Genetic and environmental variation in serum lipoproteins in relation to coronary heart disease

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SUMMARY Variation in the serum concentration of VLDL, IDL, LDL, and HDL was studied in (a) 192 survivors of myocardial infarction under the age of 50 living in the north-east of Scotland; (b) 250 relatives, mostly first degree; and (c) 259 unrelated individuals, comprising mostly spouses and their relatives.

The biochemical characterisation of the lipids, which were separated by preparative ultracentrifugation, included (a) determination of total serum cholesterol and triglyceride; and (b) determination of the content of cholesterol in the four fractions, and also of triglyceride in the VLDL fraction.

In male survivors of infarction there is a significant negative regression of VLDL and a significant positive regression of HDL on peak aspartate aminotransferase activity in blood samples taken within 24 hours of the incident. In later samples correlations were not evident.

Serum lipoprotein concentrations have been compared in samples taken at different times after the incident. The average value of samples taken within 24 hours is similar to the mean scores for LDL and VLDL of repeat samples taken, on average, 9 months later.

The incidence of hyperlipidaemia in samples taken within 24 hours of the infarction is about the same for total cholesterol and triglyceride and LDL or VLDL. Respectively, 18.3%, 16.1%, and 7.5% of the survivors exceed the 90th centile value for either LDL, VLDL, or both fractions, while, for the 95th centile limit, the corresponding figures are 8.6%, 9.7%, and 2.2%.

In samples taken within 24 hours of the incident, the LDL concentration is, on average, 20 to 30% higher, and the VLDL concentration 50 to 60% higher, than the controls. The corresponding HDL difference in males, though negative, is trivial and statistically insignificant, but in females the difference is greater and significantly so.

VLDL levels are substantially increased in regular cigarette smokers in both sexes, especially in males. HDL levels tend to be lower, though not significantly so. The frequency of persons who are or have been regular cigarette smokers is higher in index cases than in controls.

Correlation analysis of individual variation of controls and index case relatives indicates a substantial level of independence between the major lipoprotein fractions. There is no correlation between LDL and HDL. There are positive correlations between VLDL, IDL, and LDL, though when IDL is held constant by multiple regression, the correlation between VLDL and LDL is removed. There is a low but consistent negative correlation between VLDL and HDL cholesterol.

About a quarter of the variance of VLDL in males is accounted for by multiple regression on measures of body fatness, that is, relative weight and subscapular skinfold thickness. In females only about 10% of the variance is thus accounted for, and only in males do LDL levels show correlated changes with fatness. There are no significant differences for either relative body weight or subscapular skinfold thickness between the means of first degree relatives of survivors of infarction and controls.

The concentrations of VLDL and LDL in first degree relatives of survivors of infarction are significantly higher than the control means. HDL shows no significant difference.

The evidence for genetic variation in serum lipoprotein is based on the polygenic model and the analysis of the correlation between parents and offspring and the correlation between sibs. From

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the pooled regression on single parents, heritabilities for HDL, LDL, and VLDL work out as 0.67 ± 0.21 , 0.36 ± 0.18 , and 0.23 ± 0.20 , respectively. The lower heritability of VLDL is consistent with the variance of repeat measurements on the same control individuals, which is much higher for VLDL than for LDL or HDL. The high correlation between VLDL and measure of fatness, for which heritability estimates are statistically insignificant, as well as the association between raised VLDL concentration and smoking, also provide confirmatory evidence of the major importance of non-genetic causes in the variation of VLDL.

The results are discussed in relation to the origin of the effects of smoking, variation in proneness to coronary disease, and the biological significance of differences in HDL concentration.

Raised levels of serum cholesterol and/or triglyceride are often associated with coronary heart disease, but the origin of the lipid differences and the physiological relations which underlie the statistical association with coronary disease remain obscure. The role of heredity in proneness to heart disease has been suggested by comparisons of incidence in the first degree relatives of survivors of a heart attack (Slack, 1969, 1975). It has been claimed that increased levels of either cholesterol or triglyceride, or both lipids, in families of survivors may involve three separate genes (Goldstein *et al.*, 1973 a, b; Hazzard *et al.*, 1973). The present study is chiefly concerned with evidence for genetic variation in the serum concentration of the major lipoprotein fractions. The analysis refers to survivors of myocardial infarction under the age of 50, living in north-east Scotland, their first and second degree relatives, and a control group which comprises the spouses of the survivors and the corresponding relatives of the spouse.

In addition to the serum lipoprotein levels, many other items of information have been collected, including records of age, weight, height, and subscapular skinfold thickness, various kinds of clinical evidence, survival rate and the occurrence of heart disease in relatives, smoking habits, etc. Six hundred individuals were involved in the genetic study, which included 91 index cases, 250 index case relatives, and 259 controls.

Materials and methods

THE SAMPLE

The index cases were drawn from patients of either sex under 50 admitted to Aberdeen hospitals, for whom a diagnosis of coronary heart disease was made according to the definitions laid down by WHO in the Technical Report Series No. 168, 1959.

Acute myocardial infarction is defined as one of the following: (1) unequivocal electrocardiogram of recent infarction; the development of abnormal Q wave, with or without associated ST changes (and with a typical or suggestive history); (2) equivocal

electrocardiographic evidence of recent infarction (ST changes) with or without a typical history; (3) normal electrocardiogram with abnormally high level(s) of appropriate serum enzyme(s) and typical history.

In addition to the index cases included in the initial survey we have continued to record the lipoprotein scores of myocardial infarction patients under the age of 50, and this further group comprises 86 males and 15 females.

The index relatives, mostly first degree, include 105 males and 145 females. As a matter of policy, blood samples were not collected from persons below the age of 16. Since progeny of spouses are also first degree relatives of index cases, to preserve the age distribution of the control series, blood samples were also obtained from 69 individuals unrelated either to one another or to persons included in the survey. These blood samples were drawn from members of the hospital technical staff and also from the local police force. The entire group comprises 99 males and 160 females. The age distribution was similar in controls and index case relatives.

Availability of blood samples from relatives of spouses or index cases was determined by whether or not they were resident in north-east Scotland. For our study area, about 75% of present residents were born in the district so the population can be regarded as comparatively static.

SERUM LIPIDS

The biochemical characterisation of lipids in fasting serum included: (1) observations of turbidity and 'creaming' for determination of chylomicrons; (2) determination of total serum cholesterol and triglyceride; (3) electrophoresis on cellogel membrane; (4) separation by ultracentrifugation of the $S_f > 20$, $S_f 12$ to 20, $S_f 0$ to 12, and the residual HDL fractions. The corresponding upper density limits are, respectively, 1.0067, 1.020, and 1.063, so that $S_f > 20$ refers to values < 1.0067 , and HDL to values > 1.063 . The four fractions of increasing density are referred to as VLDL, IDL, LDL, and HDL, respectively; (5) determination of cholesterol

on all such fractions and of triglyceride on the VLDL fraction.

These measurements cover the criteria for lipoprotein typing discussed in the WHO Memorandum 'Classification of Hyperlipidaemia and Hyperlipoproteinaemias' (1970).

Blood samples were collected after an overnight fast of 12 to 14 hours. Chylomicrons were noted in a small proportion of cases (4.6%), and all such instances have been excluded from the analysis of lipoprotein fractions, since they probably represent breaches of the fasting rule. The few individuals (1.1%) who had been treated for diabetes or who were receiving drugs designed to lower lipoprotein levels were also excluded. Breakage of centrifuge tubes during separation of lipoproteins led to loss of a few records, and there were also occasional missing values for other variables.

