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Plasma Lipids in Twins

Environmental and Genetic Influences

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Summary

A study on 205 pairs of male and female twins, aged from 18 to 34 years, showed significant heritabilities for total and high density lipoprotein cholesterol and for triglycerides. Significant effects of shared environment were also found for total and HDL cholesterol, possibly to a greater extent in women than in men. Triglycerides showed greater variance in men but a model specifying different sized environmental and genetic parameters in the two sexes gave a good fit and indicated that the factors influencing plasma triglycerides are the same in men and women although the effects they produce are scaled differently.

Key words: *Cholesterol – Environment – Genetics – High density lipoprotein cholesterol – Triglyceride – Twins*

Introduction

Since plasma cholesterol is one of the known risk factors for coronary heart disease (CHD) but high density lipoprotein cholesterol (HDL-C) seems to have a protective effect, a knowledge of the environmental and genetic influences which determine the total concentration of cholesterol and concentrations of the lipoprotein fractions is relevant to the epidemiology of atherosclerosis. The fact that movement of individuals between countries, and deliberate dietary changes, can change plasma cholesterol and also change CHD risk [1–3] would seem to indicate

that environmental factors acting in adult life are of great importance. However, when one considers a single comparatively homogeneous group such as our 'Western' society, the relative contributions of inheritance, childhood environment and adult environment may appear in a different light. Moreover, the reduction in plasma total cholesterol usually achieved by changes to diet is only about 10% [3,4].

Many family and twin studies have been carried out on rather small samples, giving wide confidence limits to the estimated proportions of variance due to different causes, but most have concluded that a singificant genetic effect exists [5]. However, Christian et al. [6] reported unequal variances between monozygotic (MZ) and dizygotic (DZ) twin pairs, leading to conceptual and computational difficulties and to the conclusion that heritability is low but that shared environmental (possibly pre-natal) influence is important and persists at least into middle age. Secondly, many studies have either used male subjects only or have considered men and women together in the analysis of their results. Men and women certainly differ in their plasma lipids and the relative importance of genetic and environmental factors may differ too.

Most studies on cholesterol have also included measurement of plasma triglycerides, and some have measured the individual lipoprotein fractions. Because the implications of high density lipoprotein (HDL) and low density lipoprotein (LDL) for coronary heart disease risk are in opposite directions, it is desirable to break down the total cholesterol into at least its two major components, HDL cholesterol and NonHDL cholesterol (which is mostly LDL).

We have studied plasma total cholesterol, high density lipoprotein cholesterol and triglycerides in young adult male and female twins. Here we report the results of fitting different environmental and genetical models in an attempt to account for the observed differences between people in these lipid levels.

Subjects and Methods

Subjects

Pairs of monozygotic (MZ) and dizygotic (DZ) twins, aged between 18 and 34 years (mean 23.1), were recruited from the Australian NH & MRC Twin Registry for a study of alcohol metabolism and susceptibility to intoxication [7]. Both members of a twin pair attended on the same day. 89 twins (50 men and 39 women) attended on more than one occasion, with an interval between visits of 1 to 17 months (mean 4.5 months), and the results of these 89 are used to assess the repeatability of the measurements within an individual.

All twins were typed with 15 blood group antisera and for alpha-1-antitrypsin (Pi). Twins were diagnosed as DZ on the basis of a difference in sex, at least one marker locus or, in a few cases, large differences in height, colouring or other morphological features. In remaining cases of doubtful zygosity several more genetic markers were typed. It is possible, however, that there are a few pairs diagnosed as MZ who on still further typing would prove to be DZ.

Of the 205 twin pairs for whom measurements were available, there were 42 MZ female, 42 MZ male, 44 DZ female, 38 DZ male and 39 DZ pairs of opposite sex

(DZOS). There were no substantial differences in age distribution between the five zygosity groups.

Methods

The twins were instructed to have either no breakfast, or at most a breakfast of toast with no buffer or margarine and tea or coffee with no milk no later than 8.00 a.m. Blood was taken at about 10 a.m., before any alcohol was administered. 15 ml of venous blood, taken into a tube containing heparin, was centrifuged within 2 h of collection and plasma was kept at 4°C for up to 2 days before analysis. An aliquot of the heparin plasma was transferred to a tube containing EDTA and kept at 4°C until analysis for HDL cholesterol.

