

To the Graduate Council:

I am submitting herewith a thesis written by Marilyn Julie Bush entitled "High-Density Lipoprotein Status of Adolescent Females and Its Relation to Diet and Selected Cardiovascular Risk Factors." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

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HIGH-DENSITY LIPOPROTEIN STATUS OF ADOLESCENT FEMALES  
AND ITS RELATION TO DIET AND SELECTED  
CARDIOVASCULAR RISK FACTORS

A Thesis  
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## ABSTRACT

A sample of 78 black and white female subjects, age  $16 \pm 0.5$  years, was evaluated for plasma concentrations of high-density-lipoprotein cholesterol (HDL-C) and the relationship of these concentrations to dietary variables and selected cardiovascular risk factors. Blood samples were drawn from fasted subjects. A heparin-Mn<sup>2+</sup> solution was used to precipitate non-high-density Apo-B associated lipoproteins in the plasma, and the resultant supernatant fluid was analyzed for high-density-lipoprotein cholesterol by direct determination. Total cholesterol was also measured in plasma by a direct determination method. Nutrient intake was assessed by 24-hour dietary recall. Anthropometric variables evaluated in relation to HDL-C included weight, height, tricep skinfold, arm circumference, ponderal index, relative weight, and an obesity index based on factorial analysis of weight, tricep skinfold, arm circumference and height. Other factors evaluated in relation to HDL-C were blood pressure, activity level, smoking frequency, alcohol consumption, and use of oral contraceptives. Total cholesterol values from previous assessments of the same population were compared with the present values to identify a trend in the lipid profile of the population over a period of seven years.

No significant difference was found between mean HDL-C values for blacks and whites. Mean HDL-C values for both races were near the upper end of the normal range (30 to 70 mg/dl), while total cholesterol mean values were near the lower end of the normal range

(120 to 230 mg/dl). Intakes of total protein, animal protein, vegetable protein, total carbohydrate and starch were significantly inversely related to concentration of HDL-C. None of the other factors evaluated were significantly associated with HDL-C. Total cholesterol data collected at four different ages revealed a trend of rising total cholesterol levels until adolescence, at which time these levels fell sharply.

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## CHAPTER I

### INTRODUCTION

Plasma lipid levels have been the subject of profuse research in recent years due to their implications in cardiovascular disease. The literature indicates that elevated levels of plasma cholesterol are highly correlated with death from coronary heart disease. Conversely, increased levels of high density lipoproteins (HDL) appear to offer protection against atherosclerotic disorders, as indicated by the strong inverse relationship between plasma levels of HDL and mortality from cardiovascular disease. This information has prompted numerous investigations attempting to discover any relationship between HDL and other cardiovascular disease risk factor variables. Elucidation of the role of HDL in lipid metabolism has produced a clearer picture of possible effect of HDL upon the risk of coronary heart disease. Assessment of HDL values at different ages throughout the life span may offer some suggestions as to the etiology of cardiovascular problems which may occur later in life. Comparison of HDL status with other known or suspected risk factors will lead to a more comprehensive understanding of the predisposition to atherogenesis.

Adolescence is a nutritionally stressful period in the life cycle. Rapid growth places emotional and physical demands upon the body which, if not properly met, may result in permanent damage to the growing child. Diseases of middle-age often have their origins in nutritional habits developed in childhood and adolescence. Other environmental factors

present during this age period, such as activity levels, smoking and alcohol consumption, may also affect the health status of the individual in later years.

It is intended in this research to evaluate the HDL status of a population of adolescent females and to make comparisons between HDL values and those for total cholesterol, blood pressure, activity level, smoking frequency, alcohol consumption and several indices of obesity. The association of HDL values with the level of dietary fat, protein, and carbohydrate will be determined. Black and white subjects will be compared as to HDL values. The clinical values obtained for this population will be compared to those obtained in other epidemiological studies using similar analytical techniques. To ascertain a possible trend in lipid changes in this group, total cholesterol values from assessments of previous years, as well as those for the present study, will be compared.

It is expected that there will be an inverse relationship between HDL levels and blood pressure, dietary carbohydrate and protein, smoking frequency and obesity; a positive relationship between HDL and total cholesterol, activity level and alcohol consumption; no relationship between HDL and dietary fat. Blacks would be expected to have slightly higher HDL values than whites. Longitudinal lipid trends in females would be expected to indicate rising total cholesterol levels until adolescence, at which time it would fall dramatically.

## CHAPTER II

### REVIEW OF LITERATURE

#### Cholesterol Metabolism

Recent advances in the cellular biology of cholesterol metabolism have provided better insight into the control of plasma cholesterol levels in man than has been possible in the past. Efficient mechanisms for the removal of cholesterol from plasma depend upon receptors which are located on the surface of cells in the liver and extrahepatic tissues (1). These receptors bind circulating lipoproteins that transport cholesterol in the bloodstream. In this manner, lipoproteins are taken up and degraded by cells, yielding their cholesterol for cellular use (2). One class of these important transporters of cholesterol is the high-density lipoproteins (HDL), which are small aggregates of lipids and proteins which circulate in the lymph and blood plasma. About half the mass is protein, mainly the A apoproteins. The major lipids of HDL are cholesterol esters, cholesterol and phospholipids, principally lecithin (3,4). Once the attachment of the HDL is made to the receptor site of the target cell, unesterified cholesterol within the cell moves into the HDL, followed by detachment of the HDL from the cell with its new cholesterol cargo. This function allows HDL to remove cholesterol from tissue, thus reducing the total sterol burden of body tissues (5,6). Swartz et al. (4) found labelled free cholesterol from HDL more rapidly incorporated into biliary cholesterol than cholesterol from low-density lipoprotein (LDL). They

concluded that high levels of HDL would foster the efficient removal of tissue cholesterol and its subsequent elimination from the body by the liver.

#### Implications of Plasma HDL Levels

A number of studies have implicated HDL as an antiatherogenic factor (3,5,7). Direct evidence for an inverse relationship between HDL-cholesterol concentration and the prevalence of clinical coronary heart disease, independent of other plasma lipoproteins, has been provided by the Honolulu Heart and Cooperative Lipoprotein Phenotyping Studies (3). The Tromso Heart (9) and Framingham Studies (10) subsequently demonstrated that this inverse relationship precedes the clinical manifestation of coronary disease. More recently, angiographic studies have confirmed that the severity of existing coronary atherosclerosis is inversely related to HDL-cholesterol concentration (11). Gordon et al. (7) obtained lipid and lipoprotein values on subjects in an age range of 49-82 years. In the subjects who subsequently developed coronary heart disease, the major potent lipid factor was HDL-cholesterol, which had an inverse association with the incidence of coronary heart disease in either males or females. Other investigators correlated the HDL-cholesterol levels directly with the extent of arteriographically determined coronary occlusion or stenosis. A statistically significant inverse correlation between HDL-cholesterol values and occlusion score was seen in the majority of subjects (12).

