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THE EFFECTS OF GENERATION AND GENDER ON THE JOINT DISTRIBUTIONS OF LIPID AND APOLIPOPROTEIN PHENOTYPES IN THE POPULATION AT LARGE

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Abstract—The generation and gender effects on the joint distributions of total plasma cholesterol (Total-C), ln triglycerides (lnTrig), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), apolipoproteins AI (Apo AI), AII (Apo AII), and E (lnApo E) were studied in 184 male grandparents (MGP), 242 female grandparents (FGP), 237 male parents (MP), 235 female parents (FP), 202 male children (MC), and 200 female children (FC). Homogeneity of variance tests revealed that lipid variances were gender and/or generation specific while apolipoprotein variances were homogeneous across strata. In the absence of heterogeneity of variance, significant heterogeneity in LDL:lnTrig and lnTrig:Apo AII covariation was found between genders in the parental generation. In the presence of heterogeneity of variance, significant heterogeneity of correlation between genders and/or across generations was found for the HDL-C:LDL-C, Total-C:LDL-C, Total-C:lnTrig, lnTrig:LDL-C, Total-C:lnApo E and HDL-C:lnApo E bivariate distributions. Analyses of principal components revealed that the generation and gender specific cohorts have similar eigenvalues but distinct eigenvectors for the first two principal components underlying the seven dimensional lipid and apolipoprotein distribution. We conclude that the amount of variability explained by the first two principal components is the same across cohorts but how the interindividual variability is distributed among the lipid and apolipoprotein traits is generation and gender specific. This study documents the role that variance and covariance might play in determining risk of disease for special subgroups of the population at large. It also demonstrates how variances and covariances between risk factors traits characterize life processes of aging and sexual dimorphism. This study argues that future biometrical genetic and epidemiological studies of coronary artery disease must take into account age and gender effects on interindividual variability and covariability of risk factors.

Cholesterol Triglyceride HDL-cholesterol Apolipoprotein-AI Apolipoprotein-AII Apolipoprotein-E

INTRODUCTION

Discrete genetic lesions, such as familial hypercholesterolemia [1, 2], explain only a small fraction of the cases of coronary artery disease (CAD) that aggregate in families. Most CAD in the general population is associated with levels of risk factors in the normal range of variability [3, 4]. In the majority of cases, an individual's risk of developing CAD is the consequence of complex interactions among the effects of many genetic and environmental factors [5].

Quantitative levels of risk factors for CAD that are measures of lipid metabolism and hemostasis are determined by these interactions. Most investigations of genetic and environmental causes of the development of CAD in the population at large have focused on separate analyses of interindividual differences in levels of each of these continuously varying traits [6, 7]. Separate univariate analyses do not take advantage of the information about CAD risk that is provided by knowing interindividual differences in the relationship between measures of lipid metabolism and hemostasis that define a multivariate profile. Just as the discretely defined hyperlipidemias may be distinguished by a well defined relationship between the traits of lipid metabolism [8], we hypothesize that a significant fraction of the variation in risk of CAD among individuals in the general population may be associated with variations in the relationships between otherwise normal levels of risk factor traits. In other words, heterogeneity of covariability between risk factor traits among subgroups of the population may contribute to determining interindividual differences in risk for CAD.

In general, studies of the multivariate distribution of risk factor traits are necessary for establishing how the effects of genetic and environmental variation combine to determine risk of CAD. Specifically, the predictive value of genetic variability at a particular gene locus involved in lipid metabolism will be enhanced by considering its effects on interindividual differences in the relationships between traits that measure lipid metabolism. For example, in addition to affecting trait levels, inherited variation of the apolipoprotein E molecule is associated with profound differences in the magnitude and direction of the correlation between total cholesterol, betalipoprotein, and triglyceride levels [9]. Although the hyperlipidemias are characterized by a well defined relationship between triats, little is known about the relationship between covariability among these risk factor traits in the so-called normal range and risk of CAD in the population at large. Because of the importance of the relationships between biological risk factor traits that each have a genetic component, it seems obvious that our understanding of the link between genes and CAD endpoints will be enhanced by the considerations of the effects of genetic variation on the interindividual differences in the relationship between levels of such traits.

This paper reports our studies to determine effects of generation and gender on interindividual variability and covariability in the lipid metabolism profile defined by plasma levels of total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and the apolipoproteins AI, AII, and E. In addition, we report the effects of differences in phenotypic variability among generation and gender specific cohorts on the observed correlations between these traits. Since age and gender are strongly associated with risk of CAD, this information of the generation and gender specific differences in the variability and covariability among risk factor traits provides a necessary first step towards relating such covariability to risk of CAD in the general population. In a subsequent paper we will evaluate the impact of genetic variability on the multivariate profiles of the age and gender specific cohorts reported here.

SAMPLE

As part of the Rochester Family Heart Study (RFHS), 276 multigeneration pedigrees were ascertained through elementary school children. The RFHS sample includes a total of 2002 individuals. Moll *et al.* [10] give details of the sampling methods that were used. To investigate the heterogeneity in means, variances, and covariances of lipid metabolites among gender and generation groups we stratified the sample into six cohorts—grandfathers, grandmothers, fathers, mothers, male children and female children. Within-cohort covariability due to genetic relatedness was removed by considering only one individual from each set of related individuals within any one cohort. Two individuals not measured for age and height were also excluded. Women taking hormones were not excluded since this study focuses on characterizing the multivariate profiles of cohorts representative of the population at large. Future studies will examine the effects of exogenous hormones, lipid lowering drugs, and other environmental factors on interindividual lipid and apolipoprotein variability and covariability.

Statistical inferences about the distribution of lipid traits in the six generation and gender secific cohorts rely on the assumption of multivariate normality. In order to reduce skewness we removed individuals with total cholesterol (Total-C), ln triglyceride (lnTrig), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), apolipoprotein AI (Apo AI), AII (Apo AII),

and ln E (lnApo E) values more than 2 standard deviations from their generation and gender specific means. Eight individuals were removed prior to invoking the truncation criterion because they have extreme outlier values of triglyceride and/or apolipoprotein E (i.e. triglyceride levels > 1900 mg/ml and/or Apo E levels > 20 mg/dl which are at least 5 standard deviations from the overall sample mean). In addition, triglyceride and Apo E distributions were transformed before the truncation procedure since these distributions were extremely skewed ($g_1 > 1.5$) and leptokurtotic ($g_2 > 2.5$). After considering logarithmic transformation and a variety of power transformations we decided that to be consistent with the literature the natural logarithm ($\log_e = \ln$) was the most appropriate transformation. The lnTrig and lnApo E distributions showed a marked reduction in skew ($g_1 < 0.58$) and kurtosis ($g_2 < 0.68$) which was reduced further by the truncation procedure.

After removal of outliers by the truncation procedure described above, the cohorts of unrelated persons considered in this study include a total of 1300 individuals—184 male grandparents (MGP), 242 female grandparents (FGP), 237 male parents (MP), 235 female parents (FP), 202 male children (MC), and 200 female children (FC). The number of women taking hormones (17 FC, 6 FP, and 5 FGP) is small compared to the number not taking hormones and indicates that our sample is more characteristic of females not taking exogeneous hormones. Summary statistics for age, height, weight, and body mass index ([kg/cm^2] × 1000) in these six cohorts are presented in Table 1. Since the RFHS sample represents a cross section of the Rochester, Minnesota population, there was some age overlap between generations which can be seen by examining the minimum and maximum age values. The average age ranged from 15.23 (years) for male children to 68.75 (years) for MGPs. Age overlap between generation specific cohorts involved less than 10% of the total number of individuals. The height, weight, and body mass index (BMI) distributions among these cohorts were representative of other North American populations [11]. In general, the males were taller, weighted more, and had larger BMIs than females in the same generation. Specifically, the mean height and weight of MGPs were significantly ($p < 0.05$) larger and the variance of BMI was significantly smaller than for FGPs. In the parental generation, males had significantly larger age, height. weight, and BMI means than females. However, variability of age and height of MPs was significantly smaller. In children, males were significantly taller, variability of height and weight significantly greater and variability of BMI significantly smaller than females.

LABORATORY METHODS

All blood samples were collected in EDTA by venipuncture. Total plasma cholesterol and triglyceride levels were measured by standard enzymatic methods (Beckman kits) [12,13]. HDL-C was measured following precipitation of Apo B containing lipoproteins with polyethylene glycol [14]. LDL-C was calculated using the modified Freidenwald formula (LDL-C = TC − (HDL-C + 0.16 Tg)) [15]. Aliquots of plasma were frozen at −70°C for apolipoprotein determinations. Levels of Apo AI, AII,

Table 1. Summary statistics for age, height, weight and BMI (623 males, 677 females)

		Mean		SD		p-Values	
Variable	Cohort	Male	Female	Male	Female	t-Test means	F-Test variance
Age (yr)	GP[a]	68.75	67.77	7.65	8.08	0.20	0.22
	P	43.62	41.58	6.89	6.14	<0.01	0.04
	C	15.23	15.39	5.08	4.75	0.75	0.17
Height (cm)	GP	174.33	160.62	6.13	5.68	<0.01	0.13
	P	177.80	164.92	6.18	5.52	<0.01	0.04
	C	162.49	158.30	18.00	13.84	0.01	<0.01
Weight (kg)	GP	83.46	69.07	13.23	12.62	<0.01	0.24
	P	85.60	68.93	12.77	14.44	<0.01	0.02
	C	55.62	53.20	17.95	15.65	0.15	0.03
BMI (kg/cm^2 × 1000)	GP	2.74	2.68	0.40	0.46	0.11	0.02
	P	2.71	2.54	0.36	0.57	<0.01	<0.01
	C	2.04	2.08	0.35	0.40	0.34	0.03

[a]GP = grandparent, P = parent, C = child.

and E were measured in 6 replicates of each sample using radioimmunoassays developed and standardized in the Mayo Atherosclerosis Research Laboratory [16–18]. This included the use of quality control plasmas to correct for day to day variability in the assays as well as the use of primary pure apolipoprotein standards as described [16, 18].

