FACULTY RESEARCH GRANT FINAL REPORT

SCREENING FOR HYPERLIPOPROTEINEMIA

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INTRODUCTION

The major reason for measuring blood lipids is because their elevation, hyperlipoproteinemia, has been associated with increased risk of premature heart disease (1,2,8). Cardiovascular disease is responsible for over 55% of all causes of death in the United States. Most disturbing is the fact that it seems to affect not only men and women over age 65, in whom it accounts for over 45% of death, but also the young, particularly the young male (11).

There are a few studies which indicate that coronary atherosclerosis begins early in life. In autopsies of black and white males and females between the ages of 15 and 19 years, the coronary arteries showed fatty streaks in 71% to 83%, and raised atherosclerotic lesions in 7% to 22% (12). U.S. soldiers killed at a mean age of 22 years showed some evidence of coronary vessel atherosclerosis in 77% of the cases in the Korean conflict (3) and 45% of the cases in the Viet Nam War (8). These findings suggest that detection for hyperlipoproteinemia or coronary atherosclerosis should start early in life.

The lipoproteins are termed alpha (HDL), pre-Beta (VLDL) and Beta (LDL) lipoproteins based on these electrophoretic properties. The HDL (high density lipoproteins), LDL (low density lipoproteins) and VLDL (very low density lipoproteins) designations are related to density gradient centrifugation properties of the lipoproteins.

The different electrophoretogram patterns result from qualitative and quantitative difference in lipoproteins which are indicative of normal and pathological conditions (5). Normal and abnormal classification patterns are indicated in Figure 1.

Although the pathogenesis of atherosclerosis is not completely understood the risk of its development is thought to be increased by certain types of

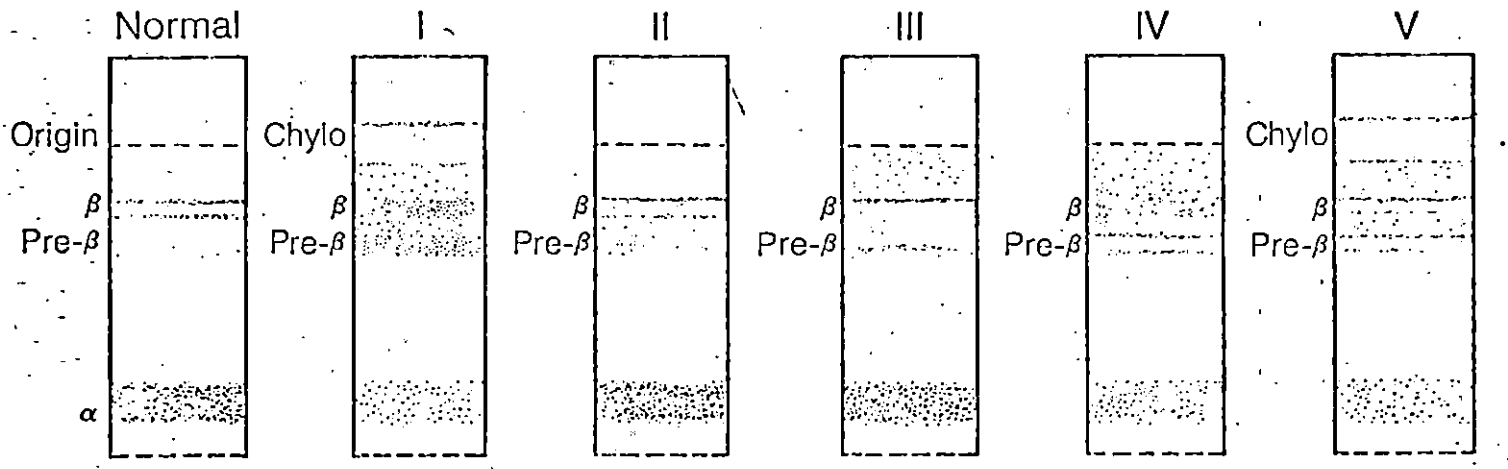

Figure 1 : Phenotyping hyperlipoproteinemia (5)

hyperlipoproteinemia. Therapy to correct or control hyperlipoproteinemia might decelerate the rate of development of atherosclerosis and thus lessen the incidence of complications such as ischemic heart disease, myocardial infarction and stroke (9). Therefore early identification of hyperlipoproteinemia is important.

