Title

Quantitative and qualitative relationships among very low density lipoproteins in 1677 consecutive lipid clinic patients

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QUANTITATIVE AND QUALITATIVE RELATIONSHIPS AMONG VERY LOW DENSITY LIPOPROTEINS IN 1677 CONSECUTIVE LIPID CLINIC PATIENTS

Apolipoprotein E Polymorphism, Apolipoprotein B and Double Pre-beta Lipoproteinemia

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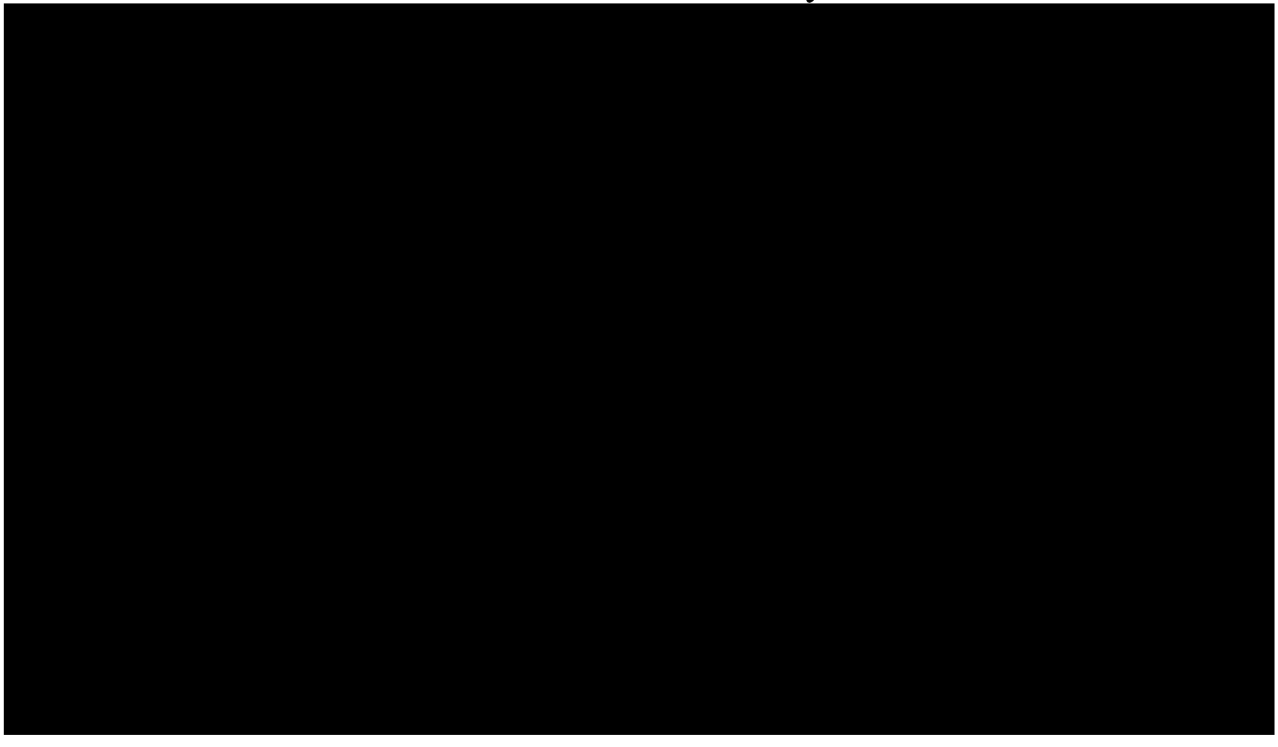
submitted April 1, 1991 in partial satisfaction of the
requirements for the M.D. with Thesis Program
University of California San Francisco



ABSTRACT

We investigated relationships between triglyceride-rich lipoproteins and coronary artery disease by retrospectively examining detailed lipid profile and chart review data on 1677 consecutive patients referred to the UCSF Lipid Clinic. We tested the hypothesis that quantitative and qualitative properties of very low density lipoproteins (VLDL) correlate with specific lipoprotein, demographic and clinical variables. Of particular interest was the isoelectric focusing pattern of apo E and the agarose gel electrophoretogram for double pre-beta (DPRE) lipoproteinemia.

The population was comprised of 751 women (mean age 46 yrs) with an average total cholesterol (TC) of 316 mg/dl and 925 men (mean age 50 yrs), with an average TC of 298 mg/dl. Lipid diagnosis was 22% primary hypercholesterolemia (IIa), 19% combined hyperlipidemia (IIb), 3% dysbetalipoproteinemia (III), 15% endogenous lipemia (IV), 4% mixed lipemia (V), 5% elevated Lp(a) and 32% with no lipid abnormality. The overall coronary artery disease (CAD) prevalence was 26%, with the greatest prevalence in dysbetalipoproteinemia, combined hyperlipidemia and endogenous lipemia.

The apo E 4-4 phenotype was more prevalent in primary hypercholesterolemia in whereas apo E 3-2 and E 4-2 were most prevalent in patients with lipemia. The overall prevalence of DPRE was 25%, with increased prevalence in female, Black, apo E 4-4 and hypercholesterolemic patients. Quantitatively, DPRE was associated with increased VLDL-C/VLDL-T, HDL-C, percent of apo B in VLDL apoprotein mass and with decreased triglyceride (TG).

Patients with CAD not only had elevated TC, LDL-C (effect greater in women) and lower HDL-C (effect greater in women), but also elevated TG and total apo B. There was no association with VLDL-C/VLDL-T ratio or % apo B (particle diameter of VLDL). In the subset of patients with TG>170 mg/dl, CAD was associated with elevated LDL-C (in women only) and elevated total apo B (in women only), with no difference in % apo B or TG.

Future analysis will cover over 2000 patients and many additional genetic, biochemical and clinical variables. We will study the independence of determinants of DPRE and CAD and their interrelationships using multivariate models.

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INTRODUCTION

Atherosclerosis remains the most significant disease afflicting the Western population. In the United States, over 400,000 individuals die of coronary artery disease each year. It is well established that lipoproteins transport cholesterol into the artery wall and contribute to the cholesterol found in atheromata. Multiple large prospective studies (Framingham, MRFIT, etc) have established the role of low density lipoprotein (LDL) cholesterol in atherogenesis, but this is clearly not the only lipoprotein involved in atherogenesis.[1] Not only LDL[2], but also the Lp(a) lipoprotein[3], and most recently very low density lipoprotein (VLDL) have been found in the artery wall (Joseph Rapp and John Kane, unpublished results). Variations in the composition and structure of lipoproteins are dependent on genetic and environmental factors, and may affect interindividual susceptibility to atherosclerotic disease. There is ample evidence that some triglyceride (TG) rich lipoproteins, particularly VLDL remnants, have atherosclerotic potential, based on observations in dysbetalipoproteinemia (type III), chronic renal failure, hypothyroidism and diabetes. Thus, studying the quantitative and qualitative properties of VLDL may reveal new insights into the pathologic processes of lipid metabolism that lead to atherogenesis.

This analysis reflects an interest in clarifying the role of TG-rich lipoproteins in atherogenesis. Failure to show the independent effects of hypertriglyceridemia on coronary artery disease (CAD) in large population-based epidemiologic studies may be influenced by heterogeneity in TG-rich lipoproteins, particularly in relation to atherogenic potential.[4] Also, most studies are unable to exclude post-prandial chylomicronemia as a source of hypertriglyceridemia.

Reasons for this Study

A specific goal of this analysis is to identify characteristics of VLDL that may stratify hyperlipidemic (especially hypertriglyceridemic) patients on the basis of underlying genetic and metabolic function, leading to the analysis of subgroups with respect to CAD prevalence and risk.

Most lipid studies are done in large groups of healthy persons, or very small groups of hyperlipidemic persons. Our large sample size of hyperlipidemic patients, and our

exclusion of the heterogeneous etiologies of secondary hypertriglyceridemia, may elucidate novel factors that influence the composition of VLDL.

The large sample size of hyperlipidemic subjects may also prove useful in exploring outlier patients with uncharacterized genetic diseases,[5] and in studying the interactive effects of diseases that are infrequent in the general population.[6]

Review of Lipoprotein Particles

Ultracentrifugal characteristics and electrophoretic migration are the two parameters by which lipoprotein families are commonly separated and classified. Viewed through the electron microscope, lipoproteins are spherical particles of varying diameter.

TABLE 1 – LIPID PARTICLE SIZE AND MOBILITY

Family	Electrophoretic Mobility	Density	Particle Size (Å)
chylomicrons	remain at the origin	< 0.950	800-10,000
VLDL	pre-beta	0.950-1.006	300-700
IDL	pre-beta to beta	1.006-1.019	300
LDL	beta	1.019-1.063	250
HDL	alpha	1.063-1.210	190-220

The alpha, beta, pre-beta, sinking pre-beta, double pre-beta nomenclature arose out of comparison of lipoprotein migration to the migration of alpha and beta globulins. Presence of a sinking pre-beta band corresponds to a qualitative assessment of Lp(a) level.

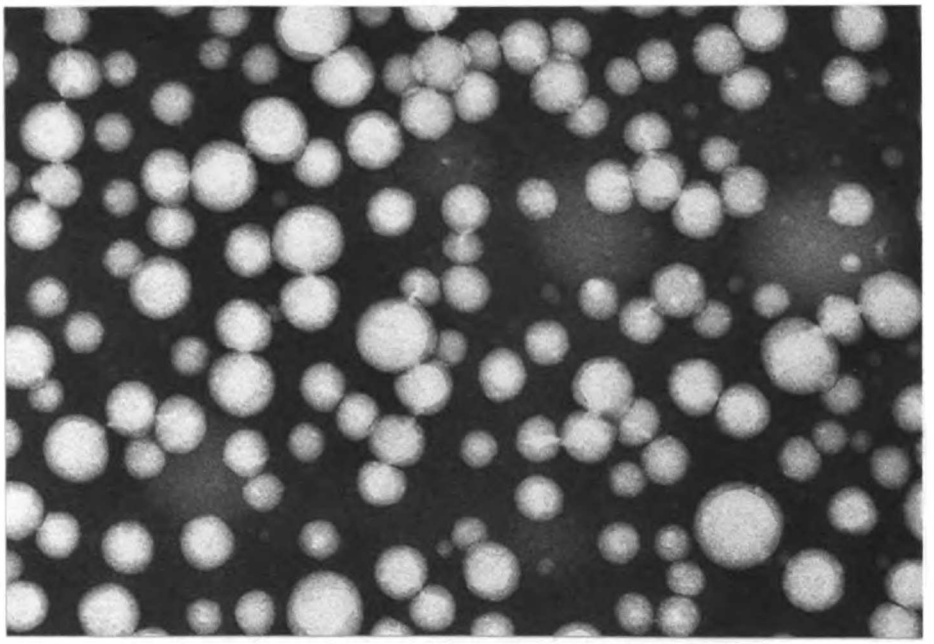


FIGURE 1 – ELECTRON MICROGRAPH OF PARTICLE SIZE DISTRIBUTION

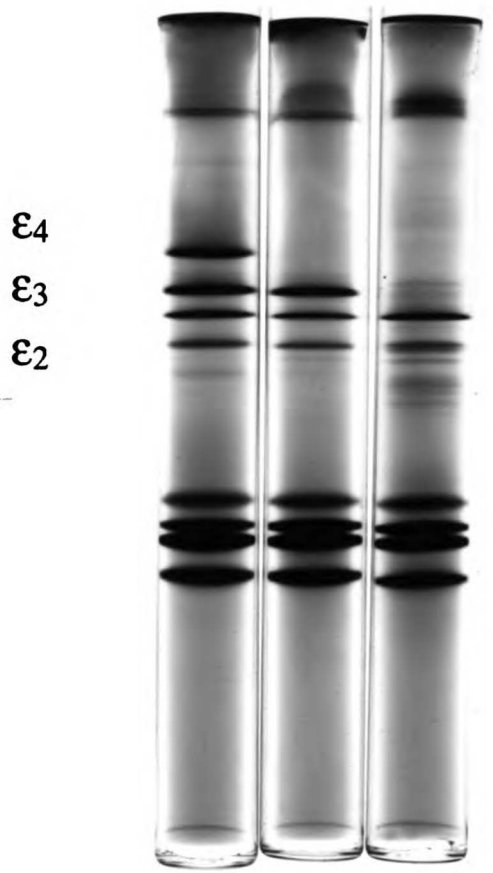


FIGURE 2 – SAMPLE APO E ISOELECTRIC FOCUSING GEL

Apo E

The hepatic recognition and metabolism of VLDL is influenced by apolipoprotein E (apo E) isoform. Apo E is a part of VLDL remnants, chylomicron remnants, and a subgroup of high density lipoprotein (HDL). Apo E is a mediator of LDL receptor endocytosis and thus modulates metabolism of atherogenic cholesterol containing particles. The apo E phenotype may also have an effect on the coordinated metabolism of cholesterol and triglycerides.[7]

The gene locus for apo E is polymorphic. Three common alleles (epsilon 2, epsilon 3, epsilon 4) give rise to 3 homozygous (E2-2, E3-3, E4-4) and three heterozygous phenotypes (E3-2, E4-2, E4-3). The apo E polymorphism, first detected by Utermann, was characterized by Rall and Weisgraber as single amino acid substitutions at two sites (Figure 2). Evidence of the impact of apo E polymorphism on atherosclerosis has recently been reviewed.[8]

VLDL Heterogeneity (Double Pre-beta)

The preponderant population of VLDL has pre beta mobility in agarose gel electrophoresis at pH 8.4. An additional population of VLDL with slower mobility is sometimes observed, and its presence has been named double pre-beta (DPRE) lipoproteinemia (Figure 3).[9] There are no published data on DPRE in large normolipidemic or hyperlipidemic populations. This phenomenon is relatively frequent, and is distinct from the broad beta band seen in type III patients. Rare variant patterns such as beta+pre-beta, broad pre-beta, and slow pre-beta were classified as DPRE in our analyses.

Normal pre-beta VLDL are processed through the lipolytic cascade, resulting in progressively smaller, cholesterol enriched lipoproteins in the transformation from VLDL remnants to IDL to finally LDL. The double pre-beta phenomenon has been hypothesized to reflect the presence of remnant VLDL in plasma.[9] The role of remnant particles in atherogenicity continues to be debated.[10]

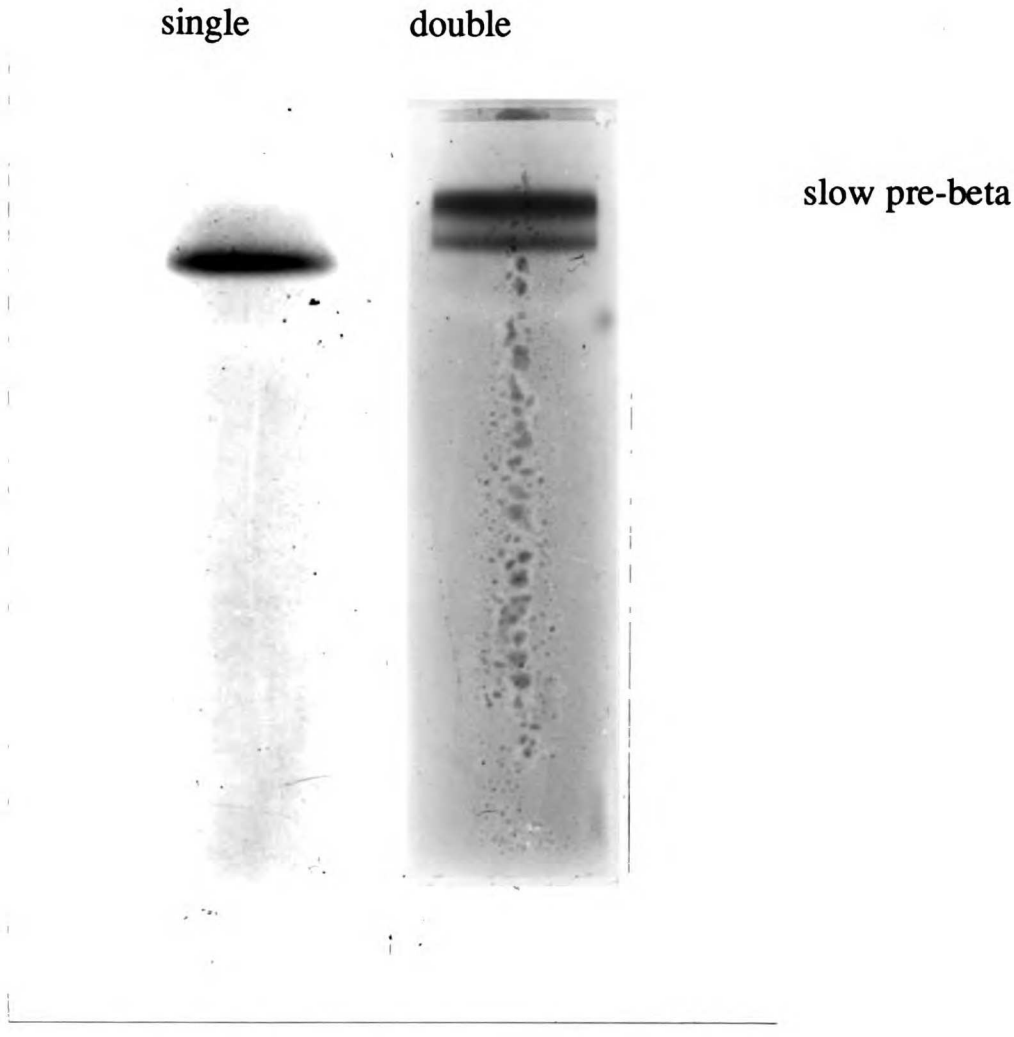


FIGURE 3 – SAMPLE GEL SHOWING SINGLE VS DOUBLE PRE-BETA VLDL

Only in rare cases such as lipoprotein lipase deficiency or apo CII deficiency is the biochemical cause of hypertriglyceridemia understood. Studies differ on whether the overproduction of VLDL, defective VLDL removal or reduced fractional catabolic rate of VLDL accounts for hypertriglyceridemia. It is apparent that hypertriglyceridemic VLDL are biochemically different than normolipidemic VLDL and it has been observed that hypertriglyceridemic VLDL are frequently cholesteryl ester rich.[11]