A review by Lewis and Naito (13) described the relationship of hypertension, lipids and lipoproteins to atherosclerosis. They referred

to results of experiments on rats that suggested that hypertension alone or hyperlipidemia under certain selected conditions can induce atherogenesis, and emphasized the importance of genetic factors. These authors reported that lipid and lipoprotein deposition in the arterial wall is accelerated by increased blood pressure, and that if plasma lipid and the LDL concentrations are also high, the process is further accelerated. Evidence that is slowly accumulating, stated the authors, suggests that atheromatous lesions may regress if the precipitating factors, including hypertension and hyperlipidemia, are controlled. The role of HDL in lipid metabolism has strong implications for a beneficial effect.

HDL has also been studied concomitantly with other risk factors thought to be involved in cardiovascular disease, such as other lipid levels, blood pressure, physical activity, smoking, alcohol consumption, obesity, and dietary levels of fat and carbohydrate. Williams et al. (14) found HDL to be inversely related to smoking, relative weight, blood pressure, carbohydrate consumption, and serum triglyceride level, and positively related to levels of physical activity, total cholesterol and alcohol consumption. The ratio of HDL to total cholesterol showed similar significant relationships to the above variables, except that the ratio was negatively correlated with total cholesterol. Prevention programs have been developed to intervene in the various risk factors associated with coronary heart disease. In a report on one such program, the authors described a 6 percent increase in average HDL-cholesterol concentration in a group of men receiving multifactor

intervention. Multiple regression analysis of these data indicated that increased plasma HDL levels occurred when plasma triglyceride levels fell, smoking decreased, and habitual alcohol intake increased. Increases in the concentration of HDL cholesterol also tended to accompany adherence to a fat-controlled diet, reduction in LDL-cholesterol and loss of body weight (15). Similar conclusions were reached by a group of researchers involved in the Multiple Risk Factor Intervention Trial (16). In an investigation of the association of HDL and cardiovascular mortality, data were adjusted for age and other risk factors. The inverse relation of coronary mortality to HDL-cholesterol emerged as the dominant factor (17).

Evidence for a relationship between obesity and plasma HDL levels is conflicting. Several investigators have found an inverse relationship between obesity indices and HDL-cholesterol, including those mentioned previously (14-16, 18, 19). However, Berchtold et al. (19) found the relationship to be significant only in women, while others found significance in both sexes (20). Although the negative correlation between obesity and HDL is apparent in both whites and blacks, it appears to be most pronounced among whites, with the highest correlations observed in white males (21). When HDL was divided into subfractions, HDL<sub>2</sub> and HDL<sub>3</sub>, it was discovered that the subfraction responsible for the negative correlation between fatness measures and HDL levels was the HDL<sub>2</sub> subfraction (18). The major difference between these two subfractions of HDL is that HDL<sub>3</sub> carries a larger cholesterol load, much of which it eventually contributes to VLDL; HDL<sub>3</sub> thus becomes HDL<sub>2</sub>, which contains relatively less cholesterol (22).

The effect of weight reduction on levels of HDL-cholesterol has also been investigated. In one such study, a short-term fast produced a significant rise in HDL-cholesterol (23). Similarly, when a group of grossly obese (183 percent relative weight) subjects were assessed after an average weight reduction of 16 kilograms, most were found to have higher levels of HDL-cholesterol than at initial assessment before diet therapy (24). Conversely, another group of obese subjects were found to have decreased levels of HDL-cholesterol after a 10-week weight reduction program. Although HDL-cholesterol decreased with increasing relative weight, it also decreased with increasing rate of weight loss. The authors suggested that negative kilocaloric balance produces a decrease in HDL-cholesterol that in prospective studies may obscure the inverse relationship between HDL-cholesterol and indices of obesity (25). When Garrison et al. (26) compared the ratio of total cholesterol to HDL-cholesterol in lean and grossly obese 20 to 29-year old men, they found substantial differences between the mean ratio for these two groups. They suggested that since other data have shown HDL-cholesterol to be the best single indicator of coronary heart disease, the atherogenic potential of obesity appears to be greater than would be suggested by the relatively weak association between obesity and total cholesterol or any single lipoprotein-cholesterol value. In a report based on a follow-up of the Framingham Study (10) mentioned previously, it was stated that a greater relative weight was associated with a high incidence of coronary heart disease, atherothrombotic brain infarction and congestive heart failure (27).

Exercise is often recommended as a tool for weight reduction, usually in conjunction with kilocalorie restriction. Weltman et al. (28) evaluated the effects of kilocalorie restriction and/or mild exercise on serum lipids, including HDL-cholesterol. Mild exercise alone and with kilocalorie restriction caused a decrease in LDL-cholesterol without affecting HDL-cholesterol, which contributed to a lower, more favorable LDL:HDL ratio. Other investigators have observed that even moderate exercise elevated HDL-cholesterol in most subjects studied (29-31). These results were not dependent on type of exercise; however, aerobic exercise appeared to be more beneficial than anerobic. Men employed in occupations which require vigorous activity tend to have higher HDL-cholesterol levels than those who are largely sedentary at work (32, 33). When male coronary-disease patients were engaged in regular exercise training, they showed significant increases in plasma HDL-cholesterol (34). The high HDL-cholesterol levels of physically well-trained people are probably accounted for, at least partly, by the increased lipoprotein lipase activity that has been observed in these subjects, and the concomitant rapid turnover of triglyceride-rich lipoprotein (35).

There are several other factors which may have implications for varying HDL-cholesterol levels among populations. The relationship between HDL and alcohol ingestion has been alluded to previously. Several studies have confirmed a strong positive correlation between alcohol consumption and HDL-cholesterol values (36-38). Investigations of the effect of progesterones and androgens on HDL-cholesterol levels

indicate that these steroids lower the concentration of HDL (39-41). These observations may be consequence to women taking oral contraceptives, and may also be related to the fall in HDL-cholesterol observed in males reaching puberty (42). Patients with existing clinical ischaemic heart disease consistently have lower levels of HDL than healthy subjects (43,44).

HDL-cholesterol concentration has been associated with diseases other than those of cardiovascular origin. Maturity-onset diabetics who are not insulin-dependent demonstrate a lower concentration of HDL-cholesterol than diabetics treated with insulin (45-47). A similar HDL profile has been observed in patients with cystic fibrosis (48).

#### Dietary Factors Affecting HDL Status

Dietary cholesterol has been investigated extensively to discover its relationship with HDL status. In one animal study, rabbits were used to determine the compositional and metabolic changes induced in HDL due to cholesterol feeding. While no change was observed in total HDL-cholesterol, there was a significant decrease in the protein content of HDL. The authors also described an accelerated HDL turnover due to cholesterol feeding (49). Ershow et al. (50) demonstrated in rhesus monkeys the existence of an important interaction between dietary fat and cholesterol. Only when saturated fat was fed with cholesterol was there a substantial rise in the plasma cholesterol concentration. The interaction resulted in a redistribution of lipoprotein cholesterol such that the largest percentage was transported as LDL rather than HDL. Human studies indicated that there was no significant rise in

HDL-cholesterol when cholesterol intake was increased (51-53). However, Mahley et al. (54) described an enhancement of HDL binding site activity by the feeding of cholesterol. Investigation of cholesterol absorption and synthesis, and fecal steroid excretion during cholesterol overloading revealed two partially effective compensatory mechanisms: a decrease in cholesterol biosynthesis (feedback inhibition), and an increase in bile acid excretion (55).