STATISTICAL METHODS

The goal of the statistical analyses in this study was to estimate and test hypotheses about the heterogeneity of variances and covariances among the six generation-gender specific cohorts. Forward stepwise regression [19] was used to select the set of concomitants which removed the maximum amout of variability in each truncated lipid and apolipoprotein variable within each cohort. The independent variables used in the forward stepwise regression include age, age^2, age^3, height, height^2, height^3, weight, weight^2, weight^3, BMI, BMI^2, and BMI^3. Using an alpha level of 0.05 as the cut point for inclusion, the variables which were selected because they made a significant contribution in at least one of the cohorts for at least one the lipid or apolipoprotein traits were age, age^2, height, height^2, weight, weight^2, BMI, and BMI^2. In instances where BMI or BMI^2 contributed significantly to be regression we found that weight, weight^2, height and height^2 did not contribute significantly. Although this variable selection procedure does not focus on the identification of which subset of variables plays an important role in which cohort, it provides a method for removing the maximum amount of variability due to concomitant variability within cohorts. A multiple regression equation including the set of selected independent variables was used to adjust each of the biological traits separately for each gender in each of the three generations. The residuals from this regression equation were used in all subsequent analyses.

Statistical inferences about homogeneity of variance and covariance among cohorts rely on the assumption of normality. To verify that each of the truncated, adjusted, and transformed lipid and apolipoprotein variables was normally distributed the Lilliefor's test for normality was performed [20]. The null hypothesis of normality was accepted for all 42 cohort-specific variables at the $p > 0.10$ significance level.

Appropriate statistics exist for testing the equality of symmetric variance–covariance matrices. We employed one that utilizes the generalized variances (i.e. the determinant of the variance–covariance matrix) in the Bartlett's homogeneity of variance test statistic [21]. Statistical theory does not exist for testing homogeneity of covariance between two traits without also considering the magnitude of the trait variance. In order to detect heterogeneity of trait variance that may be underlying heterogeneity of the variance–covariance matrices, we used the univariate Bartlett's test statistic [21].

When there was evidence for heterogeneity of trait variance across strata we tested for homogeneity of the Pearson product–moment correlations as a measure of the covariance–variance interaction which can give rise to differences among cohorts [22]. The tests for homogeneity of variance, variance–covariance, and correlation coefficients were constructed using the MATRIX programming language available from SAS [23].

A substantial fraction of the variances and covariances in the 21 bivariate distributions was significantly heterogeneous across cohorts. This result suggested to us that trivariate, tetravariate, etc. tests for homogeneity of variance–covariance matrices would be inappropriate. Principal component analyses using the correlation matrices provided a method to examine the internal dependency structure underlying the multivariate (seven dimensional) distribution [24]. The correlation matrices were used for these analyses because of scale differences between variables (i.e. logarithm transformed variables vs untransformed variables). To determine if the variation explained by the first two principal components was equivalent across strata, we tested for homogeneity of the eigenvalues using the following test statistic:

$$M = 1/2\Sigma n_i(\log_e \lambda_i - [\Sigma n_i \log_e \lambda / \Sigma n_i])^2$$

where n_i is the sample size of the ith cohort and λ_i is the eigenvalue of the ith cohort. This test statistic is approximately distributed as a chi-square distribution with $k - 1$ *df* where k is the number of cohorts [24].

RESULTS

Biological interpretations of the effects of age and gender on lipid metabolism

Plasma lipoprotein metabolism depends on a complex metabolic pathway which involves many gene product mediated reactions which

transport and catabolize the lipid components of lipoprotein particles for distribution to peripheral tissues and excretion. Metabolic pathways, such as lipoprotein metabolism, are known to be under homeostatic control [25–31]. These homeostatic control mechanisms regulate steady state levels of metabolites, the variance in metabolite concentrations, and the covariation between traits levels [25–27, 30, 31]. Since the strength and sensitivity of these control mechanisms is effected by genetic and environmental factors, the homeostatic properties of metabolic systems are expected to be different across genders and generations [25, 28–30]. The modulation of lipoprotein levels through sex hormone levels is one example of the metabolic sexual dimorphism which results from the genotypic difference between males and females [32–35]. The weakening of the homeostatic controls in many metabolic systems during senescence is one example of the changes in metabolic control which occur during the life history of an individual or population [30, 36–41]. Research in plant and animal genetics has found evidence that generation and gender differences in phenotypic variation (or covariation) is attributable to differences in genetic, gene by environment interaction, and/or environmental variation (or covariation) [42–48]. To stimulate further interest in the biological significance of the second moments of phenotypic distributions we have discussed the results from this study in terms of the role of homeostasis in explaining epidemiological observations.

Contribution of age, height, weight, and BMI to trait variability

The amount of trait variation attributable to variation in concomitants ranged from 1.83% for LDL-C in MGPs to 22.47% for lnTrig in FPs. There were substantial differences across cohorts in both the total amount of interindividual variation explained and the relative contribution of each of the concomitants (data not shown). The contribution of concomitants to Total-C, HDL-C, and LDL-C variability was the greatest in MC and FPs whereas the contribution to lnTrig variability was the greatest in MPs and FPs. Among the apolipoprotein distributions, the contribution of concomitants was greatest in the MC and FC.

Lipid levels

The gender and generation specific mean lipid levels (Table 2) ranged from 148.77–214.70 (mg/dl) for Total-C, 4.43–4.95 (ln mg/dl) for lnTrig, 40.39–51.38 (mg/dl) for HDL-C, and 88.99–140.55 (mg/dl) for LDL-C. The range of mean levels we observed were characteristic of other age and gender specific cohorts from North American populations [11, 51]. Overall, children had the lowest mean levels, grandparents had the highest mean levels, and parents had intermediate mean levels of these lipids. The only exception to this trend was the lower than expected HDL-C levels in MGPs and MPs. Traditional tests for the equality of means across strata (univariate and multivariate) were not performed because the statistical assumption of homogeneity of variance underlying those tests were violated.

Lipid variances

In Table 3, we present the generation and gender specific variances, covariances, and correlations for adjusted lipid levels. The lipid variances ranged from 389.8 to 1167.9 for Total-C, 0.09 to 0.14 for lnTrig, 53.00 to

Table 2. Mean levels for lipids and apolipoproteins across gender and generation

		Mean				Mean	
		Males	Females			Males	Females
Total-C (mg/dl)	GP[a]	204.85	214.70	Apo AI (mg/dl)	GP[a]	134.76	149.26
	P	191.38	179.37		P	130.66	140.74
	C	148.81	148.77		C	127.7	128.35
lnTrig (ln mg/dl)	GP	4.95	4.89	Apo AII (mg/dl)	GP	33.04	34.77
	P	4.83	4.52		P	34.03	34.36
	C	4.43	4.43		C	32.73	32.76
HDL-C (mg/dl)	GP	41.41	51.38	lnApo E (ln-mg/dl)	GP	1.70	1.77
	P	40.39	50.59		P	1.53	1.48
	C	45.57	46.82		C	1.38	1.40
LDL-C (mg/dl)	GP	138.97	140.55				
	P	128.92	120.69				
	C	88.99	94.46				

[a]GP = grandparent, P = parent, C = child.

Table 3. Lipid variances (diagonal elements), covariances (elements of upper triangle) and correlations (elements of lower triangle)

		Total-C		lnTrig		HDL-C		LDL-C	
		Males	Females	Males	Females	Males	Females	Males	Females
Total-C	GP[a]	1095.91	1167.92	2.67	4.42	61.78	61.11	972.81	1014.10
	P	949.44	647.12	4.05	5.23	28.78	2.90	838.22	643.71
	C	485.03	389.87	1.95	0.85	35.56	40.66	430.34	342.63
lnTrig	GP	0.224**	0.363**	0.13	0.13	−1.46	−1.46	0.92	2.97
	P	0.354**	0.553**	0.10	0.14	−1.28	−1.44	2.13	6.90
	C	0.297**	0.134	0.09	0.10	−0.69	−1.24	1.36	2.29
HDL-C	GP	0.198**	0.174**	−0.431**	−0.399**	89.15	106.02	9.39	−11.00
	P	0.127	0.012	−0.467**	−0.395**	53.95	95.82	4.56	−108.25
	C	0.219**	0.232**	−0.322**	−0.435**	53.00	78.69	−6.91	−50.62
LDL-C	GP	0.954**	0.953**	0.084	0.268**	0.032	−0.034	949.30	969.42
	P	0.961**	0.912**	0.202**	0.669**	0.022	−0.399**	801.32	769.28
	C	0.947**	0.866**	0.225**	0.355**	−0.040	−0.285**	417.17	401.35

*$p < 0.05$; **$p < 0.01$.
[a]GP = grandparents, P = parents, C = children.

106.02 for HDL-C, and 401.35 to 969.42 for LDL-C. Results from the homogeneity of variance tests across generations and between genders are presented in Table 4(A). Total-C variation was generation dependent in males and females and gender dependent in parents. lnTrig variation was generation dependent in males only. HDL-C variation was generation dependent in males and gender dependent in children and parents. LDL-C variation was generation dependent in males and females. Significant differences in variance across generations were attributable to an increase in variance with increasing age. The significant difference between genders was attributable to increased Total-C variance and decreased HDL-C variance in males when compared to females in the same generation.

From a biological point of view, the observed generation effect on variance may indicated that the homeostatic control mechanisms are weakening with age resulting in increased deviation from the mean value. The observed gender effects on variance may indicate that influences of sexual dimorphism are more pronounced in the parent generation than in the child and grandparent generation.