The specific aims of this investigation were as follows:

1. Measurement of the lipid profile of faculty and students at Morehead State University that desire the analysis.
2. Inform the participants of the results of their lipid profile analysis.
3. Promote awareness of the dangers of hyperlipoproteinemia, particularly to individuals who are obese and/or have family histories of cardiovascular diseases.
4. Encourage individuals, especially high risk persons, to have a lipid profile analysis once a year.
5. Compare the lipid profiles (cholesterol, triglycerides, and electrophoretograms) of the participants with respect to:
    a. age
    b. sex
    c. occupation
    e. height-weight relationship
    f. family history of cardiovascular diseases
    g. blood pressure

The comparisons of the lipid profiles of participants with respect to their age and sex were primary considerations of this study.

A preliminary investigation involving the use of a questionnaire (ATTACHMENT A) revealed approximately 100 faculty members and 100 students that desired to participate in this study by donating a sample of blood. The investigator is extremely grateful to the 137 individuals that did donate.

STAFF IDENTIFICATION

In addition to the applicant four graduate students from the Department of Biological Sciences were engaged in this study. They are as follows:

1. Mr. Jansen Diener
2. Mr. Erich Hess
3. Miss Jan McCorkle
4. Mr. Terry Stine

METHODOLOGY

The lipid profile analysis included serum cholesterol and triglyceride determinations, eletrophoresis of plasma lipoproteins, and observation of plasma with respect to clearness and/or creamy layer formation. The blood samples represented a fasting blood level. This means the participants did not ingest any food or drink for at least 12 hours prior to the withdrawal of the blood sample.

The blood samples were obtained using vacutainers and the samples added to two tubes. One tube contained EDTA (ethylenediaminetetra-acetate) and was used for plasma preparation. The other tube was used for serum preparation. A hospital certified phlebotomist, Ms. Jan McCorkle, performed the venipunctures and obtained the blood samples.

One portion of the plasma was stored overnight (12 hours) at 4^{o} C and then observed for clearness and/or creamy layer formation. If the plasma was clear the person very likely did not have any increase in chylomicrons or VLDL (very high density lipoproteins). If the plasma was not clear or had a creamy layer it indicates the person had not fasted or that a lipid clearing problem exists. This data can be correlated with results in the electrophoretic portion of the analysis.

The electrophoretic procedure that was used is basically that of Chin and Blankenhorn (6). Controls were analyzed.

The electrophoretograms were analyzed with a densitometer at the Good Samaritan Hospital School of Medical Technology Laboratory. Therefore I am very appreciative of the cooperation with the hospital and Mrs. Patricia Motley, a laboratory director of that facility.

Serum cholesterol and serum triglyceride levels were determined spec-

trophotmetrically. A Beckman DB spectrophotometer with a temperature control accessory was utilized. Controls and standards were used.

The cholesterol analysis was basically that of Liebermann-Burchardt (7). Blood bilirubin levels can interfere with this analysis and were checked.

The triglyceride determination was essentially that of Hantzsch (7).

There were several questions which were answered by the participants. An example of the questionnaire form used is noted as Attachment B.

RESULTS AND DISCUSSION

The screening procedure for hyperlipidemia performed for this epidemiologic study involved volunteer faculty members and student participants who were found to be generally asymptomatic. As a first step in evaluating the subjects for hyperlipidemia, determinations of fasting cholesterol and triglyceride concentrations showed approximately 40% of them to be hyperlipidemic. Frederickson has suggested that

> "hyperlipidemia deserving some attention exists when cholesterol concentration exceeds 220 mg/100 ml or triglyceride concentration exceeds 140 mg/100 ml. This rule is applicable to all patients under age 55." (5)

Combining these criteria with Zelis's scheme (13) for diagnosis of hyperlipidemia (see Figure 2), 55 of the 137 subjects comprising this study were found to be measurably hyperlipidemic "deserving some attention." Of this 40% of hyperlipidemics, over one fourth fell into the 'non-optimal cholesterol' levels but acceptable triglyceride levels; these are the probable Type IIA hyperlipidemics, the incidence of which apparently increases with age. Just under a fourth of the hyperlipidemics are probable Type IV with non-optimal cholesterol levels and elevated triglycerides. Only 2 individuals had significantly elevated cholesterol levels along with elevated triglycerides; these are probable Type IIB. Four young people had elevated triglyceride levels with optimal cholesterol concentrations. It should be mentioned that for the higher age groups, over 70% of those screened were hyperlipidemic indicating that elevated cholesterol and triglyceride levels are often part of the aging process.