The effects of dietary fats on plasma lipoprotein levels have been the bases of much research, especially the effects of polyunsaturated versus saturated fat. The results of this research are sometimes conflicting. One investigation reported that a high polyunsaturated:saturated fat ratio in the diet results in a significant decrease in the plasma LDL-cholesterol:HDL-cholesterol ratio due to increased HDL-cholesterol (56), while another study under similar conditions, found no change in the LDL-cholesterol:HDL-cholesterol ratio (56). This ratio has been implicated in cardiovascular risk: the higher the ratio, the greater the risk (5). Hjermann et al. (58) observed several effects from feeding a diet with a high polyunsaturated:saturated dietary fat ratio to a group of middle-aged men: a significant increase in HDL concentration; a decrease in total plasma cholesterol; a decrease in triglyceride levels; an increase in HDL-cholesterol:total cholesterol ratio. When Shepherd et al. (59) investigated the effects of dietary intakes of polyunsaturated and saturated fat on the composition of HDL, they observed that polyunsaturated fat ingestion resulted in a significant fall in the palmitate and stearate content of HDL

triglyceride, cholesteryl esters and phospholipids with a concomitant rise in the linoleate content of these components. The polyunsaturated diet also produced a fall in the HDL<sub>2</sub>:HDL<sub>3</sub> ratio. Although similar results in compositional changes were observed in another study (60), the rate and amount of removal of cholesterol from cells was found to be independent of these changes.

Carbohydrate is another dietary factor which may influence HDL status. When rats were fed diets in which carbohydrate was supplied by sucrose or starch, the sucrose diet resulted in less plasma HDL but greater very-low-density lipoprotein (VLDL) than the starch diet (61). Similarly, humans fed a high sugar diet displayed a decrease in HDL concentration which was restored to pre-treatment levels when the subjects returned to their normal diets (62, 63). Cham et al. (64) investigated the effect of a high energy, low carbohydrate diet on HDL levels. They observed a significant rise in serum cholesterol with no change in HDL-cholesterol, indicating the distribution of cholesterol in LDL.

Dietary protein appears to exert some influence on plasma HDL concentration and composition. Rhesus monkeys fed low protein diets manifested higher levels of VLDL and HDL<sub>2</sub> than controls (65). Rabbits fed diets containing animal protein exhibited higher total cholesterol and higher lipoprotein levels than those fed vegetable protein diets. However, the greatest lipoprotein increase occurred in LDL and the lowest in HDL (66). In human studies, the source of protein, whether from red meat, fish, poultry, eggs or vegetable oil, made no significant difference in HDL concentration (67-69).

Other dietary factors have also been investigated. A significant redistribution of cholesterol in plasma lipoproteins was observed following ingestion of large doses of D,L-alpha-tocopherol (70). In persons with low plasma levels of HDL-cholesterol, a complex response occurred, which included elevation of the plasma HDL-cholesterol levels. Another group of researchers (71) observed an increase in HDL when men were fed a diet supplemented with oat bran, and restricted in cholesterol and animal fat. However, when the inclusion of oat bran was the only change in the diet, HDL concentration remained the same as pre-treatment levels.

In a comprehensive dietary investigation, Khaltaen et al. (72) reported several relationships between diet and HDL-cholesterol in two groups of men--one group being treated for cardiovascular disease and one control group. In the former group, HDL-cholesterol was inversely related to polyunsaturated fat and carbohydrate intake. In the control group, there was no relationship between HDL-cholesterol and dietary carbohydrate. In both groups, HDL-cholesterol was not related to total cholesterol, weakly (inversely) related to cholesterol in lipoproteins and significantly (inversely) related to triglycerides in plasma.

#### Age-Race Specific HDL Status

Surveys of HDL status have been conducted among several age groups. Most have investigated the middle-aged and elderly, and have consistently found HDL to be positively correlated with age among the elderly (3, 5, 71). Nicholson et al. (73) suggested that those with lower HDL-cholesterol

have been removed from the population by coronary heart disease. Research pertaining to HDL status among children and adolescents is limited. Berenson et al. (74) reported a sharp rise in HDL concentration between birth and school age. These levels then remained relatively stable from school age through age 14. They also observed an inverse relationship between HDL and obesity, with a consistently significant relationship in older children, and between HDL and blood pressure. The Lipid Research Clinics Prevalence Study (75) revealed a decrease in HDL-cholesterol among males during adolescence, but not among females; this decrease continues throughout the post-adolescent (16 to 19 years) period. Similar results were reported by other authors (74, 76), who suggested that these changes could be related to both age and maturation, and likely reflected the influence of sex hormones on lipoprotein metabolism.

Race differences are consistently observed in evaluation of population HDL status (76 -78). Morrison et al. (76) defined associations of age, sex and race with plasma lipoproteins among a group of schoolchildren, ages 6 to 17 years. During ages 6 to 11 there were no significant changes in HDL in any of the sex-race-specific groups, and no consistent trends were observed. For ages 12 to 17 in both white and black male subjects, mean values were lower than that for ages 6 to 11. Comparing white females in age groups 6 to 11 and 12 to 17, HDL levels did not differ, but the older children had lower LDL values. There were no significant differences in plasma lipid and lipoprotein levels between older and younger black

female subjects. In both racial groups, during ages 6 to 11, females had lower HDL values than males, but during ages 12 to 17 there were no significant male-female differences in lipoproteins. For both age groups, and for both sexes, black children had higher HDL values than white children.

The relationship of HDL-cholesterol with other clinical chemistry measurements was investigated in The Princeton Schoolchildren Study (79). It was observed that HDL-cholesterol was inversely correlated with serum uric acid, and positively associated with serum glutamic oxaloacetic transaminase. These findings may have implications for improved indirect determination of serum HDL-cholesterol. Other authors reporting on this study (80) investigated a subsample of 927 hypercholesterolemic schoolchildren. In this group, black children were more likely than white children to have elevations of HDL. The authors suggest that elevated levels of HDL-cholesterol may explain apparent hypercholesterolemia in at least 16 percent of children, ages 6 to 17.

Evaluation of HDL status has acquired new importance with the emerging concept that this lipoprotein class may confer protection from premature atherogenesis. A more complete inventory of mean values for HDL for different age groups, and regular assessment of this lipoprotein, may add vital information to the health profile of the individual.

## CHAPTER III

### METHODS

#### A. General Plan

Approximately 80 female subjects, age  $16 \pm 0.5$  years, were obtained through Knoxville City and Knox County schools to participate in this investigation of high-density lipoprotein status. These girls were a subsample of a larger sample of approximately 120 females participating in the Southern Regional Nutrition Project entitled "Nutritional Health of Adolescent Females," a five-year study focusing on females, ages 12 to 16 years. The 16-year-old subjects were evaluated in a previous longitudinal study, beginning at the age of  $9 \pm 0.5$  years in 1974. Data were again collected on this population in 1975 and 1976. Racial distribution of the present sample is approximately 35 percent white and 65 percent black. All participants were given oral and written information about the study and procedures to be employed. Written consent was obtained from subjects and their parents or guardians. Dietary intakes, various anthropometric measurements, medical history information, records of physical activity levels and fasted venous blood samples were collected and analyzed as described in the following sections for evaluation in this investigation.