From a risk prediction point of view, the generation and gender effects on lipid variances provide additional information on the fraction of individuals in each cohort which exceed a particular high risk threshold value. If the null hypothesis (H_0) of homogeneity of variance were true, then cohort differences in the fraction of individuals at high risk would be attributable to differences in mean levels only. On the other hand, if the alternative hypothesis (H_1) is demonstrated, then cohort differences in the proportion of individuals at high risk is the consequence of differences in both mean and variance. In Fig. 1 we have illustrated the impact of means and variances (pooled and cohort specific) on the fraction of individuals in each cohort that exceed the standard high risk thresholds for Total-C and HDL-C. In both examples, the means plus or minus 3 standard deviations are plotted for each cohort under the null hypothesis (H_0) and the alternative hypothesis (H_1). In Fig. 1(a), a threshold of 240 mg/dl Total-C was used to discriminate between individuals at high and low risk. We calculated under the alternative hypothesis (H_1) that 14.5% of the MGP, 23.0% of the FGP, 5.7% of the MP, and 0.8% of the FP will be at high risk of CAD. Under the null hypothesis (H_0),

Table 4. Homogeneity of variance, variance–covariance matrices and correlation coefficients among lipids across gender and generation

(A) Homogeneity of variance

Null hypotheses	*df*	Total-C	lnTrig	HDL-C	LDL-C
		(χ^2 values)			
MGP = FGP = MP = FP = MC = FC[a]	5	99.20**	16.53**	46.76**	74.74**
MGP = MP = MC	2	32.64**	11.80**	17.87**	34.24**
FGP = FP = FC	2	64.61**	4.44	4.82	40.45**
MGP = FGP	1	0.21	0.01	1.55	0.02
MP = FP	1	8.57**	0.01	19.14**	0.10
MC = FC	1	2.84	1.44	7.76**	0.07

(B) Homogeneity of bivariate variance–covariance matrices (χ^2-values) where superscript b and c denote tests of correlation coefficients

Null hypotheses	*df*	Total-C: lnTrig	Total-C: HLD-C	Total-C: LDL-C	lnTrig: HDL-C	lnTrig: LDL-C	HDL-C: LDL-C
				(χ^2 values)			
MGP = FGP = MP = FP = MC = FC	15	142.21**[c]	155.76**[c]	195.19**[b]	68.73**[c]	168.19**[b]	161.22**[b]
MGP = MP = MC	6	47.16**[c]	52.05**[c]	55.29**[c]	31.01**[c]	50.40**[c]	52.54**[c]
FGP = FP = FC	6	88.77**[b]	75.98**[c]	88.23**[b]	11.00	87.63**[b]	70.78**[b]
MGP = FGP	3	2.67	1.94	0.78	2.20	3.95	2.01
MP = FP	3	23.62**[b]	29.46**[c]	51.99**[b]	24.73**[c]	51.58**	37.88**[b]
MC = FC	3	7.26	11.05*[c]	37.32**[b]	8.53*[c]	3.26	12.65**[c]

*$p < 0.05$; **$p < 0.01$.

[a]MGP = male grandparent, FGP = female grandparent, MP = male parent, FP = female parent, FC = female child, MC = male child.

[b]Heterogeneity of the vector of correlation coefficients, $p < 0.05$.

[c]Homogeneity of the vector of correlation coefficients, $p > 0.05$.

the fraction of individuals crossing the risk threshold is overestimated in children and female parents and underestimated in male parents and grandparents. In Fig. 1(b), a threshold of 30 mg/dl HDL-C was used to discriminate between individuals at high and low risk. We calculated under the alternative hypothesis (H_1) that 11.3% of MGP, 1.9% of FGP, 7.9% of MP, 1.8% of FP, 1.6% of MC, and 2.9% of FC will be at high risk of CAD. Under the null hypothesis (H_0), the proportion of individuals crossing the risk threshold is overestimated in children and male parents and underestimated in female parents and grandparents. These figures illustrate one method for using the information provided by means and variances to characterize the relationship between lipid levels and risk of CAD. If a graduated risk curve were known for these cohorts, then a Taylor series expansion about the mean of the cohorts would provide a good estimate of the fraction of individuals at risk of CAD. Other measures, such as the covariation between traits, should enhance the precision of the estimates of the proportion of individuals which are in different risk categories.

Lipid covariances and correlations

For any metabolic system, its steady state or homeostatic set point is determined by the covariation or correlation between the traits in the system. For normally distributed physiological traits, individuals in the population may exist in any one of five types of physiological states—the normal state, the compensated hyperfunctional state, the compensated hypofunctional state, the uncompensated hyperfunctional state, and the uncompensated hypofunctional state [30]. These different physiological states are expected have different risks of disease. For this reason we have examined the covariances and correlations among lipid traits in each cohort and tested for differences across generation and gender.

In cases where we had already noted significant heterogeneity of variance, tests for homogeneity of variance–covariance matrices are uninformative. We therefore relied on tests of the homogeneity of correlation coefficients across strata to reveal which members of the subset of bivariate distributions were generation and/or gender specific [Table 4(B)]. Given the results from the the tests for homogeneity of variance four types of outcomes are reported in Table 4(B):

(i) homogeneity of variance and homogeneity of variance–covariance matrices,
(ii) homogeneity of variance and heterogeneity of variance–covariance matrices,

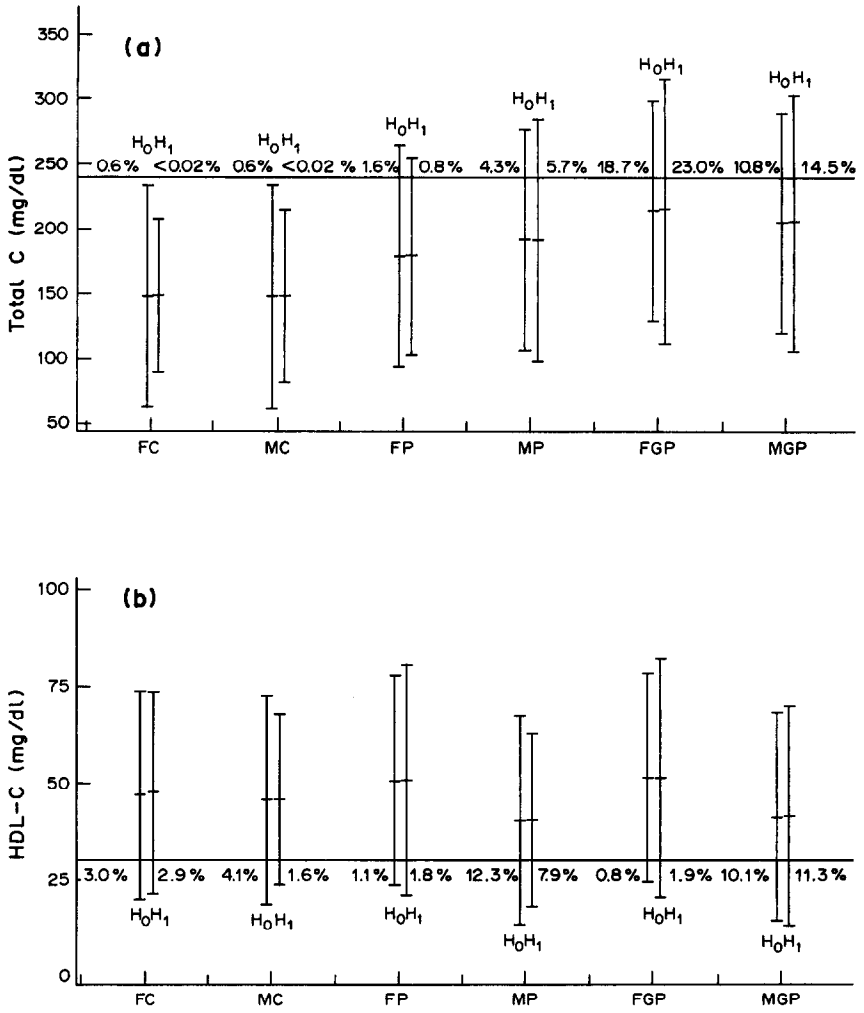

Fig. 1. (a) Total-C means ± 3 SD and risk threshold (H_0-pooled variance: H_1-cohort specific variance). (b) HDL-C means ± 3 SD and risk threshold (H_0-pooled variance: H_1-cohort specific variance).

(iii) heterogeneity of variance and homogeneity of correlation, and
(iv) heterogeneity of variance and heterogeneity of correlated.

We emphasize that the test statistics for the homogeneity of correlation coefficients have greater power to detected a given difference between correlation estimates when the values being compared are large, say ($r > 0.5$), than when the correlations are small, say $r < 0.5$.

There were only two instances where homogeneity of variance (type (i) and (ii) outcomes) allowed us to make definite inferences about covariance differences between the lipid levels. First, MGPs and FGPs had homogeneous covariances between all of their lipid levels (type (i) outcome). Second, male and female parents showed significant differences in their lnTrig:LDL-C covariation (type (ii) outcome). The corresponding Pearson product–moment correlation coefficients (Table 3) of $r = 0.202$ for MPs and $r = 0.669$ for FPs also illustrate the gender differences in lnTrig:LDL-C covariation.

In instances where heterogeneity of variance was demonstrated, the tests for homogeneity of the correlation coefficients indicated that Total-C:HDL-C and lnTrig:HDL-C correlations were similar across all strata (type (iii) outcome). The weighted average Total-C:HDL-C and lnTrig:HDL-C correlations were $r = 0.156$ and $r = -0.410$, respectively. On the other hand, we observed heterogeneity of correlations (type (iv) outcome) in the Total-C:LDL-C, Total-C:lnTrig, HDL-C:LDL-C, and lnTrig:LDL-C bivariate distributions across generations in females and between genders in the parental generation. Across

generations, we found that FPs had significantly stronger correlations for HDL-C:LDL-C (FP:$r = -0.399$ > FGP:$r = -0.034$, FC:$r = -0.285$), Total-C:lnTrig (FP:$r = 0.553$ > FGP:0.363, FC:$r = 0.133$), and lnTrig:LDL-C (FP:$r = 0.669$ > FGP:$r = 0.268$, Fc:$r = 0.355$) than female grandparents and children. In contrast, FGPs had significantly larger correlation between Total-C:LDL-C (FGP:$r = 0.953$ > FP:$r = 0.912$, FC:$r = 0.866$) than female parents and children but these differences in correlation are relatively small and probably not biologically relevant.

