG, LDL-C, uric acid, fibrinogen and other hemostatic factors and negatively associated with HDL-C, independent of BMI and glucose level?
3. Are the baseline values and changes in dietary factors (total calories, carbohydrate, protein, fat and alcohol), physical activity, smoking, and obesity (BMI, waist/hip ratio, percent body fat, and skinfolds) associated with the current (Year 10) glucose tolerance status?
4. Do CVD risk factors (BP, TC, TG, LDL-C HDL-C and uric acid) and fasting insulin measured 10 years ago at baseline, and changes in these factors which occurred during this 10 year period, differ between persons with NIDDM and IGT, and those with normal glucose tolerance?
5. Are there race and gender differences in the relationships described in Questions 1 to 4?
6. What are the prevalence rates of NIDDM and IGT in the black and white male and female participants in the CARDIA study? Are there race and gender differences in these prevalence rates?

These objectives will be accomplished by recruiting 2,000 participants during the Year 10 core examination from the four

field centers. The sample is expected to consist of 500 in each sex-race subgroup.

Background/Rationale

Diabetes has long been recognized as a major risk factor for coronary heart disease in "western" industrialized populations. Many prospective studies have demonstrated that NIDDM is strongly associated with the incidence of myocardial infarction and stroke and mortality from coronary heart disease (CHD) and CVD (1-8). Persons with NIDDM have been found to have on average higher plasma triglyceride and lower HDL-C levels than those without diabetes (9-12). Higher blood pressures and higher prevalence rates of hypertension have also been observed in patients with NIDDM, with obesity accounting for only a portion of these differences (13-16). Altered hemostatic factors have also been described in diabetes mellitus (17-20). In addition, several studies reported that men and women with IGT had higher levels of CVD risk factors than those with normal glucose tolerance (21-23).

Results from epidemiological studies have indicated that increased insulin levels are associated with high triglycerides, blood pressure, serum uric acid and lower HDL-C in non-diabetic populations (24-27). Several prospective studies have reported that fasting insulin or insulin response to glucose load is significantly related to the incidence of CVD in persons with or without diabetes, independent of the major CVD risk factors (28-31). It has been suggested that in many patients, clinical NIDDM is preceded by a long period of hyperinsulinemia (32-35). In a recent eight-year follow-up of the San Antonio Heart Study, it was reported that persons with newly developed Type II diabetes had significantly higher fasting insulin and glucose levels, total cholesterol, LDL-C, triglycerides, BMI and blood pressure and lower HDL-C at baseline than persons whose glucose tolerance remained normal (36). With the exception of blood pressure, all of these differences remained significant with adjustment for age, BMI and glucose level but were no longer significant with adjustment for insulin level. These findings suggest that the increased risk of CVD and CVD risk factor associations with NIDDM are in part due to hyperinsulinemia. However, if hyperinsulinemia is the primary determinant of CVD risk factors in persons with diabetes, then persons with IGT, in whom the average insulin level is higher than for overt diabetics, should also have higher rates of CVD than persons with NIDDM. However, this has not been the case. Thus, hyperinsulinemia alone cannot explain the increased CVD risk factor levels in diabetics. Whether hyperglycemia is associated with CVD risk factors independent of insulin levels requires clarification. On the other hand, it is also unclear whether hyperinsulinemia is related to CVD risk factors independent of glucose levels.

CARDIA has collected serial data on CVD risk factors during a ten year period of time and thus provides a unique opportunity to examine whether the levels of these risk factors have increased

during this time period for persons with glucose intolerance identified at Year 10. In addition, the data collected at Year 10 can be used to examine whether CVD risk factors are associated with glucose intolerance independent of insulin levels and whether these CVD risk factors are associated with insulin levels independent of glucose levels.

