DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

_____ is a pediatric cardiologist and Assistant Professor in Pediatrics at the University of _____. The candidate's long-term goal is to develop an independent career combining clinical research with clinical medicine. The candidate is interested in the prevention of cardiovascular disease by investigating risk factors in children and adolescents and mechanisms that influence their progress to adult atherosclerotic heart disease. _____ interests in this field developed during fellowship when _____ became interested in the relations among obesity, left ventricular mass (LVM) insulin resistance, and lipids. The proposed career development plan incorporates a multi-disciplinary program designed to provide an intense, closely mentored, patient-oriented research experience in association with a comprehensively structured didactic curriculum in epidemiology. Under the mentorship of _____ and _____, the candidate will investigate the effect of cardiovascular risk factors in adolescence on establishment of cardiovascular risk in young adulthood, while enrolled in a master's degree program in the Division of Epidemiology. This research will examine epidemiologic associations of body fatness, insulin resistance, lipids, and LVM and will test the hypothesis that body fatness and insulin resistance during adolescence predict levels of adiposity, insulin resistance, lipids, left ventricular mass, and systolic blood pressure in young adulthood. The study will be conducted in a cohort of 200 subjects recruited at mean age 13 years from the top 15% of the blood pressure distribution in a general population, and reevaluated at age 17 years. Previous studies in this cohort at age 13 have shown a difference between males and females in the response of LVM to increases in body size; and a segregation analysis in the cohort at age 17 and their parents has inferred the presence of a major gene influencing the levels of fasting insulin. Therefore, a second objective of this research will be to define gender differences in the association of left ventricular mass in young adulthood with cardiovascular risk factors in adolescence; and a third objective is to confirm the genetic results when the participants are young adults and share less of the childhood familial environment with their parents.

PERFORMANCE SITE(S) *(organization, city, state)*

University of _____
Department of _____
_____, _____

KEY PERSONNEL. See instructions on Page 11. *Use continuation pages as needed to provide the required information in the format shown below.*

Name	Organization	Role on Project
_____	University of _____	Candidate
_____	University of _____	Sponsor (mentor)
_____	University of _____	Additional Mentor

KEY PERSONNEL. (continued)

Name	Organization	Role on Project
_____	University of _____	Consultant
_____	University of _____	Consultant
_____	University of _____	Consultant

Advisory Committee:

_____	University of _____	Advisory Committee
_____	University of _____	Advisory Committee
_____	University of _____	Advisory Committee
_____	University of _____	Advisory Committee

Use this substitute page for the Table of Contents of Research Career Awards

Type to name of candidate at the top of each printed page and continuation page

RESEARCH CAREER AWARD
TABLE OF CONTENTS

(Substitute Page)

Page Numbers

Number of publications *(not to exceed six):* 1

List of key items

3 forms
Proof of U.S. Residency

Note: Type density and size for the entire application must conform to the instructions on page 6 of the general instructions.

*Include these items only when applicable.

CITIZENSHIP statement is included with the application

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory: x, N/A

Clinical: The clinic consists of 2200 sq. ft. of recently renovated space. Included are a reception area, clerical area, examination rooms, offices for interventionists, conference room, lavatory, laboratory and storage area for files and equipment. Free parking is available adjacent to the building.

Animal: x, N/A

Computer: x, N/A

Office: Office space for the principal investigator is provided by the Department of _____and is located in the _____. It consists of two adjacent offices, of which one is occupied by equipment related to tracing and digitizing echocardiograms.

Other: x, N/A

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Macintosh IIci computer with laserwriter
IBM PC AT with printer
The clinic has a modem and computer for direct communication with the Division of _____ computing service. Freezer (-20 and -70). Refrigerator.

Section II: Specialized Information

1. Candidate

a. Letters of Reference

b. Candidate's Background

c. Career Goals and Objectives: Scientific Biography

My long-term goal is to develop an independent career combining clinical research with clinical medicine. I am interested in the prevention of cardiovascular disease and believe that important scientific information in this research area can be developed by investigating risk factors in children and adolescents and mechanisms that influence their progress to adult atherosclerotic/ischemic heart disease. My research interests started during my fellowship in Pediatric Cardiology when I studied a diverse group of topics within pediatric cardiology. These resulted in primarily descriptive reports including: 1) post-mortem evaluation of coronary artery abnormalities in sudden unexpected death in the young; 2) long-term outcome in patients with pulmonary atresia and intact ventricular septum; 3) long term cardiac effects of anthracyclines in childhood cancer survivors, including evaluation of left ventricular performance and wall stress at baseline and peak exercise; 4) echocardiographic and MRI analysis conducted in the clinical research center, directed at long term outcome of individuals after surgical repair of coarctation of the aorta.

It was also at this time that I was introduced to questions about the relations between obesity, left ventricular mass, insulin resistance, and lipids in children. As a cardiologist I am aware of the epidemic proportions reached by cardiovascular disease in the adult population. As a pediatrician, I strongly believe that any effective measure against the conditions causing this disease must begin with identification and quantification of risk factors early in life and design of preventive measures during the periods of early development. It has become clear to me through participation at national and international meetings that there is much to learn about the developing heart and how early interactions with the classical risk factors for adult cardiovascular disease influence cardiac development. I am committed to making this the focus of my research.

My clinical expertise is concentrated on evaluation, diagnosis and management of children with cardiac disease. I have a strong interest and am highly skilled in echocardiography. I also have been active in the area of pediatric lipid disorders and initiated a pediatric lipid clinic at the University of _____ of which I am the Director; I envision this clinic as having great potential for patient-oriented research.

The Mentored Patient-Oriented Research Career Development Award will provide the support and time I need to develop the skills and expertise necessary to become a successful independent investigator. My work with _____, and, in particular, observing his collaborations with the Division of _____ has led me to realize that I lack some skills that are critical to a successful clinical research career. While I have gained valuable experience during the past few years on these projects, the opportunity to concentrate on developing these skills in an intense research environment will be invaluable to my progress toward an independent clinical research career.

d. Career Development/Training Activities During Award Period

Although I have a background in clinical research, my training and expertise in biostatistics and epidemiologic methodology are limited. Through my work with _____and _____ the importance of this became very clear to me. In order to become proficient in studies of disease prevention it is crucial that I acquire the skills to understand and analyze public health problems; design, implement and analyze studies; and correctly interpret study results. The Master's in Clinical Research offered in the Division of _____, School of Public Health, is ideal for these purposes, and I look forward to beginning this curriculum.

The academic environment at the University of _____ is excellent. _____, who is nationally and internationally recognized for his studies in blood pressure and has vast experience in large cohort studies, will be one of my mentors during this award period. He has been an excellent mentor for me in the past and helped guide my first steps in this field. _____ will also serve as a mentor. _____ is also nationally and

internationally recognized for his studies and is expert in epidemiologic and biostatistical methodology and in conduct and analysis of large observational studies. I feel that he and _____complement each other well with regard to the mentorship I will need in carrying out my career development plan. Both have been very generous with their time in answering my questions and I will be able to meet with them as often as necessary. In addition, I will have the opportunity to work with _____ and _____. _____is a statistical geneticist and expert in segregation analysis methodology. _____ is expert in clinical trials and cohort studies and has great experience in studies such as the one I propose.