It is known that following birth, there is a very show ascent in lipoprotein and cholesterol concentrations which continues until well into the third decade. (5) During that decade the beta lipoproteins (LDL) and prebeta lipoproteins (VLDL) begin to rise at a noticeable rate -- the individual's physical growth is ending, he or she is more sedentary, and an excess of calories easily accumulates. Although

it is usual for lipid levels to rise with increasing age, as Tables B and C show, this may not be a healthy or even a "normal" trend. (13)

In an effort to evaluate the true hyperlipidemic who has a metabolic disorder resulting in one of the five phenotypes, lipoprotein electrophoresis was performed to determine abnormal lipoprotein levels. The criteria for defining normal concentration based on percentage of all lipoprotein are those of the International Clinical Laboratories of Kentucky, Inc.:

Chylomiera	Normal 0- 2%
Beta lipoprotein	Normal 38-74%
Prebeta lipoprotein	Normal 1-37%
Alpha lipoprotein	Normal 10-38%

These ranges are for fasting plasma in a subject whose physical condition and body weight are stabilized.

Upon quantitation of the lipoprotein bands by microdensitometry with the lipoproteins expressed in relative percentage, 21 participants, about 15% of those screened, were found to have abnormal.lipoprotein levels. Table C shows that, of the 21 subjects, 7 had high levels of the alpha lipoprotein (HDL) possibly due to exogenous sources of estrogen rather than to any type of metabolic disorder. Of any lipoprotein, HDL has the most protein and is closer in weight to albumin than the other lipoproteins. It is known that women carry a higher percentage of their cholesterol in HDL than men do. This is apparently due to estrogen, for when estrogen is given to men, they, too, carry more of their total plasma cholesterol in HDL. (4) Because no harmful effects have been noted when HDL levels are increased, those subjects with increased alpha lipoprotein due to estrogen are precluded from the list of primary hyperlipidemias.

The most frequently encountered elevated lipoprtoein in this study was the beta or LDL, which forms as the result of catabolism of prebeta particles; such accumulation indicates a defect in metabolism of beta lipoproteins. This is a Type IIA disorder which is responsible for the high cholesterol level in the blood and which is the classic form of the disease recognized long ago as essential hyper-

cholesterolemia (4). Note that of all persons tested, 11 were found by the lipoprotein electrophoresis to have elevated LDL or beta lipoprotein (see Table C). Three of these, all in their 20's, had optimal plasma cholesterol levels, so that only 8 subjects proved to be Type IV disorders, 3 were verified as true hyperlipidemics. All 3 had turbid or lipemic plasma after 24-hour refrigeration; two in their forties were "heavy" while the teenager was of medium build. Obesity is often noted with Type IV. (4)

Similarly, of the 13 Probable Type IV disorders, both proved to be true hyperlipidemics from electrophoretic patterns even though neither of the normal ranges for beta or prebeta was exceeded. Type IIB describes a basic defect in LDL catabolism plus a concomitant, often marked, elevation of VLDL. To diagnose Type IIB, one must rely on elevated levels of cholesterol and triglyceride rather than on relative percentages of beta and prebeta lipoproteins.

Table D summarizes the complete findings of this study. No participants had Types I, III, or V, which are known to be uncommon.

AGE GROUP	SAMPLE SIZE N	SERUM TRIGLYCERIDE below 140 mg%; SERUM CHOLESTEROL range 210-260 mg%	%N	above 260 mg%	%N	TG above 140 mg% CHOL range 210-260 mg%	%N	above 260 mg%	%N	TG above 140 mg%; CHOL below 210 mg %	%N	FIRST EVALUATION HYPERLIPIDEMICS, % IN AGE GROUP	
10-19	18	0	0.0	0	0.0	1	5.6	0	0.0	0	0.0	1	5.6
20-29	60	7	11.9	5	8.5	0	0.0	0	0.0	4	6.7	16	26.7
30-39	21	2	9.5	4	19.0	4	19.0	0	0.0	0	0.0	10	47.6
40-49	21	4	19.1	5	23.8	6	28.6	1	4.8	0	0.0	16	76.2
50-59	16	3	18.8	5	31.2	2	12.5	1	6.3	0	0.0	11	68.8
60-69	1	0	0.0	1	100.0	0	0.0	0	0:0	0	0.0	1	100
TOTAL ALL AGES	137	16 NON-OPTIMAL CHOLESTEROL	11.7	20 PROBABLE TYPE IIA	14.6	13 PROBABLE TYPE IV	9.5	2 PROBABLE TYPE IIB	1.5	4	2.9	55	40.1

Table-B.—Incidence of Hyperlipidemia after First Step Evaluation of Serum Cholesterol and Serum Triglyceride levels.