Total cholesterol was measured on a Technicon SMAC by the cholesterol oxidase method, HDL cholesterol as described by Allen et al. [8], and triglycerides on the SMAC by the enzymatic method of Bucolo and David [9]. The HDL cholesterol was subtracted from the total cholesterol to give the value for 'NonHDL cholesterol'. Estimates of the between-run analytical error for cholesterol and triglycerides were obtained from laboratory records of precision in an external quality control scheme (Wellcome Clinical Chemistry Quality Control Programme) and for HDL cholesterol from internal quality control records of materials included in each run. To assess within-batch precision, samples of human origin were analysed twice in the same run and the within-run variance calculated from the differences.

Data analysis

Analysis of variance produced mean squares within and between twin pairs in 5 groups, by sex and zygosity. For the DZ pairs of opposite-sex the within-pair mean squares are corrected for differences in mean or variance between men and women [10]. Various models are then tested against these mean squares [10,11]. This leads to a preferred model which may include: only environmental variance specific to an individual and not shared by his/her twin (E_1); this E_1 variance plus either familial factors due to shared environment rather than shared genes (E_2) or true genetic variation due to the additive effects of genes (V_A); or all three. The model-fitting is first performed for men and women separately and then a combined model is tested; a more detailed explanation is given in [10].

Results

Transformation

The distributions of all 4 variables, particularly triglyceride, are positively skewed in both men and women (Table 1). There were also increases in the differences between results in the same person on two occasions and in the differences between a pair of MZ twins with increasing values of the variables studied, which are also indications for data transformation. Therefore $\log_{10}$ transformation was carried out for all variables.

TABLE 1

SUMMARY STATISTICS FOR RAW AND $\log_{10}$ TRANSFORMED DISTRIBUTIONS, AND TESTS FOR DIFFERENCES IN MEANS AND VARIANCES BETWEEN MEN AND WOMEN

	Cholesterol		HDL cholesterol		NonHDL cholesterol		Triglyceride	
	Raw	$\log_{10}$	Raw	$\log_{10}$	Raw	$\log_{10}$	Raw	$\log_{10}$
Males								
Mean	5.57	0.737	1.08	0.021	4.46	0.635	1.15	−0.011
SD	1.16	0.089	0.27	0.107	1.16	0.110	0.77	0.240
Skewness	0.70	0.12	0.66	−0.06	0.76	0.08	2.39	0.36
N	199		191		191		199	
Females								
Mean	5.52	0.732	1.30 ***	0.101 ***	4.16 **	0.605 **	0.87 ***	−0.100 ***
SD	1.19	0.090	0.33 **	0.111	1.08	0.110	0.42 ***	0.175 ***
Skewness	0.98	0.23	0.43	−0.19	1.10	0.00	2.37	0.59
N	212		208		207		212	

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Means and total variances

These are shown in Table 1. The distributions of total cholesterol were not significantly different in men and women, but both its fractions, HDL and Non-HDL, had different means in opposite directions. Triglyceride showed a greater mean and variance in men than in women, and these differences were significant even after $\log_{10}$ transformation. However MZ and DZ twins of a given sex did not differ significantly in their distributions.

Repeatability

Within-person (S_w^2) and between-person (S_b^2) components of variance were obtained from an analysis of variance of repeat measurements. All variables showed highly significant repeatability ($S_b^2/(S_b^2 + S_w^2)$) (Table 2). The within-person variance for triglyceride was greater in men than in women, both on the raw and log-transformed data, and HDL cholesterol was significantly more variable within women on the raw but not the transformed data.

Model fitting

The mean squares for the $\log_{10}$ transformed lipid variables by sex and zygosity, including the DZ opposite-sex pairs, are shown in Table 3. The results of the chosen models are shown in Table 4; detailed comparisons of the results for different models may be obtained from the authors.

For total cholesterol the most convincing model is that incorporating all three sources of variation, $E_1E_2V_A$, with an extremely good fit ($\chi_7^2 = 1.90$). This is true for the two sexes separately, male and female like-sex pairs together, and for all 10 meansquares including those for DZ opposite-sex pairs. Both E_2 and V_A estimates are significant overall, although it appears that E_2 is more important in women than in men. (Estimated values are 0.0018 for men and 0.0036 for women.)

When total cholesterol is divided into HDL and NonHDL fractions, a more complex picture emerges. For HDL, the $E_1E_2V_A$ model again gives the best fit overall with all 3 estimates significant, but $\hat{E}_2$ is greater than $\hat{E}_1$ or $\hat{V}_A$, accounting for 47% of the variance and the heritability is only 24%. There is a suggestion that shared environmental effects are more important in women and additive genetic effects more important in men.