At the University of _____, there are multiple opportunities to attend seminars, lectures and journal club meetings that either have direct relevance or will contribute to my understanding of epidemiology, cardiovascular risk factors and their interrelations. In addition, I will participate on a regular basis at meetings of the executive committee of the project "_____" directed by _____ and will meet bi-weekly with the cardiovascular risk group assembled by _____. These sessions provide an interactive forum that facilitates free exchange of ideas. They are extremely educational and they provide me with insights into and suggestions for my research.

The _____ (_____) at the University of _____ provides an ideal environment for patient-oriented research and allows the close collaboration of the researchers from several disciplines. Work on this research project in the _____will help me in developing the skills necessary for leadership through coordination of multiple research team members.

The resources of the Department of _____and School of Public Health will be available to me throughout the proposed project. I will be able to devote at least _____ of my time to the research efforts proposed in this grant. This is a unique opportunity because the faculty in the Division of _____ have heavy clinical demands. This protected time will allow me to immerse myself in the complexities of epidemiology and public health, and to interdigitate my clinical skills with these disciplines. My clinical, administrative and teaching responsibilities will not exceed _____ of time and effort. This amount of time in clinical medicine is important for me to maintain my skills and to stay abreast of the current literature. The Mentored Patient-Oriented Research Career Development Award will facilitate my acquisition of technical and academic skills necessary to achieve my ultimate goal, that of becoming a competent independent clinical investigator in the field of preventive cardiology.

2. Statements

a Sponsor/Mentor: _____.
Co-Mentor: _____.

b. Consultants:
1) _____
2) _____
3) _____

3. Environmental and Institutional Commitment to Candidate:

a. Description of Institutional Environment
b. Institutional Commitment to Candidate's Research Career Development

4. Research Plan

a. Specific Aims

This research is intended to examine epidemiologic associations pertaining to body fatness, insulin resistance, and other cardiovascular risk factors during adolescence and young adulthood. The primary objective is to test the hypothesis that body fatness and insulin resistance during adolescence predict levels of cardiovascular risk factors (adiposity, insulin resistance, lipids, left ventricular mass, systolic blood pressure) in adulthood. The study will be conducted in a cohort of 200 participants recruited at mean age 13 years (range, 11-14) from the top 15% of the blood pressure distribution in a general population, and reevaluated at age 17 years. Previous studies in this cohort at age 13 have shown a difference between

males and females in the response of left ventricular mass to increases in body size; and a segregation analysis in the cohort at age 17 and their parents has inferred the presence of a major gene influencing the level of fasting insulin. Consequently, a second objective of this research will be to define gender differences in the association of left ventricular mass in young adulthood with cardiovascular risk factors in adolescence. The third objective is to confirm the genetic results when the participants are young adults and share less of the childhood familial environment with their parents.

Specific Aim #1: longitudinal analyses

To obtain measurements at mean age 26 of height, weight, waist and hip circumference, skinfold thickness, body mass index, and blood pressure; blood samples for fasting insulin, glucose, and lipids; insulin clamp studies for insulin resistance; and echocardiographic measurements of left ventricular size in 200 (98 males and 102 females) normal young adults who have been followed since mean age 13, and to compare them with measurements of body size, lipids, fasting insulin and left ventricular size previously obtained in adolescence.

Hypothesis #1

Weight and body fatness at mean age 13 and changes through mean age 17 will predict degree of adiposity and levels of insulin resistance, lipids, blood pressure and left ventricular mass at mean age 26.

Hypothesis #2

Fasting insulin at mean age 17 and changes in fasting insulin from age 17-26 will predict insulin resistance, lipids, blood pressure, and left ventricular mass independent of body size in young adulthood.

Specific Aim #2: gender differences

To compare changes in cardiovascular risk between males and females from adolescence to young adulthood.

Hypothesis #3

With increasing adiposity, the increase in left ventricular mass between mean ages 13 and 26 will be proportionately larger in females than in males, conforming to cross-sectional findings at mean age 13.

Hypothesis #4

Insulin resistance in young adults will be greater in females than in males, due to greater body fat in females. This will be independent of differences in other risk factors between males and females.

Specific Aim #3: genetics of insulin resistance

To perform segregation analysis of adult child and parent fasting insulin levels and to carry out a mixture decomposition of adult child (age 26) M (insulin resistance) values.

Hypothesis #5

In segregation analysis, the environmental effect will be smaller and the genetic effect larger in the analysis which uses fasting insulin at age 26 than in the analysis (already performed) which uses fasting insulin at age 17.

Hypothesis #6

The statistical distribution of young adult M value will be composed of the sum of three separate distributions, consistent with the existence of a single Mendelian gene.

b. Background, Significance and Rationale

Research studies of the etiology of atherosclerotic cardiovascular disease support an association with insulin resistance which, in turn, is linked with obesity, hypertension and hyperlipidemia (1-4). The biologic effects of insulin, e.g., renal sodium retention (5), increased sympathetic tone (6), stimulation of vascular smooth muscle growth (7), and altered lipid metabolism (1) suggest an essential, possibly primary, role for insulin in these relations. However, it is not yet possible to entirely dissociate the influence of any one of these factors from the others in the development of cardiovascular disease.

The relation between insulin resistance and weight may be particularly relevant to the proposed associations between insulin resistance and cardiovascular risk. Obese adults have been shown to be insulin resistant when compared to normal control subjects (8), and obesity has been strongly correlated with cardiovascular risk (9). Data from the Framingham study have established an increased incidence of cardiovascular events in both men and women with increasing weight (10); body weight and mortality were directly related in the Harvard Alumni Health Study (11); and weight gain was a significant risk factor for development of diabetes mellitus in women (12). Other studies have shown sustained improvement in cardiovascular risk in association with a 10-15% weight loss maintained over time (13). A direct association between adiposity and insulin resistance has been reported in children (14, 15), as has the association between insulin resistance and both lipids (16) and blood pressure (17, 18). Weight loss is associated with a decrease in insulin concentration and an increase in insulin sensitivity in adults (19) and adolescents (20).

Increased left ventricular mass (LVM) is a powerful predictor of adverse cardiovascular events such as ischemic heart disease, dysrhythmias, and congestive heart failure (21,22). Although cardiovascular events are rarely seen during childhood, it is important to study markers such as increaseed cardiac mass in adolescence and young adulthood, because the pathologic processes associated with cardiovascular events appear to be in their early stages of activity. Framingham (23) and other studies in adults (24,25) have shown that an important cause of increased LVM is obesity. Moreover, studies performed in 475 adults showed that the effect of obesity on LVM appears to be greater in women than men (26, 27), especially in the presence of hypertension (26). Studies at mean age 13 in the subjects included in the present research proposal have suggested a greater effect of obesity on left ventricular mass in females than males (see Preliminary Studies). However, other studies in children and adolescents have reported a positive correlation between LVM and body size, that is associated with male gender (26, 28-31). In pediatric studies, in general, boys tend to have larger LV measurements and LVM than girls (28, 32,33). Although this is felt to be related to a greater muscle mass in boys (29), specific studies have not explored the independent influence of adiposity versus muscularity on LV size in males vs. females. Thus, neither the relation between obesity and cardiac size, the influence of gender on this relation, nor the impact of these on adult cardiovascular disease has been well defined.