## B. Collection Methods

### Dietary Recalls

Two 24-hour dietary recalls were collected by the investigator and four other trained interviewers. The first recall was obtained at the school which the subject attended. Each girl was called from class and interviewed individually in a conference room. The second recall was collected at the hospital on the day the blood sample was drawn. Again, each subject was consulted individually. Information was obtained for breakfast, lunch, dinner and pre-breakfast, pre-lunch, pre-dinner and post-dinner snacks. Serving sizes were reported in household measures, converted to grams and coded, along with any vitamin and/or mineral supplements, for analysis using the Extended Table of Nutrient Values.<sup>1</sup>

### Blood Pressure Measurement

Blood pressure was ascertained on the right arm with the subject in a sitting position using a blood pressure cuff of appropriate size. The procedure was conducted by trained medical personnel. A second reading was taken if the first assessment was less than 60 or more than 90 for the diastolic pressure, or less than 100 or more than 120 for the systolic pressure (81).

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<sup>1</sup>International Dietary Information Foundation, Inc., P. O. Box 38143, Atlanta, GA, 30334.

### Anthropometric Measurements

Body weight was measured on a single-beam balance and recorded in kilograms. Each subject was asked to remove shoes and all heavy outer garments. Other clothing weights were estimated, based on values obtained by the weighing of actual articles of clothing, and subtracted to yield a nude weight.

Tricep skinfold and arm circumference were taken on the same site of the right arm. Location of the site was ascertained by using an Ensure Inset-tape from Ross Laboratory. The right arm was measured from the lateral margin of the acromial process of the scapula to the tip of the olecranon. The midpoint over the tricep was marked. With the arm hanging loosely, the skinfold was lifted parallel to the long axis of the bone approximately 1 centimeter away from the site of the measurement and Lange<sup>2</sup> calipers were applied to the midpoint. The measurement was taken three times and the average was recorded in millimeters. Arm circumference was determined with an Ensure Inset-tape applied to the midpoint of the right arm and was recorded in centimeters. Height was measured without shoes with a GPM anthropometer<sup>3</sup> and was recorded in centimeters.

### Activity Level

An exercise/activity level questionnaire was used to ascertain physical activity scores for each subject (82). Each girl was

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<sup>2</sup>Cambridge Scientific Industries, Inc., Cambridge, MD, 21613.

<sup>3</sup>GPM Anthropological Instruments, Pfister Import-Export, Inc., 450 Barell Avenue, Carlstadt, NJ, 07072.

questioned regarding her participation in 20 different physical activities, including an "other" category for activities not listed on the questionnaire. Information was obtained about the frequency of the activity (whether it was weekly, monthly or yearly), whether participation was seasonal (less than six months of the year), and the intensity level of the activity. Based on this information, a single activity score was obtained for each subject by coding the variables as follows: frequency of activity--1=yearly, 2=monthly, 3=weekly; participation--1=seasonal, 2=nonseasonal; intensity level--1=light, 2=moderate, 3=heavy. Ratings for intensity level were based on kilocalorie/minute expenditure (83-85). These three values were summed for each activity. The totals for all of the activities in which the subject participated were then summed to obtain the single score used in the analysis.

#### Smoking, Alcohol Consumption and Oral Contraceptive Use

A medical history questionnaire was used to obtain the subjects' habits regarding the above variables. Each girl was questioned individually as to whether she smoked, and, if so, how many cigarettes per day. Alcohol consumption was ranked in order of frequency on a scale of 0 (never) to 7 (2 times or more per day). Wine, beer and hard liquor were included in the assessment. Use of oral contraceptives was coded as used or not used.

#### Venous Blood Samples

Blood samples were taken from the right antecubital vein of fasting subjects with the use of heparinized vacutainer tubes.

Approximately 20 ml of blood was drawn from each subject. Tubes were labelled with the subject's name and subject number and held at room temperature. Whole blood was then centrifuged at 2000 X g and 4° using a Beckman Model J-6B Centrifuge.<sup>4</sup> The resultant plasma was then removed with a Pasteur pipet and placed in separate, appropriately marked vials for total cholesterol and high-density lipoprotein analyses. Total cholesterol samples were frozen, while high-density lipoprotein samples were immediately treated to precipitate beta-lipoproteins, and analyzed for HDL-cholesterol the following day as described below.

#### C. Analyses of Blood Plasma

##### Precipitation of Non-high-density Apo-B-associated Lipoproteins

High-density lipoproteins were isolated by combining plasma with a heparin-manganese reagent, incubating at room temperature and centrifuging to selectively precipitate non-high-density apo-B associated lipoproteins. This was followed by measurement of cholesterol in the supernatant to give an estimation of high-density-lipoprotein cholesterol in the plasma. The method is derived from Albers et al. (86) and supported by research from other investigators (87-92). This is the method of choice used by the Lipid Research Clinics Program of the National Institute of Health (93).

Reagents. 1. Manganese Chloride Solution 1.06M: 209.78 g  
 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  was weighed out, dissolved in a small

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<sup>4</sup>Beckman Industries, Inc., Fullerton, CA, 32634.

amount of distilled water, and then diluted to 1 liter volume with distilled water.

2. Sodium Chloride 0.15M: 8.77 g NaCl was weighed out, dissolved in 500 ml of distilled water, and brought to 1 liter volume with distilled water.
3. Heparin 40,000 units/ml: heparin purchased contained 159 units/mg; therefore, 0.252 g of heparin was weighed out and dissolved in 1 ml 0.15M saline by vortexing. This was prepared fresh for each run.
4. Combined Heparin-Manganese Reagent: 0.6 ml of the heparin solution was added to 10 ml 1.06M  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  and mixed by inversion. This reagent was prepared fresh for each run.

Method. Plasma samples were allowed to warm to room temperature. After mixing, 1 ml of plasma was transferred to a 15 ml conical centrifuge tube using an Oxford Sampler Micro Pipet.<sup>5</sup> Duplicates were run on all samples. One-tenth of a milliliter of the heparin-manganese reagent was pipetted by graduated glass pipet into each centrifuge tube. A precipitate formed immediately. Each tube was vortexed lightly, covered with parafilm and allowed to stand at room temperature for 10 minutes. The tubes were then centrifuged in the Beckman Model J-6B Centrifuge at 1500 X g and 4° for 30 minutes.

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<sup>5</sup>Oxford Laboratories, 1149 Chess Drive, Foster City, CA.

At this time, a hard pellet had formed in the bottom of the tube. The supernatant fluid was removed with a Pasteur pipet. High-density-lipoprotein cholesterol was determined on the supernatant fluid according to the total cholesterol method described below.