AGE GROUP	SAMPLE SIZE N	PARTICIPANTS WITH ABNORMAL LIPOPROTEIN LEVELS; %N		ABNORMAL LIPID LEVELS DUE to ESTROGEN (E) or DIABETES (D)	ELEVATED BETA LIPOPROTEIN		ELEVATED PREBETA LIPOPROTEIN		ELEVATED ALPHA LIPOPROTEIN		ABNORMAL LIPOPROTEIN LEVELS CONSISTENT WITH ELEVATED CHOL and/or TG	
)-19	18	2	11.1	1 E	0	0.0	1	5.6	1	5.6	1	5.6
)-29	60	7	11.6	2 E	5	8.3	0	0.0	2	3.4	2	1.5
)-39	21	3	14.3	1 E	2	9.5	0	0.0	1	4.8	2	9.5
)-49	21	5	23.8	1 E	2	9.5	2	9.5	1	4.8	4	19.0
)-59	16	4	25.0	3 2E,D	2	12.5	0	0.0	2	12.5	3	18.8
)-69	1	0	0.0	-	0	0.0	0	0.0	0	0.0	0	0.0
OTAL LL AGES	137	21	15.3	8	11	8.0	3	2.2	7	5.2	12	8.8

able C. Incidence of Electrophoretic Findings of Abnormal Lipoprotein Levels Correlated with Age and Correlated with Elevated Cholesterol and Triglyceride Concentrations.

Figures 3 and 4 graphically illustrate the magnitude of hyperlipidemia in this sample population.