Cholesterol estimation was based on the saponification and extraction technique of Abell *et al.* (1952), followed by colour development using the stabilised Liebermann-Burchard reagent of Kim and Goldberg (1969). Triglyceride estimation was carried out by the commercial enzymatic technique of Boehringer Mannheim, based on the studies of Eggstein and Kreutz (1966). It should be noted that free glycerol is included in the estimates of total serum triglyceride and contributes about 10 mg/100 ml to the total.

Repeatability of estimates over a wide range of lipid concentrations fell well within the limits specified by the Lipid Standardisation Program of the US Centre for Disease Control, Atlanta, Georgia (Phase 1).

STATISTICAL ANALYSIS

Although the lipoprotein fractions are metabolically interrelated and share certain constituents, they manifest considerable independence in the variation of their serum concentration. Hence they may be handled as separate traits for comparison with other physiological variables and for genetic analysis, while similarities of behaviour can be described in terms of correlation and regression analysis.

Age correction

It is well known (Lopez *et al.*, 1967; Gofman *et al.*, 1968; Goldstein *et al.*, 1973a) that scores of total cholesterol and total triglyceride increase with age to a maximum at 40 to 50 years, followed by a variable decline. Authors vary in the procedure for age correction. We have adopted the pragmatic approach of fitting a curve of the form $y = a + b_1x + b_2x^2$ separately for each sex, where b_1 and b_2 refer to the regression coefficients, and x refers to the age. Where the addition of the squared term failed

Table 1 Statistically significant regression coefficients derived from fit of curve of the form $y = a + b_1x + b_2x^2$ to log transformed lipoprotein scores (mg/100 ml) of males and females separately where x refers to age

Variable	Male		Female	
	b_1	b_2	b_1	b_2
VLDL cholesterol	—	—	0.00539	—
VLDL triglyceride	—	—	0.00410	—
IDL cholesterol	0.01556	-0.00014	0.00563	—
LDL cholesterol	0.01333	-0.00013	0.00233	—
HDL cholesterol	—	—	—	—
Total cholesterol	0.01024	-0.00009	0.00590	-0.00004
Total triglyceride	0.00168	—	0.00437	—

to produce a significant reduction in the variance, it has been ignored and the coefficient of linear regression on x has been used. In some instances, for example, VLDL in males, the intrinsic variability is so high that even the linear regression coefficients are statistically quite insignificant, in which case no correction has been applied. Table 1 shows the coefficients calculated for log transformed control records.

Scale

Total triglyceride and VLDL, the chief vehicle of triglyceride, show positive skew distributions, and a log transformation makes the distribution more normal without entirely removing the skewness. Hence there are clear advantages in the use of the log scale for triglyceride values generally.

For cholesterol, comparisons of observed and expected age-corrected values at 5 centile intervals for total cholesterol on either the log or arithmetical scale yield χ^2 of 5.0 and 7.9, respectively, for 16 degrees of freedom, while for the LDL cholesterol fraction the corresponding log and arithmetic scale yield χ^2 values of 8.3 and 13.8. For total cholesterol on the log scale neither the estimate for skewness (-0.082) nor kurtosis (0.110) approach the limits of significance for a sample of 250 individuals, while, on the arithmetical scale, both measures of departure from normality are highly significant, 0.546 and 0.541 for skewness and kurtosis, respectively. Therefore, for total cholesterol there is a clear case for preferring the log scale.

For the LDL fraction, the decision is more difficult. Though the log scale gives a formally better estimate of agreement between observed and expected, judged by the relatively weak χ^2 test, there is significant skewness and kurtosis on both scales. Thus, for the log scale, skewness and kurtosis work out at -0.64 and 1.47, respectively, compared with 0.41 and 0.59 on the arithmetical scale. However, we have chosen to use the log scale for the convenience of directly comparing proportional differences in other variables.

One minor consequence of the use of the log versus the arithmetical scale is that corresponding centile values will be marginally higher on the former. Thus, in our control population, the 90th and 95th centile values derived from the mean and standard deviation of the log transformed data work out at 301 and 324 mg/100 ml, respectively, for total cholesterol, and 194 and 215 mg/100 ml for LDL cholesterol, these being the antilogs of the original figures. The corresponding values on the arithmetical scale work out at 296 and 312 mg/100 ml for total cholesterol and 190 and 203 mg/100 ml for LDL. Thus, if we apply the 90th and 95th centile values as pragmatic criteria for hyperlipidaemia, the use of the log scale means that the centile values are increased by a few percent.

The quoted centiles were derived from the mean and standard deviation of the transformed data. Comparison with the absolute values obtained by ranking the values in descending order indicated only trivial differences. When considering the centiles, it must be remembered that the antilogs of the transformed values cannot be directly compared with the untransformed values without minor adjustment (Goldstein *et al.*, 1973a). Nevertheless, they provide a general guide to the more important trends and differences.

Variability

The variance of the lipoprotein scores between individuals of the control population is the result of both genetic and different kinds of environmental or other non-genetic effects. Such non-genetic effects may be formally partitioned into: (1) variation between estimates on individual aliquots within tests; (2) variation between identical aliquots in tests carried out at different times. These two components contribute to what we term error variance; and (3) variation between blood samples drawn from the same individual at different times, that is, within-individual variation. A number of control individuals provided blood samples on two separate occasions; the mean time between samples was 7.2 ± 1.2 months.

Variation within individuals will also include variation between ultracentrifugal separations of lipoproteins such as could be measured by independent fractionation of samples drawn on a single occasion. This component has not been estimated sufficiently often to provide a valid estimate, but it is likely to be minor relative to the other sources of variation between samples. Differences between individuals as a result of genetic causes, as well as environmental effects not accounted for by the within-individual variation referred to under (3), make up the fourth component.

Estimates of the four components are available from determinations of total cholesterol and total triglyceride. Variation resulting from 'error variance' is trivial (0.7% or less), and combining it with the within-individual variation accounts for 13 and 25% respectively, of the variation of total cholesterol and triglyceride. Triglyceride is subject to considerably higher non-genetic variation than cholesterol, and the within-individual variation is about ten times higher than for total cholesterol, and some four times higher than for the combined genetic and residual environmental effects. In the repeat measurements of the lipoprotein fractions, VLDL is about ten times as variable as HDL on the log scale, while LDL is generally similar to HDL in this respect.

Results

CONTROL POPULATION

Using the regression coefficients listed in Table 1 the log transformed values have been adjusted to age 45. The means, standard deviations, and centiles for the lipoprotein fractions, as well as total cholesterol and total triglyceride, are listed in Table 2, which also includes the antilogs of the means. At age 45 the mean values for males and females are not significantly different, except for HDL which is significantly higher in females by an average of 17%. There are striking differences in the standard deviations; thus the value for VLDL is some three times greater than for HDL.