The abnormal lipid profile associated with atherogenesis (elevated total cholesterol, LDL-cholesterol and triglycerides, and low HDL-cholesterol) is related to obesity and insulin resistance. The Beaver County Lipid Study in young adults (mean age 22 years) has reported positive and significant correlations between BMI and LDL-C and triglycerides (34). Weight loss was associated with improvements in lipids, blood pressure, and fasting insulin (13). The relation between weight and abnormal lipids is also present during childhood. Waist-hip ratio has been positively correlated with serum cholesterol and LDL-C in four year old children (35); and body size has been shown to be a significant correlate of blood pressure and lipids in older children and adolescents (17, 36,38). An increase in obesity during childhood is related to changes in lipids and lipoproteins that are consistent with a more atherogenic lipid profile. Children examined at age 5-12 in the Bogalusa Study and re-examined five years later had significant correlations between change in triceps skinfold thickness and change in cholesterol, triglycerides, LDL-C, HDL-C, and VLDL-C (39); and in two separate Bogalusa cohorts evaluated after an eight year period of observation increases in weight were accompanied by adverse changes in lipids and lipoproteins (40). Similarly, in subjects examined initially at 8-18 years in the Muscatine study and again during their third decade a direct association was found between development of obesity and adult cholesterol levels (41). Insulin influences lipid metabolism via regulation of very low density lipoprotein (VLDL) production by the liver (42). Hyperinsulinemia is

associated with hypertriglyceridemia not only in obesity (1,43,44) but also in individuals with normal weight (45), and it is inversely correlated with HDL-cholesterol (1). Hyperinsulinemia and insulin resistance are characterized by an atherogenic lipoprotein profile (46,47) and insulin resistance is associated with asymptomatic atherosclerosis (48) independent of obesity or hypertension. The CARDIA study of 4576 young adults reported a weight-independent association between insulin and lipids (49). While it is currently impossible to entirely dissociate the influence of insulin from obesity on lipid levels, it is clear that an association exists between insulin and lipids that is independent of the association with obesity.

The insulin resistance (or multiple metabolic) syndrome (insulin resistance, non insulin dependent diabetes, dyslipidemia, obesity, and hypertension) is determined, at least in part, by genetic determinants (50-52) and there is evidence for a genetic influence on individual components of the syndrome. A strong genetic influence on blood pressure has been demonstrated in early childhood (53) with intensification in the presence of other risk factors (54). Fasting insulin, blood pressure and lipids are closely related in young adult offspring of hypertensive parents (55), and a parental history of NIDDM and hypertension is associated with increased levels of insulin resistance in their children (56). The aggregation of lipid levels within families has been previously recognized (57) and forms the basis for current lipid screening recommendations in children (57). Data obtained from this study cohort in adolescence showed a significant relation between insulin, lipids and blood pressure, as well as a significant relation for these factors between adolescents and their parents (see Preliminary Studies). Also in this cohort at age 17 a segregation analysis of fasting insulin in children and their parents strongly suggests the presence of a major gene (see Preliminary Studies).

1) Significance of the Research

Extensive anthropometric, blood pressure, and echocardiographic measurements on this population are available to us from age 13, and fasting insulin and lipid levels are available in most subjects from age 17. With these previous data, the proposed research provides an opportunity to determine the relationships among cardiovascular risk factors at the childhood-adolescent-adult transition, i.e., the putative earliest point in the development of cardiovascular risk, and to assess etiologic relations between early indicators of insulin resistance and establishment of risk in young adulthood. Specific gender-related and genetic analyses will further define the role of these risk factors. It is reasonable to suggest that understanding these epidemiologic relationships at earliest development and prior to the onset of overt disease may lead to strategies for reducing cardiovascular risk.

c. Preliminary Studies and Results

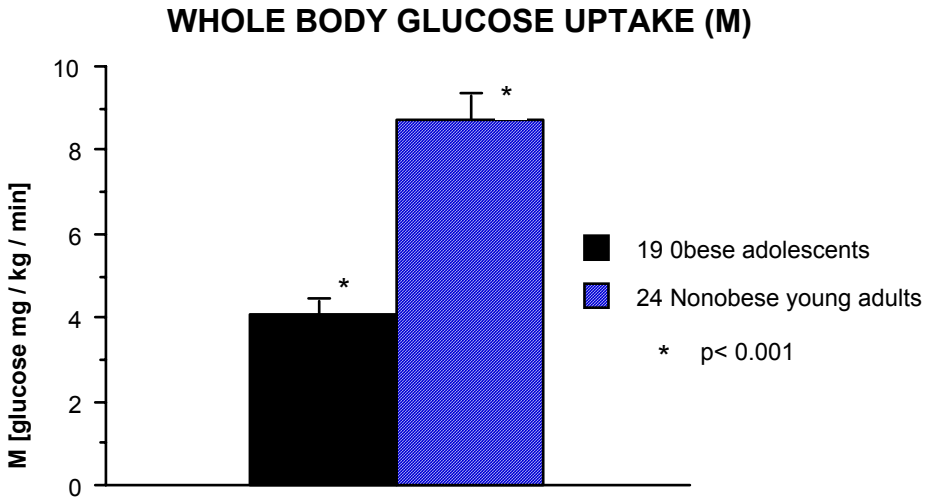
1) Results from a different study sample:

The relationship between insulin resistance and abnormal lipid profile in obese adolescents

Studies utilizing the euglycemic insulin clamp technique in normoglycemic individuals have suggested that insulin resistance can be linked with lipid and lipoprotein abnormalities. Insulin resistance has been associated with elevated fasting and post prandial insulin levels, and has been hypothesized to play a major role in dyslipidemia in individuals with normal glucose tolerance as well as those with impaired glucose tolerance, and non insulin dependent diabetes.

In a collaborative study with _____, Division of _____, University of _____, we examined whether lipid abnormalities occur in normoglycemic, obese adolescents and are associated with insulin resistance (reference number - manuscript appended). The relationship between lipid levels and insulin resistance was assessed in 82 obese adolescents (mean weight = 69.9±2.5 kg, mean % fat = 37.4±1.1%), by comparing fasting insulin and sum of the insulin values after an oral glucose tolerance test to those from 40 nonobese adolescents (mean weight = 44.3±2.9 kg, mean % fat = 20.1±1.0%). Whole body glucose uptake during euglycemic hyperinsulinemia (M value) was performed in a subset of 19 of the obese adolescents and compared with another control group, 24 nonobese young adults. The obese adolescents had significantly elevated LDL-cholesterol and triglycerides and lower HDL-cholesterol when compared with the nonobese subjects. M values were significantly depressed (i.e., increased degree of insulin resistance) in the obese compared with the nonobese subjects (see Figure 1).

Figure 1



Among the variables representing insulin resistance (fasting insulin, sum of insulin during oral glucose tolerance test, and M), the strongest correlation with the abnormal lipid profile was found for the M value. In stepwise multiple regression analysis, the M value was the only variable entered into the relationship for the dependent variables triglycerides and LDL-cholesterol, while both M value and fasting insulin entered for HDL-cholesterol.

Thus, in this small sample, the degree of insulin resistance in obese adolescents is correlated with the levels of triglycerides, LDL-cholesterol and HDL-cholesterol.