Some results suggest that the catabolism (lipolysis) of VLDL-TG is under genetic control, whereas the VLDL-TG production rate reflects factors such as total caloric intake and alcohol intake in addition to genetic determinants. It is likely that hypertriglyceridemia often develops on the basis of VLDL overproduction in individuals who have a genetically low VLDL triglyceride removal (lipolytic) capacity.[12] Recent literature discusses the significance of elevated VLDL [13] and the metabolic heterogeneity in VLDL.[14]

LDL Heterogeneity

Plasma LDL consists of multiple particle species which constitute a particularly complex spectrum in type III hyperlipoproteinemia and in hypertriglyceridemia.[15] Several studies suggest that small, dense LDL (low in cholesterol) raise the risk of CAD.[16] A recent study also presented evidence that LDL subclass patterns are genetically controlled.[17] The LDL in familial combined hyperlipidemia has been shown to be heterogeneous, with a preponderance of small, dense, lipid poor LDL as compared with LDL from normal subjects.[18]

Total and Percent Apo B

Apo B containing lipoproteins vary in size and in the content of cholesterol, triglycerides and apolipoproteins. Epidemiologic evidence suggests that all major apo B containing lipoproteins are atherogenic, but to unequal degrees.[19] The percent apo B refers to the tetramethyl urea insoluble fraction of apo B, and is inversely related to the VLDL diameter. The majority of apo B is in d>1.006 fraction.[20]

Risk Factors and Lipoprotein Metabolism

A few factors have been strongly and repeatedly correlated with the risk of CAD in the general population: male gender, smoking, obesity, diabetes mellitus, physical inactivity and diet. Statements about the "independence" of risk factors are highly contingent upon

the formal specifications of statistical testing. All variables in the analysis must be known, as should all the possible fits to be considered, the criteria for inclusion and exclusion, and the sample characteristics of the data set. Risk factors may be additive, synergistic or fully multiplicative. They may mediate effects independently, through other factors, or both. Multiple risk factors are also known to cluster in individuals. Even an "independent" risk factor may not be a causal one because a more direct biochemical marker may be as yet undiscovered.[21] Little is published about the influence of risk factors and the total cholesterol (TC) level on CAD in a large hyperlipidemic population.

One limitation of certain multiple regression analyses are that quantitative lipid variables are treated without respect to the known physical structure of lipoproteins. Although we know little about the mechanisms of interaction between multiple environmental risk factors in atherosclerosis, we do know the fundamental structure and metabolic pathways of lipoproteins. For example: some lipid regressors in a multivariate regression model may exist as integral components of the same particle, and their proportions rather than absolute value may be relevant; there are known inverse exchange relationships between certain lipoprotein species; and the diameter of a particle may be a function of the level of another species. We intend to explore the use of structurally based constraints in future multivariate analysis.

The Hyperlipidemic/Hypertriglyceridemic Population

Most epidemiologic studies, including Framingham, Seven Nations, and MRFIT (350,000 middle-aged men) present risk curves that cut off at a TC of 300 mg/dl. Few studies extrapolate the relative risk of CAD to patients with TC>300 mg/dl, TG>170 mg/dl, or both.

Both hypercholesterolemia and hypertriglyceridemia have multiple causes, and patients may cluster into multiple aberrant physiological states, with unequal atherogenic potential. Elevated triglycerides are associated with several diseases including diabetes, obesity and chronic renal failure. It should be recognized that triglycerides (all fractions) and VLDL-C have a large intraindividual variability and are known to follow a highly skewed distribution in the general population.

In an influential review, Hulley suggested that controlling for TC and HDL-C eliminates the residual correlation between TG and CAD.[22] Since then, studies on the independent effect of TG continue to be in conflict. Most studies do not measure individual lipoprotein species, and may miss high risk subpopulations. Also, several statistical problems are

known to occur during attempts to control for interrelated parameters in multivariate risk models.[23]

Framingham data showed that triglycerides are an important risk factor in women over 50 years old.[24] A Swedish prospective longitudinal study also found TG to be the main risk factor for myocardial infarction (MI) in women over 50 years old.[25] In their analysis of the 19-year Stockholm Prospective Study, the authors emphasized that combining angina with MI might hide TG effects on MI alone.[26] Recent evidence suggests hypertriglyceridemia with a normal TC may be associated with CAD if apo B is elevated.[27]

SPECIFIC HYPOTHESES

1. **Qualitative variation in VLDL, such as DPRE, correlates with the demographic characteristics and lipoprotein levels of this population.**
2. **Apo E phenotype is associated with the demographic characteristics and lipoprotein levels of this population.**
3. **The cholesterol content (VLDL-C/VLDL-T) of VLDL correlates with the presence of CAD in this population.**
4. **The particle diameters of VLDL (inversely proportional to % apo B) correlates with presence of CAD in this population.**

STUDY DESIGN

Patient Population

The patient population is a series of approximately 1700 consecutive patients referred to the UCSF Lipid Clinic between 1985 and 1990 for biochemical and clinical evaluation. Lipoprotein composition analysis, gel electrophoresis (isoelectric focusing to determine apo E phenotype, and agarose gel electrophoresis to observe for the presence of DPBL, chylomicronemia, broad beta VLDL and sinking pre-beta) and apo B immunonephelometry is performed in the evaluation of all patients. Measurements are made with patients in the fasting state and off of lipid lowering medications if possible.

A retrospective chart review was performed by retrieving available medical records from UCSF (see Appendix 1) with the assistance of the UCSF Summary Time Oriented Record (see Appendix 4). Data on multiple variables including age, sex, ethnicity, history of documented coronary artery disease and peripheral vascular disease, hypertension, diabetes, medications, family history, presence of xanthoma was gathered, verified and entered into a relational database (see Appendix 2).

This analysis was done using conventional and exploratory statistical techniques implemented on a Mac IIci system using several commercially available statistical programs. For this exploratory study analysis was done in a simple univariate or cross-tabular fashion. Future work will examine the complete data set, expected to be over 2000 patients and several additional variables, in a comprehensive multivariate fashion.

TABLE 2 – DESCRIPTIONS OF LABORATORY VARIABLES OF INTEREST

Abbrev.	Variable Description	Units
DPRE	Presence of double pre-beta band The slow component of DPRE is thought to represent cholesterol rich remnant–like particles that may be treated differently by foam cells.	Y/N
apo E	Apo E phenotype Six major isoforms. In this population E2-2 are generally type III.	2-2, 3-2, 3-3, 4-3, 4-4
Lp(a)	Lipoprotein (a) Visually assessed on gel electrophoresis as sinking pre-beta, "trace" threshold is at about 11 mg/dl.	trace, 1-4+
Chylos	Chylomicrons Visually assessed, >50 mg/dl shows as trace.	N, trace, Y
TC	Total-C	mg/dl
logTG or TT	logTotal-T	mg/dl
LC	LDL-C	mg/dl
VC	VLDL-C	mg/dl
VT	VLDL-T	mg/dl
HC	HDL-C	mg/dl
VC/VT	VLDL-C/VLDL-T Atherogenicity may be potentiated by cholesteryl ester enrichment and particle size of VLDL. This ratio may rise with longer residence time in plasma.	mg/dl
HC/VC	HDL-C/VLDL-C Used as a potential index of efficiency of transfer, a lower ratio may equate with efficient transfer, (and a high ratio may give a less atherogenic VLDL). This relates to the idea is that more HDL-C makes it easier to transfer HDL-C into VLDL.	–
HC/(VC/VT)	HDL-C/(VLDL-C/VLDL-T) Used as a potential "thermodynamic index" , inversely proportional to transfer but adjusted for HDL-C level. A higher index may equate with difficulty in transferring cholesteryl ester into TG rich lipoproteins.	mg/dl
% apo B	Percent apo B in VLDL (the TMU insoluble fraction) Shown to inversely correlate with particle size diameter of VLDL. Small particles may more effectively penetrate the endothelium; tends to mean VLDL will be cholesterol enriched.	%
T apo B	Total apo B in d>1.006 g/cm^3 (LDL/IDL) This fraction is mostly dominated by LDL but has more IDL in patients with high levels of remnant particles.	mg/dl
T apo B/LC	Total apo B/LDL-C Used as a measure of size of LDL particles (and IDL), used to test hypothesis that small dense LDL may be more atherogenic, although this effect may indirectly mediated by VLDL.	–

C = Cholesterol, T = Triglyceride, H = HDL, L = LDL, V = VLDL. All values are measured in human serum.

Potentially confounding variables with known strong associations with lipoprotein levels include gender and age. Serious metabolic diseases that significantly distort lipoprotein profiles include: dysbetalipoproteinemia (confirmed apo E 22 isoform and the presence of broad beta VLDL band), chronic renal failure and untreated hypothyroidism. Medications that significantly alter lipoprotein profiles include: HMG-CoA inhibitors, nicotinic acid, bile acid resins, clofibrate and probucol. Factors that have mild to moderate effects on lipoprotein profiles include: ethnicity, obesity, cigarette smoking and diabetes mellitus (type I and II).

Variables that were collected in the chart review but not included in the current analysis include: physical exam findings for xanthoma (tendinous, tuberous, arcus, eruptive, planar, xanthelasma); medications: steroids, beta-blockers, estrogens (synthetic or natural), progestins, aspirin, diuretics, thyroid hormone; alcohol consumption (separately for wine, beer, liquor); exercise level (none, minimal, regular/aerobic); diet (broad categories: ADA, low fat, vegetarian, fish oil supplemented); detailed atherosclerotic disease history (age at CABG, MI, angioplasty, angiogram, stroke, peripheral vascular disease, and number of events); history of high TC, high TG, CAD, pancreatitis, stroke or sudden death in first degree relatives, and free text of co-existing disease listed in "other" category. No data was available for reason for referral or familial relationships within the clinic.

TABLE 3 CRITERIA FOR LIPOPROTEIN PHENOTYPE CLASSIFICATION

Generic Designation	Elevated Species	Syn	LDL-Chol.	TG	Apo E	Chyl
Primary Hypercholesterolemia	LDL	IIa	>200	<175		
Combined Hyperlipidemia	LDL+VLDL	IIb	>170	>200		
Dysbetalipoproteinemia	beta - VLDL	III			2-2	
Endogenous Hyperlipemia	VLDL	IV	<140	>200		
Mixed Hyperlipemia	VLDL+chyl	V		>500		>tr
Hyper Lp(a) Lipoproteinemia	Lp(a)					

For the purpose of grouping, we used a classic dyslipidemic classification system.[28] Current nosology is changing in response to discovery of a multitude of monogenic and polygenic disorders[29]. The few patients with E 2-2, or a combination of disorders (not including hyper Lp(a) lipoproteinemia), were grouped together as "COMPLEX" and excluded from analyses when appropriate.

Patients with primary hypertriglyceridemia were selected based on TG >170 mg/dl. Familial dysbetalipoproteinemia was separated on the basis of apolipoprotein E phenotyping (E 2-2). All patients with E 2-2 have detectable beta-VLDL in plasma.

The VLDL-T:HDL-C exchange phenomenon was not considered in these analyses.[30] Also, the HDL measurement is known to be influenced by the Lp(a) fraction, at about a 1:1 effect, so that a trace presence of Lp(a) on electrophoresis might add 11 mg/dl to HDL.

The particle size of VLDL is measured indirectly. Apo B is insoluble in tetramethyl urea, allowing estimation of surface area, particle diameter and free cholesterol content of VLDL. The percentage apo B increased uniformly from 13.5% to 44% of total protein with decreasing particle size of VLDL (917 Å to 367 Å).[31]

METHODS

Patient Selection

The population served by UCSF Lipid Clinic is primarily from referrals by primary care physicians, both at UCSF and from a referral basin extending to the Oregon border, Reno, and Fresno (approximately 4 million people). There is also self-referral for a variety of factors. Reasons for referral include new or difficult to manage hyperlipidemia, premature atherosclerotic disease, strong family history of atherosclerotic disease, first degree relative of patient with known or suspected familial hypercholesterolemia (FH).

Data Collection

Data regarding demography, presence of CAD, habits, medications, co-existing disease and family history were collected by retrospective review of the medical record and first visit patient questionnaire (Appendix 1 and 2). Cardiovascular nurses were trained to review the clinical record for the specified parameters, ignoring information following the first visit. Interobserver and intraobserver reliability was examined using a blinded

technique, and qualitative comparisons were noted. When significant discrepancies occurred retraining and refinement of instruction was performed. For the purpose of this study the clinical CAD data was simplified to CAD present (angina or greater) or absent.

Laboratory Methods

Laboratory specimens for lipoproteins profile and gel electrophoresis were drawn at the time of the first visit . Because these tests were not performed for the purpose of this study some patients did not receive a full complement of tests. Apo B related measurements are the ones most frequently omitted.

Blood samples were drawn after a minimum fast of ten hours. The blood was allowed to clot at room temperature and the serum was separated by ultracentrifugation. Lipoprotein classes, VLDL, LDL, and HDL were separated sequential preparative ultracentrifugation ay 105,000 x g. Cholesterol and triglyceride contents of the fractions were measured by an autoanalyzer technique.[30] The apolipoprotein E phenotype was determined by isoelectric gel focusing of proteins from the $d < 1.006$ g/cm^3 fraction. [32] The lipoproteins of whole serum, and of both the infranatant and supernatant fractions at $d=1.006$ g/cm^3 analyzed by agarose gel electrophoresis. The content of apo B in the $d < 1.006$ g/cm^3 fraction was measured by nephelometry and the content of apo B among the apoproteins of VLDL was determined by its insolubility in 4.2M tetramethyl urea. [31]

The electrophoretic diagnosis of double pre-beta was established visually. The Apo E phenotype was both established visually and determined by gel scanning densitometry in a subset of patients.

Statistical Analysis

The frequency distribution of all variables was examined. The triglycerides (TG, LDL-T, VLDL-T) were transformed ($\log_{10}$) to reduce skewness. Exploratory data analysis using DataDesk Professional was used to search and describe outliers and to verify extreme values. The SuperANOVA software was used to generate means tables for various groups and to plot means against categorical variables. Scatterplots and histograms were created using Systat. The chi-square statistic was examined for cross-tabulations. P values are not reported because intercorrelation effects have not yet been examined.

RESULTS

General Demographics

The age distribution at first visit showed a small pediatric population, with about 90% of patients between 18-70. The median age for women was 52.7 years and for men 45.8 years.

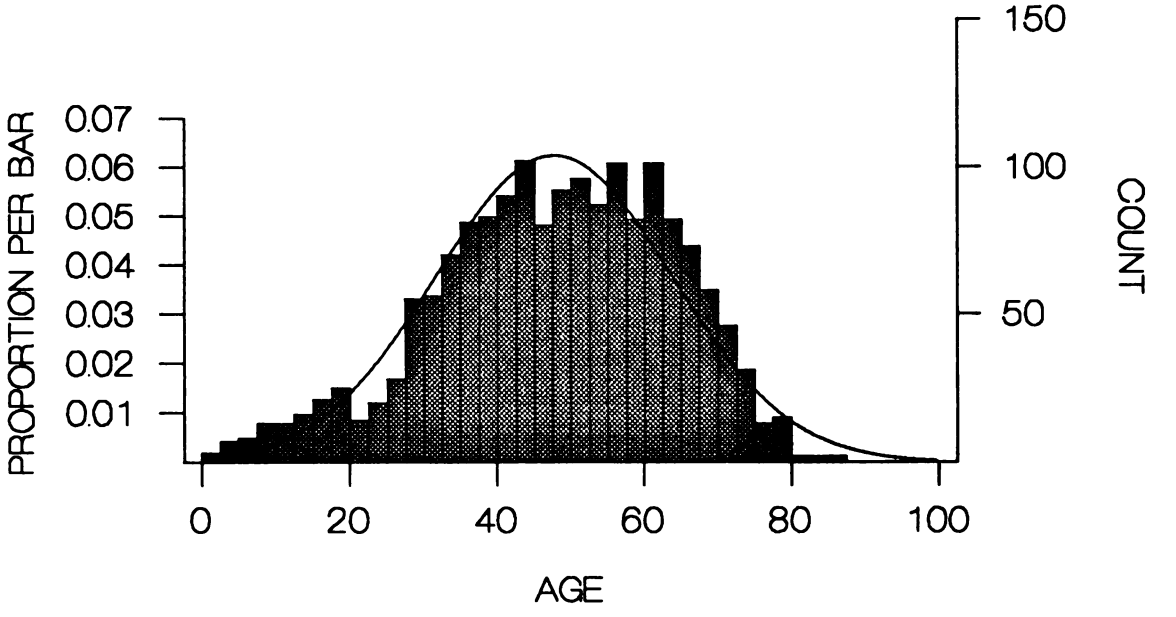


FIGURE 4 – HISTOGRAM OF AGE AT FIRST CLINIC VISIT

About 220 new patients per year were seen in the UCSF Lipid Clinic between 1984 and 1990. For clerical reasons data has not yet been entered for half of 1988, half of 1990 and all of 1991.