#### Total Cholesterol Determination

Total cholesterol was measured by direct determination,<sup>6</sup> utilizing a chemical reaction based upon the classical Lieberman-Burchard reaction which involves the C<sup>5</sup> double bond in the sterol molecule (94,95). Although the reaction may be falsely elevated by bilirubin in excess of 3.0 mg/dl (94), this amount is well above the normal total serum bilirubin range of 0.2 to 1.4 mg/dl (96). An abnormal level of bilirubin would not be expected in a population of healthy subjects.

This same procedure was used on the supernatant derived from the isolation of high-density lipoprotein described above to determine high-density-lipoprotein cholesterol.

- Reagents.
1. SR Direct Cholesterol Reagent: a solution (v/v) of glacial acetic acid (41.5 percent), acetic anhydride (41.5 percent), sulfuric acid (13.6 percent) and phosphoric acid (3.4 percent).
  2. Direct Cholesterol Standards: 200 mg/dl and 400 mg/dl: 0.2 g percent (w/v) and 0.4 g percent (w/v) cholesterol in glacial acetic acid; diluted

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<sup>6</sup>SR Direct Cholesterol Test Set, Stanbio Laboratory, Inc., 2930 Houston Street, San Antonio, TX, 78202.

with acetic acid to obtain standards of 100, 200 and 300 mg/dl for total cholesterol determinations, and 20, 50 and 100 mg/dl for HDL-cholesterol determinations.

Method. Plasma samples were thawed and allowed to warm to room temperature. The direct cholesterol reagent was also warmed to room temperature. Test tubes were appropriately labelled for duplicate blanks, standards as described above, and samples. One-tenth of a milliliter of distilled water was pipetted into the blank tubes; 0.1 ml of direct cholesterol standards into the standards tubes; 0.1 ml of plasma into the samples tubes. Six milliliters of SR Direct Cholesterol Reagent were then pipetted into all tubes and vortexed. All test tubes were then placed in a 37° water bath for 20 minutes to allow the color to develop. Upon removal from the water bath, the contents of the tubes were mixed thoroughly by inversion and the absorbance of the various solutions was determined using the Beckman Model 34 Spectrophotometer<sup>7</sup> at 625nm against the reagent blank.

Calculations for total cholesterol and HDL-cholesterol. Total cholesterol and HDL-cholesterol in milligrams per deciliter were determined according to the following formula:

$$\frac{\text{absorbance of unknown}}{\text{absorbance of standard}} \times \text{concentration of standard} = \text{mg/dl}$$

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<sup>7</sup>Beckman Industries, Inc., Fullerton, CA, 32634.

Normal values for total cholesterol were accepted as being between 120 and 230 mg/dl. Normal values for HDL-cholesterol were accepted as being between 30 and 70 mg/dl (97).

#### D. Treatment of Data

Prior to the application of statistical tests, new variables were created from anthropometric data previously collected. The ponderal index of each subject was calculated according to the following formula: height (in inches) divided by the cube root of weight (in pounds). Percent desirable weight was determined by actual weight divided by desirable weight (according to Metropolitan Life Insurance Company charts<sup>8</sup>) and expressed as relative weight. An obesity index was assigned to each subject by factorial analysis of body weight, tricep skinfold, arm circumference and height.

All statistical tests were calculated by computer using the Statistical Analysis System.<sup>9</sup> Pearson correlation coefficients (98) were calculated for HDL-cholesterol values as compared to dietary saturated fat, polyunsaturated fat and total fat; dietary sucrose and total carbohydrate; dietary starch and fiber; dietary protein, including animal and vegetable protein; smoking; systolic and diastolic blood pressure values; total cholesterol values; anthropometric measures, including body weight, tricep skinfold, arm circumference,

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<sup>8</sup>Metropolitan Life Insurance: New weight standards for men and women. Stat Bull Metropolitan Life Insurance 40, 1-4, 1959.

<sup>9</sup>SAS Institute, Inc., P. O. Box 10066, Raleigh, NC, 27605.

relative weight, obesity index and ponderal index. Spearman correlation coefficients (98) were calculated for HDL-cholesterol levels as compared to activity level and alcohol consumption. Analysis of variance (98) was calculated for HDL-cholesterol versus oral contraceptive use and for HDL-cholesterol versus race. Tukey's method (98) was applied to total cholesterol data collected on these subjects at ages 9, 10, 11 and 16 to compare differences among mean values for those ages.

## CHAPTER IV

### RESULTS

#### A. High-Density-Lipoprotein Cholesterol Levels

The mean levels of high-density-lipoprotein cholesterol (HDL-C), total cholesterol (TC), and the ratio of HDL-C to total cholesterol (HDL-C:TC) are reported for both races studied in Table 1. An analysis of variance was performed to test the effect of race on mean levels of HDL-C. The results indicated that the means were not significantly different. In the total population, 32 subjects demonstrated HDL-C values above the normal range of 30 to 70 mg/dl, while none of the subjects had HDL-C values below this range. Total cholesterol values were below the normal range of 120 to 230 mg/dl for 12 of the subjects, while none of the girls had values above this range. A comparison for the percentiles for HDL-C and TC between the present research population and that of the Princeton Family Lipid Program is shown in Table 2.

#### B. Intakes of Dietary Variables and Mean Values for Blood Pressure and Anthropometric Measurements

Mean values for dietary intakes of the nutrients investigated in this research are displayed for both races in Table 3. An analysis of variance was performed on each dietary variable to determine if there were significant differences between dietary intake between whites

TABLE 1

Means of HDL-cholesterol, total cholesterol,  
and HDL-cholesterol:total cholesterol for  
black and white girls 16 years of age

| Indices                 | Race                          |                   |
|-------------------------|-------------------------------|-------------------|
|                         | Black<br>(n=49)               | White<br>(n=29)   |
|                         | <u>mg/dl</u>                  | <u>mg/dl</u>      |
| HDL-cholesterol (HDL-C) | 63.88 $\pm$ 2.52 <sup>1</sup> | 67.66 $\pm$ 4.72  |
| Total cholesterol (TC)  | 149.45 $\pm$ 4.39             | 148.27 $\pm$ 5.73 |
| HDL-C:TC, %             | 44.04 $\pm$ 2.00              | 47.35 $\pm$ 3.54  |

<sup>1</sup>Mean  $\pm$  SEM

TABLE 2

Comparison of percentiles for HDL-cholesterol and total cholesterol of the research group with those of the Princeton Family Lipid Program for black and white girls 16 years of age

| Percentile | HDL-cholesterol |           |       |           | Total cholesterol |           |       |           |
|------------|-----------------|-----------|-------|-----------|-------------------|-----------|-------|-----------|
|            | White           |           | Black |           | White             |           | Black |           |
|            | UT              | Princeton | UT    | Princeton | UT                | Princeton | UT    | Princeton |
|            | mg/dl           |           | mg/dl |           | mg/dl             |           | mg/dl |           |
| 5th        | 35              | 37        | 36    | 37        | 101               | 126       | 102   | 129       |
| 10th       | 37              | 39        | 41    | 41        | 114               | 130       | 106   | 139       |
| 50th       | 64              | 51        | 65    | 57        | 138               | 159       | 146   | 165       |
| 90th       | 102             | 65        | 85    | 72        | 190               | 189       | 180   | 193       |
| 95th       | 110             | 72        | 92    | 75        | 195               | 201       | 212   | 204       |

<sup>1</sup>Morrison et al. (1978) *Pediatric* 62, 990-995.