To provide a comparison between our results and the values reported for another population of similar age distribution, Table 3 shows the values quoted by Goldstein *et al.* (1973a, b) for the means of total cholesterol and total triglyceride compared with our corresponding values. In the Seattle study,

Table 2 Average serum lipoprotein concentrations (mg/100 ml) of controls adjusted to age 45, standard deviations and centiles, sexes combined or adjusted to male equivalent (HDL)

Lipoprotein fraction	Log scale		Antilogs			
	Mean	δ	Centile		Mean	
			90th	95th	90th	95th
VLDL cholesterol	1.138	0.318	1.546	1.661	14	35
VLDL triglyceride	1.699	0.318	2.107	2.222	50	128
IDL cholesterol	0.811	0.232	1.108	1.193	6	13
LDL cholesterol	2.128	0.125	2.288	2.334	134	194
HDL cholesterol	1.741	0.101	1.870	1.907	55	74
Total cholesterol	2.367	0.087	2.479	2.510	233	301
Total triglyceride	2.035	0.189	2.277	2.346	108	189

Means are based on 243-247 individuals; antilogs are rounded to nearest unit.

Table 3 Average scores (mg/100 ml) of total serum cholesterol and total serum triglyceride in different populations. All values are adjusted to age 45

Study	Cholesterol		Triglyceride	
	Male	Female	Male	Female
Aberdeen	229	235	88	89
Seattle ¹	220	220	94	97

¹The Seattle figures are taken from the paper by Goldstein *et al.* (1973a).

cholesterol values were not transformed to a log scale. We have quoted the antilog of the means of our transformed scores. These work out at 229 and 235 mg/100 ml compared with the Seattle values of 220 for either sex. We have also estimated the mean of total cholesterol without transforming to a log scale but correcting for age by similar multiple regression. The mean values work out at 233 and 240 mg/100 ml for males and females, respectively. Thus the score for total cholesterol in our population is a little higher than that of the Seattle population.

In the case of total triglyceride the scores were log transformed in both studies, though the antilogs of the means are quoted in Table 3. Our figures have been adjusted to allow for the difference in procedure for estimating triglyceride. The values for the two studies do not differ appreciably; thus serum lipid concentrations are rather similar in the two populations.

It may be noted that the 90th and 95th centile values quoted of 301 and 324 mg/100 ml for total cholesterol and 189 and 222 mg/100 ml for total triglyceride are higher than the corresponding values quoted in the Seattle study of 270 and 285 mg/100 ml for cholesterol and 147 and 165 mg/100 ml for triglyceride.

INDEX CASES

Post-incident concentrations

The significance of the serum concentration of a particular lipoprotein fraction in a patient who has recently suffered a myocardial infarction depends upon when the sample was taken after the incident. Though plasma cholesterol concentration does not change during the first 24 to 26 hours after the incident (Dodds and Mills, 1959; Fyfe *et al.*, 1971; Kirkeby, 1972), changes occur thereafter, while other constituents like total catecholamines, free fatty acids, cortisol, and glucose, may show differences within an hour of the event (Vetter *et al.*, 1974). Dodds and Mills (1959) reported that the Sf > 20 fraction differs from the others in not showing a decline during the first few days after an incident. Deegan and Hayward (1965) reported a fall to a

minimum at about 5 to 6 days followed by a steady increase thereafter. Fyfe *et al.* (1971) reported that serum triglyceride tended to fall after admission, but not greatly so, while Kirkeby (1972) claimed that there was at first little change in the fasting triglyceride concentration, though later there was a rise. Individuals probably differ in the sequence of lipoprotein changes after an incident, and when reports are based on small numbers this adds to the uncertainty.

Though, as Dodds and Mills (1959) have observed, only records before and after the incident inspire unqualified confidence, post-incident records may not be wholly uninformative with respect to pre-infarction concentrations. This aspect has been examined as follows:

- (1) Fasting blood samples have been classified according to the time after the incident at which they were collected, namely up to 24 hours, between 25 and 36 hours, and after 36 hours.
- (2) In a proportion of the index cases in the main survey, an additional blood sample was taken an average of 9.2 months after the incident, that is, at a time when the effect of the infarction on serum lipoprotein concentration might be expected to have worn off.
- (3) For 58 of the 80 males who provided samples within 24 hours of the incident, we have records of the severity of the infarction in terms of peak activity of aspartate amino-transferase (International Units per litre) so that we can test whether lipoprotein concentrations are correlated with the severity of the incident.

Table 4 shows the mean values for VLDL, LDL, and HDL as percentage deviations from the control means, according to when the blood sample was collected. The scores of the index cases have been adjusted only for age and include the evidence from both the survey and the post-survey patients. Males show statistically highly significant average VLDL deviations, which work out at 59, 105, and 53%, respectively, for the groups in which blood samples were collected at successively longer intervals after infarction. In females, the sample sizes are smaller and only for the first period, up to 24 hours, is the similar difference statistically significant. For LDL in males, there is a highly significant positive deviation of 24% above the control mean for the first period, while the means of the samples taken at later times do not significantly differ from the control mean. Possibly at some time between 25 and 36 hours changes occur which promote a lowering of the LDL concentrations in the serum, and this may

Table 4 Average percent deviation from control means of major lipoprotein fractions according to the interval of time the blood sample was collected after the incident

	Males			Females		
	Deviation (%)	N	P	Deviation (%)	N	P
VLDL						
—24 hours	+ 59.4	80	<0.001	+48.1	13	<0.01>0.001
25–36 hours	+104.6	26	<0.001	–25.6	3	>0.5
>36 hours	+ 53.0	19	<0.02>0.01	+55.2	3	>0.2
LDL						
—24 hours	+ 24.0	80	<0.001	+33.0	13	<0.001
25–36 hours	+ 1.6	26	>0.5	+13.4	3	>0.40
>36 hours	+ 9.3	17	>0.2	+26.5	3	<0.10>0.05
HDL						
—24 hours	– 4.2	80	<0.2>0.1	–27.7	13	<0.001
25–36 hours	– 1.5	26	>0.5	–12.4	3	<0.2>0.1
>36 hours	– 6.9	17	>0.2	–29.1	3	<0.02>0.01

The percentage difference is derived from the antilogs of the means of the log transformed records.

contribute to the relatively lower values after the 24-hour period. For the corresponding differences in females, the mean deviation is 33% for the first period, while the other differences, though positive, do not significantly differ from the control mean. Finally, for HDL, the three average deviations in males are negative, do not exceed a few percent, and are hence statistically insignificant. In females, however, the reductions are considerable: 28% for the first period and 12 and 29%, respectively, for the later ones. In the first period the difference is statistically significant and, in spite of the small numbers, the same is true of the difference for the third period. The apparent sex difference in degree of reduction of HDL merits further study.

For the comparisons between samples taken at the time of the incident and those taken several months later, possible confusion arises from the differences in treatments, for example, atromid, weight reducing diets, etc., to which some of the patients were exposed. For VLDL in males, though the concentrations in untreated tend to be higher than in treated persons, the differences are statistically insignificant and have been ignored. For 23 paired records, subtracting the average of the second from that of the first sample gives a value of 0.05 ± 0.06 , a statistically insignificant difference. If the effects of infarction on lipoprotein concentration have worn off by the time of the second sample, this suggests that the VLDL concentration in the first 24-hour period may not be greatly different from that of the preinfarction concentration. For LDL, there is no indication of differences according to post-incident treatment and the average difference between first and second samples is trivial, consistent with the view that samples taken within 24 hours of the incident may reflect preinfarction concentrations. For HDL, there is evidence of a significant effect of treatment, which is associated with relatively higher

HDL levels. For the 7 untreated males, the average concentration in the second sample is approximately 10% lower than in the first.