TABLE 4 – FREQUENCY DISTRIBUTION OF CATEGORICAL VARIABLES

	Count	Percent
Gender		
F	751	44.8
M	925	55.2
Total	1676	

Double Pre-beta		
Double	369	25.3
Single	1088	74.7
Total	1457	

Apo E		
22	27	1.9
32	149	10.7
33	795	57.3
42	33	2.3
43	317	22.9
44	66	4.7
31	2	0.14
53	2	0.14
Total	1391	

CAD		
NO	1243	74.1
YES	434	25.9
Total	1677	

Lipid Dx		
CMPLX	47	2.8
MIX LIP	70	4.1
LP(a)	89	5.3
END LIP	258	15.4
COMB	315	18.8
PRIM HC	360	21.5
NORM	538	32.1
Total	1677	

	Count	Percent
Hypertension		
NO	1223	72.9
YES	454	27.1
Total	1677	

Diabetes I		
NO	1655	98.7
YES	22	1.3
Total	1677	

Diabetes II		
NO	1581	94.3
YES	96	5.7
Total	1677	

Ethnicity		
Middle Eastern	32	2.4
South East Asian	48	3.6
Black	60	4.5
Hispanic	60	4.5
North Asian	118	9.0
White	990	75.7
Total	1308	

Smoking		
NO	1024	61.1
YES	653	38.9
Total	1677	

Renal Disease		
NO	1623	96.8
YES	54	3.2
Total	1677	

	Count	Percent
Estrogen Use		
NO	1564	93.3
YES	113	6.7
Total	1677	

Diuretic Use		
NO	1454	86.7
YES	223	13.3
Total	1677	

Beta Blocker Use		
NO	1454	86.7
YES	240	14.3
Total	1677	

Hypolipidemic Use		
NO	1465	85.7
YES	240	14.3
Total	1677	

Lp(a) qualitative		
trace	43	3.0
1+	85	5.9
2+	58	4.0
3+	12	0.8
4+	2	0.1
Total	1677	

Chlomicronemia		
NO	1376	91.7
trace	36	2.4
YES	88	5.8
Total	1677	

TABLE 5 – SUMMARY OF RELATIONSHIPS AMONG CATEGORICAL VARIABLES

	Gender	DPRE	Apo E	CAD	Lipid Dx
DPRE yes/no	F>M in double	•			
Apo E (6)	no effect	up in E 4-4 >> E 3-2 > E 4-3; less in E 3-3, E 4-2	•		
CAD yes/no	M>F	no effect	increased in E 2-2, E 4-2, down in E 4-4	•	
Lipid Dx (7)	M>F in lipemias	up in IIa and Lp(a); less in IIb and lipemias	E2 in lipemias E4 in IIa	III > IIb > IV > IIa > nl	•
HTN yes/no	no effect	no effect	no effect	two-fold increase	up in lipemias > III > IIb; less in IIa
Diabetes II yes/no	no effect	down in diabetes	? more in E 3-3 vs E 4-3	two-fold increase	up in V > IV; less in IIa
Ethnicity (6)	no effect	up in Blacks less in M. Eastern, N. Asian	no effect	no significant effect	no effect
Smoking yes/no	M>F	no effect	no effect	two-fold increase	up in lipemias, III; less in normals

Only associations with large chi-square statistics (corresponding to $p < .01$ or $p < .001$) are reported as effects.

The primary quantitative variables of interest, TC, logTG, LDL-C, HDL-C, VLDL-C, logVLDL-T, VLDL-C/VLDL-T, HDL-C/VLDL-C, HDL-C/(VLDL-C/VLDL-T), %apo B, Total apo B and Total apo B/LDL-C were split into MEN/WOMEN (Table 6), CAD absent/present (Table 7) and DPRE single double (Table 8).

TABLE 6 – LIPID PROFILE SUMMARY STATISTICS ± MF

Identities	N	Mean	Median	Std.Dev	5th%ile	95th%ile
MEN						
age	921	45.89	45.83	14.56	18.45	68.99
TC	925	298.22	278.00	119.35	180.30	485.00
logTT	925	2.28	2.23	0.38	1.79	3.02
LC	884	176.67	167.50	79.59	67.25	327.00
VC	887	59.99	31.00	104.62	7.00	213.00
VT	887	240.54	110.00	491.06	25.00	849.20
HC	882	45.32	44.00	14.80	26.00	70.00
vc/vt	887	0.30	0.27	0.14	0.16	0.53
HC/VC	882	2.49	1.37	3.71	0.15	8.37
hc/(vc/vt)	882	170.54	157.91	77.92	76.73	318.41
%ApoB	724	43.23	43.10	7.20	31.35	54.98
TApoB	541	1.17	1.11	0.42	0.61	1.96
WOMEN						
age	746	49.91	52.75	17.40	16.84	73.64
TC	751	316.61	305.00	106.12	185.20	472.40
logTT	750	2.22	2.17	0.38	1.69	2.96
LC	714	193.77	185.50	80.84	74.25	326.75
VC	717	48.25	24.00	87.56	4.00	165.50
VT	717	200.66	83.00	501.99	15.00	718.80
HC	715	55.74	54.00	18.43	29.00	90.00
vc/vt	717	0.31	0.28	0.15	0.16	0.55
HC/VC	714	4.73	2.26	8.95	0.20	15.66
hc/(vc/vt)	714	207.31	179.27	114.56	86.03	408.00
%ApoB	549	43.90	44.00	7.04	32.60	55.85
TApoB	436	1.30	1.27	0.47	0.59	2.05

TABLE 7 – LIPID PROFILE SUMMARY STATISTICS ± DPRE

Identities	N	Mean	Median	Std.Dev	5th%ile	95th%ile
Single Pre-beta						
age	1087	47.55	48.75	15.65	17.82	70.83
TC	1088	299.89	285.50	115.29	180.00	463.55
logTT	1087	2.30	2.26	0.39	1.77	3.04
LC	1069	174.73	170.00	72.75	59.50	301.50
VC	1075	60.55	30.00	108.13	6.00	226.00
VT	1075	267.55	119.00	570.84	22.00	902.00
HC	1069	47.55	45.00	16.76	25.00	76.00
vc/vt	1075	0.27	0.25	0.10	0.16	0.43
HC/VC	1068	3.15	1.43	6.30	0.14	11.60
hc/(vc/vt)	1068	194.90	173.50	99.61	86.59	371.04
%ApoB	930	42.81	43.00	6.92	31.56	54.05
TApoB	713	1.19	1.15	0.41	0.59	1.90
Double Pre-beta						
age	367	50.31	51.25	14.62	27.95	72.30
TC	369	329.34	313.00	108.14	202.00	505.50
logTT	369	2.15	2.10	0.32	1.73	2.78
LC	369	208.44	195.00	89.25	86.00	362.00
VC	369	44.35	23.00	77.37	6.00	158.50
VT	369	138.97	65.00	304.31	18.50	505.00
HC	369	55.62	53.00	17.30	33.00	88.00
vc/vt	369	0.37	0.34	0.18	0.20	0.61
HC/VC	369	3.58	2.33	3.87	0.21	11.13
hc/(vc/vt)	369	167.98	149.63	79.69	73.79	336.15
%ApoB	314	45.28	45.20	7.21	33.45	56.75
TApoB	245	1.36	1.33	0.51	0.73	2.16

TABLE 8 – LIPID PROFILE SUMMARY STATISTICS ± CAD

Identities	N	Mean	Median	Std.Dev	5th%ile	95th%ile
CAD Absent						
age	1235	45.11	44.92	16.64	15.40	70.77
TC	1243	303.39	287.00	117.97	176.20	471.80
logTT	1242	2.23	2.17	0.39	1.72	2.99
LC	1183	182.22	175.00	79.42	68.20	322.40
VC	1185	53.45	25.00	103.33	5.00	188.50
VT	1185	218.35	88.00	513.80	17.00	786.40
HC	1182	51.03	49.00	18.02	26.00	83.00
vc/vt	1185	0.30	0.27	0.15	0.17	0.53
HC/VC	1181	3.93	1.93	7.47	0.17	13.16
hc/(vc/vt)	1181	191.22	170.46	101.05	81.04	373.86
%ApoB	925	43.50	43.50	7.09	31.83	54.97
TApoB	726	1.21	1.18	0.44	0.58	1.97
CAD Present						
age	433	55.13	55.58	11.17	36.03	72.58
TC	434	315.07	295.00	101.12	192.75	496.75
logTT	434	2.33	2.29	0.35	1.84	3.01
LC	416	190.15	177.50	83.54	75.85	352.05
VC	420	58.31	35.00	78.69	8.00	199.90
VT	420	234.66	120.00	442.61	26.00	757.35
HC	416	47.06	45.00	14.74	27.00	75.15
vc/vt	420	0.30	0.28	0.14	0.15	0.60
HC/VC	416	2.27	1.29	3.27	0.16	7.69
hc/(vc/vt)	416	175.28	158.96	86.80	78.46	319.93
%ApoB	348	43.58	43.25	7.26	32.04	55.36
TApoB	251	1.27	1.14	0.47	0.73	2.15

Histograms with superimposed normal curves (based on sample mean and standard deviation) were created for each measured quantitative variable in the lipid profile (Figures 5A, 5B, 5C, 6A, 6B, 6C, 7A and 7B).

Mean values (± SEM) of TC, logTG, LDL-C, HDL-C, VLDL-C, logVLDL-T, VLDL-C/VLDL-T, HDL-C/VLDL-C, HDL-C/(VLDL-C/VLDL-T), %apo B, Total apo B and Total apo B/LDL-C were plotted against apo E phenotype (excluding E 4-2) for men and women separately (Figures 8 A-L). This analysis was repeated using only patients with TG>170 (Figure 9 A-L).

Mean values (± SEM) of TC, logTG, LDL-C, HDL-C, VLDL-C, logVLDL-T, VLDL-C/VLDL-T, HDL-C/VLDL-C and HDL-C/(VLDL-C/VLDL-T) were plotted against apo E henotype (excluding E 2-2 and E 4-2) for patients with single and double pre-beta separately (Figure 10 A-I). Apo E 4-2 was excluded from the analysis because of small sample size. Apo E 2-2 was excluded from analysis regarding DPRE, because broad beta and DPBL are mutually exclusive.[33]

TC was highly elevated in E 2-2, since these are mostly type III hyperlipidemic patients. LogTG was also highest in E 2-2, with E 2-2>E 3-2>E 3-3=E 4-3>E 4-4. LDL-C was highest in E 4-4, with E 4-4=E 4-3=E 3-3>E 3-2>E 2-2. HDL-C was higher in women than men across all phenotypes. VLDL-C was sharply elevated in E 2-2, with E 2-2>>E 3-2>E 3-3=E 4-3=E 4-4. LogVLDL-T was elevated in E 2-2, with E 2-2>E 3-2>E 3-3=E 4-3>E 4-4.

The ratio VLDL-C/VLDL-T was dramatically elevated in E 2-2, with other isoforms equal. HDL-C/VLDL-C was generally higher in women, with E 2-2<E 3-2=E 3-3=E 4-3<E 4-4. The ratio HDL-C/(VLDL-C/VLDL-T) was highest in E 2-2, with E 2-2>E 3-2>E 3-3=E 4-3=E 4-4. Percent apo B was elevated in E 2-2, but otherwise constant across apo E isoform and gender. Total apo B was highest in E 4-4, with E 4-4=E 4-3=E 3-3>E 3-2>E 2-2.

In the subgroup of patients with TG>170 mg/dl the same effects were generally observed. HDL-C/VLDL-C was very low, regardless of apo E isoform. Of note is that among hypertriglyceridemic E 4-4, no increase in HDL-C was seen in women.

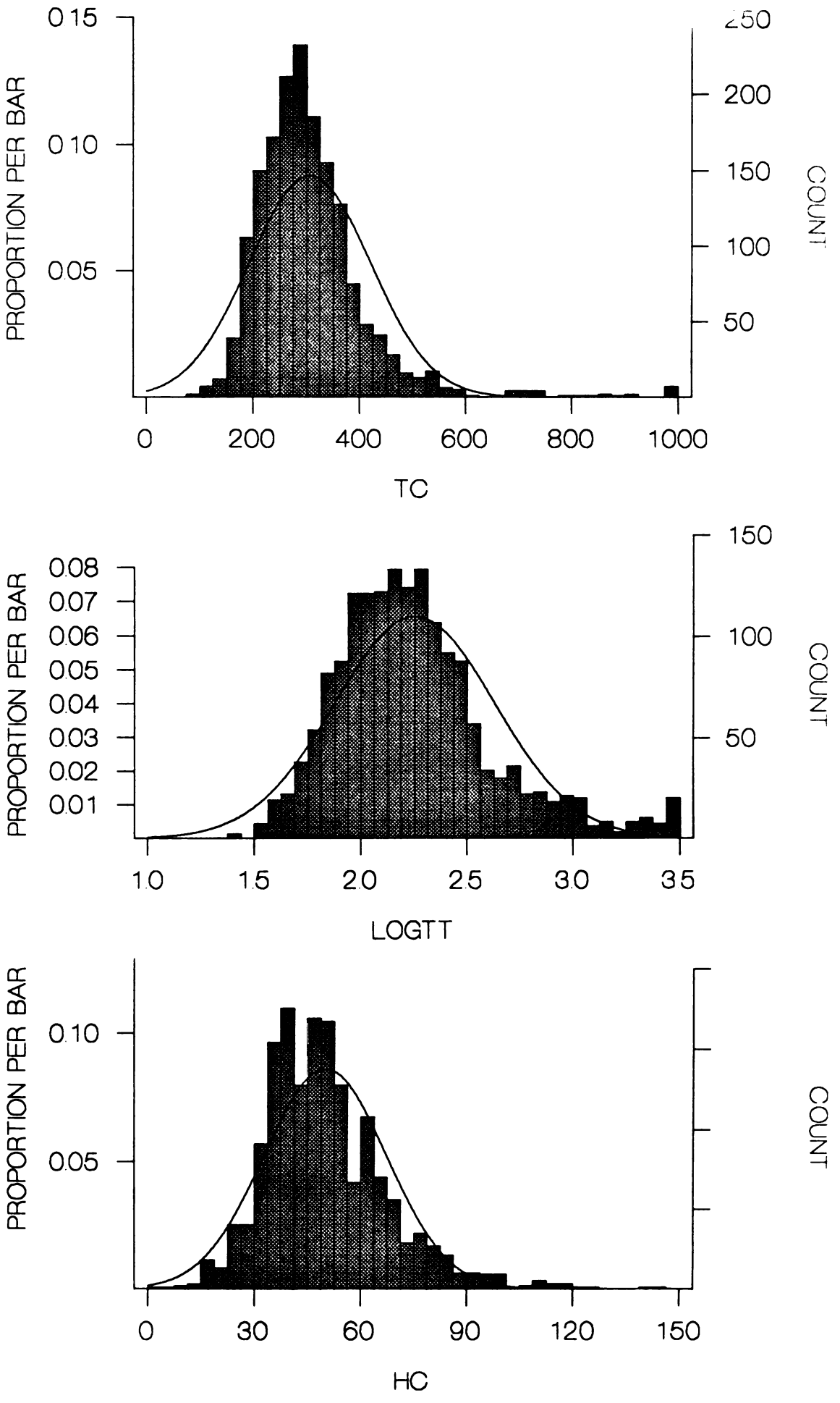
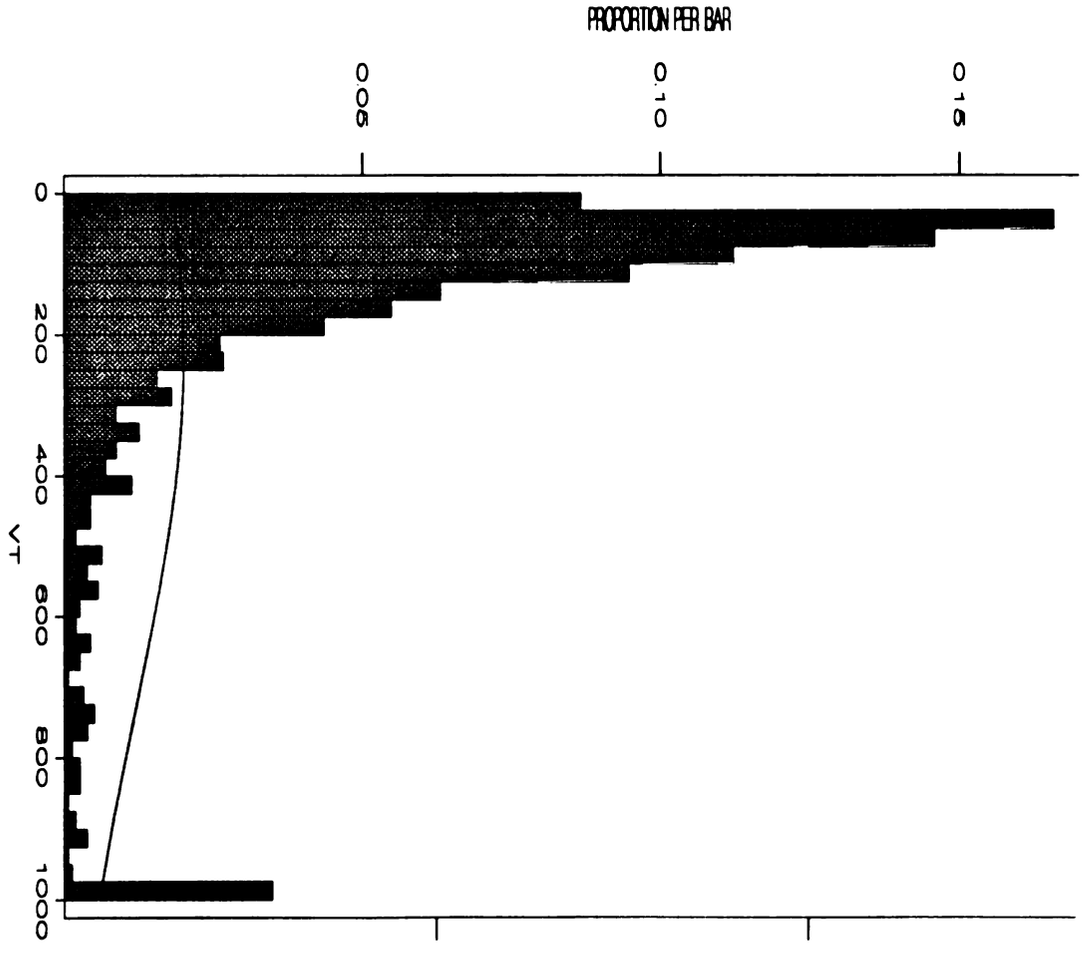
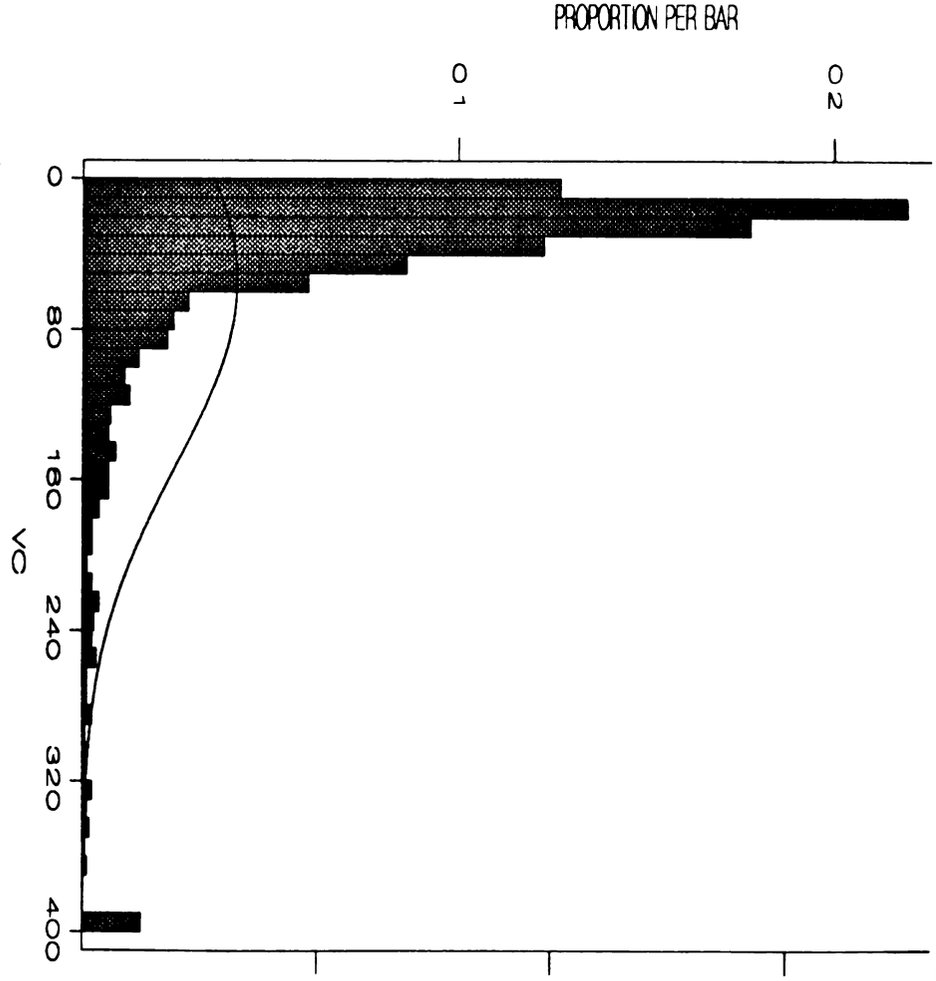