2) Results from the cohort proposed for this study

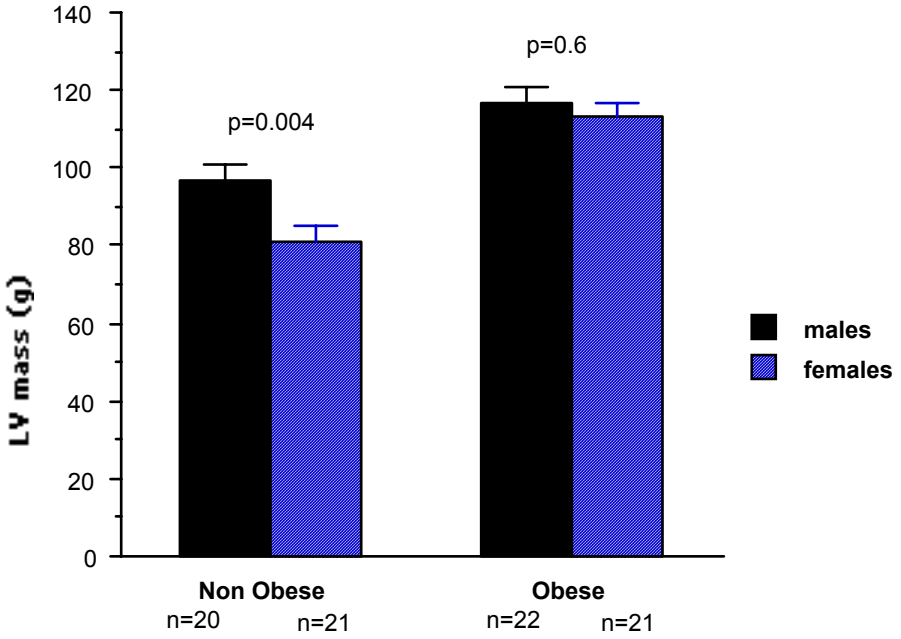
a) Obesity and female sex influence left ventricular size in children

Echocardiographic measurements of left ventricular posterior wall thickness, chamber size (left ventricular internal dimension) and mass were performed in 210 children aged 11-14 yrs. Children were stratified into quintiles of body mass index. Comparisons were made between the highest (obese) and lowest (nonobese) quintiles (BMI (mean ± standard error): 29.4±0.7 v 17.2±0.1 p=0.0001). Systolic blood pressure differed significantly between quintiles (males: 131±2 v 119±2 p=0.0001; females: 123±2 v 118±2 p=0.03). Echocardiographic measurements were made using the American Society of Echocardiography criteria. Comparisons of left ventricular size and mass between obese (highest quintile of BMI) and nonobese (lowest quintile of BMI) in the table below were adjusted for height, systolic blood pressure and sexual maturity (Tanner score).

	Male			Female		
	Obese n=22	Nonobese n=20	P	Obese n=21	Nonobesen =21	P
Left ventricular wall thickness(mm)	7.0±0.2	6.6±0.2	0.10	6.7±0.2	5.8±0.2	0.007
Left ventricular internal dimension (mm)	51.4±0.7	48.7±0.2	0.01	49±0.7	45±0.7	0.0004
Left ventricular mass (g)	116.6±3	98.1±3.3	0.0006	108±3.7	74±3.4	0.0001

In this study, obesity in children, independent of height, systolic blood pressure, and sexual maturity, was associated with increased left ventricular (LV) size and mass. These findings are consistent with previous reports showing a direct relationship between body size and LV size in children and a larger LV size in males than females. However, it was found that LV mass was significantly greater in boys than in girls only in the lowest (non obese) quintile of BMI, whereas in the highest (obese) quintile LV mass was similar in males and females; this is depicted in the graph below.

Figure 2



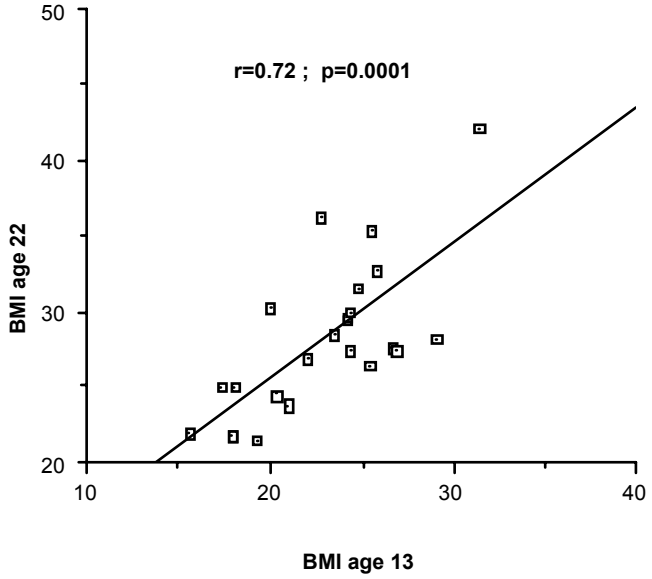
The results were similar when waist circumference was substituted for BMI in the analyses. Thus, these data suggest that body fatness has a particularly adverse effect on cardiac size in females.

It is not clear why these gender differences exist between children of the upper and lower BMI quintiles. It is possible that the greater LV mass/BMI relation in non obese males is due to a greater muscle mass, whereas in obese children the equalization of the LV mass/BMI relation between males and females may be due to a disproportionate increase in fatness in females as they gain weight. The relative importance of the differences in changes in LV mass and body fatness in males versus females as they mature from adolescence to young adulthood is not known.

b) Adiposity at age 13 is a predictor of adiposity, insulin resistance and abnormal lipids at age 22

The purpose of this study was to determine whether adiposity in children predicts insulin resistance and abnormal lipids in young adults. The children had blood pressure, weight and height measured at age 13.3 ± 0.3 years. 24 of them (7 males and 17 females) were reevaluated at age 21.8 ± 0.3 years, at which time the measurements were repeated, a euglycemic insulin clamp was performed, and fasting lipids were measured. All values are expressed in mean ± SEM. Data were analyzed by linear regression analysis. Body mass index (BMI) in childhood (22.8 ± 0.8) was highly correlated with BMI in young adulthood (28.3 ± 1.02) (r = 0.72; p = 0.0001). As shown in Figure 3, although only 2 of the 24 subjects at age 13 had a BMI>27, at age 22, eleven subjects had a BMI>27.

Figure 3



The Figures below show the regression analyses of the M value on BMI at age 22 (Figure 4), and of M value at age 22 (Figure 5) on BMI at age 13.