For comparison of the effects of the severity of the infarction with the lipoprotein concentration, the peak aminotransferase activity has been converted to the log scale. As shown in Table 5, there is a highly significant negative correlation in males between the peak activity level and VLDL, measured either by the triglyceride or cholesterol moieties. The regressions on activity work out at -0.42 ± 0.15 and -0.40 ± 0.15 , respectively, for VLDL triglyceride and cholesterol. The regression of total triglyceride on activity works out at -0.25 ± 0.09 . For HDL, the association is positive and statistically significant at 0.11 ± 0.05 . There is a high negative correlation of -0.47 between VLDL and HDL. Only for LDL cholesterol is there a lack of significant association with activity. For the much smaller sample of females there are signs of a different relationship. Though statistically insignificant, the correlation between activity and VLDL triglyceride is positive, while

Table 5 Relations between peak serum aspartate aminotransferase activity and lipoprotein concentration in blood samples collected within 24 hours of infarction

	Males			
	r	b	N	P
VLDL	–0.36	-0.42 ± 0.15	58	<0.01>0.001
LDL	0.16	0.07 ± 0.06	58	>0.20
HDL	0.30	0.11 ± 0.05	58	<0.05>0.01
	Females			
	r	b	N	P
VLDL	0.07	0.07 ± 0.31	10	>0.5
LDL	0.64	0.20 ± 0.09	10	<0.05>0.01
HDL	–0.35	-0.08 ± 0.03	10	<0.05>0.01

Aspartate activity is expressed as log IU/l.

the regression of HDL on activity is negative and significantly so. For LDL also, there is a significant positive correlation and a regression of 0.20 ± 0.09 . Larger numbers are required to test the validity of this apparent sex difference.

Thus, for males, within the first 24 hours of the incident, it appears that the greater the severity of the infarction, the lower the concentration of VLDL and the higher the concentration of HDL. At later periods the relationship disappears. Thus, for samples taken within the 25- to 36-hour period, the regressions on aspartate for VLDL and HDL are -0.01 ± 0.32 and -0.04 ± 0.09 , respectively.

Incidence of hyperlipidaemia

It is also useful to record the incidence of abnormality in terms of hyperlipidaemia, for which the 90th and 95th centiles of the single-tailed distribution may be taken as arbitrary criteria for the occurrence of more or less extreme abnormality.

For VLDL and total triglyceride the percentages quoted in Table 6 are based exclusively on samples collected within the first 24 hours of the incident. For LDL, from the preceding discussion, we should be entitled to include estimates taken several months after the incident, but, to provide a reliable comparison with instances in which both the VLDL and LDL concentrations in the same individual exceed the centile values, only samples taken within 24 hours of the incident have been used.

The frequency of hyperlipidaemia is virtually the same whether either VLDL or LDL concentrations or the records of total triglyceride or total cholesterol are used. The frequency of survivors under the age of 50 in which both lipids exceed one or other centile is higher than the product of the frequency with which one alone exceeds the corresponding centile limit, but not greatly so. Combining the frequencies and sexes, 42% of the index cases are

Table 7 *Percentage deviation of mean serum lipoprotein concentrations of first degree relatives from the mean concentration of controls*

Lipoprotein fraction	Difference (%)	P
VLDL	+ 16.8	<0.01 >0.001
IDL	+ 9.0	>0.05
LDL	+ 8.9	<0.01 >0.001
HDL	+ 2.1	>0.05

The percentages are based on the antilogs of the means, which are derived from data adjusted to age 45 and corrected for smoking. HDL values of females are adjusted to male equivalents. Sexes are averaged for all fractions.

hyperlipidaemic at the 90th centile level, compared with 11.5% of controls, while at the 95th centile the corresponding values are 20.5 and 4.3%.

INDEX CASE RELATIVES

Table 7 summarises the differences percent between the mean lipoprotein concentration of first degree relatives of the index cases in the survey and the means of the control population. The percentages are based on the antilogs of the transformed data, adjusted to age 45, and corrected for smoking habit, as described in the next section. The deviations are positive and significantly so for VLDL and LDL. In spite of the higher average VLDL score of the relatives, the average HDL deviation is also positive, though statistically insignificant, and provides a contrast with the usual inverse relationship between VLDL and HDL.

Familial differences of the kind noted here may be compared with the evidence for higher levels of serum cholesterol and triglyceride reported by Patterson and Slack (1972) and Nikkila and Aro (1973). The correlation between index case and spouse is relevant here. For comparisons where a sufficiently reliable estimate for the serum concentration in the index case is available, the correlations for VLDL, LDL, and HDL work out at the statistically insignificant values of -0.36 (23), 0.06 (62), and 0.02 (62), respectively; the figures in parentheses refer to the number of paired observations for each comparison. Since we might expect the day to day similarity of diet and general environment of the pairs of spouses to be greater than that of first degree relatives, living often in different households, the evidence of Table 7 favours a genetic contribution to the familial differences.

SMOKING HABITS

Before dealing with the relations between the lipoprotein concentrations and other variables, we consider the effect of cigarette smoking, which is the most obvious environmental variable. Persons have been classified into three categories, namely, those

Table 6 *Percentage incidence of hyperlipidaemia in survivors of infarction under age 50*

	90th centile		95th centile	
	Index cases	Controls	Index cases	Controls
LDL	18.3	3.4	8.6	1.7
VLDL	16.1	6.4	9.7	1.7
Both	7.5	1.7	2.2	0.9
Total	41.9	11.5	20.5	4.3
TC	18.3	3.6	10.5	2.8
TT	17.2	7.9	10.5	3.6
Both	9.7	3.2	2.1	2.0
N	93	246	93	246

Sexes are combined. TC and TT refer, respectively, to total serum cholesterol and total serum triglyceride. LDL and VLDL refer to estimates of the cholesterol and triglyceride content, respectively. The evidence for the index cases is derived exclusively from blood samples collected within 24 hours of infarction.

Table 8 *Effect of smoking: percentage deviation of mean concentration of serum lipoprotein fractions in regular smokers from the mean score of persons who have never smoked*

	Male		Female	
	Deviation (%)	P	Deviation (%)	P
VLDL	33.7	<0.01	22.8	<0.01
LDL	1.1	>0.05	11.9	<0.05
HDL	-0.7	>0.05	-4.8	>0.05

who have never smoked cigarettes or only occasionally so, persons who were regular smokers but have stopped, and persons who are regular cigarette smokers. The average serum lipoprotein concentration of present and former smokers did not significantly differ, and so the values for these two groups have been combined for comparison with non-smokers. The difference between smokers and non-smokers is approximately equivalent in controls and index case relatives, and so the data were combined to arrive at the differences listed in Table 8.

In regular smokers, the VLDL levels are substantially and significantly higher in both sexes, by about 30% in males and 25% in females. The proportional increase is equally evident in the concentration of either VLDL cholesterol or VLDL triglyceride. The LDL concentration is significantly higher in women smokers, but not so in men. For HDL cholesterol, though the differences are statistically insignificant in either sex, the smokers tend to have lower values, reflecting the familiar inverse relationship between VLDL and HDL cholesterol. Lipoprotein concentrations dealt with in the subsequent analysis have been corrected for smoking habit.