Between genders, in the parent generation, the significant heterogeneity in correlation coefficients was attributable to stronger correlations in females for HDL-C:LDL-C (FP:$r = 0.399$ < MP:$r = 0.022$) and Total-C:lnTrig (FP:$r = 0.553$ > MP:$r = 0.354$) and a stronger Total-C:LDL-C correlation in males (MP:$r = 0.962$ > FP:$r = 0.912$). In the child generation, only the Total-C:LDL-C correlation was significantly different across gender (MC:$r = 0.947$ > FC:$r = 0.866$). In general, the statistically significant differences in the Total-C:LDL-C correlations across cohorts may not be biologically relevant because the actual differences in correlation are small.

From a biological perspective, the generation effects on lipid covariances and correlations indicate that either the genetic covariances or environmental covariances between lipid traits must be changing with age. Consequently, the distribution of individuals within each of the five possible physiological states—normal, compensating or uncompensating hyperfunctioning or hypofunctioning—is shifting with age. The gender effects on the lipid covariance and correlations also provides evidence that the genetic or environmental covariances are sexual dimorphic.

From a risk assessment point of view, knowledge about the covariances or correlations enables one to partition the bivariate space defined by combinations of risk factors into high, medium and low risk categories. In Fig. 2 we illustrate that gender differences in HDL-C:LDL-C and lnTrig:LDL-C covariation is associated with differences in the proportion of individuals at risk of CAD. In Fig. 2(a), the 95% confidence ellipses for HDL-C and LDL-C levels in MPs and FPs show mean, variances, and covariance differences across genders and their impact on risk. Using risk thresholds of 30 mg/dl HDL-C and 180 mg/dl LDL-C, it is evident that a greater proportion of MPs are in the HDL-C and LDL-C medium risk range than FPs because of the lack of correlation. However, if males had a strongly negative HDL-C:LDL-C correlation, like the females, then a much greater proportion of the MP cohort would cross into the high risk domain. In Fig. 2(b), the 95% confidence ellipses for lnTrig and LDL-C levels in MPs and FPs also show that means, variances, and covariances combine to determine differences in fraction of individuals at risk. Using risk thresholds of 5.25 ln mg/dl lnTrig and 180 mg/dl LDL-C, there is a greater fraction of MPs in the medium and high risk ranges than FPs. However, in contrast to the HDL-C:LDL-C example, this figure illustrates that the interaction between means, variances, and covariances predicts that some proportion of the FP cohort will be in the high risk category.

Apolipoprotein levels

The AI, AII, and E apolipoproteins have distinct functions in lipoprotein metabolism. The Apo AI and AII are cofactors of lecithin-acyl transferase in HDL particles [49]. The Apo E is a protein component of chylomicron, VLDL, and some HDL particles and functions as a ligand for the lipoprotein uptake by the liver and peripheral tissues with E-receptor and B–E receptors [50]. The mean levels of Apo AI, AII, and E presented in Table 2 range from 127.70–149.26 (mg/dl) for Apo AI, 32.73–34.77 (mg/dl) for Apo AII, and 1.38–1.77 (ln mg/dl) for lnApo E. Apo AI and lnApo E mean levels were lowest in the children, intermediate in the parents, and highest in the grandparents. Apo AII mean levels were highest in the FGPs and FPs and lowest in the MC.

Apolipoprotein variances

Since apolipoproteins are gene products which mediate the catabolism of lipoprotein particles, variability in their levels must be controlled by gene regulation [25, 27–30]. Differences in variance across generation or gender could imply differences in the homeostatic control of gene regulation in response to aging or gender specific metabolic processes. The cohort specific variances, covariances, and correlations for the apolipoprotein variables are presented in Table 5. The apolipoprotein variances ranged from 163.62–309.77 for Apo AI, 12.15–15.74 for Apo AII, and 0.11–0.13 for lnApo E. In contrast to the substantial

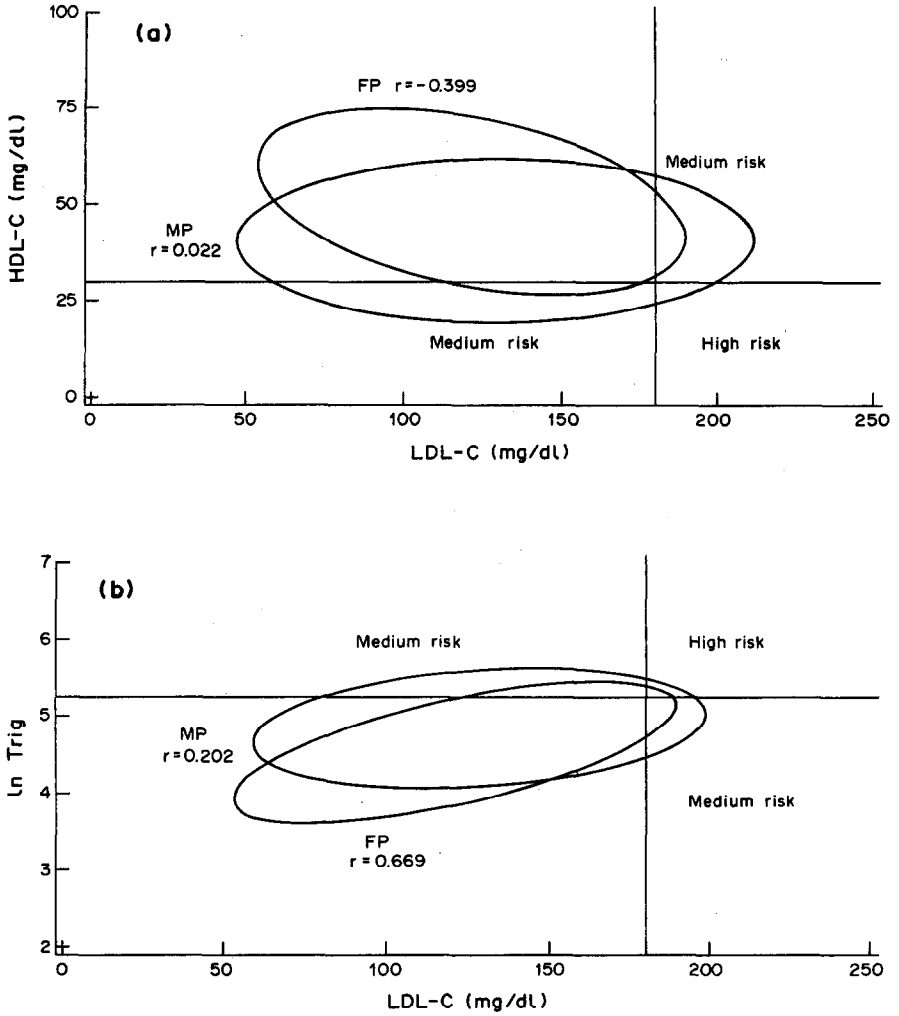

Fig. 2. (a) Ninety-five percent confidence ellipses for HDL-C and LDL-C levels in male and female parents. (b) Ninety-five percent confidence ellipses for lnTrig and LDL-C levels in male and female parents.

heterogeneity of variance noted for the lipid distributions among cohorts, tests for the homogeneity of variance (Table 6) revealed that only Apo AI variability was significantly different across generations in males and between genders in the parental generation. The significant effect of generation in males was associated with increasing variability in Apo AI levels with increasing age. The significant effect of gender in the parental generation was attributable to larger variability in Apo AI levels in females than in males.

From a biological point of view, the generation and gender effect on Apo AI variances but not Apo AII or lnApo E variances may be a consequence of differences in the regulation of Apo AI, Apo AII, and Apo E genes. These results may also indicate that the homeostatic control of Apo AI gene expression weakens with age in males but not females and is different in male and female parents. The homogeneity of Apo AII and lnApo E variance across cohorts does not imply that the proportion of variation explained by genetic and environmental factors are the same across cohorts. It only implies that the total phenotypic variation is constant.

Apolipoprotein covariances and correlations

The covariation and correlation between apolipoproteins (Table 5) was also more homogeneous across cohorts than the covariation and correlation among lipids traits. Heterogeneity in the Apo AII:lnApo E variance–covariance matrices across all six cohorts, in the absence of evidence for heterogeneity of variance, was the only exception (Table 6). This heterogeneity of covariance was not substantial enough to significantly affect the correlation coefficients. Tests for the homogeneity of correlation coefficients indicated that all correlations,

Table 5. Apolipoprotein variances (diagonal elements), covariances (elements of upper triangle) and correlations (elements of lower triangle)

		Apo AI		Apo AII		lnApo E	
		Males	Females	Males	Females	Males	Females
Apo AI	GP[a]	309.77	259.93	33.02	24.28	0.08	0.39
	P	188.19	249.44	20.97	24.48	−0.11	0.16
	C	163.62	191.29	18.97	20.50	0.65	0.49
Apo AII	GP	0.473**	0.410**	15.74	13.49	0.04	−0.04
	P	0.404**	0.445**	14.32	12.15	0.02	0.13
	C	0.400**	0.423**	13.75	12.26	0.27	0.20
lnApo E	GP	0.012	0.75	0.027	−0.033	0.13	0.11
	P	−0.023	0.031	0.014	0.113	0.12	0.11
	C	0.145*	0.108	0.206**	0.176*	0.12	0.11
		Apo AI		Apo AII		lnApo E	

*$p < 0.05$; **$p < 0.01$.
[a]GP = grandparents, P = parent, C = child.

including Apo AI:Apo AII and Apo AI:lnApo E correlations, were homogeneous across all cohorts. The average correlation for Apo AI:Apo AII was $r = 0.425$, for Apo AI:lnApo E was $r = 0.056$, and for Apo AII:lnApo E was $r = 0.080$. It should be noted that the MC have a statistically significant Apo AI:lnApo E correlation ($r = 0.145$) and MC and FC have statistically significant Apo AII:lnApo E correlations (MC:$r = 0.206$, FC:$r = 0.176$) while the other cohorts show no significant correlation between these traits. The homogeneity of covariance across generations or genders indicates that the relationship between apolipoprotein levels is stable throughout life and not influenced by gender differences. Also, homogeneity of covariance across cohorts implies that these bivariate distributions do not provide additional risk information beyond the information about high risk subgroups which is available from examining the univariate means and variances.