For NonHDL cholesterol (which is highly correlated with total cholesterol) all 3 sources of variation are also significant. Once again the E_2 estimate is rather lower in men than women, but the combined model with the same estimates for both sexes is very acceptable on the χ^2 test ($\chi_7^2 = 1.64$, $P = 0.977$). This model yields a heritability estimate of 0.54 ± 0.13, and 27% of the variance is due to shared environmental factors.

For triglycerides, considering the sexes separately, neither the E_1E_2 nor E_1V_A model can be rejected but the results for men and women cannot be combined to give a simple model with good fit. The overall population variance was about twice as great in men as in women and the within-person variance differed even more, so it seemed likely that a more complex model allowing different sized parameter estimates for men and women might be needed.

TABLE 2

REPEATABILITY WITHIN INDIVIDUALS AT REPEAT VISITS, AS INTRACLASS CORRELATION COEFFICIENT, r_i, AND WITHIN-INDIVIDUAL VARIANCE, S_w^2. TESTS OF SIGNIFICANCE OF REPEATABILITY AND MALE/FEMALE DIFFERENCES IN WITHIN-INDIVIDUAL VARIANCE

	Cholesterol		HDL cholesterol		NonHDL cholesterol		Triglyceride	
	Raw	Log_{10}	Raw	Log_{10}	Raw	Log_{10}	Raw	Log_{10}
Males								
r_i	0.83 ***	0.84 ***	0.65 ***	0.67 ***	0.81 ***	0.80 ***	0.36 **	0.46 ***
S_w^2	0.215	0.0073	0.018 †	0.016	0.220	0.0125	0.215 ††	0.163 †††
N	48		46		46		48	
Females								
r_i	0.89 ***	0.89 ***	0.67 ***	0.67 ***	0.76 ***	0.79 ***	0.51 ***	0.56 ***
S_w^2	0.191	0.0053	0.036 †	0.022	0.180	0.0091	0.051 ††	0.053 †††
N	39		35		35		39	
Both sexes								
r_i	0.86 ***	0.86 ***	0.73 ***	0.87 ***	0.79 ***	0.79 ***	0.42 ***	0.54 ***
S_w^2	0.204	0.0064	0.26	0.018	0.203	0.011	0.213	0.114
N	87		81		81		87	

** $P < 0.01$; *** $P < 0.001$.

Tests for unequal variance components in men and women: † $F_{35,46} = 2.00$, $P < 0.05$; †† $F_{46,35} = 4.21$, $P < 0.01$; ††† $F_{46,35} = 3.08$, $P < 0.01$.

TABLE 3

MEAN SQUARES WITHIN AND BETWEEN PAIRS, BY SEX AND ZYGOSITY

Log_{10} transformed data.

	df	Total cholesterol	df	HDL cholesterol	df	NonHDL cholesterol	df	Triglyceride
MZ males								
Between	41	0.014220	38	0.016232	38	0.022857	41	0.117586
Within	42	0.001605	39	0.002719	39	0.002412	42	0.030397
MZ females								
Between	42	0.016225	41	0.018610	40	0.022333	42	0.035503
Within	43	0.001452	42	0.004174	41	0.002092	43	0.014143
DZ males								
Between	37	0.011503	36	0.023000	36	0.016816	37	0.068233
Within	38	0.003779	37	0.005933	37	0.005829	38	0.038462
DZ females								
Between	43	0.013417	42	0.023024	42	0.018443	43	0.044181
Within	44	0.003174	43	0.005150	43	0.004376	44	0.018108
DZ opposite-sex								
Between	38	0.010092	38	0.018163	38	0.017773	38	0.041158
Within [a]	38	0.004025	38	0.004383	38	0.006090	38	0.024764

[a] One degree of freedom removed for mean sex differences.

TABLE 4

SUMMARY OF RESULTS OF MODEL-FITTING TO log-TRANSFORMED DATA, SHOWING SELECTED MODELS, GOODNESS OF FIT, AND ESTIMATES OF COMPONENTS OF POPULATION VARIANCE ASCRIBABLE TO INDIVIDUAL ENVIRONMENT (E_1), FAMILY ENVIRONMENT (E_2), AND ADDITIVE GENETIC EFFECTS (V_A)