TABLE 3  
Mean dietary intakes of selected nutrients for black  
and white girls 16 years of age

| Dietary variable    | Black<br>(n=49)               | White<br>(n=29)    |
|---------------------|-------------------------------|--------------------|
|                     | g                             | g                  |
| Total protein       | 73.69 $\pm$ 3.75 <sup>1</sup> | 68.62 $\pm$ 6.03   |
| Animal protein      | 52.72 $\pm$ 3.10              | 46.60 $\pm$ 4.63   |
| Vegetable protein   | 19.31 $\pm$ 0.92              | 20.52 $\pm$ 1.87   |
| Total carbohydrate  | 256.82 $\pm$ 12.94            | 238.61 $\pm$ 18.78 |
| Starch              | 79.98 $\pm$ 4.70              | 77.42 $\pm$ 7.49   |
| Crude fiber         | 2.59 $\pm$ 0.21               | 2.73 $\pm$ 0.27    |
| Total sucrose       | 102.12 $\pm$ 7.12             | 91.19 $\pm$ 8.66   |
| Total fat           | 88.92 $\pm$ 5.29              | 89.40 $\pm$ 7.05   |
| Polyunsaturated fat | 14.80 $\pm$ 0.99 <sup>a</sup> | 18.26 $\pm$ 1.33   |
| Saturated fat       | 32.81 $\pm$ 2.19              | 31.81 $\pm$ 3.06   |
| Cholesterol         | 0.33 $\pm$ 0.03               | 0.32 $\pm$ 0.04    |

<sup>1</sup>Means  $\pm$  SEM

<sup>a</sup>Means between races are significantly different ( $p < 0.05$ ).

and blacks. Polyunsaturated fat was the only dietary variable in which there was a significant difference in dietary intakes between the races. Table 4 shows mean values for systolic and diastolic blood pressures and anthropometric measurements collected for this population.

#### C. Relationship of HDL-C to Dietary Variables

Correlation coefficients ( $r$ ) for HDL-C values and the dietary variables investigated are shown in Table 5. A probability level of  $<0.05$  was considered significant. At this probability level, there was a significant negative correlation between HDL-C values and the following dietary variables: total protein, animal protein, vegetable protein, total carbohydrate, and starch. Although the  $r$  value was not significant, HDL-C levels were more negatively correlated with dietary intake of saturated fatty acids than with polyunsaturated fatty acids. As expected, there was no significant correlation between total dietary fat or dietary cholesterol and HDL-C. The ratio of HDL-C to TC was not significantly correlated with any of the dietary variables considered.

#### D. Relationship of HDL-C to Anthropometric Measures and Blood Pressure

The values of HDL-C were not significantly correlated with any of the obesity indices studied, as shown by the correlation coefficients reported in Table 6. However, of all the obesity indices investigated,

TABLE 4

Mean values for systolic and diastolic blood pressures and  
anthropometric measurements for black and white girls  
16 years of age

| Variable                        | Black<br>(n=49)                | White<br>(n=29)   |
|---------------------------------|--------------------------------|-------------------|
| Systolic blood pressure, mm Hg  | 120.16 $\pm$ 1.54 <sup>1</sup> | 116.21 $\pm$ 2.32 |
| Diastolic blood pressure, mm Hg | 75.53 $\pm$ 1.15               | 75.83 $\pm$ 1.03  |
| Ponderal index                  | 12.52 $\pm$ 0.10               | 12.83 $\pm$ 0.13  |
| Body weight, kg                 | 58.90 $\pm$ 1.52               | 56.53 $\pm$ 2.03  |
| Height, cm                      | 159.89 $\pm$ 0.87              | 162.70 $\pm$ 1.19 |
| Tricep skinfold, mm             | 18.18 $\pm$ 1.01               | 17.59 $\pm$ 0.98  |
| Arm circumference, cm           | 26.26 $\pm$ 0.48               | 25.64 $\pm$ 0.50  |
| Relative weight                 | 111.31 $\pm$ 2.78              | 104.76 $\pm$ 3.38 |
| Obesity index                   | 154.68 $\pm$ 2.61              | 151.69 $\pm$ 3.22 |

<sup>1</sup>Means  $\pm$  SEM

TABLE 5

Pearson correlation coefficients (r) for HDL-cholesterol  
(HDL-C) and HDL-cholesterol:total cholesterol  
(HDL-C:TC) values as compared to  
dietary variables for girls  
16 years of age

| Variable<br>(n=78)  | HDL-C               | HDL-C:TC |
|---------------------|---------------------|----------|
| Total fat           | -0.075              | 0.016    |
| Saturated fat       | -0.204              | -0.043   |
| Polyunsaturated fat | -0.075              | -0.016   |
| Total protein       | -0.256 <sup>a</sup> | -0.152   |
| Animal protein      | -0.229 <sup>a</sup> | -0.111   |
| Vegetable protein   | -0.253 <sup>a</sup> | -0.218   |
| Cholesterol         | -0.110              | -0.081   |
| Total carbohydrate  | -0.305 <sup>b</sup> | -0.141   |
| Starch              | -0.283 <sup>a</sup> | -0.217   |
| Crude Fiber         | -0.082              | -0.217   |
| Total sucrose       | -0.159              | 0.056    |

<sup>a</sup>p<0.05

<sup>b</sup>p<0.01

TABLE 6

Pearson correlation coefficients (r) for HDL-cholesterol (HDL-C) and HDL-cholesterol:total cholesterol (HDL-C:TC) values as compared to obesity indices and systolic and diastolic blood pressure for girls 16 years of age

| Variable<br>(n=78)       | HDL-C               | HDL-C:TC |
|--------------------------|---------------------|----------|
| Ponderal index           | -0.081 <sup>1</sup> | -0.004   |
| Body weight              | 0.058               | 0.006    |
| Tricep skinfold          | -0.166              | -0.180   |
| Arm circumference        | 0.004               | -0.032   |
| Relative weight          | 0.079               | -0.001   |
| Obesity index            | -0.016              | -0.053   |
| Systolic blood pressure  | 0.053               | -0.002   |
| Diastolic blood pressure | 0.018               | -0.047   |

<sup>1</sup>All r values were nonsignificant ( $p > 0.05$ ).

HDL-C was most negatively correlated with tricep skinfold measurement. Table 6 also gives the  $r$  values for systolic and diastolic blood pressure. Neither of these parameters were significantly related to HDL-C levels. Correlation coefficients were also determined for the ratio of HDL-C:TC and all obesity indices, systolic and diastolic blood pressures. None of these parameters were found to be significantly correlated with the ratio. As with HDL-C levels, the ratio was most negatively correlated with tricep skinfold than with any other obesity measurement.