Lipid and apolipoprotein covariation and correlation

Characterizing the covariation and correlation between an individual's apolipoprotein and lipid levels is of particular importance because it reflects the relationship between levels of gene products and intermediate traits that are measures of lipid metabolism that may link genetic and environmental variation to variation in CAD risk. The correlations (Table 7) between the apolipoproteins and lipids can be divided into two classes based on the average correlation across cohorts. The first class includes those traits which were statistically significantly correlated, $r > 0.2$, and the second class includes those traits that are marginally correlated, $r < 0.2$. The traits involved in each class are consistent with known physiological relationships between the lipids and apolipoproteins. The significantly correlated variables include HDL-C and the protein constituents of the HDL particle, Apo AI and Apo AII, with weighted average correlations of $r = 0.714$ and $r = 0.309$, respectively. Other members in the class of strongly correlated gene products and intermediate phenotypes include Total-C with each of the apolipoproteins and lnTrig with lnApo E. The class of bivariate distributions with marginally significant correlations were of less interest, but still noteworthy, because specific cohorts that show deviations from the remaining cohorts may indicate qualities that characterize high or low risk profiles. This set of correlations included lnTrig with Apo AI and with Apo AII, LDL-C with each of the apolipoproteins, and HDL-C lnApo E.

There are several instances where homogeneity of variance (type (i) and (ii) outcomes) allowed us to make definite inferences about covariance differences between apolipoproteins and lipids across cohorts (Table 8). First, for females the covariances of lnTrig with each of the apolipoproteins and HDL-C with each of the apolipoproteins were homogeneous across generations. Second, MC and FC had homogeneous covariances between Total-C, lnTrig, or LDL-C and each of the apolipoproteins. Third, MPs and FPs showed similar magnitudes of covariation in their LDL-C:Apo AII, LDL-C:lnApo E, and lnTrig:lnApo E distributions. In contrast, there were significant differences in lnTrig:Apo AII covariation between MPs and FPs. The lnTrig:Apo AII correlations in MPs and FPs were $r = -0.050$ and $T = 0.180$, respectively. Also, between genders, MGPs and FGPs showed approximately equal

Table 6. Homogeneity of variance, variance–covariance matrices among apolipoproteins across gender and generation

Null hypotheses	df	Homogeneity of variance (χ^2 values) Apo AI	Apo AII	lnApo E	df	Homogeneity of variance-covariance matrix superscripts where b and c denote tests of correlation coefficients (χ^2 values) Apo AI: Apo AII	Apo AI: lnApo E	Apo AII: lnApo E
MGP = FGP = MP = FP = MC = FC[a]	5	29.56**	4.84	3.58	15	35.51**[c]	37.76**[c]	18.99**[c]
MGP = MP = MC	2	22.69**	0.92	0.21	6	22.89**[c]	26.39**[c]	6.24
FGP = FP = FC	2	5.62	0.79	0.11	6	7.32	6.53	6.40
MGP = FGP	1	1.61	1.24	2.01	3	2.42	4.16	3.63
MP = FP	1	4.65*	1.58	0.52	3	7.52	5.48	3.48
MC = FC	1	1.22	0.66	1.02	3	2.29	2.42	1.62

*$p < 0.05$; **$p < 0.01$.
[a]MGP = male grandparent, FGP = female grandparent, MP = male parent, FP = female parent, MC = male child, FC = female child.
[c]Homogeneity of the vector of correlation coefficients, $p > 0.05$.

covariation in all lipid and apolipoprotein bivariate distributions, except HDL-C:Apo AI covariation which was significantly greater in MGPs than in FGPs. Given that the correlations between HDL-C and Apo AI are not very different (FGP:$r = 0.750$, MGP:$r = 0.787$), it seems unlikely that the observed statistical significance is biologically relevant.

In instances where heterogeneity of variance was demonstrated, the tests for homogeneity of correlation coefficients provided evidence for heterogeneity in Total-C:lnApo E and HDL-C:lnApo E correlation (type (iv) outcome) across generations in females and males, respectively. Across generations in females we found that FPs had a substantially stronger Total-C:lnApo E correlation (FP:$r = 0.303$) than FC and FGPs (FC:$r = 0.201$, FGP:$r = 0.071$). Across generations in males, we found that MC had a significant, positive HDL-C:lnApo E correlation (MC:$r = 0.217$) while MPs and MGPs had weak, negative correlations (MP:$r = -0.111$, MGP:$r = -0.021$).

To summarize these results, we observed homogeneity of covariance (type (i)) or homogeneity of correlation (type (iii)) among all six cohorts in the bivariate distributions of Total-C:Apo AI (weighted average $r = 0.253$), Total-C:Apo AII (weighted average $r = 0.269$), lnTrig:Apo AI (weighted average $r = -0.044$), lnTrig:lnApo E (weighted average $r = 0.229$), HDL-C:Apo AII (weighted average $r = 0.309$), LDL-C:Apo AI (weighted average $r = 0.027$), LDL-C:Apo AII (weighted average $r = 0.165$), and LDL-C:lnApo E (weighted average $r = 0.150$). In contrast we observed significant heterogeneity in HDL-C:Apo AI covariation between genders in grandparents, in lnTrig:Apo AII covariation between genders in parents, in Total-C:lnApo E correlations across generations in females, and in HDL-C:lnApo E correlations across generations in males.

Summary of lipid and apolipoprotein covariances and correlations

Table 9 presents a summary of the generation and gender effects on the covariation and correlation between lipid and apolipoprotein traits. The table is separated into generation effects (lower triangle) and gender effects (upper triangle). The *Y* indicates a statistically significant difference across cohorts, the "?" indicates that variance and covariance differences are confounded, and the blanks indicate homogeneity of covariance across cohorts. This table

Table 7. Person product–moment correlations and (covariances) between lipids and apolipoproteins

		Total-C		lnTrig		HDL-C		LDL-C	
		Males	Females	Males	Females	Males	Females	Males	Females
Apo AI	GP[a]	0.224**	0.284**	−0.165*	−0.014	0.787**	0.750**	0.088	0.069
		(151.18)	(155.66)	(−1.04)	(−0.08)	(130.73)	(124.58)	(47.95)	(34.83)
	P	0.197**	0.216**	−0.150*	0.005	0.696**	0.719**	0.082	−0.096
		(83.50)	(86.72)	(−0.77)	(0.03)	(70.17)	(111.16)	(31.95)	(−42.23)
	C	0.347**	0.221**	0.037	0.020	0.685**	0.623**	0.128	−0.102
		(98.85)	(60.27)	(0.14)	(0.09)	(63.82)	(76.44)	(33.56)	(−28.40)
Apo AII	GP	0.341**	0.266**	0.032	0.194**	0.393**	0.289**	0.244**	0.150*
		(44.81)	(33.42)	(0.04)	(0.25)	(14.72)	(10.93)	(29.77)	(17.11)
	P	0.229**	0.162*	−0.050	0.180**	0.324**	0.272**	0.188**	0.038
		(26.72)	(14.41)	(−0.07)	(0.23)	(9.00)	(9.28)	(20.15)	(3.65)
	C	0.399**	0.235**	0.136	0.177*	0.306**	0.281**	0.299**	0.087
		(32.96)	(16.22)	(0.15)	(0.20)	(8.27)	(8.73)	(22.64)	(6.09)
lnApo E	GP	0.191**	0.071	0.293**	0.200**	−0.021	0.065	0.121	0.001
		(2.27)	(0.79)	(0.04)	(0.02)	(−0.07)	(0.22)	(1.34)	(0.01)
	P	0.271**	0.303**	0.350**	0.259**	−0.111	−0.013	0.208**	0.283**
		(2.91)	(2.55)	(0.04)	(0.03)	(−0.28)	(−0.04)	(2.05)	(2.60)
	C	0.263**	0.201**	0.147*	0.097	0.217**	0.200**	0.176*	0.096
		(2.05)	(1.30)	(0.02)	(0.01)	(0.56)	(0.58)	(1.26)	(0.62)

*$p < 0.05$; **$p < 0.01$.

[a]GP = grandparents, P = parents, C = children.