Figure 5 A, B, C – Histograms of TC, logTG, HDL-C

Figure 6 A, B, C – Histograms of VLDL-C, VLDL-T, VLDL-C/VLDL-T





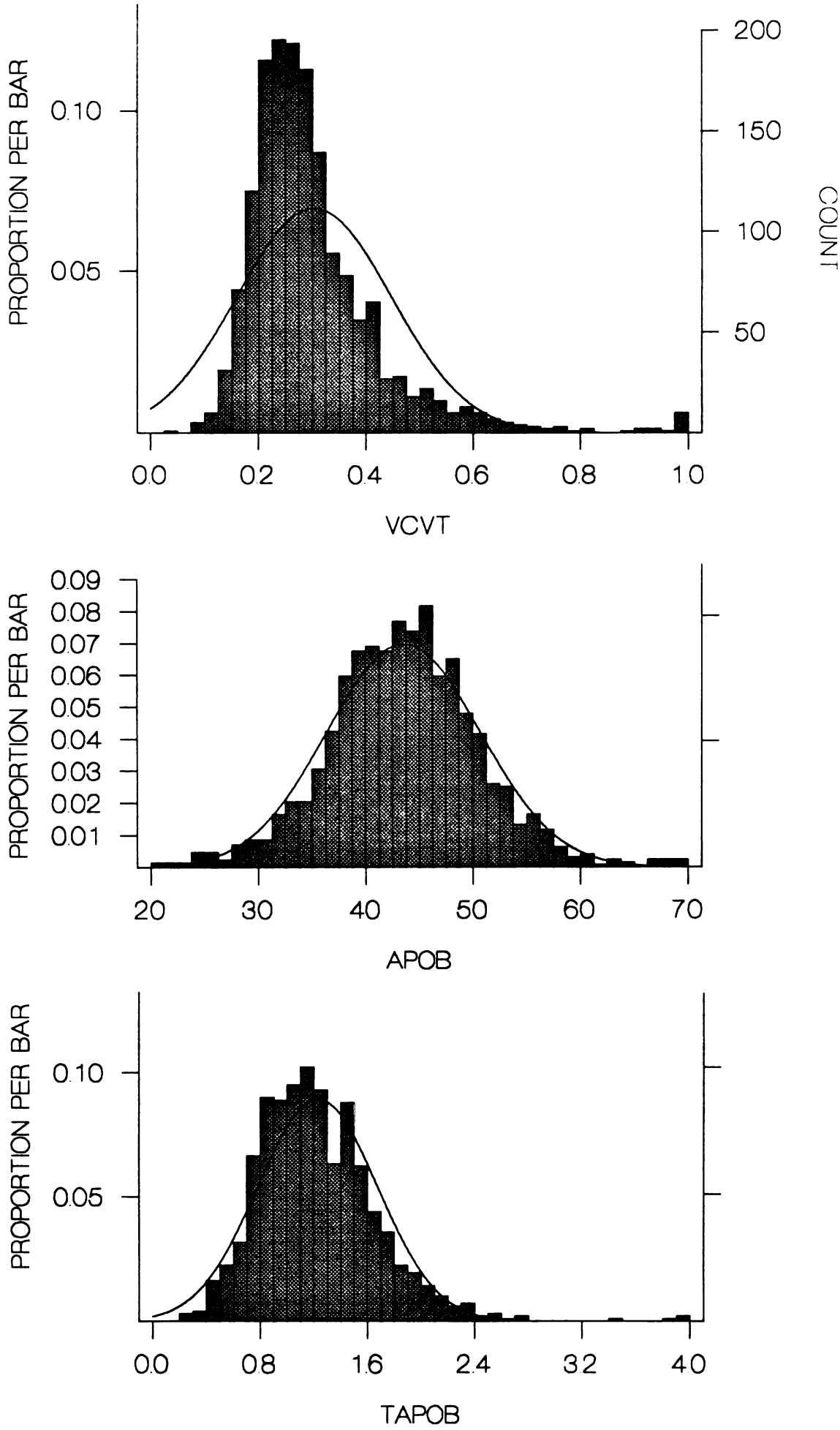


Figure 7 A, B – Histogram of Percent apo B, Total apo B

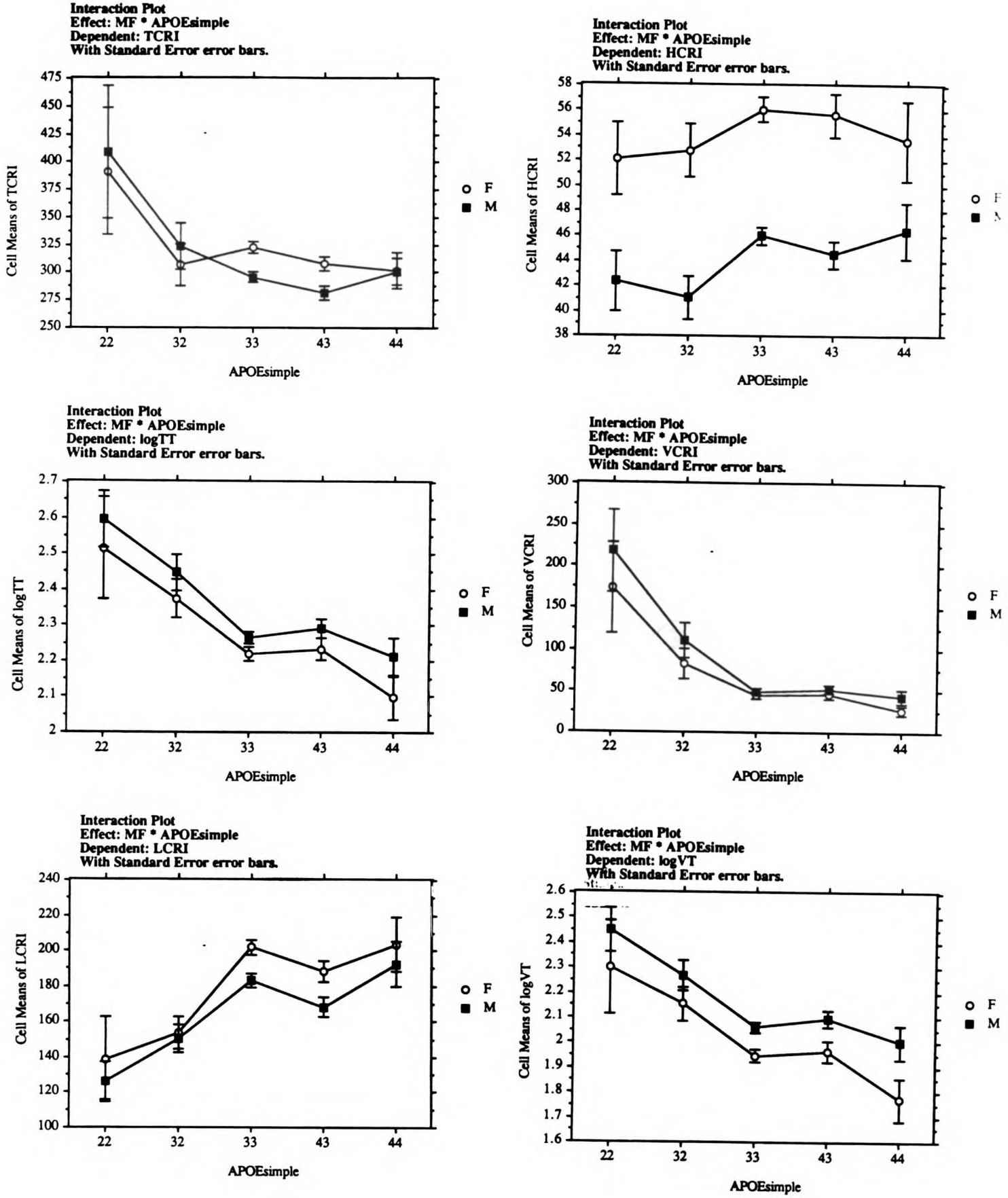


Figure 8 A-F – Line Graphs of Lipid Profile vs Apo E ±M/F, all TG

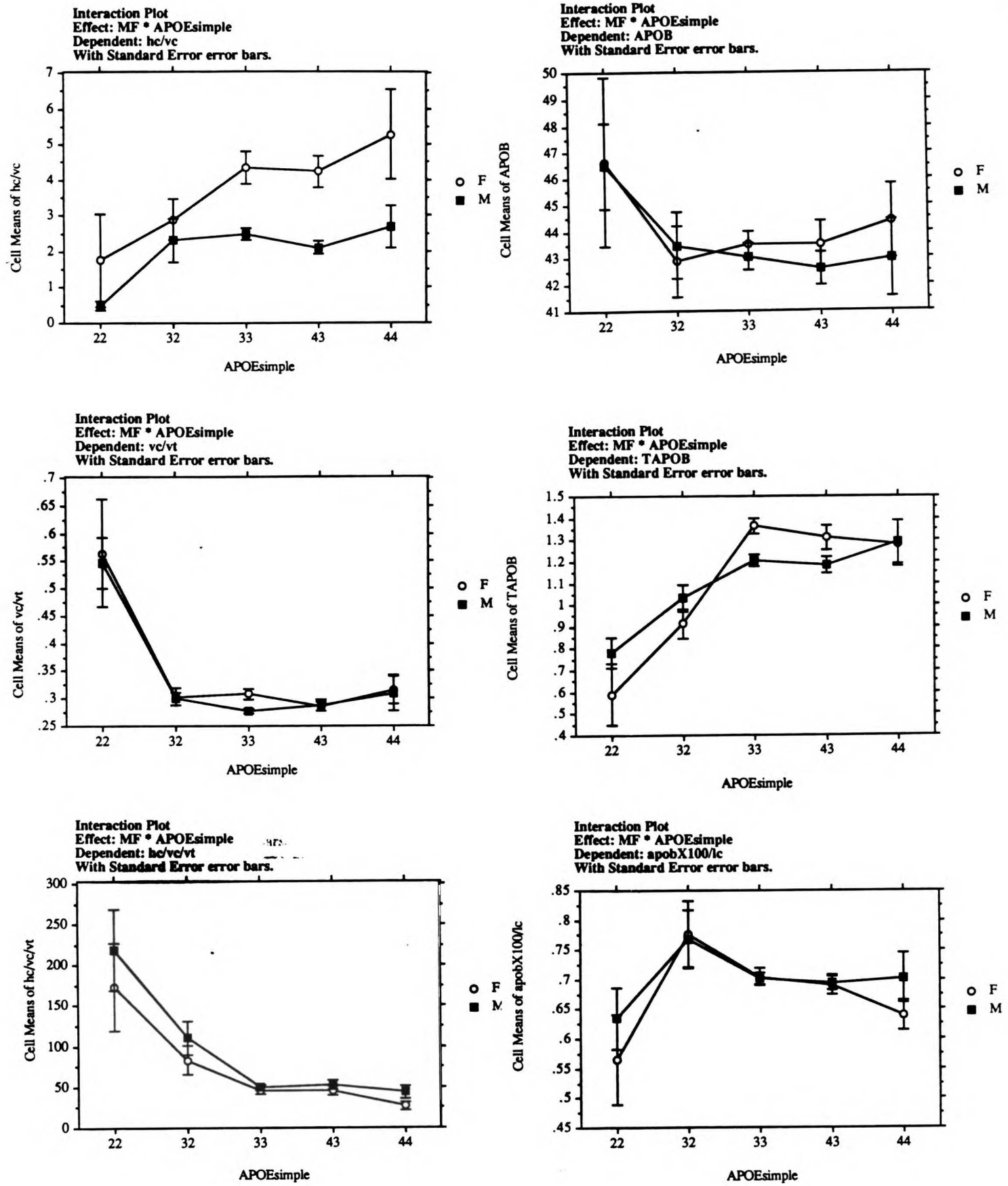


Figure 8 G-L – Line Graphs of Lipid Profile vs Apo E ±M/F, all TG

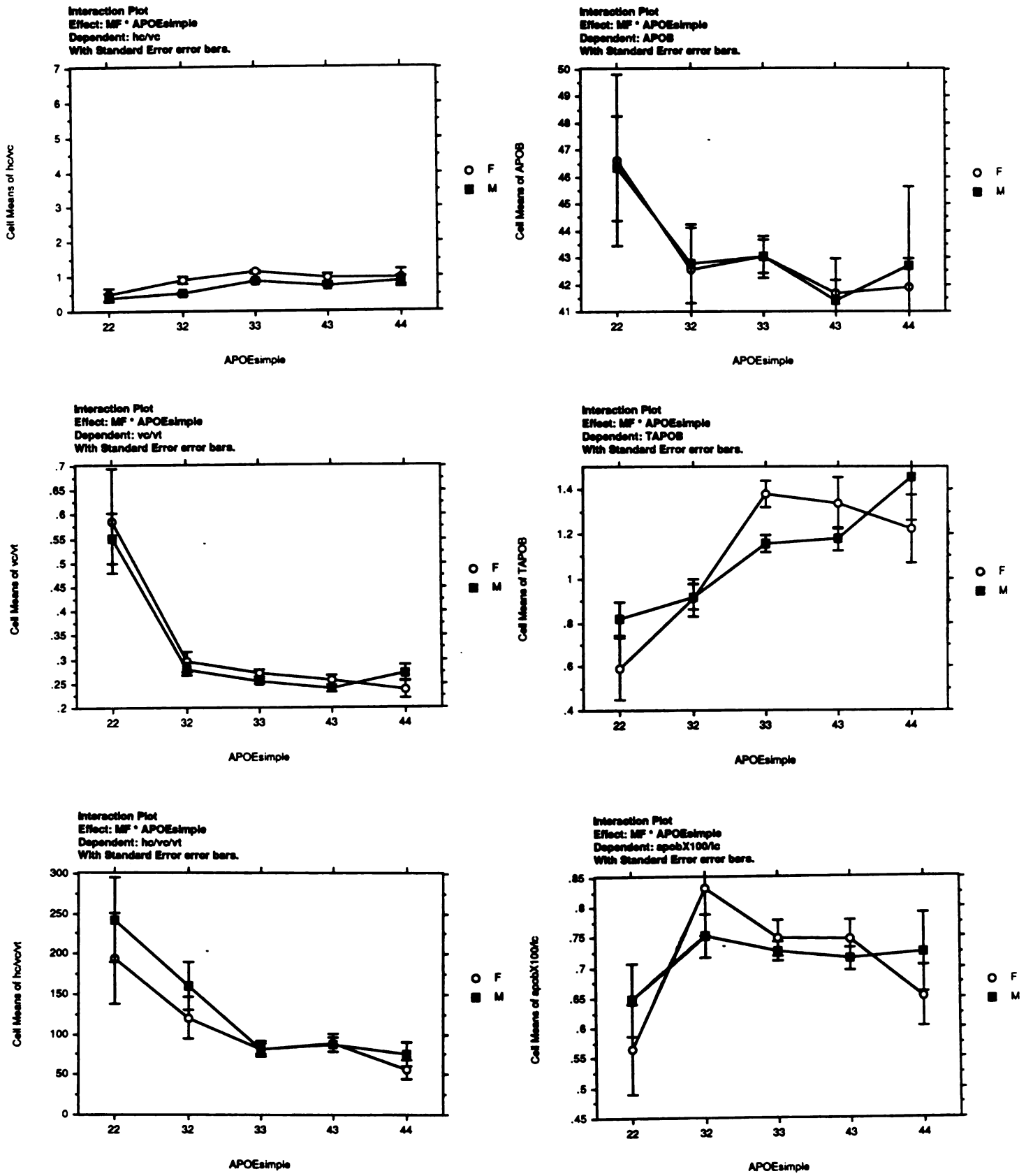


Figure 9 G-L – Line Graphs of Lipid Profile vs Apo E ±M/F, TG>170

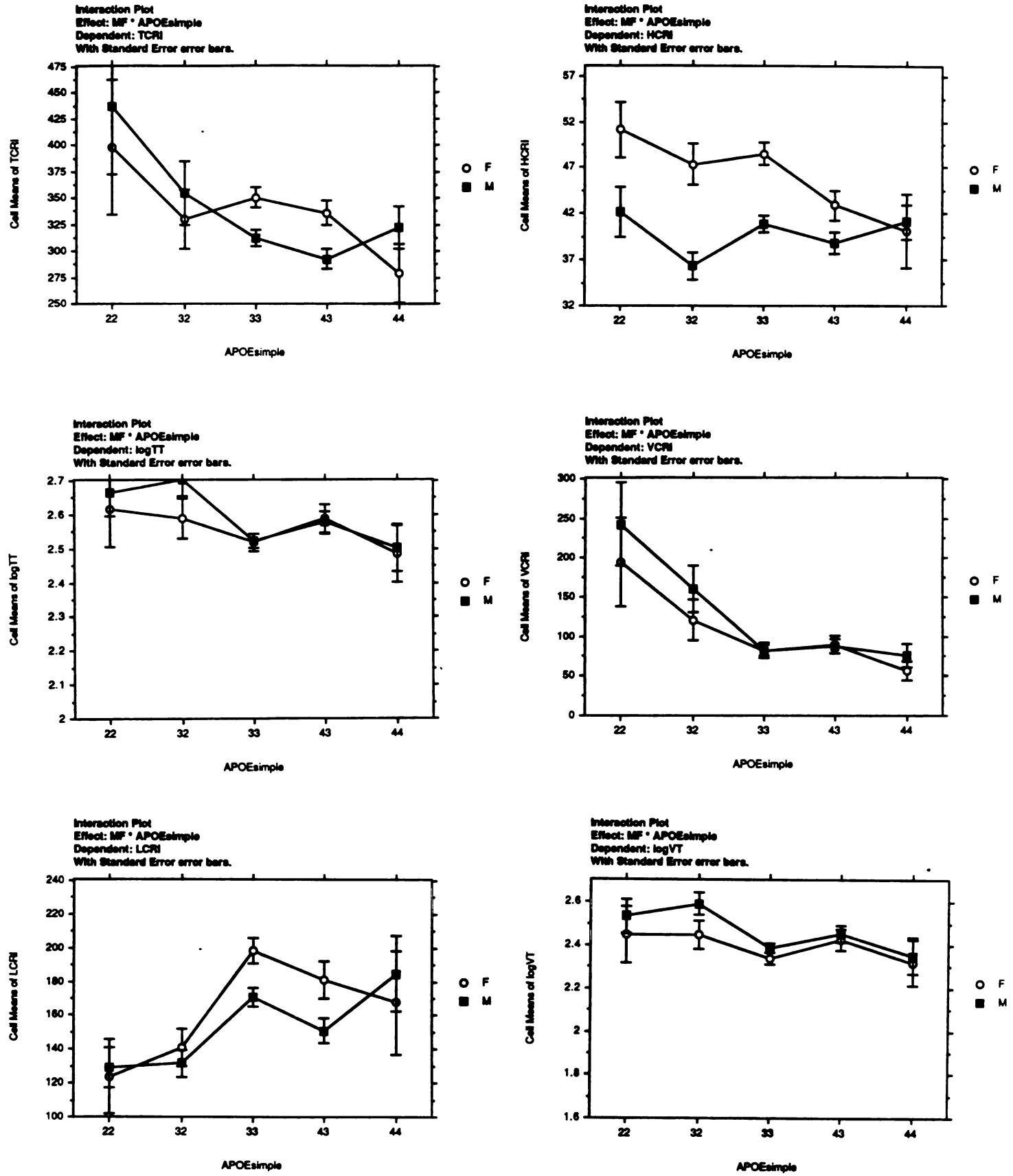


Figure 9 A-F – Line Graphs of Lipid Profile vs Apo E ±M/F, TG>170

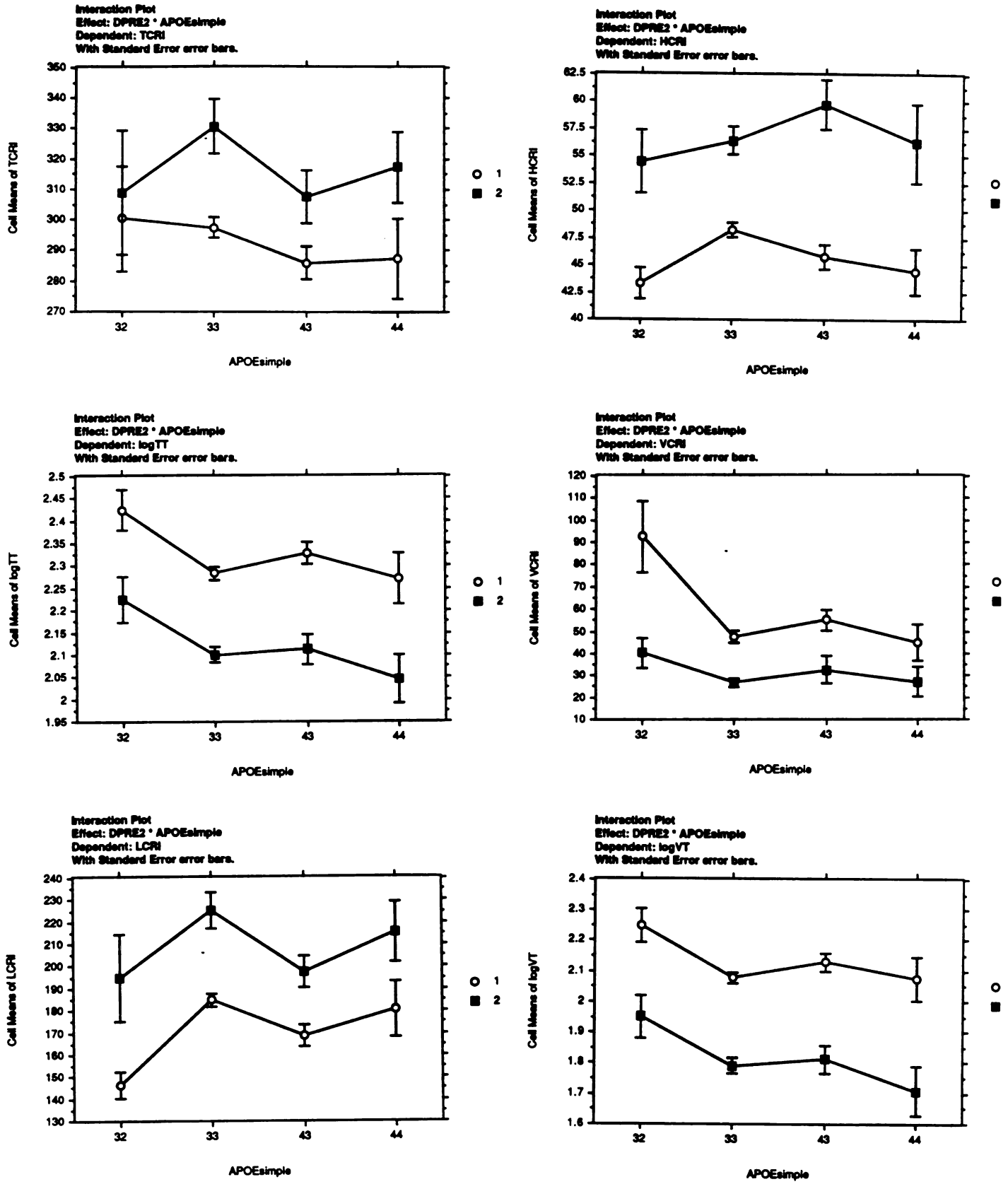


Figure 10 A-F – Line Graphs of Lipid Profile vs Apo E ± DPRE, all TG

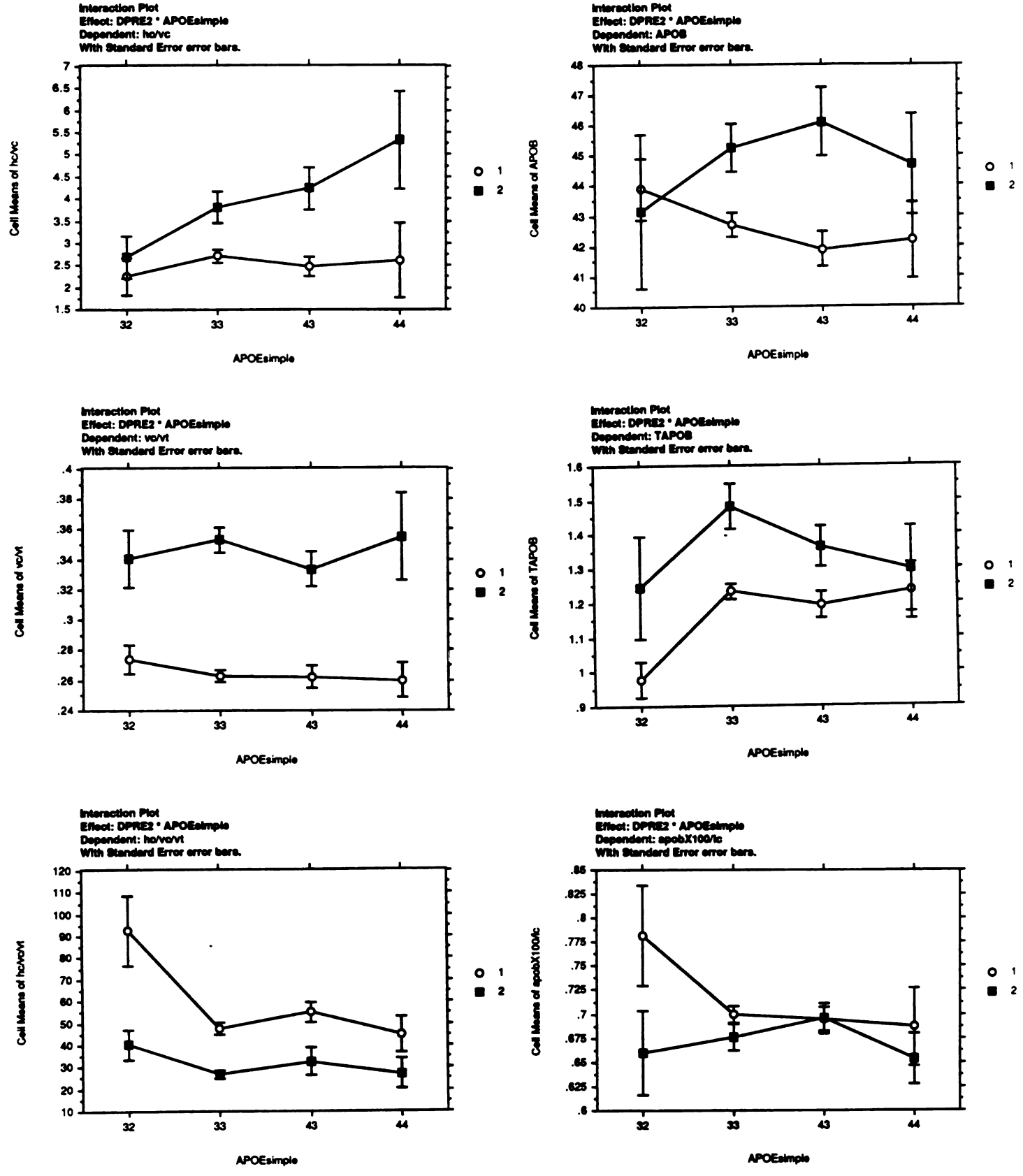


Figure 10 G-L – Line Graphs of Lipid Profile vs Apo E ± DPRE, all TG

DISCUSSION

Associations with DPRE

DPRE has never been studied in a large hyperlipidemic population. The prevalence of DPRE was dramatically increased in primary hypercholesterolemia and decreased in the lipemias (40.6% vs 11.8% in endogenous lipemia and 21% in mixed lipemia). From kinetic studies it is known that VLDL are converted to remnant particles by lipoprotein lipase. Small diameter VLDL remnants are probably converted to LDL by the effects of hepatic lipase, and others are abstracted into liver by the LDL receptor (in FH or hypothyroidism). The DPRE phenomenon describes the coexistence of a VLDL population of normal pre-beta mobility with one of decreased electrophoretic mobility. Studies by Pagnan suggest that these particles are probably the cholesteryl ester rich .[9]

DPRE was also associated with female gender, apo E 4-4 isoform, Lp(a) and Black race. DPRE prevalence was low in type II diabetics and North Asian and Middle Eastern patients. It is striking that although HDL-C is higher in women than in men, HDL-C is also elevated in the presence of DPRE in both sexes. The HDL association persists within the primary hypercholesterolemia phenotype.