Of male index cases, 87.7% were classed as smokers, though most had given up the habit after their infarction. Of male controls and index case relatives combined, 72.8% were classed as smokers and the difference is statistically significant ($P < 0.02 > 0.01$). For the smaller sample of female index cases, the proportion of smokers was 88.2% compared with 46.8% in female controls and index relatives, a highly significant difference ($P < 0.001$). The smoking habit is common in our population and the average number of cigarettes smoked per day by regular smokers worked out at 19.3 ± 0.03 and 16.0 ± 1.0 , respectively, in men and women, whose mean ages when they started smoking were 16 and 18 years, respectively.

CORRELATION BETWEEN LIPOPROTEIN FRACTIONS

Table 9 summarises the phenotypic correlations between serum concentrations, after correction for differences in age and smoking habit. The values are

Table 9 *Correlation between lipoprotein fractions*

	Males		Females	
	Controls	Index relatives	Controls	Index relatives
VLDL-IDL	0.22**	0.44**	0.51**	0.35**
VLDL-LDL	0.24**	0.19*	0.07	-0.01
VLDL-HDL	-0.30**	-0.18*	-0.39**	-0.12
IDL-LDL	0.37**	0.32**	0.14*	0.23**
IDL-HDL	-0.14	-0.32**	-0.27**	0.01
LDL-HDL	0.12	0.00	-0.03	-0.03
N	94-95	93-96	149-152	133-136

* and ** indicate significance at the 0.05 and 0.01 levels of probability respectively.

shown separately for controls and the index case relatives, as well as sex. For VLDL the estimates refer to triglyceride and, for the other fractions, to the cholesterol content. The average correlation between the cholesterol and triglyceride contents of VLDL is so high, 0.95 in males and 0.86 in females, while the regression of one on the other approaches unity on the log scale, that the correlations for VLDL cholesterol merely duplicate the figures for triglyceride and have therefore been omitted.

The chief features are as follows.

- (1) In males there is a highly significant correlation (0.2 to 0.5) between VLDL and IDL, not unexpected in view of the close metabolic relations between the two fractions. There appears, however, to be a sex difference since correlation is not evident in females.
- (2) There is consistently negative correlation between HDL cholesterol and both VLDL and IDL in both sexes.
- (3) There is no correlation between LDL and HDL, the chief vehicles of cholesterol.

With respect to the relations between VLDL, IDL and LDL, multiple regression has shown that, when IDL is held constant, LDL and VLDL are not correlated, implying a substantial degree of independent variation between these major fractions. The relations between VLDL, IDL, and HDL differ since, when IDL is held constant, there remains significant negative correlation between HDL and VLDL.

RELATIONS BETWEEN LIPOPROTEINS AND MEASURES OF FATNESS

We have two measures of body 'fatness', namely relative body weight ($\text{weight (kg)/height (cm)}^2 \times 100$) and subscapular skinfold thickness (mm). An index of 0.28 or above for relative weight is often regarded as evidence of obesity, but, as for hyperlipidaemia, the limits of normality are arbitrary. Distribution of relative weight is skewed but not sufficiently so to

make transformation mandatory, whereas log transformation greatly improves the normality of the otherwise skew distribution of skinfold thickness. Both variables show age-related changes. For relative weight, linear regression is adequate, but for skinfold thickness the regression on age is significantly curvilinear in males and nearly so in females. The coefficients used to correct to age 45 are listed in Table 10. Comparisons according to smoking habits provided no significant evidence of differences in skinfold thickness in either sex, or in relative weight in males, but, in females, the relative weight was marginally lower by 0.01 ($P < 0.05 > 0.01$) in smokers, compared with non-smokers.

The average deviations from the control means for both measures of fatness for index cases and their relatives are set out in Table 11. There is no evidence of a familial difference in fatness; the average scores for the index case relatives barely differ from the

control means in either sex. For all available male index cases the average difference is higher, especially for relative body weight, though not significantly so.

If we compare those index cases whose LDL value exceeds the 90th centile value in a blood sample taken within 24 hours of the incident or after an average interval of 9.2 months, the average deviation from the control mean for both relative weight and skinfold thickness is statistically insignificant. But, for the average difference of those index cases whose VLDL value exceeds the 90th centile value in samples taken within the first 24 hours of the incident, a clear indication of greater body fatness is found, since the differences both for relative weight and subscapular skinfold thickness are statistically significant. There are too few females to make such subdivision worthwhile. Thus, in males at least, the hypertriglyceridaemic survivors of myocardial infarction manifest higher degrees of body fatness without necessarily being obese according to conventional criteria.

Table 12 includes the correlations between the alternative lipoprotein fractions and relative weight and skinfold thickness, respectively, as well as the coefficients of regression of lipoprotein on either variable, for controls and index relatives separately, according to sex. The estimates for both controls and index case relatives are consistent for the alternative measures of fatness. The correlations of VLDL and IDL with either measure of fatness are positive and significant and the values are generally higher in males than in females, with higher regression coefficients. For HDL, all the correlations are negative, though all but one fall below the level of statistical significance. The negative association with HDL is probably related to the positive association with VLDL.

Since relative weight and subscapular skinfold

Table 10 Regression of relative body weight and log subscapular skinfold thickness on age in the control population

Sex	Relative body weight	
	b	r
Male	0.00075 $\pm$ 0.00018	0.39
Female	0.00099 $\pm$ 0.00019	0.38

Sex	Skinfold thickness		
	b ₁	b ₂	r
Male	0.0152 $\pm$ 0.0029	-0.00014 $\pm$ 0.00003	0.37
Female	0.0076 $\pm$ 0.0029	-0.00005 $\pm$ 0.00003	0.29

For subscapular skinfold thickness b₁ and b₂ refer to the partial regression coefficients on x and x² where x refers to age, while r refers to the multiple correlation. The regressions are based on 99 males and 158 females.

Table 11 Deviation of average relative weight and subscapular skinfold thickness from control means for index relatives, index cases, and hyperlipidaemic index cases

Category	Relative weight			Skinfold thickness		
	Deviation from control mean	N	P	Deviation from control mean	N	P
Males						
Index relatives	0.001	104	>0.5	-0.013	104	>0.4
Index cases—all	0.009	70	<0.10>0.05	0.013	70	>0.4
high LDL	0.010	21	>0.20	0.022	21	>0.4
high VLDL	0.015*	25	<0.05>0.01	0.079*	25	<0.05>0.02
Females						
Index relatives	-0.002	144	>0.4	-0.023	144	>0.2
Index cases—all	0.006	18	>0.5	-0.023	18	>0.4
Control means						
Male	0.244 $\pm$ 0.003	97	—	1.197 $\pm$ 0.017	97	—
Female	0.242 $\pm$ 0.003	159	—	1.255 $\pm$ 0.015	159	—

High LDL and high VLDL refer to values above the 90th centile point. Relative weight refers to (weight (kg)/height (cm²)); subscapular skinfold thickness refers to log mm.