Table 8. Homogeneity of bivariate lipid and apolipoprotein variance–covariance matrices where superscripts b and c denote results from test of homogeneity of correlation coefficients

Null hypotheses	*df*	Total-C: Apo AI	Total-C: Apo AII	Total-C: lnApo E (χ^2 values)	lnTrig: Apo AI	lnTrig: Apo AII	lnTrig: lnApo E
MGP = FGP = MP = FP = MC = FC[a]	15	134.63**[c]	114.56**[c]	117.35**[c]	53.58**[c]	34.44**[b]	27.20**[c]
MGP = MP = MC	6	61.77**[c]	42.20**[c]	35.67**[c]	37.61**[c]	16.78**[c]	15.03*[c]
FGP = FP = FC	6	69.38**[c]	66.29**[c]	76.00**[b]	10.15	5.26	6.67
MGP = FGP	3	2.08	2.10	3.57	3.82	4.51	2.61
MP = FP	3	13.82**[c]	9.87*[c]	9.99*[c]	7.91*[c]	8.20*[b]	1.48
MC = FC	3	6.11	5.16	3.74	2.69	2.30	2.78

Null hypotheses	*df*	HDL-C: Apo AI	HDL-C: Apo AII	HDL-C: lnApo E (χ^2 values)	LDL-C: Apo AI	LDL-C: Apo AII	LDL-C: lnApo E
MGP = FGP = MP = FP = MC = FC	15	75.03**[b]	56.43**[c]	69.07**[b]	114.49**[c]	90.17**[c]	91.23**[c]
MGP = MP = MC	6	27.22**[c]	18.05**[c]	30.54**[b]	57.51**[c]	39.06**[c]	36.12**[c]
FGP = FP = FC	6	8.91	5.62	10.44	50.87**	42.19**	51.34**
MGP = FGP	3	8.84*	4.79	4.36	1.65	2.05	3.39
MP = FP	3	23.27**[c]	23.32**[c]	21.08**[c]	8.43*[c]	4.01	1.75
MC = FC	3	15.48**[c]	9.28*[c]	9.18*[c]	6.66	5.12	1.60

[a]MGP = male grandparent, FGP = female grandparent, MP = male parent, FP = female parent, FC = female child, MC = male child.
[b]Heterogeneity of the vector of correlation coefficients, $p < 0.05$.
[c]Homogeneity of the vector of correlation coefficients, $p > 0.05$.

illustrates that there were significant generation effects and gender effects on lipid covariation in the Total-C:lnTrig, Total-C:LDL-C, lnTrig:LDL-C, and HDL-C:LDL-C bivariate distributions. There were significant generation effects on the Total-C:lnApo E and HDL-C:lnApo E distributions and significant gender effects on HDL-C:Apo AI and lnTrig:Apo AII distributions. No generation or gender effects were found in any of the apolipoprotein covariances or correlations. Table 9 also illustrates that a substantial proportion of the bivariate distributions have confounded variance and covariance differences across cohorts. One method to resolve the confounded nature of these bivariate distributions is to examine the relationship between the correlation coefficients and the geometric means of the variances (i.e. the denominator of the correlation coefficient) across cohorts. This method resolves approximately half of the confounded differences across cohorts (data not shown) into variance dominated or covariance dominated difference in correlations across cohorts. The development of statistical procedures for testing covariance differences (exclusively) would eliminate this problem.

Principal component analyses

Since lipid metabolism is an integrated assemblage of lipid and apolipoprotein traits which function as a unit, the ability of the lipid metabolic pathway to respond to environmental changes depends upon the nature of the relationships among traits. Defining the extent to which the levels of lipid and apolipoprotein traits are interrelated is therefore a means of identifying the sets of traits which may cumulatively result in predisposition or resistance to CAD. Principal components analysis is one method for identifying the sets of covarying lipid and apolipoprotein traits. The eigenvalues and eigenvectors associated with the first two principal components are presented for each cohort in Fig. 3. The eigenvalues represent the variances of the principle components determined by orthogonal weighted functions of the seven adjusted lipid and apolipoprotein variables. The test results indicate that the eigenvalues for both components were homogeneous across all strata. The first and second principal components combined explained approximately 64% of the total sample variance in each cohort. Although there is no specific test for heterogeneity of eigenvectors across strata, the magnitude and direction of the eigenvector weightings for each principal component provide a profile for comparison (Fig. 3).

In all cohorts, the first principal component explained approximately 35% of the total cohort variance. Figure 3(a) illustrates that the eigenvector weightings for the first component are approximately the same across cohorts except for FPs. In all cohorts except FPs, the first principal component was dominated by the positive weighting of Total-C, LDL-C, Apo AI, Apo AII, and HDL-C. In contrast, FPs had the most distinct profile in that LDL-C, Total-C,

Table 9. Generation and gender effects on covariance correlation (Y = significant effect, ? = confounded variance–covariance effect, blank = no effect)

Generation effect		Gender effect: TOTAL-C	InTRIG GP	InTRIG P	InTRIG C	HDL-C GP	HDL-C P	HDL-C C	LDL-C GP	LDL-C P	LDL-C C	APO AI GP	APO AI P	APO AI C	APO AII GP	APO AII P	APO AII C	InAPO E GP	InAPO E P	InAPO E C
TOTAL-C				Y			?	?		Y	Y		?			?			?	
InTRIG	M	?					?	?		Y			?				Y			
	F	Y																		
HDL-C	M	?	?							Y	?	Y	?	?		?	?		?	?
	F	?																		
LDL-C	M	?	?			?							?							
	F	Y	Y			Y														
APO AI	M	?	?			?			?											
	F	?							?											
APO AII	M	?	?			?			?			?								
	F	?							?											
InAPO E	M	?	?			Y			?			?								
	F	Y							?											

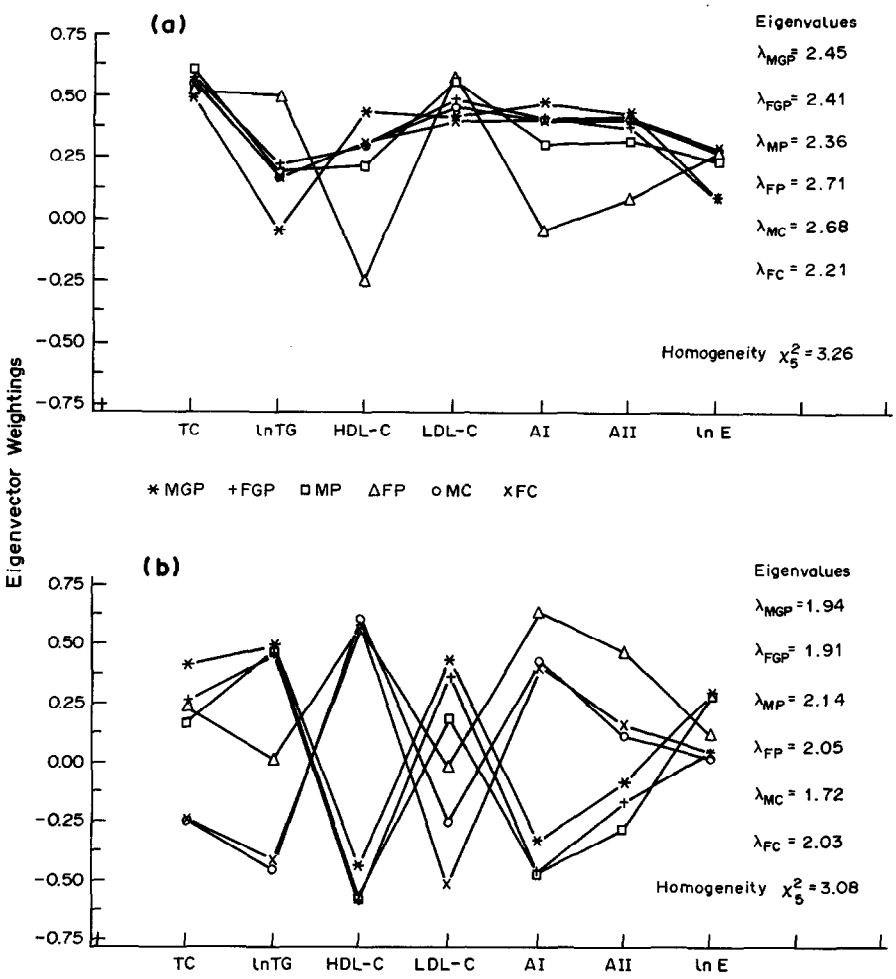

Fig. 3. (a) First prinicipal component. (b) Second principal component.

and lnTrig—in this rank order—were the dominant positive contributors in the eigenvector.

The second principal component explained an additional 28% (approximately) of the total cohort variation. In this principal component [Fig. 3(b)] there is negative covariance between HDL-C:lnTrig, HDL-C:LDL-C, and lnTrig:Apo AI. The eigenvector weightings of this principal component [Fig. 3(b)] clearly separates the six cohorts into two classes. The second component of MGPs, FGPs, and MPs show a positive association between Total-C, lnTrig, LDL-C, and lnApo E and a negative association with HDL-C, Apo AI, and Apo AII. FPs, MC, and FC have eigenvectors with an opposite orientation to the MGP, FGP, and MP type of eigenvector. Although the third through seventh principal components also exhibited heterogeneity across cohorts, they are not presented here because, separately, they each represent less than 15% of the multivariate variation.

DISCUSSION

Mean levels of lipids and apolipoproteins

Our data agree with other investigations of the impact age and gender have on mean levels of measures of lipid metabolism [52–56]. Between genders, women tend to have higher concentrations of the molecules, such as HDL-C and Apo AI, associated with decreased risk to CAD and men tend to have higher levels of metabolites, such as Total-C and LDL-C associated with increased risk of CAD. In our study, we found only two exceptions to this pattern of gender differences: FC show substantially higher levels of LDL-C than MC and FGPs have higher levels of Total-C than MGPs. In the Bogalusa Heart Study, Freedman *et al.* [56] also report higher LDL-C concentrations in FC vs MC. Tyroler *et al.* [53] report that older females have higher Total-C levels than males of comparable age.

Age-associated increases in mean levels of lipid measures are well established [57] and may represent a metabolic drift as homeostatic control mechanisms weaken with age [40, 58]. In our study, the mean levels of the lipids and apolipoprotein levels, with the exception of Apo AII, dramatically increased across generations. This age-dependent rise in levels has also been demonstrated in longitudinal studies [58, 59]. An age-dependent profile of lipid levels is most likely due to a combination of environmental (e.g. changes in diet and exercise) and biological factors (e.g. changes in the biological activity of gene products or hormone production associated with aging). Biochemical studies indicate that the increase in total Total-C could be due to increased LDL-C production, increased VLDL-C production and decreased fractional clearance of LDL-C, or decreased LDL-C-receptor activity [60]. Further investigations are needed to determine the genetic and environmental causes of this age-dependent risk factor profile.

Generation and gender specific variability

Our results indicate that heterogeneity of variance across generation and gender is highly significant for the lipid traits but not for the apolipoprotein traits. There are at least four possible explanations for the variance differences across cohorts: (i) differences in additive genetic variance, (ii) differences in environmental variance, (iii) differences in gene by environment interaction, and (iv) some combination of (i), (ii), and (iii) [45, 46]. In other words, the differences in variation across cohorts will emanate from external, internal, or interaction between internal and external forces.