DPRE was associated with significantly increased TC, LDL-C, HDL-C, VLDL-C/VLDL-T and HDL-C/VLDL-C (dramatic in E 4-4 and E 4-3). These observations and the higher %apo B (in VLDL) in DPRE is consistent with the second band in DPRE being a small remnant particle. Thus, with a long residence time apo B remains present even when C proteins and perhaps apo E have left the particle. DPRE was associated with significantly decreased TG, VLDL-C (dramatic in E 3-2), VLDL-T and HDL-C/(VLDL-C/VLDL-T) (dramatic in E 3-2). All effects were seen independent of gender and apo E isoform unless otherwise noted.

The association of DPRE with primary hypercholesterolemia may be explained by patients with LDL receptor defects, because the LDL receptor also plays a role in VLDL remnant endocytosis. Conversely, defects in intravascular lipolysis might negatively associate with DPRE if degradation of VLDL cannot proceed to the remnant step. The association between Lp(a) and DPRE may reflect the dependence of Lp(a) removal on LDL receptor activity. The metabolic basis for the association of DPRE with increased female gender and HDL-C is less clear. This may reflect subtle effects of HDL speciation on the lipolytic cascade.

In both Italian and Finnish lipid clinic subjects, apo E4 was associated with increased prevalence of double pre-beta.[34] This effect was confirmed in our large population, but the percentage of DPRE in our E4 subjects was still only 37.9% (n=66). Pagnan and colleagues did not report the gender related effect on the prevalence of DPRE, or lipoprotein in correlates.

Apo E

The apo E phenotype was found to vary significantly across lipoprotein phenotype. The epsilon 2 allele was associated with lipemias and the epsilon 4 allele was associated with primary hypercholesterolemia. Association of the apo E phenotype with CAD and type II diabetes was not statistically significant.