Table 12 Association between serum lipoprotein concentration, relative body weight (RW), and subscapular skinfold thickness (ST) in controls and index case relatives

	Correlation (r)				Regression (b)			
	Controls		Index relatives		Controls		Index relatives	
	RW	ST	RW	ST	RW	ST	RW	ST
Males								
VLDL	0.38**	0.41**	0.37**	0.43**	3.29 ± 0.84	0.73 ± 0.17	2.96 ± 0.78	0.79 ± 0.17
IDL	0.11	0.06	0.25**	0.32**	0.77 ± 0.74	0.09 ± 0.15	1.49 ± 0.59	0.42 ± 0.13
LDL	0.32**	0.29**	0.32**	0.23**	1.02 ± 0.32	0.19 ± 0.07	0.87 ± 0.27	0.14 ± 0.06
HDL	-0.06	-0.14	-0.02	-0.18	-0.18 ± 0.32	-0.09 ± 0.07	-0.04 ± 0.26	-0.10 ± 0.06
TC	0.33**	0.28**	0.42**	0.30**	0.84 ± 0.25	0.14 ± 0.05	0.81 ± 0.18	0.13 ± 0.04
TT	0.37**	0.40**	0.39**	0.45**	2.10 ± 0.54	0.45 ± 0.11	1.92 ± 0.42	0.50 ± 0.10
N	93		95					
Females								
VLDL	0.38**	0.29**	0.11	0.25**	1.80 ± 0.67	0.50 ± 0.14	0.88 ± 0.67	0.41 ± 0.41
IDL	0.10	0.08	-0.01	0.06	0.58 ± 0.46	0.09 ± 0.10	-0.04 ± 0.57	0.08 ± 0.12
LDL	0.16*	0.20*	0.03	0.01	0.55 ± 0.28	0.14 ± 0.06	0.10 ± 0.32	0.01 ± 0.07
HDL	-0.15	-0.21**	-0.16	-0.07	-0.37 ± 0.20	-0.12 ± 0.04	-0.56 ± 0.29	-0.05 ± 0.06
TC	0.17*	0.18*	0.01	0.05	0.39 ± 0.20	0.08 ± 0.04	0.03 ± 0.20	0.02 ± 0.04
TT	0.22**	0.27**	0.17	0.25**	1.08 ± 0.39	0.27 ± 0.08	0.77 ± 0.37	0.23 ± 0.08
N	149		132					

b, regression of lipoprotein fraction on either relative weight or skinfold thickness; * and **, significance at the 0.05 and 0.01 levels of probability respectively.

thickness are so highly correlated (0.61 in males, 0.66 in females), it is of interest to know how much of the variation of the serum lipoproteins can be accounted for by the combined variation of these alternative measures of body fatness. This is shown in Table 13, in terms of the percentage sums of squares of the lipoprotein fractions.

A substantially higher proportion of the variance is accounted for by differences in fatness in males than in females, except possibly for HDL which shows only a minor association. Of particular interest is the greater correlation between fatness and IDL in male index cases compared with controls. In males, differences in fatness account for 20 to 27% and about 12% of the variation of VLDL and LDL, respectively.

GENETIC DIFFERENCES

With respect to application of the polygenic model, Goldstein *et al.* (1973b) and Hazzard *et al.* (1973)

Table 13 Percentage sums of squares of lipoprotein fractions accounted for by multiple regression on relative weight and subscapular skinfold thickness

	Males	
	Controls	Index relatives
VLDL	19.3	26.7
IDL	1.2	12.4
LDL	11.5	12.9
HDL	2.2	3.1
Females		
VLDL	8.9	8.7
IDL	1.1	0.6
LDL	5.0	0.1
HDL	4.4	2.8

advanced a hypothesis in favour of separate monogenic determination of either raised cholesterol or raised triglyceride, or a rise of both lipids, and suggested that the three putative genes concerned are of major importance in hyperlipidaemia relative to polygenic determination, which is of secondary importance. This hypothesis was based on a comparison of total cholesterol and triglyceride scores on a selected sample of index cases and their families. Confident recognition of monogenic effects, which are superimposed on continuously distributed polygenic variation, is notoriously difficult and requires a very large number of families unless the supposed monogenic effects are very striking (Morton and MacLean, 1974). Our study is not on this scale, neither was it designed to test for monogenic effects, but we have looked for any evidence of this kind. Since HDL cholesterol does not contribute to hyperlipidaemia, we are left with VLDL and LDL levels for comparison. IDL accounts for too small a fraction of the cholesterol or triglyceride concentration to make much difference.

For cholesterol, there is evidence of a relatively higher variance among first degree relatives of survivors, while for the five available sib groups in which the index case exceeds the 95th centile value for LDL cholesterol, 6 individuals exceed the 95th centile value and 8 do not. For individuals above and below the centile, the mean values for LDL work out at 2.427 ± 0.034 and 2.181 ± 0.042 , respectively. This looks like the effect of monogenic segregation on LDL serum concentration.

The survivors of myocardial infarction may include a proportion of persons who carry a gene

with a comparatively major effect on LDL, but their scores may overlap the tail end of the distribution of polygenic differences. Since we lack evidence from an independent biochemical test to distinguish between such types, for analysis of the resemblances between relatives for LDL we have excluded those survivors whose LDL score exceeds the 95th centile value and also their relatives. This may involve truncation of the upper range of the polygenic distribution, but should not affect the regression of progeny on parent. It makes little difference in practice whether or not we exclude these individuals since they are few in number, but there is merit in dealing with variation which is virtually entirely polygenic in origin.

For triglyceride, our data provide neither evidence of increased variance on the part of first degree relatives of the index cases, referred to in Table 6, which exceed the 95th centile value for total triglyceride, nor convincing evidence of bimodality in plots of cumulative frequency on normal probability paper, nor evidence of apparent monogenic effects in the distribution of either total triglyceride or VLDL triglyceride values. Thus, for the sib groups for which the corresponding index case exceeds the 95th centile value for the VLDL fraction, none of the 12 sibs exceeds this arbitrary limit. Hence, there is no case for excluding index case relatives in the analysis and we have used the polygenic model for VLDL and also HDL.

The main questions to be answered are:

- (1) to what extent can the normal range of variation of the lipoprotein fractions be attributed to polygenic variation?
- (2) do the lipoprotein fractions differ in this respect?
- (3) do alternative estimates of resemblance between first degree relatives agree?

Since we have evidence from three generations, namely the index case and spouse generation and that of their parents and also of their children, we can calculate the correlation of offspring with either father or mother for the index/spouse generation and also for their children. Alternatively, the relationship can be expressed as regression on a single parent. In view of the qualifications about the lipoprotein concentration in blood samples taken at the time of the incident, we have excluded records of the index case from the analysis of resemblances between relatives.

An alternative estimate of genetic contribution to the phenotypic variance may be derived from the intraclass correlation between groups of sibs. The ratio of the between-family component of variance to the sum of the within-family and between-family components estimates the intraclass correlation.

Provided that maternal effects are nil, dominance, epistasis, and assortative mating are unimportant, and there are no special environmental conditions common to sibs which would inflate the intraclass correlation estimates, the correlation between parent and offspring and the intraclass correlation estimates should agree within the limits of sampling. To obtain an estimate of heritability, which measures the proportion of the phenotypic variation due to the average or additive effects of genetic variation, the regression estimates must be doubled.

The regressions of offspring on either father or mother offer no evidence of maternal effects. On general grounds, we might anticipate that dominance and epistasis will be unimportant in the variation of characters like the serum lipoprotein fractions, which probably vary about intermediate optima, and so we do not expect inflation of the intraclass correlation due to dominance effects. As we have already seen, there is no evidence of assortative mating.