Many studies have reported that the prevalence of NIDDM and IGT are significantly higher in blacks than in whites (37-39). This difference cannot be explained by the higher prevalence of obesity in blacks (37). In addition to different measures of obesity, CARDIA has collected many lifestyle characteristics during the 10 year period. These data can be used to examine lifestyle factors such as physical activity and dietary factors, that may be associated with the black-white difference in glucose intolerance/hyperglycemia.

Due to the young age of the CARDIA participants, the number of NIDDM cases at year 10 is likely to be small. Thus, the questions described above may not be answered for NIDDM alone. However, the results of the oral glucose tolerance tests (OGTT) can be used as baseline data for determining the incidence of NIDDM in future follow-up of these 2000 men and women. The questions described above can then be examined for with the new NIDDM cases.

Approach/Method

Recruitment

The proposed OGTT will be conducted by all four field centers during the core examination. For each center, the recruitment goal is to enroll half of the Year 10 participants into the study. When each participant is contacted by phone for scheduling the Year 10 Examination, he/she will be informed about the substudy. Information on current treatment for diabetes will be obtained. Those who are currently on insulin for diabetes will be excluded from the substudy. The recruitment will be monitored and adjusted throughout the study period to ensure that there will be at least 500 participants from each sex-race subgroup.

Method

Each participant will be asked to fast for 12 hours and refrain from smoking for two hours prior to the examination. The blood specimen for fasting glucose and insulin can be drawn with the regular phlebotomy component. Eight ml of blood will be drawn at baseline, using two vacutainers (one 3 ml grey top for glucose and one 5 ml red top for insulin). The participant will be asked to drink 75 grams of glucose water (TRU GLU). The participants will then be instructed to report to the phlebotomy station one hour and fifty minutes after the load of glucose water. The exact time for the 2-hour blood draw will be written on an adhesive label

to remind the participant and clinic staff. An additional 8 ml of blood will be drawn at two hours post-load. During the two hours waiting time, core examination components can proceed as usual. The participant should continue to fast until after the 2-hour blood draw. Plasma (serum) will be separated from blood, frozen, and shipped on dry ice to the designated central laboratories for glucose (insulin) analysis.

Glucose tolerance will be classified as diabetes, IGT, or normal based on WHO criteria (40). The WHO criteria for diabetes require that the fasting plasma glucose be ≥ 140 mg/dl or the 2-hour glucose be ≥ 200 mg/dl if fasting plasma glucose is <140 mg/dl; the criteria for IGT require that the fasting plasma glucose be <140 mg/dl and the 2-hour value fall between 140 and 199 mg/dl; and the criteria for normal glucose tolerance require that both the fasting and 2-hour values be <140 mg/dl.

Laboratory Methods

Glucose

Glucose measurements will be performed by Dr. Thomas Cole at Washington University Lipid Research Center Core Laboratory (through a subcontract with Dr. Ronald Gingerich at Linco Research, Inc.) on a Technicon Chemistry Autoanalyzer using hexokinase-ultraviolet method. The combination of hexokinase and glucose-6-phosphate dehydrogenase has long been recognized as the method of choice in the specific measurement of glucose (41). In 1974, it was recommended as a reference method and "Product Class Standard" (42). This method was used in the determination of glucose in the CARDIA samples during the Year 0 and the Year 7 examinations.

Samples for glucose determination will be shipped to Linco Research, Inc., logged-in, organized and stored at -70°C prior to shipment on dry ice via local courier to the Lipid Core Laboratory for analysis. Each specimen is entered into the computer system upon arrival in the Lipid Core Laboratory and is assigned a unique specimen number. Permanent electronic and hardcopy databases are maintained. Results from the main analyzer are transferred to the database by electronic interface. Each result is checked for possible errors by two automated programs: the first checks for values outside preset limits and orders repeat testing if necessary and the second is a "delta" check which uses historical data for a particular subject to test whether the current results agrees with the last previous result within preset limits. Results are released only if the run is "in control" and both checks are passed. Results will be sent to the Coordinating Center via diskette on a monthly basis.