Figure 4

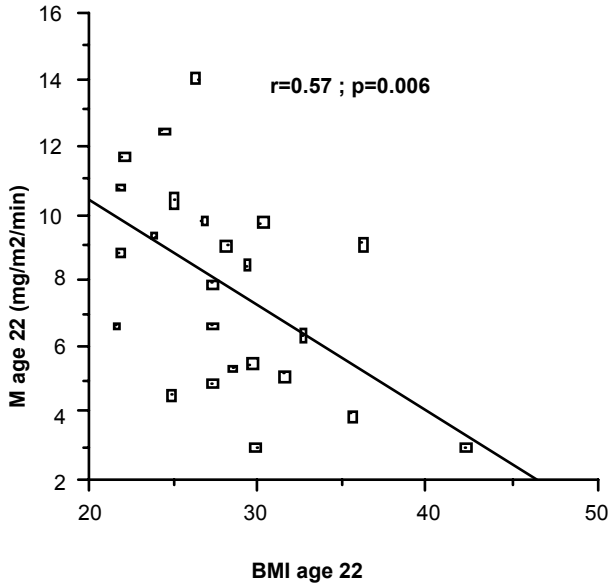
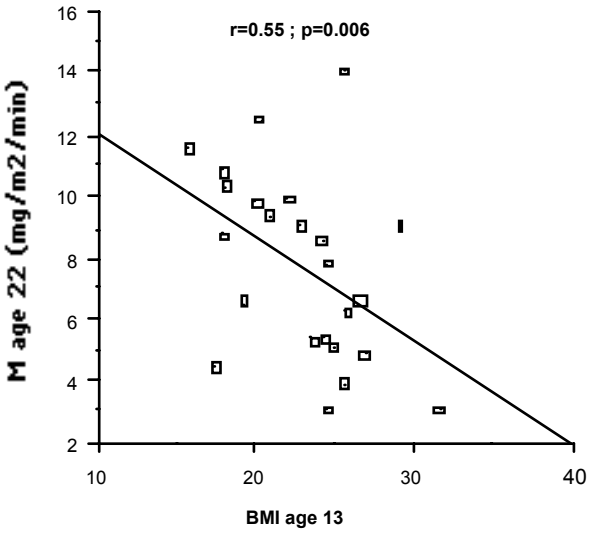


Figure 5



These data suggest that: despite the low frequency of obesity at age 13, higher BMI at age 13 predicted obesity at age 22; at age 22 insulin resistance was directly correlated with adiposity; and in the relatively nonobese population higher BMI at age 13 was predictive of insulin resistance at age 22.

Childhood BMI was not only highly correlated with young adult insulin resistance (r=0.55, p=0.006), but also with total cholesterol (r=0.68, p=0.0006), and LDL-cholesterol (r=0.70, p=0.0003). These data confirm that adiposity in childhood is a strong predictor of young adult adiposity and that cardiovascular risk factors such as insulin resistance and hyperlipidemia in young adulthood are related to the degree of adiposity established as early as age 13.

c) Relation of Fasting Insulin to Blood Pressure and Lipids in Adolescents and Parents

The children were 16.7 ± 0.1 years (range: 14-18 years) at the time of this study. A fasting early morning blood sample was obtained from 183 of the 210 children (87 boys, 96 girls) and 241 of their parents (143 mothers, 98 fathers) for fasting insulin and lipids. Fasting insulin was significantly correlated with systolic blood pressure in the adolescents (r=0.29, p=0.00001) and also in the parents (r=0.20, p=0.0076) before and after adjustment for BMI. Fasting insulin was correlated significantly with cholesterol, triglycerides, HDL-C, and LDL-C in the adolescents. It was correlated only with triglycerides and HDL-C in mothers and fathers. After adjustment for BMI, the correlations between fasting insulin and lipids in the children were not significant. Associations between parents' and children's values are shown in the table below.

Pearson Correlation Coefficients (r) Between Parents' and Children's Fasting Insulin, Lipids, and Systolic Blood Pressure Before and After Adjustment for BMI

		Adjustment for BMI			
		Before		After	
Variable	Statistic	Mother (n = 143)	Father (n = 98)	Mother (n = 143)	Father (n = 98)
Insulin	r	0.18	0.29	0.23	0.36
	p	0.03	0.006	0.005	0.003
Total Cholesterol	r	0.38	0.12	0.35	0.08
	p	0.0001	0.23	0.0001	0.43
Triglycerides	r	0.34	0.25	0.36	0.13
	p	0.0001	0.01	0.0001	0.19
LDL-C	r	0.42	0.17	0.38	0.17
	p	0.0001	0.11	0.0001	0.10
HDL-C	r	0.20	0.29	0.19	0.22
	p	0.02	0.05	0.03	0.03
Systolic Blood Pressure	r	0.15	0.13	0.15	0.09
	p	0.07	0.21	0.07	0.56

Significant correlations were found between the children and fathers fasting insulin, triglycerides and HDL-C, whereas significant correlations were found for fasting insulin and all lipids between mothers and children, and these remained significant after adjustment for BMI (except for children's and father's triglycerides - see table). A significant relation was shown for children's systolic blood pressure (dependent variable) regressed on mother's fasting insulin and systolic blood pressure. These results show 1) a significant relation between fasting insulin and both lipids and systolic blood pressure in adolescents and 2) a significant relation for these factors between adolescents and their parents. Although weight appears to play an important role in this relation during adolescence, genetic and environmental factors other than those mediated via weight appear to be operative in the control of insulin metabolism within families.

d) Genetic Studies:

A segregation analysis (58) of fasting insulin was performed on this cohort (16.7 ± 0.1 years) and their parents by _____, Division of _____, _____. Using maximum likelihood methods a model allowing Mendelian transmission only and a model allowing environmental transmission only was tested against a general model that incorporated both sets of variables. The Mendelian model was accepted (p=0.51) and the environmental model was rejected (p=0.00002), leading to the inference of a major gene. The frequency of the low (L) allele was estimated to be 0.75 and the frequency of the high (H) allele was 0.25, the mean fasting insulin value of the LL genotype was 13.7, of the LH genotype was 20.0, and of the HH genotype was 32.5. The model simulataneously adjusted for the effects of sex, age, and BMI. As

maximum likelihood methods are sensitive to outliers, they were removed. The major gene accounted for 46% of the total variation, the covariates accounted for 20% of the total variation and 34% was due to noise.

d. Research Design and Methods

1) Participants

a) Young Adults

The cohort of participants consists of approximately 200 subjects who will be aged 25-27 years in 1999. These participants originally were recruited in 1985-1986 (at ages 11-14 years) as participants in the "Sodium -Potassium Blood Pressure Trial in Children" (59). Blood pressure screening was conducted in 19,452 (93% of all enrolled) 5-8th grade students in the _____ and _____ public schools during regular school days. Blood pressure was measured twice on the right arm with students in the seated position by trained personnel using a standard clinical sphygmomanometer and following a standardized protocol (60). All children whose systolic blood pressure (mean of two measurements) equaled or exceeded the 70th percentile of the sex and age-specific blood pressure distribution, as derived from the screening, had their blood pressure measured a second time under identical conditions. After rescreening of all black, white and Hispanic children, the top 15 percent of the blood pressure distribution (n=3,223) were further screened for eligibility and willingness to participate; 231 were enrolled, in a four year blood pressure intervention trial. Blacks represented 17.6% and Hispanics 2.8% of the total children screened, and their representation in the group of 231 was 12.8% and 1.6%, respectively. The participants were seen in clinic four times each year for 4 years. At each of the clinic visits, data were obtained for height, weight and blood pressure. Once each year a complete set of anthropometric data were obtained, including height and weight, waist and hip circumferences, and triceps and subscapular skinfold measurements. At the end of four school years, fasting blood samples were obtained for insulin, glucose and lipids; in addition, body size measurements and fasting blood samples for glucose, insulin and lipids were obtained from the parents of the participants. Of the 231 initial participants we have maintained contact by telephone and postcards with approximately 200 who are now young adults and have expressed willingness to participate in this study.

b) Parents of the Young Adults

Parents will be seen once in the clinic for anthropometric and blood pressure measurements, and in the Clinical Research Center for fasting insulin, lipids and glucose.