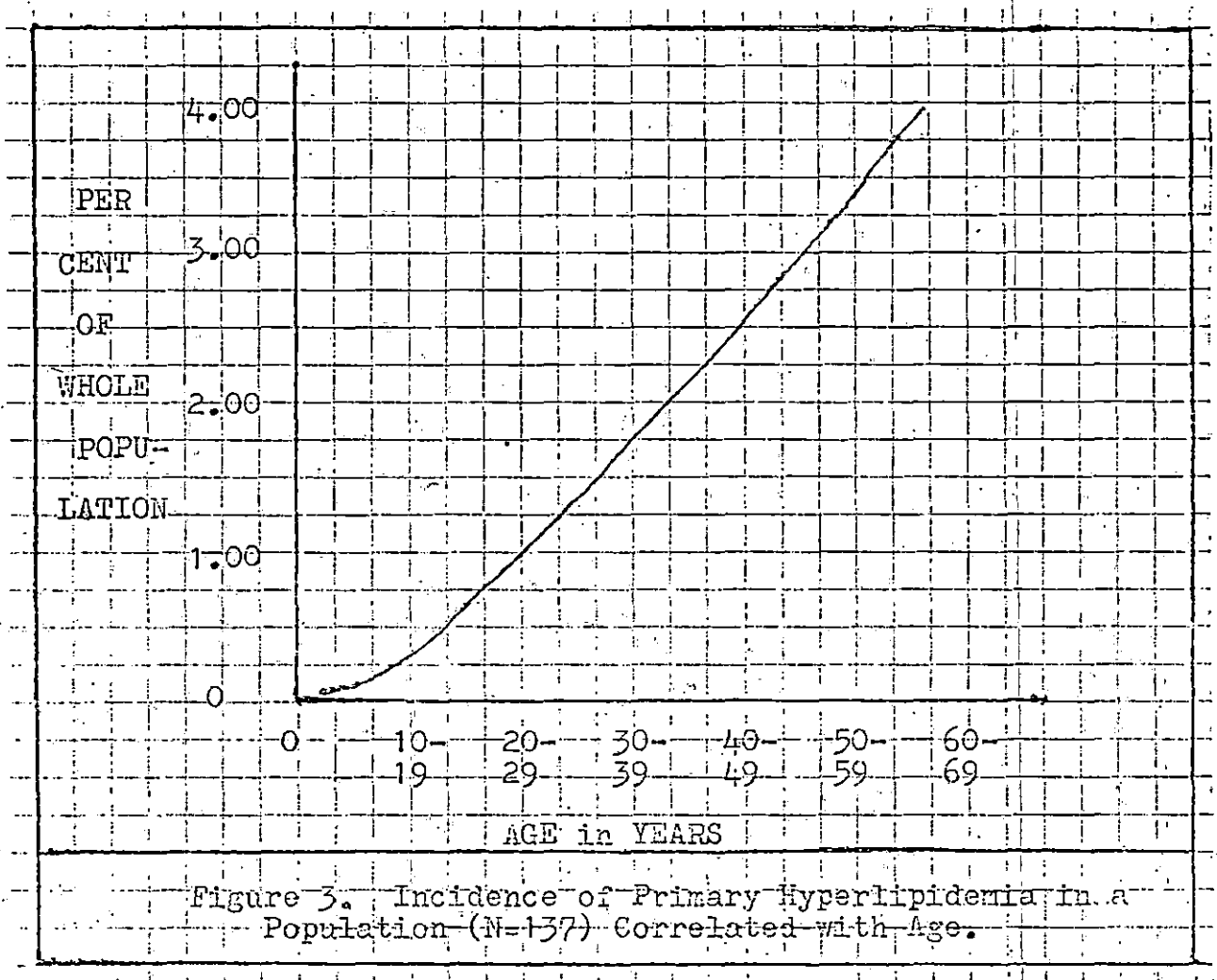

Figure 3. Incidence of Primary Hyperlipidemia in a Population (N=137) Correlated with Age.

Table D summarizes the complete findings of this study. No participants had Types I, III, or V, which are known to be uncommon.

AGE GROUP	N	PRIMARY HYPERLIPIDEMIAS					
		TYPE IIA	%N	TYPE IIB	%N	TYPE IV	%N
10-19	18	0	0	0	0	1	5.6
20-29	60	2	3.3	0	0	0	0
30-39	21	2	9.5	0	0	0	0
40-49	21	2	9.5	1	4.8	2	9.5
50-59	16	2	12.5	1	6.3	0	0
60-69	1	0	0	0	0	0	0
TOTAL ALL AGES	137	8	5.8	2	1.5	3	2.2

Table D. Incidence of Primary Hyperlipidemia Confirmed by Lipoprotein Electrophoresis and Correlated with Age.