The association of epsilon 2 with hyperlipemia is interesting because apo E2 is not a functional ligand for the LDL receptor and accumulation of remnant particles might be expected. The absence of epsilon 2 association with DPRE, however, suggests that the lipemia association may involve processes preceding the formation of electrophoretically slow VLDL. The association of epsilon 4 with hypercholesterolemia has been observed previously.

Hypothetical mechanisms for apo E2 related hypertriglyceridemia and apo E4 related hypercholesterolemia have been proposed.[35] In 473 healthy women, E3-2 --> E3-3 --> E4-3 progression was seen for TC, LDL-C, and apo B with no change in trends after menopause.[33] Apo E polymorphism has been shown to be independent of apo B levels.[36] In one Japanese study, frequency of E phenotype was identical in FH (92), non-FH hyperlipidemia (426) and normals. VLDL-T and VLDL-C was higher in E3-2 than in E3-3. The E3-2 incidence was higher in type III and V phenotypes.[37]

In another Japanese study, apo E phenotype and lipoprotein phenotype were found to be related. The prevalence of E4 was increased in Type IIa and IIb, V and FH; whereas the prevalence of E2 was increased with Type III and IV.[38] More apo E4 was found in FH patients (30.0%) than in normolipidemic subjects (15.5%). TC, TG and LDL-C were higher, in FH patients with apo E4. The prevalence of ischemic heart disease (IHD) was significantly higher in FH patients with apo E4 (73.3%) than in those without apo E4 (31.4%). No significant difference was noted in age and in the prevalence of obesity, diabetes, hypertension and smoking between the FH groups with and without apo E4.[39] Nigerian and American Blacks have a higher frequency of E4 (.31 and .26) compared with Caucasians.[40]

Our population included two patients with E 3-1 and two with apo E 5-3 phenotypes. The two rare phenotypes of apo E, E1 and E5, have recently been described. A family with 3 heterozygotes and two homozygotes for E1 is described.[41] A single hyperlipoproteinemic individual with E5/3 is described.[42] The reported incidence of E5 and E1, based on 1209 subjects was .002 and .001, and effect of E5 was like E4, and E1 was like E2.[43]

Prevalence of CAD vs Lipoprotein Profile and Phenotype

CAD was prevalent in combined hyperlipidemia (34.6%), "complex" patterns (42.6%), and endogenous lipemia (31.8%) compared with primary hypercholesterolemia (22.8%) and "normolipidemics" (20%). The "normolipidemics" with CAD (n=106) may be an interesting subgroup to analyze. It is of note that primary hypercholesterolemia patients had an average age of 44.8 yrs, younger than that of combined hyperlipidemia (IIb) patients (53.0 yrs). Although affected by referral bias, the high prevalence of CAD among endogenous lipemia patients in this population is striking, and suggests the need to better characterize hypertriglyceridemic subgroups.

Classical risk factors such as age, male gender, hypertension and smoking were associated with increased prevalence of CAD in this population. The association of CAD with elevated LDL-C and decreased HDL-C is consistent with numerous population based studies. The association of CAD with elevated VLDL-T will be interesting to examine in a multivariate model. Similarly, it will be interesting to note the independent utility of the HDL-C/VLDL-C ratio in predicting presence of CAD (mean of 2.27 in CAD present vs 3.93 in CAD absent). The current results provide preliminary evidence that efficiency of cholesteryl ester transfer may be associated with CAD.

No difference in percent apo B or apo B/LDL-C was noted between CAD patients and non CAD patients. Total apo B, however was slightly elevated in CAD patients (1.27 vs 1.21), in both men and women. Among the subgroup with TG>170 mg/dl a similar result was found.

GENERAL LIMITATIONS

Bias

Although it is a consecutive case series, our study was similar to a cross-sectional design in that we have not examined any follow-up data. In case series there is always difficulty in

choosing an appropriate control group. We have limited our observations to comparisons within hyperlipidemic and normolipidemic patients in our clinic and have not attempted to construct a matched control group from outside the clinic. Clearly, we have no ability to demonstrate the order of causality of observed associations. Also we expect a "survivor" bias, in that patients who are still alive after CAD events are enriched, compared with patients who suffered fatal coronary events.

The format of our patient questionnaire and chart review form led to some difficulty in distinguishing missing and true negative data. This would dilute discriminatory ability between groups. Interrater reliability during chart review was good, but some disagreement remained in distinguishing mild cases of CAD, hypertension and diabetes.

The largest source of bias and variability in our sample is probably the reason for referral. Referral to the clinic is primarily for work up and management of hyperlipidemia, usually diagnosed by the primary care physician through TC, TG and sometimes HDL-C and LDL-C measurement. It is important to note that patients are not referred to the clinic on the basis of apo E phenotype, apo B measurement, or the presence of DPRE as these laboratory tests are not available outside specialized medical centers. There are also patients with "normal" lipids referred for work up of premature or familial atherosclerotic disease. The clinic also recruits first degree relatives of patients with suspected FH. The second largest potential confounder is age. In this analysis no formal attempt to control for age was made, and this will clearly be necessary in future analyses.

Laboratory Error

Optimal use of any laboratory test depends upon on awareness of analytical variance, intraindividual variance, and interindividual variance. Biological interindividual variation in TC, HDL-C and total apo B is significant. These parameters were recently examined in a study of 10 samples each of 12 patients, with an interindividual variance of 17% for TC and 25% for total apo B.[44] In the CVRI laboratory, during the period of this study, analytical variance was only 2.5 mg/dl for TC and 4.5 mg/dl for TG. These factors may have greater implications for lipid screening in low prior probability of disease individuals than for the evaluation of known dyslipidemics or patients with CAD.

Most comparative studies have used the Friedwald formula for the calculation of LDL-C. We measured each lipoprotein fraction directly using quantitative ultracentrifugation. The Friedwald formula can be used confidently when TG > 2 g/l[45] but fails when TG 2-4 g/l (72% accurate) and 4-6 g/l (39% accurate).[46] Most studies cannot directly identify type III

patients and post-prandial chylomicronemia. We have not examined the reproducibility of the double pre-beta phenomenon across time; its presence could conceivably vary depending upon the metabolic state of the individual.

Effects of Medications and Habits

Diuretics are known to induce small increases in TG and even smaller increases in TC. Beta-blockers may reduce HDL-C. High alcohol consumption and cigarette smoking may elevate TG levels.[47]

Statistical Methods

We have employed very simple descriptive statistics to characterize this complex data set. The chi-square statistic was examined for each contingency table. It was recognized that with the multiple comparisons examined, reasonable threshold for statistical significance would be at least in the $p < .01$ -.001 range (using a simple Bonferroni type adjustment). Formal procedures for multiple group comparison were not applied, given the exploratory nature of the study. The effects of apo E phenotype and gender, and apo E phenotype and DPRE were grossly examined with plotted means ± SEM bars, and some univariate ANOVA (without post-hoc tests for individual pairs).

Several statistical methods have been applied to complex multivariate lipoprotein data including: logistic discriminant analysis (CABG vs control, showed apo E improved classification),[48] factor analysis (CAD case-control) [49] and discriminant function analysis.[50] Multivariate analysis of correlated risk factors was used to separate genetic and environmental influences on HDL-VLDL, VLDL-LDL and HDL-LDL correlations in groups of related individuals.[51]

FUTURE DIRECTIONS / TESTABLE HYPOTHESIS

Age Effects

Studying the age dependence of apo E phenotype effects can clarify how metabolic regulation is affected by aging. The effect of apo E phenotype on lipid and apo B levels has even been studied in neonates.[52]

Gender and Menopause

We have collected but not analyzed post-menopausal estrogen usage data for female patients. It has been shown in 435 healthy white women, after controlling for the effects of confounding variables (age, body mass index and smoking status), that TC, LDL-C, TG and apo B remained significantly increased in postmenopausal women as compared with premenopausal women.[53] Estrogens have been reported to not mask the allelic effects of the apo E locus.[33]

Obesity

We have collected but not analyzed height and weight measurements. We intend to study reported relationships between apo E, obesity and hypertriglyceridemia. Hypertriglyceridemia is the most frequent lipid abnormality associated with obesity, and this association may be modulated by apo E. The prevalence of E 4-3 (29.7%), E 4-4 (8%), and E 4-2 (2.1%) in subjects with TG>200 and 120% ideal body weight was significantly higher than in controls E 4-3 (14%), E 4-4 (2.7%), and E 4-2 (0.9%). The E4 allele has been reported to increase the risk of hypertriglyceridemia among obese individuals.[54 55] Obesity (with a mean of 131% ideal body weight) and non-obese controls were examined for apo E phenotype and hyperlipoproteinemia. E2 and/or E4 individuals were more susceptible to hyperlipoproteinemia (100% vs 47%).[56]

Diabetics

Our population has nearly one-hundred type II diabetics that we will examine as a subgroup. Several small studies report interrelationships between lipids in diabetic subjects. Diabetic VLDL contains more apo E than hyperlipidemics or normals (17.3%, 13.4%, 12.4% respectively).[57] NIDDM may predispose E2 carrying subjects to hyperlipoproteinemia .[58] NIDDM subjects with apo E4 alleles tend to be hypercholesterolemic: IIa (51%), IIb (38%), normals (16%).[59]

Stroke

We have collected but not analyzed data on a subgroup of stroke patients. A case control study suggested that VLDL-T, VLDL-C (especially its cholesteryl ester level), TG and apo B are elevated in stroke survivors, and HDL-C is down. The hypothesis was forwarded that cholesteryl ester is excessively transferred from HDL to VLDL in cerebral infarction

patients (n=37 males).[60] Rossner reported that VLDL-C/VLDL-T was higher in cerebral infarction vs controls.[61]

Outlier Analysis

We intend on examining all patients approximately ± 4 SD from the mean on any of the continuous variables or ratios. This would be the opposite approach from data "trimming" of continuous distributions before computing correlation coefficients. Another approach would be to examine the extreme 0.5% of cases.[62]

If no obvious explanation is found for the extreme case, we will pull the clinical records and study individual patients for the presence of uncharacterized genetic defects or unusual lipoprotein effects of coexisting diseases (liver transplant, etc.). The relational database makes it easy to search for multiple combinations of genetic variables, such as non apo E 2-2 dysbetalipoproteinemia.

Multivariate Analysis

We intend to apply methods of discriminant function methods to answer several interesting questions raised by this analysis. For example, which set of variables best predicts DPRE or apo E phenotype, and how accurate are these predictions. Then we would attempt to examine predictors of CAD in this population after adjusting for known effects. This will be challenging given the cross sectional nature of the data, and we will interpret such results cautiously. However, to our knowledge, the opportunity to examine this wide range of quantitative and qualitative variables among a large hyperlipidemic population is unprecedented.

We intend on using multivariate methods to explore correlates of VLDL particle size. In a case-control study of non-fatal MI, using multivariate logistic regression analysis, HDL-C and TG was found to associate with small dense LDL subclass pattern.[16] Some studies have compared components of small VLDL in hypertriglyceridemic post-infarction patients vs controls, leading to the conclusion that enrichment with cholesteryl esters of small VLDL from type IV hypertriglyceridemic patients is caused by lipid transfer from LDL and high density lipoprotein (HDL).[11]

Lipid Intercorrelations

We have not systematically examined the multiple linear and non-linear correlations between our major lipid variables: TC, logTG, LDL-C, HDL-C, VLDL-C, logVLDL-T,

VLDL-C/VLDL-T, HDL-C/VLDL-C, HDL-C/(VLDL-C/VLDL-T), %apo B, Total apo B and Total apo B/LDL-C. An example of such a correlation is presented in Figure 12. We intend to compare these relationships with those published in large normal and CAD studies, and to test for subgroups that do not obey the same correlative relationships.

For example, it has been noted that as TG are increased, VLDL and VLDL-T/VLDL-C rise, and LDL and HDL decrease.[63] In the Milwaukee Cardiovascular Data Registry of 5216 white adults presenting for angiograms, the HDL-C:TG correlation coefficient was=-0.39 to -0.51 .[64] Correlations may be due to physical exchange phenomenon and complex metabolic derangements. The known inverse relationship between HDL-C and TG has been characterized in normals, but has not been examined as a function of phenotype, gender or other demographic variables.

Several authors have used lipoprotein ration in an attempt to better predict outcome variables. In a study of angiographically proven CAD in Blacks, no variables predicted CAD in men, but apo AI/B and TC/HDL-C (in upper 50%, TC>208) did in women.[65] In another study of male survivors of MI vs controls (n=64 and 60), looking at the subpopulation with normal TC and TG, TC/apo B, HDL-C/apo B and apo B were reported as useful in classifying the two groups.[66]

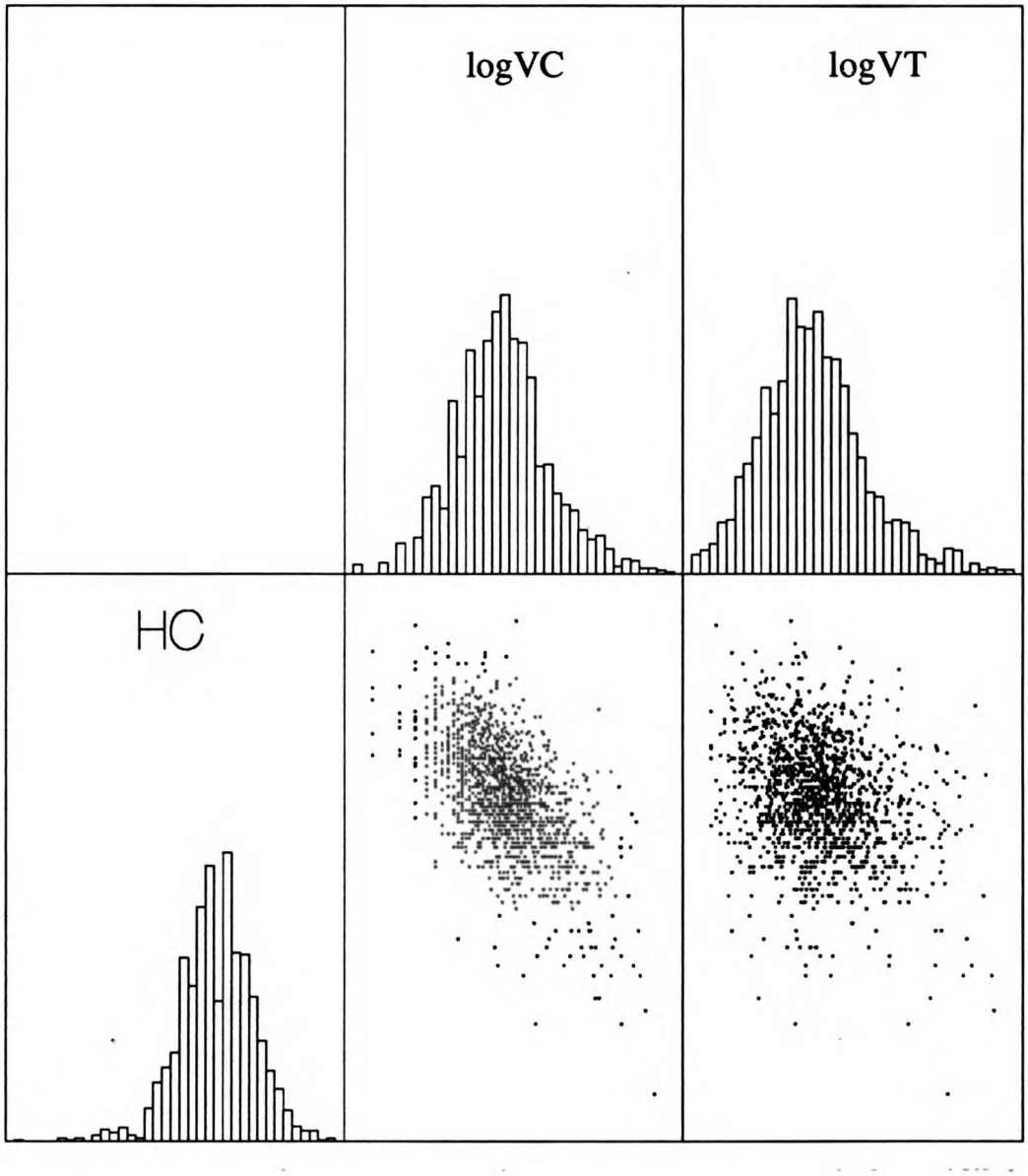


FIGURE 12 – EXAMPLE SCATTERPLOT BETWEEN HDL-C:LOGVLDL-C AND HDL-C:LOGVLDL-T

Cluster Analysis

We wish to explore several methods of cluster analysis to search the data set for previously undetected multidimensional grouping. Cluster analysis does not require *a priori* definition of subgroups. This methodology may be useful in studying threshold effects and the complex interdependence of variables. The Recursive Partition and Amalgamation method (RECPAM) was recently applied to the classification of type I diabetics and yielded interesting results.[67]

Computer Aided Diagnosis / Artificial Intelligence

Our database would be ideally suited for the validation and optimization of expert classification. Existing programs have generally been tested on only few hundred patient cases. A Canadian group developed a microcomputer based classification program for interpreting lipoprotein electrophoretic data, based on a need to improve phenotyping accuracy for a low volume test in their hospital.[68] In 1984, a Frankfurt group began work on a more sophisticated knowledge-based approach to lipid diagnosis. They developed the Pro MD shell in Prolog and wrote over 900 rules with forward reasoning and Bayesian logic. Recently, there has been an interest in "critiquing" and advice systems for the medical management of chronic diseases. Systems for hypertension, diabetes and lipid disorders have begun to implement complex longitudinal guidelines for drug intervention.[69]

Comprehensive Longitudinal Database

Although not part of this analysis, our database tracks follow-up lipid measurements on all patients. Eventually, we hope to include medication study protocols to aid in clinical trial data management. We could then examine determinants of response to medications, such as genetic polymorphism.[70] Our new clinic questionnaire will also include reason for referral and familial relationships to other patients seen in the clinic.