Previous publications include a number of reports of seasonal variation in cholesterol and/or triglyceride values (Thomas *et al.*, 1961; Doyle *et al.*, 1965; Fuller *et al.*, 1974; Leren *et al.*, 1975; Warnick and Albers, 1976). Such reports are not consistent as to whether it is triglyceride or cholesterol or both which chiefly vary, they refer mostly to males, and it is quite uncertain how far cyclical, physiological changes and/or dietary alterations contribute to the reported differences. In the collection of blood samples from members of individual families, it is quite impractical to randomise the sampling with respect to season, and so samples other than from index cases are often collected at about the same time. Familial and possible seasonal differences are confounded and, if the latter exist, they may contribute to the resemblance between sibs and the correlations between offspring and parents in some cases. The numbers of unrelated individuals in the study are not great enough, neither are they so distributed as to provide a reliable basis for correction for possible seasonal differences, and so the problem has been dealt with in the following way. The year has been divided into six successive two-monthly periods and the records falling within these individual periods have been used to estimate components of variance within and between families for the spouse and index case parent generation, index sibs, spouse, and sibs, and the children of index case and spouse, respectively.

Comparison of the pooled within-sib group variance according to whether samples collected at different times of the year are combined or analysed separately suggests that only for HDL is the former variance significantly greater than the latter ($P < 0.05$). Though it makes little difference to the

Table 14 Correlation between parent and offspring, regression on father or mother, and sib correlations for VLDL, LDL, and HDL

	VLDL	LDL	HDL
df	90	93	82
Parent-offspring correlation	0.12	0.21	0.33**
Regression on offspring on parent	0.11 ± 0.11	0.18 ± 0.09	0.34 ± 0.10
Sib correlation			
δ ² _w	0.0801	0.0107	0.0061
df	129	124	137
δ ² _b	0.0148	0.0048	0.0036
df	227	217	217
r _t	0.16	0.31*	0.37**

δ²_w and δ²_b refer respectively to the within sib-group and between sib-group components of variance.
* and ** indicate significance at the 0.05 and 0.01 levels of probability, respectively.

final estimates, we have preferred to deal, for all fractions and categories, with the within-period estimates which were then combined to produce average estimates of δ²_w, the component of variation within families, and δ²_b, the component of variation between families, weighting by the degrees of freedom.

For the regressions on parent, allowing for variation in family size (Falconer, 1963), a common slope has been fitted by pooling the sums of squares and cross-products, since statistically significant heterogeneity of slope was not encountered either between periods of the year or between type of comparison. The regression and correlation coefficients are quoted in Table 14.

Since the comparisons include regressions on father and mother for two successive generations, the estimates will be partly correlated, though this will be least so for members of the spouse and index case generation and their respective parents, since mortality ensured that there was only a partial overlap in the families used to compute the regressions on either father or mother. Only the standard error of the coefficients is likely to be moderately affected by such correlation.

From the average coefficients quoted in Table 14 we may infer:

(1) There is consistency between the intraclass correlations, the regression estimates, and the corresponding estimates of correlation between parent and offspring for VLDL and HDL. In LDL, though the sib is higher than the parent-offspring correlation, little weight can be attached to this difference. Though ideally correlation and regression estimates should coincide, in practice identity is not expected. Thus, there is no convincing evidence that special environmental effects, common to groups of sibs, are important.

(2) The lipoprotein fractions differ in the magnitude

of the parent/offspring correlation, with the highest value of 0.33 in HDL, the lowest value of 0.12 in VLDL, and an intermediate value of 0.21 in LDL. (3) The within-sibship variances differ, with the lowest value of 0.006 in HDL, 0.011 in LDL, and the highest value of 0.080 in VLDL. That VLDL shows evidence of parent-offspring correlation, in spite of the very high within-sibship variance, suggests that the phenotypic expression of either a given genetic or environmental difference may be relatively greater for VLDL than for the other fractions, and so the enhanced variation of VLDL should probably not be simply equated with greater environmental variation.

(4) From the regression of offspring on parent we can derive heritabilities for HDL, LDL, and VLDL of 0.67 ± 0.21, 0.36 ± 0.18, and 0.23 ± 0.20, respectively, which estimate the proportions of the respective phenotypic variances which are additively genetic. Kannel (1976) has reported that alteration of serum lipoprotein concentration by dietary changes does not alter the ranking of individuals, so there is practical evidence in favour of additivity between genetic and environmental effects of the kind commonly considered to be important for variation in lipoprotein levels. The less important genotype-environment interaction is, the greater our confidence that estimates of heritability measure the relative importance of genetic and environmental causes of variation.

A very different picture is presented by the corresponding comparisons for relative weight and skinfold thickness (Table 15) in which the season of the year was ignored. Here there is no evidence of statistically significant correlation between parent and offspring, but there is a low, but statistically significant, correlation between sibs for either measure of fatness, suggesting some environmental component which tends to make sibs more alike than would otherwise be expected. Though we may doubt that genetic differences play no role in the variation of body fatness, especially in the more pronounced cases of obesity, for the ordinary range

Table 15 Fatness comparisons between relatives

	Relative weight	Skinfold thickness
df	109	117
Parent-offspring correlation	0.017	0.100
Regression on parent	0.014 ± 0.080	0.072 ± 0.068
Sib correlation		
r _t	0.162*	0.116*
δ ² _w	0.00114	0.02914
df	242	226
δ ² _b	0.00022	0.00369
df	255	258

Symbols as in Table 14.

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of variation of body fatness in the general population non-genetic causes of variation grossly predominate. Hence, the phenotypic correlations between lipoprotein concentrations and either relative weight or skinfold thickness are probably of environmental origin. It will be recalled from Table 13 that body fatness accounts, on average, for some 20% or more of the variation of VLDL, less for LDL, and only for a trivial fraction in HDL. Such differences are consistent with the ranking of the heritability estimates.

We are thus led to the conclusion that polygenic differences play an important part in the normal variation of LDL, and especially HDL, cholesterol concentrations. For VLDL, non-genetic variation is relatively much more important. Given such differences between the lipoprotein fractions, heritability estimates derived from either total cholesterol or total triglyceride are misleading.

Discussion

The properties of the lipoprotein fractions discussed in the present study are in general agreement with recent reports by Carlson and Ericsson (1975), Olsson (1975), and Olsson and Carlson (1975).

The cigarette smoking habit appears to be an important environmental variable, since it is associated with raised VLDL levels in both sexes. In comparisons of total triglyceride concentration between non-smokers and smokers, Erikssen and Enger (1978) have reported higher concentrations in the latter; the deviation from the average score of non-smokers rose to 15% for the heaviest smokers. The physiological origin of the association between smoking and triglyceride concentration may be complex and involve both the direct effect of smoking *per se* and also a difference in life style, in which the level of social drinking may play a part. Thus, Barboriak *et al.* (1977) have reported, for a group of patients with angina pectoris and/or a previous myocardial infarction, a positive association between smoking, serum triglyceride concentration, and also the level of alcohol consumption. The evidence of Avogaro and Cazzolato (1975) in relation to alcohol consumption and serum triglyceride concentration may be relevant in this context.