Insulin

Insulin will be measured at Linco Research, Inc. (Dr. Ronald Gingerich, P.I.) by radioimmunoassay (RIA) using an overnight, equilibrium incubation format. The key feature of this assay is the use of a unique antibody (43) that produces less than 0.2% crossreactivity to human proinsulin and its primary circulating split form, Des 31,32 proinsulin. These two proinsulin components constitute the majority (>95%) of circulating proinsulin-like material (44). A third component, Des 64,65 proinsulin, crossreacts with this assay, but this product makes up a very small fraction of circulating proinsulin (45). The insulin values provided by this measurement represent "true" insulin levels without significant contribution from proinsulin. Separation of bound from free ligand is accomplished by a second antibody.

The importance of true insulin measurement centers around the variability in concentration of proinsulin-like components in plasma. Common antibodies crossreact typically 30-100% with proinsulins and thus overestimate true insulin levels depending on the degree of crossreactivity and concentration of proinsulin. It was previously assumed that this error in insulin measurements was insignificant since proinsulin levels were thought to be low and exhibited a constant ratio relative to insulin. Recent data from several laboratories (45-47) show proinsulin levels vary greatly depending upon the study group. Proinsulin to insulin molar ratios range from 10-20% in normal individuals to as high as 100% (equimolar levels of insulin and proinsulin) in some type II diabetics with severe hyperglycemia (46). This variability of proinsulin levels would adversely impact insulin measurements obtained by commonly employed assays. Since the impact of such factors as weight, race, age, etc on proinsulin to insulin ratios has not been established, and since insulin is a potential risk factor in the CARDIA Study, it seems prudent to measure true insulin levels and eliminate the potential confusion of a proinsulin component.

Samples arriving from the four field centers will be logged into a registry, given a unique laboratory identification number and placed in the CARDIA -70°C freezer until analyzed. Immediately prior to assay, samples will be thawed and brought to homogeneity by inversion and gentle vortexing. After pipetting, specimens will be stored in the refrigerator (4°C) for no more than 24 hours. This will allow determination as to whether repeats are required and thus eliminate freeze-thaw cycles. Upon completion of assay, results will be recorded and checked for accuracy. Results will be reported via diskette to the Coordinating Center on a monthly basis.

Quality Control

Glucose

Internal quality control is monitored in real-time during all runs. The program consists of the use of two levels of commercially purchased control materials. A control is analyzed after every 11 samples, alternating between the normal and high control. Acceptability of a run is based upon the standard Westgard rules (48). Current controls for glucose are 75 mg/dl and 287 mg/dl with 2 standard deviations limits of 72-78 mg/dl and 278-296, respectively. Within-run precision is below 1% CV. Between-run precision is below 2% CV. Long-term consistency of measurement is evaluated through the use of monthly histograms showing mean and median values and 95% confidence intervals. External quality control for glucose is through the SURVEYS program of the College of the American Pathologists, which certifies the Core Lab.

Insulin

Internal quality control samples are run at the beginning and end of each assay. Acceptability of a run at the beginning and end of each assay. Acceptability of a run is based upon five of six QC volumes falling within ± 2 standard deviations of a rolling mean that is determined from twenty previous assays. If a QC falls outside the acceptable range, a fresh vial is hydrated for future runs. In the event that two QCs fall outside the acceptable range, the run is rejected and samples are re-analyzed. The data obtained from daily analysis of QC pools are visually inspected by Levy-Jennings plots to determine if there are trends or shifts from the average target value (49).

Study Monitoring

In addition to the quality control programs in place within each laboratory, the CARDIA Coordinating Center will monitor laboratory performance through a two-pronged system. First, univariate analyses will be conducted on glucose and insulin results. Summary statistics will be generated and out-of-range values will be investigated to assure correct recording and reporting procedures. Second, duplicate measures will be made on an approximate 14% random sample of participants for the first three months and an approximate 9% random sample thereafter. Reliability will be assessed using correlation coefficients and the technical error measurement (the square root of the pooled between measure variance as a percent of the sample mean).

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