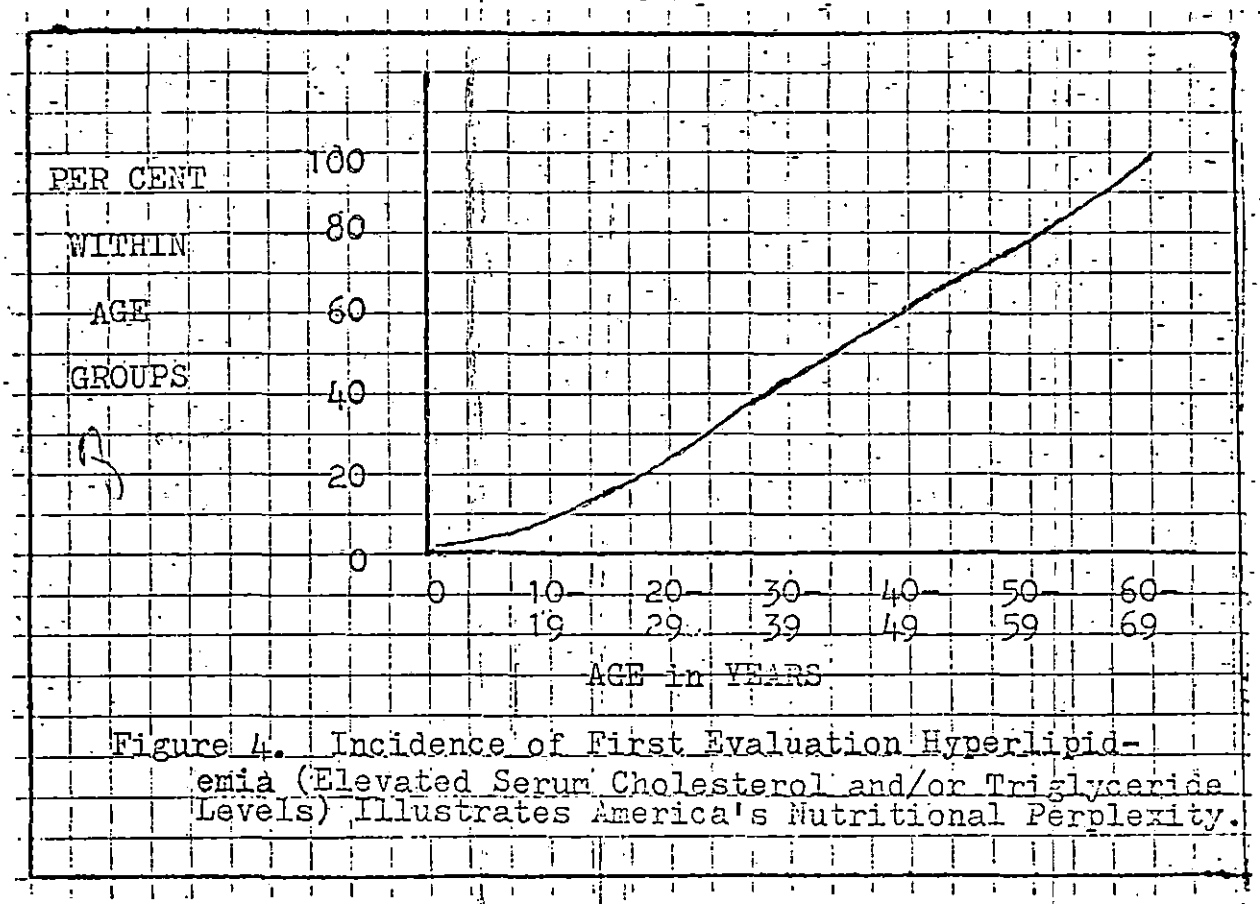

Figure 4. Incidence of First Evaluation Hyperlipidemia (Elevated Serum Cholesterol and/or Triglyceride Levels) Illustrates America's Nutritional Perplexity.

Further analysis shows that after the age of 40, the incidence of hyperlipidemia increases by a factor of 4. See Table E.

AGE GROUP	N	PRIMARY HYPERLIPIDEMICS	%N
Under 40	99	5	5
40 and over	38	8	21
All Ages	137	13	9.5

Table E. Incidence of Hyperlipodemia with age

In attempting to establish a correlation between incidence of hyperlipidemia and sex, mixed results were obtained. A significantly higher incidence of hyperlipidemia and triglyceride levels. See Table F. But by contrast, Table G shows that, for the confirmed primary hyperlipidemics, there is a somewhat higher incidence among women; this difference, however, is not significant due to the small population size and the consequent low number of hyperlipidemics.

SEX	SAMPLE SIZE N	TG below 140 mg% CHOL 210- 260 mg%	TG below 140 mg% CHOL over 260 mg%	TG over 140 mg% CHOL 210- 260 mg%	TG over 140 mg% CHOL over 260 mg%	TG over 140 mg%; CHOL optimal	%N
Female	78	11	9	4	2	1	34.6
Male	59	5	11	9	0	3	47.5

Table F. Incidence of First Evaluation Hyperlipidemia Correlated with Sex.

SEX	N	Type IIA Elevated Beta	Type IIB Elevated Beta and Prebeta	Type IV Elevated Prebeta	%N
Female	78	5	2	2	11.5
Male	59	3	1	0	8.5

Table G. Incidence of Primary Hyperlipidemia Correlated with Sex.

No pattern or correlation between hyperlipidemia and unusual or even consistent blood pressure readings could be discerned. Nor was a relationship seen between weight or body build and hyperlipidemia. Neither smoking nor consumption of alcohol could be correlated with incidence of hyperlipidemia in a meaningful way. While such correlations might exist, they were not found in this small study in which there were so few verifiable hyperlipidemics.

The incidence of verified hyperlipidemia found in this study was lower than expected by about half. This is probably due to the voluntary nature of the participation of those screened. Those who participated were aware of the occurrence of the disease, generally aware of proper diet, and sought confirmation through this study as to their state of good health. Others, who may have been aware of their own high blood pressure, overweight condition, or high cholesterol levels perhaps avoided a potentially dissatisfactory experience.