Additional Biochemical Variables

One of the most promising areas of new data is in HDL speciation. Conventional HDL species (HDL_1, HDL_2, HDL_3) may be artifactual (personal communication, John Kane), and over 10 distinct HDL species have recently been appreciated. Quantitative measurements of the first of these, pre-beta HDL, is now available in normal subjects and clinic patients. Particle size information from automated analysis of electron micrographs may soon be feasible on a large scale. Quantitative Lp(a) measurements have already been performed on over 200 patients, and may soon be included in the standard clinic testing. A recent study of 1065 consecutive individuals at a French metabolic clinic demonstrated that Lp(a) is elevated in dyslipidemia, but not influenced by type of dyslipidemia or gender.[71]

Additional Genetic Variables

We plan to add to the database RFLP data already collected on about 500 UCSF Lipid Clinic patients. Sample DNA was analyzed by cleavage with over 10 restriction enzymes,

and polymorphisms of several genes including the apo E gene, apo B gene, apo CI gene, renin gene and the AI-CIII-AIV gene complex were recorded.

SUMMARY

In summary, we present detailed biochemical, genetic, demographic and clinical data from a large lipid clinic population. Double pre-beta lipoproteinemia (a qualitative characteristic of VLDL which has never been examined in a large hyperlipidemic population) was found to correlate with gender, lipoprotein phenotype, apo E phenotype and several quantitative characteristics of VLDL and HDL. The apo E phenotype significantly influenced the lipoprotein profile. The presence of CAD was not only associated with elevated TC and LDL-C, but also elevated TG, VLDL-C, HDL-C/VLDL-C, total apo B, lipoprotein phenotype and apo E isoform. The presence of CAD was not associated with percentage apo B (indicator of VLDL size) or VLDL-C/VLDL-T.

We consider these univariate associations preliminary, and we will extend this analysis to cover over 2000 patients with many additional genetic and biochemical variables. We will examine the independence of determinants of DPRE, CAD presence and apo E phenotype and their interrelationships in a multivariate manner.

REFERENCES

1. Steinberg, D., Parthasarathy, S., Carew, T. and Khoo, T. *Beyond Cholesterol.* N Engl J Med, 1989. **320**(14): p. 915-924.

2. Spring, P. and Hoff, H. *LDL accumulation in the grossly normal human iliac bifurcation and common iliac arteries.* Exp and Mol Path, 1989. **51**(2): p. 179-185.

3. Beisiegel, U., Niendorf, A. and Wolf, K. *Lipoprotein (a) in the arterial wall.* Eur Heart J, 1990. **Aug 11**(SuppE): p. 174-83.

4. Reardon, M., Nestel, P., Craig, I. and Harper, R. *Lipoprotein predictors of the severity of coronary artery disease in men and women.* Circulation, 1985. **71**(5): p. 881-888.

5. Havel, R., Kotite, J., Kane, J. and Tun, P. *Atypical familial dysbetalipoproteinemia associated with apolipoprotein phenotype E3/3.* J Clin Invest, 1983. **72**: p. 379-387.

6. Hobbs, H.H., Leitersdorf, E., Leffert, C.C., Cryer, D.R., et al. *Evidence for a dominant gene that suppresses hypercholesterolemia in a family with defective low density lipoprotein receptors.* J Clin Invest, 1989. **84**(2): p. 656-64.

7. Boerwinkle, E., Visvikis, S., Welsh, D., Steinmetz, J., et al. *The use of measured genotype information in the analysis of quantitative phenotypes in man. II. The role of the apolipoprotein E polymorphism in determining levels, variability, and covariability of cholesterol, betalipoprotein, and triglycerides in a sample of unrelated individuals.* Am J Med Genet, 1987. **27**(3): p. 567-82.

8. Davignon, J., Gregg, R.E. and Sing, C.F. *Apolipoprotein E polymorphism and atherosclerosis.* Arteriosclerosis, 1988. **8**(1): p. 1-21.

9. Pagnan, A., Havel, R., Kane, J. and Kotite, L. *Characterization of human very low density lipoproteins containing two electrophoretic populations: double pre-beta lipoproteinemia and dysbetalipoproteinemia.* J Lipid Res, 1977. **18**: p. 613-622.

10. Zilversmit, D. *A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride-rich lipoproteins.* Circ Res, 1973. **33**: p. 633-8.

11. Tornvall, P., Hamsten, A. and Carlson, L.A. *Abnormalities of composition and of in vitro lipolysis products of human small very low density lipoproteins in hypertriglyceridemia.* Atherosclerosis, 1990. **82**(1-2): p. 125-35.

12. Sane, T. and Nikkila, E.A. *Very low density lipoprotein triglyceride metabolism in relatives of hypertriglyceridemic probands. Evidence for genetic control of triglyceride removal.* Arteriosclerosis, 1988. **8**(3): p. 217-26.

13. Halpern, M.J. and Mesquita, M.F. *Increased VLDL.* Adv Exp Med Biol, 1988. **243**(327): p. 327-32.

14. Shepherd, J. and Packard, C.J. *Metabolic heterogeneity in very low-density lipoproteins.* Am Heart J, 1987. **113**(2): p. 503-508.

15. Luc, G., de, G.J. and Chapman, M.J. *Further resolution and comparison of the heterogeneity of plasma low-density lipoproteins in human hyperlipoproteinemias: type III hyperlipoproteinemia, hypertriglyceridemia and familial hypercholesterolemia.* Atherosclerosis, 1988. **71**(2-3): p. 143-56.

16. Austin, M.A., Breslow, J.L., Hennekens, C.H., Buring, J.E., et al. *Low-density lipoprotein subclass patterns and risk of myocardial infarction.* Jama, 1988. **260**(13): p. 1917-21.

17. Austin, M.A., Brunzell, J.D., Fitch, W.L. and Krauss, R.M. *Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia.* Arteriosclerosis, 1990. **10**(4): p. 520-30.

18. Brunzell, J.D., Chait, A., Albers, J.J., Foster, D.M., et al. *Metabolic consequences of genetic heterogeneity of lipoprotein composition (lipoprotein heterogeneity).* Am Heart J, 1987. **113**(2): p. 583-588.

19. Grundy, S.M. and Vega, G.L. *Does Measurement of Apolipoprotein B Have a Place in Cholesterol Management* Arteriosclerosis, 1990. **10**: p. 668-671.

20. Sniderman, A.D. and Silberberg, J. *Is it time to measure apolipoprotein B? [see comments]* Arteriosclerosis, 1990. **10**(5): p. 665-7.

21. *Asymptomatic hypercholesterolemia: a clinical policy review. The Toronto Working Group on Cholesterol Policy.* J Clin Epidemiol, 1990. **43**(10): p. 1028-121.

22. Hulley, S., Rosenman, R., Bawol, R. and Brand, R. *Epidemiology as a guide to clinical decisions. The association between triglyceride and coronary heart disease.* N Engl J Med, 1980. **302**: p. 1383-1389.

23. Abbott, R. and Carroll, R. *Interpreting multiple logistic regression coefficients in prospective observational studies.* Am J Epidemiol, 1984. **119**: p. 830-6.

24. Castelli, W. *The triglyceride issue: a view from Framingham* Am Heart J, 1986. **112**: p. 432-437.

25. Lapidus, L., Bengtsson, C., Lindquist, O., Sigurdsson, J., et al. *Triglycerides - main lipid risk factor for cardiovascular disease in women?* Acta Med Scand, 1985. **217**: p. 481-489.

26. Carlsson, L. *Risk factors for ischemic heart disease in men and women.* Acta Med Scand, 1985. **218**: p. 207-211.

27. Sniderman, A.D. *Apolipoprotein B and apolipoprotein AI as predictors of coronary artery disease.* Can J Cardiol, 1988. **4A**: p. 24A-30A.

28. Havel, R.J. *Approach to the patient with hyperlipidemia.* Med Clin of North Am, 1982. **66**: p. 319-333.

29. Schonfeld, G. *The genetic dyslipoproteinemias--nosology update 1990.* Atherosclerosis, 1990. **81**(2): p. 81-93.

30. Meyers, L., Phillips, N. and Havel, R. *Mathematical evaluation of methods for estimation of the concentration of the major lipid components of human serum lipoproteins.* J Lab Clin Med, 1976. **88**: p. 491-505.

31. Kane, J., Sata, T., Hamilton, R. and Havel, R. *Apoprotein composition of very low density lipoprotein in human serum.* J Clin Invest, 1975. **56**: p. 1622-1634.

32. Havel, R., Kotite, J. and Kane, J. *Isoelectric heterogeneity of the cofactor protein for lipoprotein lipase in human bood plasma.* Biochem Med, 1979. **21**: p. 121-128.

33. Eichner, J.E., Kuller, L.H., Ferrell, R.E., Meilahn, E.N., et al. *Phenotypic effects of apolipoprotein structural variation on lipid profiles. III. Contribution of apolipoprotein E phenotype to prediction of total cholesterol, apolipoprotein B, and low density lipoprotein cholesterol in the healthy women study.* Arteriosclerosis, 1990. **10**(3): p. 379-85.

34. Pagnan, A., Zanetti, G., Bonanome, A., Biffanti, S., et al. *Apolipoprotein E polymorphism, serum lipids and occurrence of "double pre-betalipoproteinemia" (DPBL) in subjects from two different populations.* Atherosclerosis, 1987. **65**(1-2): p. 23-8.

35. Utermann, G. *Apolipoprotein E polymorphism in health and disease.* Am Heart J, 1987. **113**(2): p. 433-440.

36. Pairitz, G., Davignon, J., Mailloux, H. and Sing, C.F. *Sources of interindividual variation in the quantitative levels of apolipoprotein B in pedigrees ascertained through a lipid clinic.* Am J Hum Genet, 1988. **43**(3): p. 311-21.

37. Kitahara, M., Shinomiya, M., Shirai, K., Saito, Y., et al. *Frequency and role of apo E phenotype in familial hypercholesterolemia and non-familial hyperlipidemia in the Japanese.* Atherosclerosis, 1990. **82**(3): p. 197-204.

38. Eto, M., Watanabe, K. and Ishii, K. *Apolipoprotein E alleles and hyperlipoproteinemia in Japan.* Clin Genet, 1988. **34**(4): p. 246-51.

39. Eto, M., Watanabe, K., Chonan, N. and Ishii, K. *Familial hypercholesterolemia and apolipoprotein E4.* Atherosclerosis, 1988. **72**(2-3): p. 123-8.

40. Kamboh, M.I., Sepehrnia, B. and Ferrell, R.E. *Genetic studies of human apolipoproteins. VI. Common polymorphism of apolipoprotein E in blacks.* Dis Markers, 1989. **7**(1): p. 49-55.

41. Steinmetz, A., Assefbarkhi, N., Eltze, C., Ehlenz, K., et al. *Normolipemic dysbetalipoproteinemia and hyperlipoproteinemia type III in subjects homozygous for a rare genetic apolipoprotein E variant (apoE1).* J Lipid Res, 1990. **31**(6): p. 1005-13.

42. Tajima, S., Yamamura, T. and Yamamoto, A. *Analysis of apolipoprotein E5 gene from a patient with hyperlipoproteinemia.* J Biochem (Tokyo), 1988. **104**(1): p. 48-52.

43. Ordovas, J.M., Litwack, K.L., Wilson, P.W., Schaefer, M.M., et al. *Apolipoprotein E isoform phenotyping methodology and population frequency with identification of apoE1 and apoE5 isoforms.* J Lipid Res, 1987. **28**(4): p. 371-80.

44. Ford, R.P. *Essential data derived from biological variation for establishment and use of lipid analyses.* Ann Clin Biochem, 1989. **26**: p. 281-285.

45. Gonzalez, E.M., Rodriguez, F.C., Astarloa, I.R. and Lahera, E.M. *Use of serum cholesterol/triglyceride ratio to discern for which individuals the Friedewald formula can be used confidently.* Clin Chem, 1990. **36**(9): p. 1673-5.

46. Warnick, G.R., Knopp, R.H., Fitzpatrick, V. and Branson, L. *Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints.* Clin Chem, 1990. **36**(1): p. 15-9.

47. Phillips, N., Havel, R. and Kane, J. *Levels and interrelationships of serum and lipoprotein cholesterol and triglycerides: association with adiposity and the consumption of ethanol, tobacco and beverages containing caffeine* . Arteriosclerosis, 1981. **1**: p. 13-24.

48. Chivot, L., Mainard, F., Bigot, E., Bard, J.M., et al. *Logistic discriminant analysis of lipids and apolipoproteins in a population of coronary bypass patients and the significance of apolipoproteins C-III and E.* Atherosclerosis, 1990. **82**(3): p. 205-11.

49. Aursnes, I., Smith, P., Christiansen, E.N. and Berg, K. *Factor analysis of plasma lipoprotein components.* Atherosclerosis, 1988. **69**(2-3): p. 219-24.

50. Barbir, M., Wile, D., Trayner, I., Aber, V.R., et al. *High prevalence of hypertriglyceridaemia and apolipoprotein abnormalities in coronary artery disease.* Br Heart J, 1988. **60**(5): p. 397-403.

51. Vogler, G.P., Rao, D.C., Laskarzewski, P.M., Glueck, C.J., et al. *Multivariate analysis of lipoprotein cholesterol fractions.* Am J Epidemiol, 1987. **125**(4): p. 706-19.

52. Steinmetz, A., Thiemann, E., Czekelius, P. and Kaffarnik, H. *Polymorphism of apolipoprotein E influences levels of serum apolipoproteins E and B in the human neonate.* Eur J Clin Invest, 1989. **19**(4): p. 390-4.

53. Bonithon, K.C., Scarabin, P.Y., Darne, B., Malmejac, A., et al. *Menopause-related changes in lipoproteins and some other cardiovascular risk factors.* Int J Epidemiol, 1990. **19**(1): p. 42-8.

54. Gueguen, R., Visvikis, S., Steinmetz, J., Siest, G., et al. *An analysis of genotype effects and their interactions by using the apolipoprotein E polymorphism and longitudinal data.* Am J Hum Genet, 1989. **45**(5): p. 793-802.

55. Fumeron, F., Rigaud, D., Bertiere, M.C., Bardon, S., et al. *Association of apolipoprotein epsilon 4 allele with hypertriglyceridemia in obesity.* Clin Genet, 1988. **34**(4): p. 258-64.

56. Eto, M., Watanabe, K. and Ishii, K. *Apolipoprotein E polymorphism and hyperlipoproteinemia in obesity.* Int J Obes, 1989. **13**(4): p. 433-40.

57. Black, S.C., Hewett, S., Kotubi, Y., Brunt, R.V., et al. *Isoform patterns of apolipoprotein E in diabetes mellitus.* Diabetic Med, 1990. **7**(6): p. 532-9.

58. Eto, M., Watanabe, K., Sato, T. and Makino, I. *Apolipoprotein-E2 and hyperlipoproteinemia in noninsulin-dependent diabetes mellitus.* J Clin Endocrinol Metab, 1989. **69**(6): p. 1207-12.

59. Eto, M., Watanabe, K., Iwashima, Y., Morikawa, A., et al. *Increased frequency of apolipoprotein epsilon 4 allele in type II diabetes with hypercholesterolemia.* Diabetes, 1987. **36**(11): p. 1301-6.

60. Matsuda, M., Miyahara, T., Murai, A., Fujimoto, N., et al. *Lipoprotein abnormalities in survivors of cerebral infarction with a special reference to apolipoproteins and triglyceride-rich lipoproteins.* Atherosclerosis, 1987. **68**(1-2): p. 131-6.

61. Rossner, S. and Kjellin, K. *Dyslipoproteinemia in patients with ischemic cerebrovascular disease* Atherosclerosis, 1978. **30**: p. 199.

62. Eisenberg, S., Heiss, G., Friedlander, Y., Rifkind, B., et al. *Comparison of plasma lipids, lipoproteins and dyslipoproteinemia in Israel and the United States. The Lipid Research Clinics Program Prevalence Study.* Atherosclerosis, 1986. **59**(1): p. 63-74.

63. Sommariva, D., Branchi, A., Tirrito, M., Bonfiglioli, D., et al. *Relationship of serum triglyceride concentration to lipoprotein composition and concentration in normolipidemic and hyperlipidemic subjects.* Ric Clin Lab, 1988. **18**(4): p. 281-90.

64. Freedman, D.S., Gruchow, H.W., Anderson, A.J., Rimm, A.A., et al. *Relation of triglyceride levels to coronary artery disease: the Milwaukee Cardiovascular Data Registry.* Am J Epidemiol, 1988. **127**(6): p. 1118-30.

65. Ford, E.S., Cooper, R.S., Simmons, B. and Castaner, A. *Serum lipids, lipoproteins and apolipoproteins in black patients with angiographically defined coronary artery disease.* J Clin Epidemiol, 1990. **43**(5): p. 425-32.

66. Winkler, L., Schlag, B., Ostermann, G. and Dargel, R. *Apolipoproteins as risk indicators of ischemic heart disease.* Przegl Lek, 1989. **46**(7): p. 595-8.