2) Clinic and General Clinical Research Center (GCRC) Protocol for Young Adults

For logistic reasons, the protocol requires 2 separate visits, one to a clinic, the other to the GCRC. The two visits are usually several days apart. This protocol has been pilot tested in 24 participants as detailed in the preliminary studies section. Visit contents are summarized in the table below.

Schedule of measurements at the clinic and at the _____ Research Center (_____RC)

	Clinic	_____RC
Blood pressure	X	-
Anthropometry (weight, height, triceps and subscapular skinfold, waist and hip circumference)	X	-
Questionnaires (participant past medical history, family social history, family medical history)	X	-
Euglycemic insulin clamp	-	X
Serum lipids	-	X
Echocardiogram	-	X

a) Blood pressure and Anthropometry

After arriving at the clinic, participants will have their seated blood pressure measured twice, using a random zero sphygmomanometer. Anthropometric measurements (height, weight, waist and hip circumferences, and subscapular and triceps skinfold thickness) then will be obtained.

b) Questionnaires

These forms were developed for prior studies at the University of _____ and have undergone extensive evaluation and use. They include: participant past medical history, family social history, family medical history, and exercise and diet.

c) Echocardiogram

Echocardiography will be obtained using 2D-Echo guided M-mode imaging with Doppler to evaluate cardiac mass, cardiac output and cardiac function. Analyses will be conducted to determine if changes in these measurements can be correlated with changes in insulin resistance, or blood pressure. All studies will be performed in the Echocardiography Laboratory of the University of _____ utilizing Hewlett-Packard echocardiographic equipment by _____, a technician with over _____ years experience. Measurements will be made by _____. Accuracy will be determined by random selection of five percent of echocardiograms for evaluation by a second reader and for a second blinded reading by the applicant. All measurements will be made in accordance with the recommendations of the American Society of Echocardiography using leading edge to leading edge methodology (61). The transverse dimensions of the left ventricle at end diastole and at end systole will be obtained with the ultrasound beam passing through the left ventricle slightly below the tips of the mitral valve leaflets. The end-diastolic dimensions of the left ventricular cavity (LVID), posterior wall (LVPW) and interventricular septum (IVS) will be taken at the onset of the QRS complex. Left ventricular systolic dimension will be measured at the nadir of septal motion. Left ventricular cycle length for heart rate calculation also will be measured at the onset of the QRS complex. The measurements for five consecutive beats will be averaged for each participant. Left ventricular mass (LVM) will be calculated utilizing the formula $LVM = 0.80\ (1.04x(IVS+LVID+LVPW)^3-LVID^3))+0.6$, as previously recommended by Devereaux et.al. (62). Systolic function will be estimated by calculating the fractional shortening of the left ventricle (the difference between the LVID at end diastole and end systole/ LVID at end diastole). Left ventricular peak systolic wall stress (PSWS) will be estimated utilizing the formula recommended by Grossmann et.al (63) based on left ventricular end-systolic (ES) dimensions **:** PSWS = [(1.35)(systolic BP)(LVID systole)] /[(4)(LVPW systole)(1+LVPW systole/LVID systole)].

d) Euglycemic Insulin Clamp

The euglycemic clamp studies will be performed in the _____ of the University of _____. All participants will be admitted to the Center the morning of the study after fasting from 8:00 p.m. The study will begin at 7:00 a.m. With the participant in a semi-supine position, a polyethylene cannula will be placed into an antecubital vein in one arm. A scalp vein needle will be inserted into a dorsal vein of the other hand, after which that hand will be placed in a warming box at 60 degrees C to obtain arterialized venous blood samples. The participants will remain semi-supine (45 degree elevation) throughout the study. Blood will be drawn for sodium, potassium, glucose, insulin, cholesterol, triglycerides and HDL-C. After the blood samples are obtained, a constant infusion of insulin will be administered at a dose of 1mU/kg/min for 180 minutes. Concomitantly with the insulin, an intravenous infusion of 20 percent glucose will be administered by a variable infusion syringe pump (Harvard Apparatus, Holliston, Mass). Blood samples will be obtained at five minute intervals for determination of blood glucose concentration. The plasma glucose concentration will be held constant at baseline by varying the glucose infusion rate every five minutes. Since at these insulin infusion rates hepatic glucose output should be nearly completely suppressed, the amount of glucose required to maintain euglycemia will be used as the index of whole-body glucose uptake.

3) Data Processing and Management

The _____ and_____ (_____) center of the Division of _____ at the University of _____ available for use by the candidate. This center has experience over decades in data processing of large epidemiological studies. It has developed a modern data processing system of national reputation, and its use will assure quality and completeness of data. Use of the _____ simplifies creating, editing and merging clean data files. A process to accomplish these tasks has been already in place for several studies conducted by our group.

Data collected at the clinic will be visually edited, batched and sent to _____ for entry. Data from a particular form are entered and then appended to the entire data set on a VAX mainframe. The _____ and _____ (_____) in the Department of _____ at the University of _____ builds in edit and consistency checks for data entry, so all data are verified and edited and a study data file is created.

4) Quality Control

All personnel participate in training sessions prior to the study and undergo training in all measurement techniques every six months. Personnel involved in measurements are compared using Z-scores for each observer (standardized deviates comparing each observer to the average of all others) and those with significant Z-scores are retrained and retested.

Forms to be used in this study have been carefully pretested and have been used previously. All forms are precoded and are reviewed using a clinic checklist before the participant leaves the clinic.

Laboratory variability will be assessed by a 5 to 10% sample of blind duplicates sent to each lab to determine the technical error of the measurement, which is computed as $(\sum d^2/2n)^{1/2}$, where d is the difference between duplicate samples and n is the number of duplicates.

For echocardiograms, a 5% random sample will be selected for a second (blinded) reading by the candidate and for reading by a second echocardiographer. Inter and intra-observer measures of agreement will be computed using statistics such as Kappa and intraclass correlation coefficients.

5) Analysis Plans

a) Analyses of hypothesis #1 and #2

We will initially assess whether anticipated associations hold in cross-sectional data at age 26, following completion of studies performed in the clinic and in the Clinical Research Center. In these analyses, relations of insulin resistance to body fatness and other variables of interest will be characterized, and useful insights will be provided for subsequent longitudinal analyses. The expectation is that: 1. body weight and body mass index will be positively correlated with blood pressure, insulin resistance, dyslipidemia, and left ventricular size; 2. insulin resistance will be positively correlated with blood pressure,total cholesterol, triglycerides, LDL-cholesterol and left ventricular size, and negatively correlated with HDL-cholesterol, and will explain the association of body size to these factors.

Insulin resistance will be defined either as fasting insulin or as the glucose uptake during the euglycemic insulin clamp. It is expected that relations will be stronger with the more specific insulin resistance measure, glucose infused in the euglycemic insulin clamp than with fasting insulin. In each case, for descriptive purposes, correlation coefficients will be examined, and means and standard errors of blood pressure, body size, serum lipids, and left ventricular wall thickness will be examined according to categories of insulin resistance**.** Multiple regression analysis with insulin resistance as the dependent variable and body size as the independent variable of interest will be used to assess whether observed relations are independent of age, race, sex and blood pressure. General body fatness will be assessed using the body mass index (wt/ht^2), while central fatness will use waist circumference. Body fatness will also be assessed using triceps and subscapular skinfolds. Our expectation is that insulin resistance/ body fatness relations will be found to be independent of all other factors examined. Parallel analyses will be carried out for other dependent variables: blood pressure, serum lipids, and left ventricular wall thickness.