Because things can go wrong with lipid metabolism as one ages or because of genetic disorders, and because so much of what one can do (developing proper eating habits, exercising, avoiding the use of tobacco) is under voluntary control, everyone should be screened for lipid abnormalities. This might best be done by the family physician whenever the patient comes in for a physical or insurance exam, or for periodic checkups. From the standpoint of preventive medicine, more emphasis should be placed on the problem of hyperlipidemia in younger people, even in children so that low-fat diets may become a habit from an early age.

If only a single parameter were to be measured in a screening procedure foe hyperlipidemia, it would be the serum cholesterol level. It has been established that the higher the serum cholesterol level, the poorer the epidemiologic prognosis. (13) This value, when significantly elevated, was seen throughout this study to be a first signal for suspecting a susceptible individual to all the risks of hyperlipidemia.

The hyperlipidemic individual can be diagnosed from measurement of serum

cholesterol, triglycerides and other parameters such as the level of systolic blood pressure, cigarette-smoking history, postprandial glucose and electrocardiographic evaluation for left ventricular hypertrophy. These factors may be used to quantitate an individual's relative risk and the susceptible individual can then be treated according to a specific multifactorial approach; i.e., elimination of smoking, lowering of blood pressure, and dietary modification designed to achieve ideal weight. (13)

It deserves repetition that, although it is usual for lipid levels to rise with increasing age, this may not be a healthy or even a "normal" trend. It is important to note that

> "many workers in the field are now coming to believe that the average values in the U.S. population are probably higher than should be considered a "normal" level. A number of investigators feel that if these normal limits were lowered, many more hyperlipidemic persons would be detected -- and this is the basis for their warning that hyperlipidemia is affecting Americans in epicemic proportions." (13)

Long range studies involving the screening of large random samples of the population for frequency and type of lipid disorders with an eye toward inherited lipid patterns might provide long overdue data on the lipid problem we're facing in the nation today.

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ATTACHMENT A

WHAT IS THE CHOLESTEROL LEVEL IN YOUR BLOOD?

Blood levels of cholesterol and other lipids are highly significant with respect to many diseases. Elevated levels of cholesterol may lead to atherosclerosis and other cardiovascular problems. Once the atherosclerotic process begins it is extremely difficult to correct the damage which has occurred, but other damage may be prevented. Elevated levels of these lipids may be detrimental to young and old people.

I wish to analyze blood samples of faculty and students. The analysis will include investigation of cholesterol and other lipids in the blood.

If you would like to become involved in this experiment by donating ONE SAMPLE of blood please give the information requested at the bottom of the page and return it to me as soon as possible. If you desire more information please call 783-3101

Respectfully,
David J. Saxon

Dr. David J. Saxon
UPO 798 MSU

NAME _____

CAMPUS ADDRESS _____

CAMPUS PHONE _____

FACULTY _____ OR STUDENT _____

Name _____ I.D. Number

Campus mailing address _____

QUESTIONNAIRE FOR LIPID ANALYSIS PARTICIPANT

Please note that all information will be treated confidentially and that individual results of the analysis will be reported to you.

Sex (circle one) M F	Age	Occupation	
Height	Weight	Race	Blood Pressure

1. What did you eat at your evening meal last night? _____

_____

2. How many hours since your last meal or snack? _____

3. General diet information: a.) Your usual breakfast, if any, consists of

_____

b.) Your usual mid-day meal consists of _____

_____

c.) Your evening meal generally consists of _____

_____

4. Alcohol consumption - estimated: a) Beer - _____cans/week

b) Hard liquor - _____oz./week

5. Do you smoke? If so, how many packs/week? _____

General health information and family health history:

Check space if you 1.) have ever had or 2.) now have any of the following. Also indicate 3.) to the best of your knowledge, if your siblings (S), mother (M), father (F), grandmother (GM), or grandfather (GF) have ever had any of the following.

Condition:	Past	Present	Family
Heart or coronary problems			
High blood pressure			
Hardening of the arteries			
Sugar diabetes			
Thyroid problems			
Removal of ovaries-hysterectomy ..			
Lipid disease			
Kidney disease			
Severe reaction to stress			
Recent weight change (G or L)			
Hormone treatment			
Chronic infections			
Other _____			
Birth control pills or any other source of estrogen			

6. Describe any other family health history information which may be helpful or any special diet you are now following on the back of this form.