67. Ciampi, A., Schiffrin, A., Thiffault, J. and Quintal, H. *Cluster analysis of an insulin dependent diabetes cohort towards the defenition of clinical subtypes* J Clin Epidemiol, 1990. **43**(7): p. 701-715.

68. Loughlin, J., Leung, F. and Henderson, A. *Classification of hyperlipoproteinemias by computer interpretation.* Ann Clin Biochem, 1984. **21**: p. 326-331.

69. Rucker, D., Mavon, D. and Shortliffe, E. *Temporal representation of clinical algorithms using expert system and database tools.* Comp in Biomed Res, 1990. **Jun 23**(3): p. 222-239.

70. De, K.P., Stalenhoef, A.F., Mol, M.J., Gevers, L.J., et al. *Influence of apo E polymorphism on the response to simvastatin treatment in patients with heterozygous familial hypercholesterolemia.* Atherosclerosis, 1990. **83**(1): p. 89-97.

71. Boyer, H., de Gennes, J., Truffert, J. and Chatellier, G. *Lp(a) levels in different types of dyslipidemia in the French population.* Atherosclerosis, 1990. **85**: p. 61-69.

APPENDICES

LIPID CLINIC INITIAL MEDICAL HISTORY FORM

Please complete this form as well as you can. Your doctor will discuss the information with you. All information will be kept confidential. Note that each page has two sides.

GENERAL INFORMATION LOCATION: _____ DATE: _____

Name _____
Last First Middle

Birthdate _____

Sex ___ Female
___ Male

Referring Physician: Name _____

Address _____

Other physicians who are seeing you regularly:

Name _____

_____

Address(es) of physician(s) to whom you wish us to send our report:

_____

_____

Your Home Address: _____

Your day time phone: ( ) _____

Your home phone: ( ) _____

Other person who would know how to reach you:

Name: _____

Relationship: _____ Phone: ( ) _____

MEDICAL HISTORY

What is the reason for which you are referred to this clinic?

_____

_____

Have you had any of the following? If yes, please give date of onset.

Chest Pain - Angina _____

Heart Attack _____

Stroke _____

Pain in legs when walking _____

High blood pressure _____

Pancreatitis - (severe _____

Thyroid disease ___

Diabetes _____

FORM NO. 435-21 12/83

Appendix 1 – Original Patient Questionnaire

Liver disease _____

Gout _____

Kidney disease _____

Tumor(s) or Cancer _____

Have you ever had the following diagnostic tests for your heart?

Electrocardiogram (ECG) Date _____ Done where? _____

Exercise ECG Date _____ Done where? _____

Cardiac Catherization Date _____ Done where? _____

Other _____

Have you ever had surgery? _____No _____Yes

Please list: _____

_____

How much did you weigh when you were: How tall are you? _____ ft. _____ inches

20 years old _____

30 years old _____

40 years old _____

50 years old _____

Allergies: _____

Drug Sensitivities: _____

Please descibe other medical problems that you feel might be important:

_____

_____

FOR WOMEN ONLY:

Are you still menstruating? _____ No _____ Yes

If No, at what age did you have your last period? _____

Did you have a hysterectomy (womb removed)? _____No _____Yes

If Yes, at what age? _____

Have your ovaries been removed? _____ No _____Yes _____ Unsure

Are you now pregnant? _____ No _____Yes _____Unsure

HABITS

Are you a regular smoker, either past or present? _____ No _____Yes

If yes:

Average amount smoked per day? _____ For how many years? _____

What kind of tobacco do you smoke? _____

Have you smoked in the past month? _____No _____Yes

if yes, average amount smoked per day _____

if no, when did you last smoke (approximate month and year)? _____

Have you ever used alcoholic beverages regularly? _____ No _____Yes

Do you currently drink alcoholic beverages (beer, wine, gin, vodka, whiskey, rum, etc.) other than on special occasions such as weddings and holidays?

_____No _____ Yes

If yes, how much of the following do you drink during an average week:

Beer (number of 12 oz. cans or equivalent): _____

Wine (in ounces): _____

Liquor (in ounces): _____

Have you ever had a problem with heavy drinking? _____

Do you engage in regular physical activity?____ No ___Yes

If yes, what kind of activity and how often? _____

_____

_____

Do you follow any special diet?____ No ____Yes

If so, please describe briefly: _____

_____

_____

_____

_____

_____

MEDICATIONS

Have you ever taken any medication for your cholesterol or triglyceride problem?_____No _____Yes

If Yes, indicate which of the following you have taken, when it was prescribed, and for how long.

Name of Medication	When	Duration
_____ cholestyramine (questran)		
_____ colestipol (colestid)		
_____ gemfibrozil (lopid)		
_____ probucol (lorelco)		
_____ nicotinic acid (niacin, nicobid)		
_____ sitosterol (cytellin)		
_____ thyroid (choloxin)		
_____ clofibrate (Atromid - S)		
_____ estrogen		
_____ other		

Please list all the medications you currently take. (Include aspirin, vitamins, birth control pills.)

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

FAMILY HISTORY

Which of the following best describes your racial heritage?

Please check only one.

_____ European (white)

_____ Mexican (Chicano or Mestizo)

_____ African (black or Negro)

_____ Asian (Chinese, Japanese, Korean, Indochinese)

_____ Pacific Islands (Filipino, Samoan, Hawaiian)

_____ None of these

Is your father living?____ No ____Yes _____ unknown

What is your father's present age or age at death? _____

Is your mother living?____ No ____Yes _____ unknown

What is your mother's present age or age at death?_____

How many brothers and half-brothers do you have? living_____ deceased _____

How many sisters and half-sisters do you have? living_____ deceased _____

How many natural sons do you have? living_____ deceased _____

How many natural daughters do you have? living_____ deceased _____

Has any relative ever had the following conditions? If so, please complete the following:

Condition	Relationship	Living (age)	Deceased (age)	Cause of Death
_____ Heart Attack				
_____ Stroke				
_____ Diabetes				
_____ Pancreatitis				
_____ High Cholesterol				
_____ High Triglycerides				
_____ Sudden Death (unexplained causes)				

1.0 PATIENT DATA

☐ No Chart Available

*WRITE NEATLY, MINIMIZE STRAY REMARKS

Patient First Name	[redacted]	ATB Date	5/15/90
Patient Last Name	[redacted]	Coding Date	6/27/90
Unit Number	[redacted]	Coder Name	CCB
		Status	
Telephone #1 (Latest)	([redacted]		
Telephone #2	([redacted]		
City (Latest)	S.F (Ex: SF)	State	CA (Ex: CA)
Gender (M/F)	M	Date of Birth	[redacted]
Height (ft/in) (ATB)	5'7" (2.54 cm/in)	Age at Menopause	
Weight (lbs) (ATB)	139 (2.2 lb/kg)		
Ethnicity (1', 2')	1	1 = White, 2 = Black, 3 = Hispanic, 4 = Jewish	
Other Ethnicity		5 = North Asian, 6 = South East Asian, 7 = Middle Eastern	
		8 = American Indian, 9 = East Indian, 10 = unknown	

2.0 SIGNS OF OF DISEASE

☐ No Medical History

Key: Use + for Yes, 0 for No

Anytime = Any information to date in chart.
ATB = Information obtained at Across the Board Test Date.

	Anytime	ATB	other Hospital, City, State	Date
Angiogram (Anytime)	+	+		7/28??
	+/0	UCSF	other Hospital, City, State	Date

Coronary Disease (1st Event Only)	Anytime	Date Dx	or Age Dx	MD confirm
No clin evidence		/		
Angina		/		
MI	+	7/88		+
CABG	+	9/88		+
Angioplasty		/		
EKG Evidence		/		
Data Unavailable				
Stroke (CVA, TIA) (Anytime)		/		
Periph. Vasc. Dis. (Anytime)				
Hypertension (Anytime)				

Highest Resting/Office Blood Pressure (Anytime) [/] no meds [133/74] BP on meds

Diabetes (Anytime)

Type I (insulin)	[ ]	[ ]	Ever Received Insulin +/o
Type II (NIDDM)	[ ]	[ ]	Ever Took Oral Hypoglycemics +/o

Xanthomas (Anytime)

Tendinous	[ ]	[+]	Arcus	[ ]	Eruptive
Tuberous	[ ]	[ ]	Planar	[ ]	Xanthelasma

Relevant Diseases (ATB)

Renal	[ ]	[ ]	Pancreatitis
Cholestasis	[ ]	[ ]	Untreated Hypothyroidism

Other Diseases [ ] [ ]

3.0 HABITS [ ] NO Questionnaire Available

(ATB)

	+/o			
Reg. Cig. Smoker	[+]	Avg Amount	[1] (pk/day)	[35] (yrs)
Smoked past month	[+]	Avg Amount	[.5] (pk/day)	
Date Last Smoked	[/ /]		(20 cigarettes = 1 pack)	

Alcohol (ATB)

	+/o	Amount in Avg Week	
Ever Used EtOH Regularly	[+]	Beer (12)	[ ] oz
Drink EtOH other than special	[+]	Wine (4-6)	[1|3] oz
Problem with heavy drinking	[+]	Liquor (1-1.5)	[ ] oz

	1° 2°	
Diet Modification (ATB)	[1 |]	1 = none, 2 = some/minimal, 3 = low fat/cholesterol 4 = vegetarian, 5 = fish/ fish oil, 6 = other, 7=ADA
Exercise Level (ATB)	[1]	1 = none, 2 = minimal, 3 = regular/aerobic

.0 MEDICAL HISTORY AFFECTING LIPIDS

Drugs at (ATB)		Examples
HMG-CoA inhibitors		Lovastatin, Mevacor
Nicotinic Acid		Nicotinic Acid (Niacin), Niacobid
Bile Acid Resins		Colestipol, Cholestryramine (Questran)
Clofibrate		Clofibrate (Atromid), Gemfibrozil (Lopid)
Probucol		Probucol, Lorelco
Steroids		Prednisone, Dexamethasone
Estrogens (Synthetic → Conjugated (Premarin))		Estradiols, Estrols (OCP's, ERT)
Progestins		In OCP's, ERT
Aspirin		Bayer, Anacin, etc...
Ca Channel Blocker		Procardia, Cardiazem
Beta Blockers	+	Propanalol, Inderal, Lopressor, Atenolol
Diuretics		Hydrochlorothiazide, Furosemide, Lasix
Thyroid hormone		Thyroxine, Synthroid
Persantine/Dipyridamole		
Other Drugs		
Other Drugs		

ONLY MAJOR OVER the Counter Meds
NOT Vitamins, NSAID's, Anti-Hypertensives

Lab Tests (Closest to ATB)	Norm	or	Abnl	Value	Date	Unkn
T4 Free/Total				/	/ /	+
TSH	+				/ /	
Uric Acid					/ /	+
Creatinine	+				/ /	
BUN	+				/ /	
Untreated FBS	+				/ /	
Other 1					/ /	
Other 2					/ /	

5.0 RELEVANT FAMILY HISTORY (primarily positives)

☐ No Family History

(Anytime) Relation	Sex M/F	Age	L or D	Disorders (by #, key below) #, Age / #, Age / #, Age / #,
Father	M	~64	D	, / , / , / , / , / ,
Mother	F	~64	L	, / , / , / , / , / ,
Sibling 1				, / , / , / , / , / ,
Sibling 2				, / , / , / , / , / ,
Sibling 3				, / , / , / , / , / ,
Sibling 4				, / , / , / , / , / ,
Sibling 4				, / , / , / , / , / ,
Child 1				, / , / , / , / , / ,
Child 2				, / , / , / , / , / ,
Child 3				, / , / , / , / , / ,
Child 4				, / , / , / , / , / ,
Child 5				, / , / , / , / , / ,
Mat. Uncle/Aunt 1				, / , / , / , / , / ,
Mat. Uncle/Aunt 2				, / , / , / , / , / ,
Mat. Grandparent				, / , / , / , / , / ,
Mat. Grandparent				, / , / , / , / , / ,
Pat. Grandparent				, / , / , / , / , / ,
Pat. Grandparent				, / , / , / , / , / ,
Pat. Uncle/Aunt 1				, / , / , / , / , / ,
Pat. Uncle/Aunt 2				, / , / , / , / , / ,
Other				, / , / , / , / , / ,

1 = High Cholesterol
2 = High Trigylcerides
3 = CV Disease (angina, MI, ...)
4 = Pancreatitis
5 = Stroke (CVA, TIA)
6 = Sudden Death

6.0 ADDITIONAL NOTES / COMMENTS / PROBLEMS

Topic	Note

DAVID SMITH GULLION BD: 11-11-42 SEX: M DX: ↑ LDL prob (↑ TG before - on Epoit)
UNIT NUMBER: 63 56 04 Multiple type ↓ HDL

TEST RESULTS ON SPECIMEN COLLECTED OR RECEIVED ON 01-24-86

MI, angioplasty

LIPID LEVELS-MG/DL:	SERUM	VLDL	LDL	HDL	RECOVERY	
CHOL:	255	23	190	36	249	98%
TRIG:	125	84	34	8	126	101%

APOPROTEIN COMP OF VLDL (1.006T):

APO B = 37.5 % OF TOTAL PROTEIN

% DIST OF SOLUBLE PROTEIN:

SER =

ARP =

GLU =

ALA1 =

ALA2 =

ALA3 =

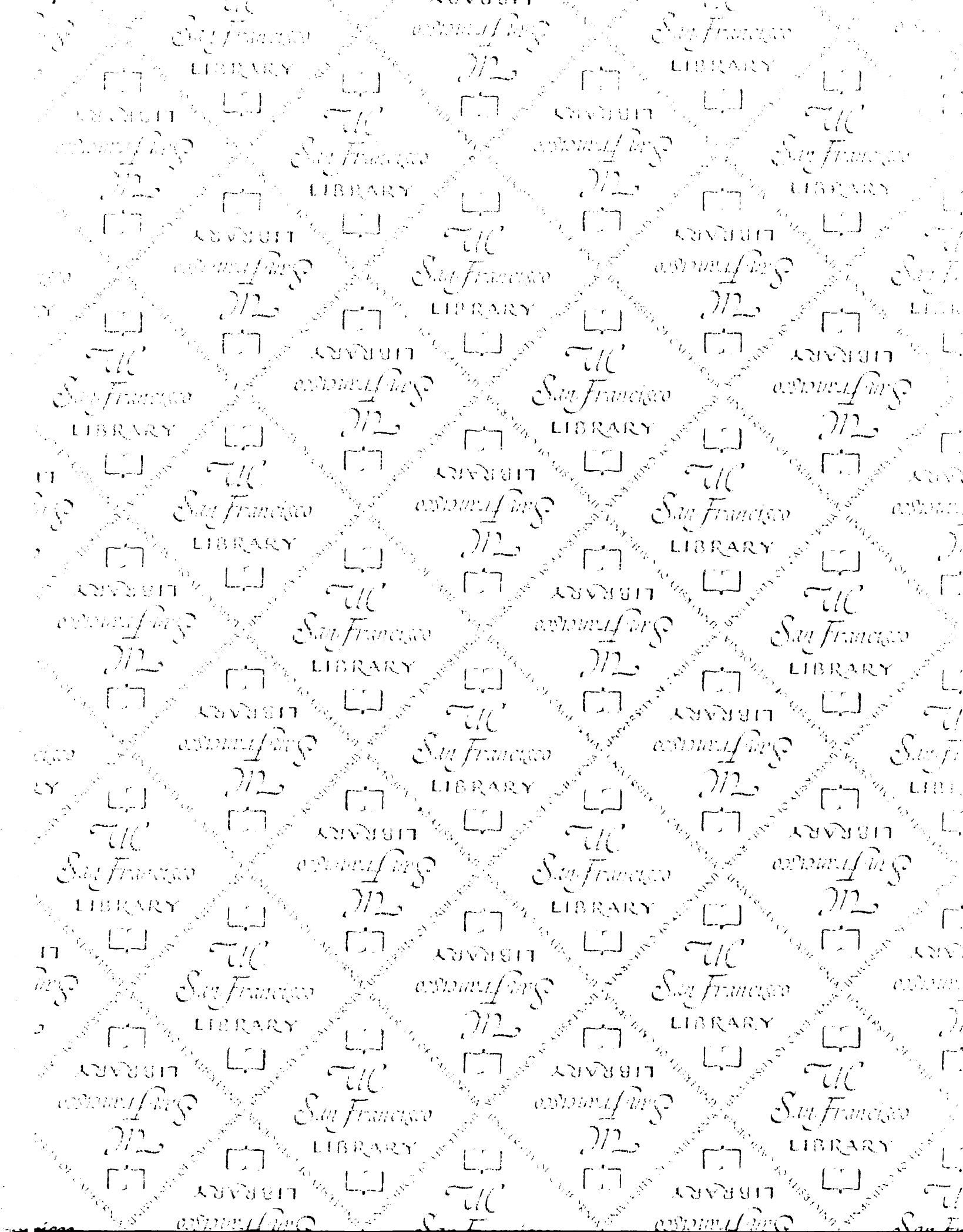
ALA4 =

E ISOFORMS:	% E1	% E2	% E3	% E4	E3/E2
W/O 2ME:	0.0	15.4	18.1	66.5	1.18
WITH 2ME:	0.0	12.6	17.4	70.0	1.38

$E_4 - E_4$

AGAROSE GEL ELECTROPHORESIS: double b/p
no sinking

Appendix 3 – Sample Lab Data Log Book Entry


San Francisco
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FOR REFERENCE

NOT TO BE TAKEN FROM THE ROOM

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