#### E. Biochemical Relationships

No significant correlation was found between HDL-C and TC values. However, there was an expected significant negative correlation ( $p=0.0002$ ) between HDL-C values and the ratio of HDL-C:TC.

#### F. Relationship of HDL-C with Other Factors

No significant correlations were found between HDL-C levels and smoking frequency, alcohol consumption and physical activity level, as reported in Table 7. According to analysis of variance, there was no significant difference in HDL-C values between those subjects who used oral contraceptives and those who did not.

#### G. Comparison of Longitudinal Total Cholesterol Data

Table 8 displays the mean values for TC for the original study population from 9 to 11 years of age and for the current study population at 16 years of age. The mean value for 11-year-olds was

TABLE 7

Correlation coefficients (r) of HDL-cholesterol values as compared to activity level, smoking frequency and alcohol consumption for girls 16 years of age

| Variable            | n  | HDL-cholesterol      |
|---------------------|----|----------------------|
| Activity level      | 75 | -0.124 <sup>1a</sup> |
| Smoking frequency   | 9  | -0.215 <sup>b</sup>  |
| Alcohol consumption | 7  | 0.182 <sup>a</sup>   |

<sup>1</sup>all r values were nonsignificant ( $p > 0.05$ ).

<sup>a</sup>Spearman correlation coefficient.

<sup>b</sup>Pearson correlation coefficient.

TABLE 8  
Mean values for plasma total cholesterol of girls  
at 9, 10, 11 and 16 years of age

| Age          | Mean (mg/dl)        | n   |
|--------------|---------------------|-----|
| 9 $\pm$ 0.5  | 147.49 <sup>a</sup> | 110 |
| 10 $\pm$ 0.5 | 144.06 <sup>a</sup> | 113 |
| 11 $\pm$ 0.5 | 172.32 <sup>b</sup> | 108 |
| 16 $\pm$ 0.5 | 149.04 <sup>a</sup> | 78  |

<sup>a</sup>Means with the same letter are not significantly different.

significantly different from mean values for the other three years. Mean values for 9-year-old, 10-year-old and 16-year-old girls were not significantly different from one another.

## CHAPTER V

### DISCUSSION

#### A. High-Density-Lipoprotein Cholesterol Levels

Contrary to results found in other studies, the means of black and white subjects were not significantly different in the present population. The Lipid Research Clinics (LRC) Prevalence Study (75) found both total plasma cholesterol and HDL-C to be higher among blacks than whites of 16 years of age. However, there were some dietary differences between the LRC population and the present research sample. In the LRC group, the mean intakes of total fat and saturated fat were lower in blacks than white, and the polyunsaturated:saturated fat (P:S) ratio was higher in blacks. The trend in the present sample was towards a lower fat intake for blacks, but with a slightly higher intake of saturated fat among blacks than among whites (see Table 3). The P:S ratio in the present population was considerably lower for blacks (.45) than for white (.56). As demonstrated in Table 2, there were differences between the Princeton Family Lipid Program (76) population and the one presently studied. Black and white subjects in the present group tended to have lower total cholesterol values than those in the Princeton group. HDL-C levels in both groups were comparable below the 50th percentile, but were considerably higher for the present group at the 50th percentile and above. Overall, blacks in the Princeton study demonstrated higher TC and HDL-C values

than whites. In the Bogalusa Heart Study (74), the mean value for HDL-C (68 mg/dl) was more similar to that of the present population (65 mg/dl).

Some of the diversity between the current study and others may be partially explained by examining the parameters investigated. Black subjects in this population evinced higher obesity indices (except ponderal index) than white subjects. Statistical analysis showed tricep skinfold to be weakly negatively correlated ( $p=0.10$ ) with HDL-C. Blacks also had slightly higher systolic blood pressure and higher dietary intakes of total protein, animal protein, total carbohydrates and total sucrose, all of which have been reported in the literature as having a negative effect on HDL-C (62-64, 66, 72). As stated earlier, total protein, animal protein and total carbohydrate showed significant negative correlations with HDL-C among this population.

#### B. Relationship of HDL-C to Dietary Variables

Most researchers agree that the dietary intake of cholesterol is not associated with either TC or HDL-C (49, 51-53). The latter assumption was verified in the present study by the lack of a significant correlation between HDL-C and cholesterol intake. While there was no significant relationship of HDL-C with any other lipid investigated, it is interesting to note that saturated fat was found to be more negatively correlated with HDL-C than polyunsaturated fat, since several investigators have reported that an increase in the P:S ratio will result in an improvement in the lipid profile, including an increase in HDL-C (56, 68, 70). Blacks in this population consumed

38 percent of their kilocalories as fat, while the total fat consumption of white girls was responsible for 40 percent of their kilocalorie intake.

There was a negative but nonsignificant correlation between HDL-C and sucrose in this study population, and a significant inverse relationship between HDL-C and starch. Hostmark (61), working with rats, found that feeding sucrose as the source of carbohydrate decreased HDL-C more than when starch was fed as the source of carbohydrate. Macdonald (63) and Yudkin et al. (62) found similar results feeding sucrose to young human subjects. Population dissimilarity may account for differences in results between these two studies and the present one. Also, the studies cited above were conducted under controlled conditions, while the 24-hour dietary recall was the measure of nutrient intake in the present study.

The finding of a negative correlation between total carbohydrate and HDL-C demonstrated by the current research population is in agreement with results reported by Khaltaen et al. (72). While Cham et al. (64) did not find a change in HDL-C levels when a low carbohydrate diet was fed to human subjects, they did report cholesterol to be redistributed predominantly in LDL. The percentage of kilocalories consumed as carbohydrate for the present group was 48 percent for blacks and 47 percent for whites.

The effects of fiber on HDL-C have not been fully explored. Anderson et al. (71) found oat bran ingestion lowered TC and LDL, but did not alter HDL-C. In the present study, no relationship was found between crude fiber and HDL-C.

A negative association between HDL-C and protein intake has been reported by several authors. Animal studies have demonstrated differences in HDL-C levels according to the source of the protein (65, 66). However, in humans the source of the protein appears to be nonsignificant (67-69). The results of the present investigation are consistent with those reported in the literature, since both animal and vegetable protein, as well as total protein was significantly inversely related to HDL-C. The percentage of the kilocalories consumed as protein was 14 percent for black girls and 13 percent for white girls.

#### C. Relationship of HDL-C to Anthropometric Measures and Blood Pressure

Although many investigators have consistently found an inverse association between obesity and HDL-C, none of the obesity indices explored in this research showed a significant relationship with HDL-C. Frerichs et al. (21) reported a negative correlation between HDL-C and obesity and sexual maturity in both sexes. The sample consisted of children who were younger (5 to 14 years) than those studied currently. Another researcher (26) described results similar to those of Frerichs et al. (21) among a group of young adults, as did Glueck et al. (20) from data on both children and adults. Several investigators have published results indicating that HDL-C levels increase after weight reduction (23, 24). In much of the research on the relationship between obesity and HDL-C grossly obese (relative weight up to 183 percent) subjects have been used. In the population examined in the present study, 19.2 percent had relative weights equal to or greater than 120 percent. Disparity in sample size between the present population (n=78) and

those referred to above (n=3,000 to 7,000) may serve to account for differences in results. Garn et al. (99) emphasized the importance of adequate sample size when ascertaining relationships between fatness and lipid levels.