For a complex system such as lipid metabolism there is a hierarchy associated with the metabolic network [61–64]. We hypothesize that the different degree of heterogeneity of variance between lipids and apolipoproteins is due to their position within the hierarchy of biological macromolecules (e.g. intermediate traits vs gene products). We expect different susceptibilities to perturbation by environmental factors at different levels in the hierarchy [63, 64]. Within this context, we would expect apolipoprotein levels to be less susceptible to perturbation by environmental variation and more susceptible to perturbation by genetic variation. In contrast, interindividual differences in lipid levels are expected to depend more on gene by environment interaction and environmental variation and less on genetic variation. The role of gene by environment interaction in determining phenotypic variation has been well established in animal and plant genetics [47, 48] but there has been very little recognition of the role that gene by environment interaction and possibly covariation may have on changes in phenotypic variance during human development and senescence [65, 66].

Given the hypothesis stated above, the observed increase in variation in lipid levels across generations could represent differential exposures to variation in diet, exercise, alcohol or cigarette consumption, and stress which each have effects on lipid metabolism. Children are expected to be exposed to a smaller range of environmental variations and therefore show less phenotypic variability. A fraction of the increased phenotypic variability in parents and grandparents may be due to the cumulative effects of exposures to environmental factors over a greater age range. In addition, the observed increase in lipid variation could be associated with differences in gene by environment interaction.

Gender differences in lipid level variation may also be products of environmental and biological factors. Men and women commonly differ in their attention to diet and exercise as well as differential attraction to alcohol and cigarette consumption or stress related activities [67]. Also, gender-specific hormone production and gender-related differences in the homeostatic mechanisms underlying the metabolic systems could affect the metabolite variability in a particular cohort [32–35]. In this cross-sectional study, the significant heterogeneity between genders in the parent generation could reasonably be explained by these biological and environmental differences. In contrast, the homogeneity of variance between MGPs and FGPs may be a product of selective survivorship in our sample. In the truncated sample used in these analyses, there were only 184 MGPs vs 242 FGPs. This ratio was approximately the same in the untruncated sample (i.e. 226 MGP, 331 FGP). The homogeneity of variance between MGPs and FGPs may also be due to diminishing sexual dimorphism.

Generation and gender specific covariability

It is widely accepted that changes in lipid levels with respect to age and gender are discriminators of high and low risk of CAD. The age and gender differences in the relationship

between lipid traits also has the potential to discriminate between high and low risk groups (Fig. 2). The bivariate correlations which were heterogeneous across strata represent a set of potential metabolic relationships which are associated with risk—especially, the correlation differences which are unique to the high risk age and gender groups, such as MPs, FGPs and MGPs. From our results we would predict that weak levels of lnTrig:LDL-C, HDL-C:LDL-C, and HDL-C:lnApo E covariation are associated with increased risk of CAD. In contrast, strong, positive lnTrig:LDL-C and HDL-C:lnApo E correlations and strong, negative HDL-C:LDL-C correlations are associated with decreased risk.

In general, our estimates of the correlations among lipids and apolipoproteins were in agreement with other studies [52–55, 68, 69]. The effects of gender and age differences on lipid correlations have been also demonstrated in several studies [54, 68–70]. These studies agree with our findings that there is no association between LDL-C and HDL-C levels in males but a significant negative association in females not taking hormones [54, 68]. The gender and generation differences in corrrelations may be due to similar or unknown environmental and/or biological factors which affected the variance of these metabolites. However, it is important to note that differences in trait covariation across cohorts must be associated with differences in environmental, genetic, or gene by environment covariation [45, 71]. In other words, the pleiotropic effects of genes or environments must be different across cohorts [72]. For example, if smoking is associated with a 15% decrease in HDL-C levels and a 10% increase in LDL-C levels in MPs, then in MGPs smoking may only be associated with 5% decrease in HDL-C levels and still a 10% increase in LDL-C levels. In genetic terms, a change or difference in genetic covariation is attributable to differences in pleiotropic effects across cohorts. In animal and plant genetics, many studies have shown that pleiotropic relationships which increase reproductive fitness change to antagonistic pleiotropic relationships during the development of senescence [41, 73, 74]. This phenomena is especially relevant in the study of CAD since the relationship between LDL-C and HDL-C levels may positively influence reproductive fitness through sex hormone levels but during senescence the relationship between these cholesterols may shift to an antagonistic pleiotropic relationship.

The principal component analyses summarizes the covariance and correlation differences across cohorts. The striking similarity between the MGP's, FGP's and MP's second principal components provides evidence that these individuals may be in the same risk category despite differences in their levels of the risk factor traits. The homogeneity of the eigenvalues across cohorts indicates that there are similar partitions of the multivariate space of lipid metabolites even when the lipid and apolipoprotein levels, variances, and covariances are heterogeneous across cohorts. In other words, the homogeneity of the first and second principal component's eigenvalues across strata suggests that distinct subspaces, such as a sphere or ellipsoid within a more complex three-dimensional space, may have their diameter or major axis (i.e. principal component) defined by several different functions of the individual variables (i.e. eigenvectors). One could hypothesize that these distinct and orthogonal subspaces are a consequence of the balance between stabilizing (homeostatic) and adaptive (dynamic or kinetic) mechanisms which work independently to ensure viability of the organism (within the context of the metabolic system under consideration) [25, 61, 63, 64].

CONCLUSION

Significant heterogeneity among cohorts in the second moments of the multivariate distribution of lipid metabolites suggests some guidelines or at least cautions for future genetic and epidemiological studies. First, males and females should be analyzed separately. This is already a heuristic for many investigators when they are examining average differences in trait levels. Variance and covariance homogeneity across genders should be demonstrated before pooling males and females together to analyze primary hypotheses about causation. Second, the age window under analysis should be defined carefully with respect to the underlying question being asked. Third, the assumption of no age and gender effects on the mean and variance of a trait should be demonstrated before the trait is analyzed to determine if a single gene (measured or unmeasured) with a significant effect on trait levels is segregating in pedigrees. Regression to the age and gender mean of a large sample of pedigrees will not remove the heterogeneity observed in this study

but only distort it. Age and gender dependent penetrance functions in genetic analyses are required to deal with this problem.

Although we have pointed out several instances where differences in the relationship between variables across cohorts may be important to assessing risk of CAD, further studies are needed to evaluate the utility of variability and covariability in predicting risk. By examining the variance and covariance profile of individuals in different clinical subgroups with CAD, we hope to define the multivariate profiles (i.e. means, variances, and covariances) which discriminate between subgroups in the population at large who have different levels of CAD risk. The current ability to measure an individual's genotype (either at the DNA or protein product level) for genes involved in lipid metabolism may also help to discriminate between subgroups in the population who have different levels of CAD risk. Further studies are needed to identify and characterize the genotypes and environments responsible for heterogeneity in the multivariate profile of risk factors among gender and age specific strata of the population at large.

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REFERENCES

1. Goldstein JL, Brown MS. The LDL receptor defect in familial hypercholesterolemia: Implications for pathogenesis and therapy. **Med Clin North Am** 1982; 66: 335–362.
2. Goldstein JL, Brown MS. Familial hypercholesterolemia. In: Stanbury JB, Wyngaardern JB, Frederickson DS, Goldstein JL, Brown MS, Eds. **The Metabolic Basic of Inherited Disease.** New York: McGraw-Hill; 1983: 672–712.
3. Berg K. Genetics of coronary heart disease. In: Steinberg AG, Bearn AG, Motulsky AG, Childs B, Eds. **Progress in Medical Genetics,** Vol V. Philadelphia: WB Saunders; 1983: 36–90.
4. Goldbourt U, Neufeld HN. Genetic aspects of arteriosclerosis. **Arteriosclerosis** 1986; 6: 357–377.
5. Davignon J, Dufour R, Contin M. Atherosclerosis and hypertension. In: Jacques G *et al.*, Eds. **Hypertension, Physiopathology, and Treatment.** New York: McGraw-Hill; 1983: 810–852.
6. Iselius H. Genetic epidemiology of common disease in humans. In: Weir BS, Eisen EJ, Goodman MM, Namkoong G, Eds. **Proc 2nd Int Conf on Quantitative Genetics.** Sunderland: Sinauer Associates; 1988: 250–269.
7. Sing CF, Boerwinkle E, Moll PP, Templeton AR. Characterization of genes affecting quantitative traits in humans. In: Weir BS, Eisen EJ, Goodman MM, Namkoong G, Eds. **Proc 2nd Int Conf on Quantitative Genetics.** Sunderland: Sinauer Associates; 1988: 250–269.
8. Brown MS, Goldstein JC, Frederickson DS. The familial hyperlipoproteinemias. In: Stanbury JB, Wyngaardern JB, Frederickson DS, Goldstein JL, Brown MS, Eds. **The Metabolic Basis of Inherited Disease.** New York: McGraw-Hill; 1978: 655–671.
9. Boerwinkle E, Visvikis S, Welsh D, Steinmetz J, Hanash SM, Sing CF. 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: 567–582.
10. Moll PP, Michels VV, Weidman WH, Kottka BA. Genetic determination of plasma apolipoprotein AI in a population-based sample. **Am J Hum Genet** 1989; 44: 124–139.
11. **Fiscal Year 1983 Fact Book, National Heart, Lung and Blood Institute.** Bethesda, Md: National Institutes of Health; 1983.
12. Barham D, Trinder P. An improved color reagent for the determination of blood glucose by the oxidase system. **Analyst** 1972; 98: 142–145.
13. Barr SE, Kottke BA, Mao SJT. Improved method for determination of triglycerides in plasma lipoproteins by an enzymatic kit method. **Clin Chem** 1981; 27: 1142–1144.
14. Izzo C, Grillo F, Murador E. Improved method for determination of high density lipoprotein cholesterol: Isolation of high density lipoproteins by use of polyethylene glycol-6000. **Clin Chem** 1981; 27: 371–374.
15. DeLong DM, DeLong ER, Wood PD *et al.* A comparison of methods for the estimation of plasma low and very-low-density lipoprotein cholesterol: The Lipid Research Clinics prevalence study. **JAMA** 1986; 256: 2372–2377.
16. Au YPT, Hallaway BJ, Kottke BA. Radioimmunoassay for apolipoprotein A-I: Use of a quality control plasma sample to decrease interassay variation. **Clin Chem** 1986; 32: 1394–1396.
17. Kottka BA, Zinsmeister AR, Holmes DR Jr, Kneller RW, Hallaway BJ, Mao SJT. Apolipoproteins and coronary artery disease. **Mayo Clin Proc** 1986; 61: 313–320.
18. Au YPT, Bren ND, Kottke BA. A rapid apolipoprotein E. radioimmunoassay using solid-phase staphylococcus protein. Use of pooled plasma as a secondary standard. **Biochem Biophys Res Commun** 1986; 138: 455–462.
19. Draper NR, Smith H. **Applied Regression Analysis.** New York: Wiley; 1982: 307–313.
20. Conover WJ. **Practical Nonparametric Statistics.** New York: Wiley; 1971: 302–306.
21. Morrison DF. **Multivariate Statistical Methods.** New York: McGraw; 1971: 152–153, 221–244.
22. Sokal RR, Rolf FJ. **Biometry,** 2nd end. New York: Freeman; 1981: 583–599.
23. **SAS User's Guide,** version 5 edn. Cary, N.C.: SAS Institute, Inc.; 1982.
24. Seber GAF. **Multivariate Observations.** New York: Wiley; 1984: 175–200.
25. Waddington CH. **The Strategy of Genes.** New York: Macmillan; 1957: 41–47.
26. von Bertalanffy L. **General System Theory.** New York: George Braziller; 1968: 156–171.
27. Heinrich R, Rapoport SM, Rapoport TA. Metabolic regulation and mathematical models. **Prog Biophys Molec Biol** 1977; 32: 1–82.
28. Johnson G. Enzyme polymorphism and metabolism. **Science** 1974; 184: 28–37.