Further analyses will be carried out with each of these latter variables as dependent variables and body size and insulin resistance both as independent variables. Because we hypothesize that the effect of obesity on these dependent variables is mediated by insulin resistance, our expectation is that, in these multiple regression analyses, insulin resistance will be predictive, but body size will not.

We will specifically assess whether the observed relations are different in race, sex, serum lipid or blood pressure strata (the latter two for left ventricular mass). Goodness of fit of regression analyses will be assessed by examining mean levels of dependent variables according to categories of independent variables.

Hypotheses #1 and #2 are that adolescent levels and changes in body size and insulin resistance are predictive of the development of adiposity and cardiovascular risk factors at age 26. The associations are predicted to parallel those seen in the cross-sectional analyses, that is, that body fatness and insulin resistance in adolescence will predict young adult obesity and insulin resistance, as well as changes from adolescence in blood pressure, serum lipids, and left ventricular wall thickness.

We recognize that, compared to longitudinal analyses, the cross-sectional analyses are in some ways stronger, and in some ways weaker estimates of the strength of relations between insulin resistance, body fatness and the cardiovascular risk factors. The great strength of the cross-sectional analyses is that they pertain to long term relations in the sense that they represent the cumulative (26 year) lifetime experience of insulin and body size. However, the cross-sectional analyses have several weaknesses. They do not assess temporality (for example, does increased insulin resistance precede or coincide with body size increase) and they do not take advantage of increased statistical power due to reduced variance of within person analyses. Longitudinal analyses are more powerful in these respects.

Preliminary analyses will examine means and standard deviations of longitudinal variables, and correlations between variables. Multiple regression analyses will use change in each of blood pressure, serum lipids, and left ventricular mass as dependent variables, and assess their associations with the independent variables baseline levels of body size and of insulin resistance. In longitudinal as in cross-sectional analyses we expect stronger relations with the euglycemic clamp measure than with fasting insulin, and we anticipate that associations with body size will be explained by insulin resistance.

We will also model change in cardiovascular risk factors (dependent variables) according to body size and insulin resistance simultaneously a) as level at age 13/17 and b) change until age 26, to examine whether adolescent levels of body size and insulin resistance are predictive of changes in the cardiovascular risk factors, independent of their changes during adolescence.

b) Analyses of hypothesis #3, #4

These analyses pertain to gender differences in the development of higher levels of insulin resistance and left ventricular mass.

Specifically, it is our expectation that the slope of change in left ventricular mass on body mass index and waist circumference will be steeper in females than in males. This analysis will be carried out by examining a regression analysis of change in left ventricular mass on body mass index and waist circumference, gender and their interactions.

We also anticipate that insulin resistance will be greater in females than in males, and that this association will be explained in multiple regression by adjustment for body fatness.

c) Analyses of hypothesis #5

Complex segregation analysis (64) will be performed on the phenotype using the computer program REGC in SAGE (58). We will compare a set of restricted models to an unrestricted model. For a single locus with two alleles, the unrestricted model assumes that up to three unobservable types exist in the data and that these types may correspond to any genetic inheritance. The three types can be denoted as AA, Aa, and aa. Corresponding to each type will be a mean value of that type. This mean is assumed to be the mean of a normal distribution. This mean will be estimated for each type along with a common standard deviation for the associated distributions. Three transmission parameters denoting the probability that an individual with a given type (AA, Aa, or aa) transmits A to an offspring, will be estimated in the unrestricted model. Different patterns among these transmission parameters will indicate whether an environmental effect or a single gene explains the data. These two possibilities, environmental (cardiovascular risk factor) or genetic, form the two subclasses of restricted models. These classes of restricted models will be tested against the unrestricted model using the unified approach of Lalouel et al. (65). The first class of models allows only random environmental effects. The value of the transmission parameters are all equal in this model, i.e. the types have no effect on the data. Variations on this model allow for polygenic inheritance and heterogeneity between generations. The second class of models assumes Mendelian transmission is the major cause of phenotypic variation. The three Mendelian transmission parameters are (1,1/2,0), i.e. the probability that AA transmits A to his offspring is 1, the probability that Aa transmits A to his offspring is 1/2, and the probability that aa transmits A to his offspring is 0. Variations on this model include restrictions on the means that represent dominant and recessive effects. Residual non-independence among relatives is subsumed into familial correlation parameters.

In humans, random mating is usually assumed. This implies that the frequencies of the types follow Hardy-Weinberg proportions (p^2: 2p(1-p): $(1-p)^2$), thus only one allele frequency, p, is required. In human populations deviations from Hardy-Weinberg equilibrium are almost unknown (66).

Effects of covariates that have been shown to be significant in simpler models or are suspected of having an effect based on prior information will be estimated simultaneously. Each restricted model will be compared with the unrestricted model using likelihood ratio statistics which are twice the difference between the natural log-likelihoods of the unrestricted and restricted models. These test statistics are approximately asymptotically distributed as a chi-square distribution with degrees of freedom equal to the difference in the number of parameters between the two models being compared. If there is no significant difference between the models then the more parsimonious restricted model is preferred. A "major gene" is said to exist when the Mendelian model is accepted and the environmental model is rejected. Effects between the two age points will be evaluated with standard statistical paired and longitudinal methods similar to those presented in the previous section of this proposal.

d) Analyses of Hypothesis #6

This hypothesis states that the young adult (age 26) M values arise from 3 separate distributions, each corresponding to one genetic type (AA, Aa, or aa) of a Mendelian trait. Although parent M values from the euglycemic clamp will be be available, it is still possible to deconvolute the histogram of young adult M values, to estimate the 3 underlying probability distributions. The program REGC can be used for this purpose. If the hypothesis is true, REGC will estimate that the observed distribution of M is well described as the sum of 3 underlying normal distributions, with varying means. such a deconvolution was shown to hold in an analysis preliminary to the segregation analysis carried out using the fasting insulin values at age 17, and is expected to hold for M values, a more direct measure of insulin resistance.

e) Detectable differences

The detectable difference in change in left ventricular mass from adolescence through young adulthood, according to level of fasting insulin at age 17 was estimated based on the detectable linear regression coefficient, using variances based on age 17 fasting insulin and pilot study left ventricular mass. The standard deviation of change in left ventricular mass was 36 grams, and of fasting insulin at age 17 was 10 microU/ml. Assuming that we observe a sample of about 200, we estimated the standard error of the regression coefficient of left ventricular mass on fasting insulin to be $36/(10*\sqrt{199}) = 0.25$ g/microU/ml. For alpha = 0.05 and power = 0.85, the regression coefficient must be at least 3 times its standard error (0.75 g/microU/ml) to be declared statistically significant. This corresponds to 7.5 g per 10 microU/ml (one standard deviation) of baseline insulin. A difference of this magnitude is of clinical interest, and the study is deemed to have adequate power.