Berchtold et al. (19) investigated the effects of suspected cardiovascular risk factors on HDL-C levels and found the most important risk factor to be diastolic hypertension, and systolic hypertension the second most important risk factor. A review by Lewis & Naito (15) stressed the interactions of hypertension, lipids and lipoproteins, and their implications for atherosclerosis. Other investigators found HDL-C to be inversely related to systolic and diastolic blood pressure (14, 74). No significant relationship between HDL-C and diastolic or systolic blood pressure was detected in the group of adolescents currently studied. Although 19 percent of the girls had blood pressure readings above the normal range for either systolic or diastolic pressure, or both, most of these differed from the norm by less than five mm Hg. The mean diastolic blood pressure among this sample was 76, and the mean systolic blood pressure was 119, values well within the normal range for this age group (31). Thus, it was not possible to ascertain any effect of hypertension on HDL-C for this population.

#### D. Biochemical Relationships

The literature regarding the relationship of HDL-C to TC is conflicting. While Williams et al. (14) reported HDL-C to be directly

related to TC in their investigation of normal men, Khaltaen et al. (72) found no such relationship in another group of normal men. Similarly, there was no significant correlation between HDL-C and TC among the present group of adolescent females. The dissimilarity among these populations as to age and sex makes it difficult to compare results.

#### E. Relationship of HDL-C to Physical Activity and Other Factors

Investigations of the effect of exercise and physical activity on levels of HDL-C have consistently detected a positive correlation between these variables. Vigorous exercise such as running and power training has been shown to significantly increase HDL-C levels (29, 30, 34, 99). Nikkila et al. (35) concluded from their data on long-distance runners that endurance training is associated with an adaptive increase of lipoprotein lipase activity in skeletal muscle and adipose tissue. Men employed in occupations requiring vigorous physical activity demonstrate higher HDL-C levels (32, 33). Huttunen et al. (31) detected an increase in HDL-C levels in subjects even after mild exercise. Although Weltman et al. (28) did not find increased HDL-C in men after mild exercise, they did find a decrease in LDL-C without an effect on HDL-C, contributing to a more favorable LDL:HDL ratio. For the sample investigated in the present research, no significant correlation was found between HDL-C and physical activity. It should be pointed out that the literature cited above involved controlled conditions rather than the use of a questionnaire, which was employed in the current study.

Alcohol consumption is another factor that has been consistently positively correlated with HDL-C levels. Barboriak et al. (37) concluded from their studies using chronic alcoholics that the dose-effect relationship between alcohol intake and HDL-C levels extends over a large range of alcohol intake. Other investigators have reached similar conclusions (36, 38). Although no significant correlation was found between alcohol consumption and HDL-C values in the present group, the sample size may have been too small to detect any effect. Less than 10 percent of the population consumed any alcohol, with most of these subjects using minimal amounts. The lack of a significant negative correlation between smoking and HDL-C may also be related to small sample size since only 11 percent of the population was using tobacco. There was, however, a negative though nonsignificant correlation ( $p=0.531$ ) between HDL-C and smoking. Other investigators have reported strong negative correlations between these two variables (14-16). Due to the small sample size for alcohol consumption and smoking, it is inappropriate to state conclusions regarding these two variables.

Although a relationship between HDL-C and the use of oral contraceptives was detected by Bradley et al. (41), no significant difference was found in this group of adolescents between those who use oral contraceptives and those who did not. In the current population, 17 percent of the subjects were either currently using oral contraceptives or had used them in the past year.

#### F. Comparison of Longitudinal Plasma Total Cholesterol Data

An attempt was made to ascertain a lipid profile of this group by evaluating plasma total cholesterol data collected in three previous years, 1974, 1975 and 1976, when the population consisted of nine-, ten- and eleven-year olds, respectively, and that collected in 1981, at sixteen years of age. The mean values for these years were compared to determine any significant differences among ages. The mean value for eleven-year olds was significantly different from the other three ages. It is interesting to note that the mean value for plasma total cholesterol rose considerably from 1975 when the girls were approximately 10 years of age, to 1976 when they were approximately 11 years of age. By 1981, when the girls were adolescents, the TC mean value had fallen significantly. Tamir et al. (75) reported a similar decrease in total cholesterol during this age period, as did Berenson et al. (74). Thus, the trend in total cholesterol levels in the present population is consistent with that described in other populations of the same age and sex.

#### G. Conclusions

Mean levels of high-density-lipoprotein cholesterol in this group of adolescent females were within the normal range, although 41 percent demonstrated individual values above the normal range. These levels were inversely related to total protein, animal protein, vegetable protein, total carbohydrate and starch. They were not related

to any of the indices of obesity investigated, nor were they significantly associated with systolic and diastolic blood pressures, physical activity level, smoking, alcohol consumption or use of oral contraceptives (although the sample size of the last three variables was considered too small for conclusive remarks). Dietary fat was not related to HDL-C values. Mean values for HDL-C were not significantly different between races. The ratio of HDL-C to TC, which is considered by many to be the best indicator of coronary heart disease risk, was not significantly correlated with any of the factors studied. There was no significant correlation between HDL-C and TC values. Longitudinal TC data indicated a pattern of rising TC levels until adolescence, when it fell sharply.

## CHAPTER VI

### SUMMARY

A biracial sample of 78 16-year-old females was evaluated for high-density lipoprotein status and its relationship to several dietary variables, blood pressure, obesity indices, smoking frequency, physical activity, alcohol consumption and use of oral contraceptives. Plasma total cholesterol values of the subjects at four different ages were examined for the existence of a trend in the lipid profile of the population.

Mean values for high-density-lipoprotein cholesterol were near the upper end of the normal spectrum for both races, while mean values for total cholesterol were near the lower end of the normal range. There was no significant difference in mean plasma HDL-C values between the races.

Dietary variables which were significantly inversely related to HDL-C included total protein, animal protein, vegetable protein, total carbohydrate and starch. None of the other factors investigated were significantly associated with HDL-C values.

Plasma total cholesterol data collected on the population at four different ages revealed a trend of rising total cholesterol levels until adolescence. During this age period, total cholesterol levels fell considerably.

The implications of the role of high-density lipoprotein as a protective factor against atherosclerosis and coronary heart

disease indicate the importance of further research on this transporter of cholesterol. As evinced in past investigations, as well as the current one, nutrient intake has consistently been associated with the level of high-density lipoprotein. Elucidation of these interrelationships is imperative if multifactor risk prevention is to be successful. The study of lipid and lipoprotein profiles at several stages during the life span may add another dimension, along with genetic information, medical history and psychological profile, to health evaluation. Preventative medicine is a wholistic approach to health, which requires current data about all facets of human well-being.

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## LITERATURE CITED

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## VITA

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