We have refrained from expressing the incidence of hyperlipidaemia in terms of the familiar types since, as both Hazzard *et al.* (1973) and Olsson and Carlson (1975) have noted, they do not correspond to biological entities and involve arbitrary truncations of continuous distributions. Arbitrary classification has been confined to the categories which exceed or fall below the 90th or 95th centiles of the single-tailed distribution. In these terms, as well

as in the difference of the average lipoprotein concentrations in the index cases compared with the control means, hyperlipidaemia is evidently important in our sample of index cases under the age of 50. Of these, 42% are hyperlipidaemic at the 90th centile and 21% at the 95th centile level, frequencies which are several times greater than the corresponding values for the control population.

From the analysis of the changes in lipoprotein concentration after an infarction, the quoted frequencies for raised LDL alone with respect to the 90th and 95th centile limits of 18.3 and 8.6%, respectively, may reflect the preinfarction concentrations. If we include instances in which the VLDL concentration also exceeds the corresponding centile limit, the figures for LDL are increased to 25.8 and 10.8%, respectively. The correlation between relatives, the apparent segregation in families of index cases which exceed the 95th centile, the significantly higher LDL concentration in relatives compared with the controls, combined with the lack of correlation between spouses, suggest that genetic differences play a major role in accounting for such instances of hypercholesterolaemia.

Carlson (1976) has drawn attention to the apparent occurrence of two forms of hypercholesterolaemia distinguished by either the presence or the absence of xanthomata. In our study, as in the Swedish study, none of the survivors of infarction manifested xanthomata. The appearance of monogenic segregation, with or without associated xanthomata, suggests the possible presence of at least two genes, allelic or non-allelic, with a major effect on LDL concentration, but which differ in other phenotypic effects. It is also worth noting that Heiberg and Slack (1977) found evidence of genetic heterogeneity in familial hypercholesterolaemia in age at coronary death.

In blood samples collected within 24 hours of infarction, the average concentration of VLDL substantially exceeds that of the controls. The 90th and 95th centile frequencies for raised VLDL alone are 16.1 and 9.7%, respectively, and, if we include individuals for whom LDL is also raised, the figures are increased to 23.6 and 11.9%, that is, roughly of the same magnitude as for LDL. For VLDL there is greater doubt as to how far such frequencies reflect the preinfarction concentrations. Mobilisation of free fatty acids may contribute to the post-infarction increase in VLDL (Vetter *et al.*, 1974). Numerically, hypertriglyceridaemia is just as important as hypercholesterolaemia among the survivors of infarction, but the genetic responsibility is certainly much less and environmental circumstances are relatively more important. The higher variance of repeat measurements on the same control individuals, compared

with LDL, the high correlations with alternative measures of fatness whose variation appears predominantly environmental, together with the striking effects of smoking, are consistent with the low estimate of heritability for VLDL.

With respect to combined hyperlipidaemia in which total cholesterol and triglyceride scores both exceed the 90th or 95th centile limit, it is not clear how far the observed frequencies truly exceed the product of the separate frequencies of raised VLDL and raised LDL. Since triglyceride and cholesterol occur in both the fractions, and in each are highly correlated in their joint occurrence, a rise of either fraction, though causing a major increase in the chief constituent, will also involve an increase in the other. For VLDL at least, in which there is an inherently high level of variation and the frequent occurrence of relatively high concentrations, this effect cannot be ignored in relation to the consequential rise of total cholesterol as well.

While for VLDL there is circumstantial evidence which supports the low estimate of heritability, the value for LDL may underestimate the genetic contribution to variation between normal individuals. LDL shows strong age-related changes, and it may be that individuals differ in this respect, especially in the younger age group, as a result of differences in rates of maturation in which there may be a genetic component. Variation of this kind may not be adequately allowed for in the regression procedure for adjusting to a common age and, if this is so, it will contribute to the error variation. Sequential records extending over a number of years are needed to evaluate this possibility.

Earlier reports have provided somewhat conflicting evidence for heritable variation in total serum cholesterol, triglyceride, or lipoprotein fractions. In twin studies, Feinleib (1976) reported no difference in the intra-pair variances between mono- and dizygotic twins, though Pikkarainen *et al.* (1966) estimated a heritability of 33% for total serum cholesterol. Heiberg (1974) reported comparatively high heritabilities for total serum lipids, as well as somewhat lower values for lipoprotein fractions, but these estimates were queried by Weinberg *et al.* (1976) for technical reasons. There are a number of other published reports which provide evidence for heritable variation especially in total cholesterol, for example, Adlersberg *et al.* (1957), Schaeffer *et al.* (1958), Osborne *et al.* (1959), Gedda and Poggi (1960), Mayo *et al.* (1969), and Martin *et al.* (1973). There is also Weibust's (1973) convincing estimate of heritability of total serum cholesterol in mice of 0.56. He was in the fortunate position of being able to validate this figure by effective artificial selection which increased or decreased the serum cholesterol

level as expected from the estimates of additive genetic variance.

If there is a substantial genetic component in the variation of LDL and HDL, we must recognise the possibility of regional differences in gene frequency which could contribute to corresponding differences in proneness to coronary disease.

The relations between VLDL and HDL merit attention. In most comparisons we find evidence of a negative correlation which, however, is generally low and often statistically insignificant. However, in the blood samples collected from males within 24 hours of infarction the correlation reaches the highly significant value of -0.47 and this is clearly related to variation in the severity of infarction as measured by the respectively positive or negative correlation of VLDL or HDL with the peak aspartate aminotransferase activity.

The most important exception to the negative association occurs in the comparison between the average concentrations in first degree relatives and controls. The former clearly exceed the latter for average VLDL concentration but do not significantly differ for HDL cholesterol.

Cholesterol provides only one of several possible indices of HDL concentration. Change in composition could arise in different ways and this has implications for the relations between VLDL and HDL cholesterol.

Thus (1) triglyceride molecules may be able to replace cholesterol ester molecules in the HDL lipoprotein core so that VLDL triglyceride is positively correlated with HDL triglyceride and negatively correlated with HDL cholesterol (Barter and Conner, 1975); (2) cholesterol may be carried on the C-apoproteins which exchange between HDL and VLDL (Eisenberg *et al.*, 1972); and (3) an increased rate of HDL catabolism may be associated with an increase in VLDL serum concentration, as might be inferred from the consequences of providing an 80% carbohydrate diet (Blum *et al.*, 1977). On present evidence the second possibility appears the most attractive.

It has been claimed that HDL plays an important role in reducing the risk of coronary disease (Miller and Miller, 1975). The evidence of Medalie *et al.* (1973) concurs with the indications from the Framingham study (Gordon *et al.*, 1977) of an inverse relation between HDL and proneness to coronary disease. HDL cholesterol has been referred to as a 'robust protective risk factor at any age in either sex, except perhaps for older women' (Kannel, 1976). If the heritability of HDL cholesterol is particularly high, then genetic variation in this fraction could be a moderate, to no small degree, familial difference in proneness to coronary heart disease and heart attack, though the present data do not provide genetic

support for this, since the average serum concentration of HDL in first degree relatives is not lower than the means of the controls. In view of the scope for inverse relations in the concentration of VLDL and HDL, it is important to enquire how often the apparent advantage of raised HDL cholesterol reflects the possibly more relevant reduction of VLDL triglyceride. Systematic comparison, in different circumstances, of VLDL concentration with the quantitative composition of HDL is needed to resolve possibly opposing tendencies which may not be evident in scores of HDL cholesterol alone.

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