5. Career Development Plan

This career development plan incorporates a multi-disciplinary program designed to provide an intense, closely mentored, patient-oriented research experience in association with a comprehensively structured didactic curriculum in epidemiology. The goal is to build on the Candidate's previous training and experience in clinical Cardiology while providing the additional epidemiologic skills required to successfully pursue a clinical research career. The Candidate's record, to date, indicates a strong commitment to an academic career. On completion of this plan she will have the ability to compete on a national basis for patient-oriented research funding, independent of her mentors.

This is a five year program in which the clinical research protocol will run concurrently with the didactic course. Attempting to schedule the didactic sessions in a solid block of time would require a two-year full time commitment by the Candidate and eliminate the possibility of any meaningful work on the research protocol. The nature of this type of patient-oriented research is such that it demands regular attention over a multi-year time frame. Thus, the program will allow the Candidate to enroll in classes spread over a four-year period and provide sufficient time to organize and conduct the clinical research. In addition, attempting to concentrate either component of this plan to a greater degree will not allow for the 25% time allotted by this award for ongoing clinical activities.

a. Didactic Component

The didactic component will be conducted in the Division of _____, _____(_____). The Candidate will be enrolled in an Interdisciplinary Graduate Program in Clinical Research. This program consists of 53 credits of course work (quarter basis) and offers an MS degree in Public Health. As noted above, the Candidate will successfully complete the course over four years. This course will provide a comprehensive educational resource that will prepare the Candidate for all aspects of patient-oriented research, including, but not limited to, the following :

1) Biostatistics: probability models, hypothesis testing, regression and correlation techniques, analysis of variance, multiple regression analysis, model selection and analysis, and others.

2) Epidemiologic Principles; general principles applicable to epidemiologic studies.

3) Clinical Trials: methodology of randomized clinical trials, including design issues, case examples, operational aspects and applications to follow-up studies.

4) Epidemiologic Methods: methods and techniques for collecting and managing research data , including sampling, response rates, forms design, training interviewers, and data preparation , entry, cleaning, and management.

5) Research Grant Writing: mechanics of grant development and writing, principles of informed consent, budget development, grant-review process.

6) Statistical Computing: analyzing biomedical data.

7) Biomedical Ethics.

8) Genetic Epidemiology: disease within relatives, inherited disease in populations, case-control family studies, twin studies, segregation analysis, gene mapping.

9) Electives.

10) Thesis.

In addition to the course work, _____ will participate in the Division of _____ graduate student research seminars.

b. Research Plan

The research plan will build on the Candidate's prior patient-oriented research experience in which she has had the opportunity to observe methodologies for protocol development, participant recruitment, and data gathering and has been trained in the insulin clamp procedure for determining insulin resistance. The Candidate will be the Principal Investigator on this grant. She will be responsible for patient recruitment and scheduling and will conduct the insulin clamps in the _____. She will ensure accurate data collection and entry and will be responsible for data analysis.

It is anticipated that the proposed protocol will be completed over five years. By balancing the didactic program with the research proposal, adequate time will be provided for the significant patient-oriented activities required to successfully complete the study.

c. Mentors

1) _____.

_____ has been a member of the Division of _____, Department of _____ since 1974. The Division has trained approximately 30 fellows during that time, and approximately 90% of them currently are in academic faculty positions.

_____ has been involved in patient-oriented research on cardiovascular risk factors for over 20 years. He is nationally and internationally recognized for his studies in blood pressure and cardiovascular risk in children and young adults. Specifically, he has had ongoing NIH funding since 1985 for large cohort studies of the type proposed in this application and currently is Principal Investigator of the study "_____". He has served on _____ and is a member of the _____ and the _____.

2) _____

_____ has been a member of the Division of _____ since 1974. Since 1988, he has been advisor or co-advisor for 19 Ph.D. students, 17 of whom are currently in academics or government research, and 8 postdoctoral fellows, all of whom are still in research.

_____ is expert in epidemiologic and biostatistical methodology and in conduct and analysis of large observational studies. He is nationally and internationally recognized for his studies of determinants of cardiac and other chronic disease and public health interventions and currently is Principal Investor on two NIH funded studies. He and _____ have worked closely together for a number of years.

d. Group Support

_____ will meet weekly with her mentor, _____, and will participate in the activities of the clinical research/epidemiology group associated with ongoing research of cardiovascular risk. This group, consisting of _____, _____, _____, _____, and _____ meets bi-weekly to review progress in current studies, review data analyses, consider new questions and areas of research suggested by these data, plan for submission of new grant proposals and funding, and plan and review manuscripts. In addition, _____ will meet regularly with _____ to review data collection and analysis questions and with _____, and _____ to discuss specialized areas of her research and course work.

e. Instruction in Responsible Conduct of Research

_____ has completed a required course, "Responsible Conduct in Research" given by the University of _____ on June 10-11, 1996. Subjects included: 1) The role of the scientist in society; 2) Environmental health and safety issues; 3) Responsibility and ethics regarding the role of the faculty member in mentoring; 4) The role of the institutional review board in ensuring appropriate participant consent, risk-benefit balance, justice, advocacy for research subjects, adverse event recording, modification of protocol; 5) Code of conduct regarding fabrication of data, plagiarism, supervision of research and assignment of authorship, and fiscal responsibility; 6) Conflict of interest.

During the period of the Award _____ will enroll in Philosophy 8320, “Ethical Issues in Human Experimentation. This course will discuss ethical protection of human subjects, definition of research, informed consent, competency, and ethics of research on vulnerable subjects such as children, prisoners, and the mentally ill.

Section III: Other Information

1. Research Plan Continued

a. Minorities and Women:

1) The subject population will be approximately 200 young adults, ages 25-27 years, equally divided between males and females, approximately 13% African-American, 2% Hispanic and 85% white, and in excellent health.

b. Human Subjects:

1) Research material obtained from individuals will consist of blood specimens, urine specimens and information recorded on a number of forms. These materials and data will be obtained specifically for research purposes.

2) Recruitment will be by letter, telephone call and personal interviews. The participants will sign informed consent and a copy of the consent will be provided to all participants and/or parents. Participants will be fully informed of all procedures to be performed and how all information will be used. Consent will be documented by signature of the participant and will be witnessed by clinic personnel. The Institutional Review Board has not authorized any modifications or waiver of the elements of consent.

3) The risks from this study are minimal. There is the potential that a small amount of pain will occur during the blood drawing. The measurements and form completion have been performed in numerous studies by our group and have not been shown to be a risk. The potential risk associated with the insulin clamp studies is minimized by performing these studies in the _____ under close medical supervision.

4) Absolute confidentiality will be maintained. All data are stored in locked compartments and are not released without consent of the participants. If data are used in scientific presentations or publications, individuals are never identified. All data will be monitored by the applicant and Sponsor at their meetings.

5) Identification of physiologic and/or biochemical factors that may be associated with the onset of cardiovascular risk offers the opportunity to initiate intervention strategies early in development in order to prevent onset of the disease. A number of basic and clinical studies have suggested that insulin resistance may be etiologically related to cardiovascular risk. Thus, determining the relation between insulin resistance, and other risk factors during childhood and adolescence may help reduce the incidence of cardiovascular disease. The very small risk involved with this study may result in great benefit, if the findings lead to a greater understanding of factors that cause cardiovascular disease.

c. Vertebrate Animals

Not applicable

d. Literature Cited

e. Consortium/Contractual Arrangements
Not applicable

2. Checklist

3. Appendix