29. Johnson G. Genetic polymorphism among enzyme loci. In: Scandalios JG, Ed. **Physiological Genetics.** New York: Academic Press; 1979: 239–271.
30. Grannis GA. The study of body-fluid composition in aging humans. II. The biogyron: concepts and examples in clinical chemistry. In: Deitz, Marcum, Eds. **Aging—It's Chemistry.** Washington, D.C.: American Association for Clinical Chemistry; 1980: 340–358.
31. Savageau MA, Sorribas A. Constraints among molecular and systemic properties: Implications for physiological genetics. **J Theor Biol** 1989; 141: 93–115.
32. Kin H, Kalkhoff RK. Changes in Lipoprotein composition during the menstrual cycle. **Metabolism** 1979; 28: 663–668.
33. Choi Y, Sugano M, Ide T. Sex-differences in the age related change of cholesterol metabolism in rats. **Mech Ageing Dev** 1988; 44: 91–99.
34. Smith DW, Huber RW. Does genotypic sex have a direct effect on longevity? **Exp Gerontol** 1989; 24: 277–288.
35. Gorbach SL, Schaefer EJ, Woods M *et al.* Plasma lipoprotein cholesterol and endogenous sex hormones in healthy young women. **Metabolism** 1989; 38: 1077–1081.
36. Kritchevsky D. Lipid metabolism in aging. **Mech Ageing Dev** 1972; 1: 275–284.
37. Kritchevsky D. How aging affects cholesterol metabolism. **Postgrad Med** 1978; 63: 133–138.
38. Choi Y, Ide T, Sugano M. Age-related changes in the regulation of cholesterol metabolism in rats. **Exp Gerontol** 1987; 22: 339–349.
39. Kreisberg RA, Kasim S. Cholesterol metabolism and aging. **Am J Med** 1987; 82(Suppl.): 54–60.
40. Woodbury MA, Manton KG. A mathematical models of the physiological dynamics of aging and correlated mortality selection. I. Theoretical development and critique. **J Gerontol** 1983; 38: 398–405.
41. Johnson TE. Genetic specification of life span: Processes, problems, and potentials. **J Gerontol** 1988; 43: 87–92.
42. Hanson WD. Genotypic stability. **Theor Appl Genet** 1970; 40: 226–231.
43. Martin NG, Eaves LJ. The genetical analysis of covariance structure. **Heredity** 1977; 38: 79–85.
44. Cheverud JM, Rutledge JJ, Atcheley WR. Quantitative genetics of development: Genetic correlations among age-specific trait values and the evolution of ontogeny. **Evolution** 1983; 37: 895–905.
45. Atcheley WR. Ontogeny, timing of development, and genetic variance–covariance structure. **Am Nat** 1984; 123: 519–540.
46. Via S, Lande R. Genotype–environment interaction and the evolution of phenotypic plasticity. **Evolution** 1985; 39: 505–522.
47. Westcott B. Some methods of analysing genotype–environment interaction. **Heredity** 1986; 56: 243–253.
48. Jinks JL, Pooni HS. The genetic basis of environmental sensitivity. In: Weir BS, Eisen EJ, Goodman MM, Namkoong G, Eds. **Proc 2nd Int Conf on Quantitative Genetics.** Sunderland: Sinauer Associates; 1988: 505–522.
49. Eisenberg S. High density lipoprotein metabolism. **J Lipid Res** 1984; 25: 1017–1058.
50. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. **Arteriosclerosis** 1988; 8: 1–21.
51. Castelli WP, Cooper GR, Doyle JT. Distribution of triglyceride and total, LDL and HDL cholesterol in several populations: A cooperative lipoprotein phenotyping study. **J Chron Dis** 1977; 30: 147–169.
52. Assmann G., Funke H, Schriewer H. The relationship of HDL-apolipoprotein A-I and HDL-cholesterol to risk factors of coronary heart disease: Initial results of the prospective epidemiological study in company employees in Westfalia. **Clin Chem Clin Biochem** 1982; 20: 287–289.
53. Tyroler HA, Heiss G, Schonfeld G, Cooper G, Heyden S, Hames CG. Apolipoprotein A-I, A-II, and C-II in black and white residents of Evans County. **Circulation** 1980; 62: 249–254.
54. Connor SL, Connor WE, Sexton G, Galvin L, Bacon S. The effects of age, body weight and family relationships on plasma lipoproteins and lipids in men, women and children of randomly selected families. **Circulation** 1982; 65: 1290–1298.
55. Donahue RP, Orchard TJ, Stein EA, Kuller LH. Apolipoproteins AI, AII and B in young adults: associations with CAD risk factors: The Beaver County experience. **J Chron Dis** 1986; 39: 823–830.
56. Freedman DS, Srinivasan SR, Webber LS, Bereson GS. Divergent levels of high density lipoprotein cholesterol and apolipoprotein A-I in children: The Bogalusa Heart Study. **Arteriosclerosis** 1987; 7: 347–353.
57. Wallace RB, Anderson RA. Blood lipids, lipid-related measures, and the risk of atherosclerotic cardiovascular disease. **Epidemiol Rev** 1987; 9: 95–119.
58. Manton KG, Woodbury MA. A mathematical model of the physiological dynamics of aging: II. Application to the Duke longitudinal study. **J Gerontol** 1983; 4: 406–413.
59. Hershcopf RJ, Elahi D, Andres R *et al.* Longitudinal changes in serum cholesterol in man: An epidemiological search for an etiology. **J Chron Dis** 1982; 35: 101–114.
60. Grundy SM, Vega GL, Bilheimer DW. Kinetic mechanisms determining variability in low density lipoprotein levels and rise with age. **Arteriosclerosis** 1985; 5: 623–630.
61. Waddington CH. **Tools for Thought.** New York: Basic Books; 1977: 48–63.
62. Mesarovic MD. Theory of hierarchial structures. In: Haken H, Ed. **Synergetics.** F.R.G.: B.G. Teubner; 1973: 255–262.
63. von Bertalanffy L. **Perspectives on General System Theory.** New York: George Braziller; 1975: 97–102, 137–148.
64. Salthe SN. **Evolving Hierarchial Systems.** New York: Columbia University Press; 1985: 153–155, 235–238.
65. Berg K. Variability gene effect on cholesterol at the Kidd blood group locus. **Clin Gen** 1988; 33: 102–107.
66. Sing CF, Moll PP. Genetics of variability of CHD risk. **Int J Epidemiol** 1989; 18(Suppl.): S183–S195.
67. Wilson PWF, Garrison RJ, Abbott RD, Castelli WP. Factors associated with lipoprotein cholesterol levels. **Arteriosclerosis** 1983; 3: 273–281.
68. Davis CE, Gordon D, LaRosa J, Wood PDS, Halperin M. Correlations of plasma high-density lipoprotein cholesterol levels with other plasma lipid and lipoprotein concentrations: The Lipid Research Clinics program prevalence study. **Circulation** 1980; 62 (Suppl. IV): 24–30.
69. Cowan LD, Wilcosky T, Criqui MH *et al.* Demographic, behavioral, biochemical, and dietary correlates of plasma triglycerides. **Arteriosclerosis** 1985; 5: 466–480.
70. Abbot RD, Garrison RJ, Wilson PWF *et al.* Joint distribution of lipoprotein cholesterol classes. The Framingham study. **Arteriosclerosis** 1983; 3: 260–272.
71. Falconer DS. **Introduction to Quantitative Genetics.** London: Longman; 1981: 112–126.
72. Lande R. The genetic covariance between characters maintained by pleiotropic mutations. **Genetics** 1980; 94: 203–215.
73. Williams GC. Pleiotropy, natural selection, and the evolution of senescence. **Evolution** 1957; 11: 398–411.
74. Rose MR, Graves JL. What evolutionary biology can do for gerontology. **J Gerontol** 1989; 44: B27–29.