Variable	Factors included in chosen model	Test for goodness of fit			Estimates			Heritability
		χ^2	df	P	E_1	E_2	V_A	
Cholesterol	$E_1E_2V_A$	1.90	7	0.965	0.0015 ***	0.0021 *	0.0043 ***	0.54±0.13
HDL cholesterol	$E_1E_2V_A$	5.24	7	0.631	0.0036 ***	0.0058 ***	0.0029 *	0.24±0.14
NonHDL cholesterol	$E_1E_2V_A$	1.64	7	0.977	0.0022 ***	0.0032 *	0.0064 ***	0.54±0.13
Triglyceride								
Men	E_1V_A	7.01	5	0.220	0.0274 ***	–	0.0311 ***	0.53±0.10
Women	E_1V_A				0.0134 ***	–	0.0137 ***	0.51±0.10

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

TABLE 5

COMPARISONS OF ANALYTICAL ERROR WITH NON-REPEATABLE VARIANCE (S_w^2) ON RAW SCALE

	S_w^2 (mmol/l)^2		Analytical error (mmol/l)^2	
	Male	Female	Between-batch	Within-batch
Cholesterol	0.215	0.191	0.040	0.0006
HDL cholesterol	0.018	0.036	0.009	0.0022
Triglyceride	0.215	0.051	0.022	0.0007

A complete model including different E_1E_2 and V_A parameters for males and females is given in reference [10]. The best models, which were not rejected at $P < 0.05$, were $E_{1M}E_{1F}V_{AM}V_{AF}V_{AMF}$ or $E_{1M}E_{1F}E_2V_{AM}V_{AF}V_{AMF}$. Although the latter had a slightly greater probability, the estimate of E_2 was small and not significant, so we prefer the former model. Both E_1 and V_A estimates were just over twice as great in men as women, so that even though the overall variances were different, the proportion due to genetic effects (heritability) was most identical: 0.53 in men and 0.51 in women.

The values of $\hat{V}_{AM}$, $\hat{V}_{AF}$, and $\hat{V}_{AMF}$ can be used to test whether the same genes are affecting a trait in men and women [10]. In this instance the calculated value for r_{V_A}, the correlation between the effects in men and women, for triglycerides is 1.36, which is not significantly different from 1.0 and indicates that the same genetic effects are operating in both sexes.

Analytical error

Since some of the values from which estimates of analytical error were derived were not readily available for log-transformation, the analytical error variances in raw form are compared against the within-person variance to see if they contribute an important amount to the outcome. It will be seen in Table 5 that analytical error is in all cases small compared to other causes of variation.

Repeatability and heritability

In general the repeatability, expressed as the intraclass correlation, sets an upper limit for heritability. Comparison of Tables 2 and 4 shows that cholesterol, HDL cholesterol, and NonHDL cholesterol have a repeatability close to the sum of the heritability and the shared environmental effect, and that for triglyceride the heritabilities are not significantly different from the repeatabilities.

Discussion

Cholesterol

In twin studies up to 1975 (summarised in ref. [6]), significant genetic effects on plasma total cholesterol were found in 7 out of 8 studies, judged by a significantly greater within-pair mean square in DZ than MZ twin pairs. Christian's group

reported [6] on a study of 514 male twin pairs aged 42–56, but found that the total variance for the DZ pairs was significantly greater than that for MZ pairs. Such a finding violates one of the assumptions of twin studies and there is no general agreement on the way to proceed. However, we found no significant differences in means or variances between MZ and DZ pairs, as can be seen from Table 3; if such differences existed they would also show up in the model-fitting as a poor fit for all models.

One subsequent study on 105 pairs of both sexes and unspecified ages [13] found significant heritability for all lipid variables, with h^2 values ranging from 0.52 for α-lipoprotein to 0.80 for triglyceride. Quantitatively, these results differ from ours; the study was carried out in Brazil, and it could be that the gene pool of their subjects differed in some significant way from that in our subjects who were predominantly of northern European descent. Our results show that, both for total cholesterol and NonHDL cholesterol (mostly LDL), the variation in the values observed in our population is about 55% due to additive genetic effects, 25% to environmental effects shared by members of a twin pair, and 20% due to non-shared environment.

The twin study method has difficulty in detecting dominant gene effects [12]. From a clinical point of view dominant genes such as that for familial hypercholesterolaemia due to LDL receptor defects [14,15] are more striking than the polygenic inheritance of moderate elevations of cholesterol, but overall the latter are a greater health problem. Models assuming dominant gene effects were also tested and not rejected in our study but they produced negative variance estimates and made no sense. The incidence of the major gene for hypercholesterolaemia is probably around 1 in 200 or less [14], so this would have little effect on total variance in a population study.

The nature of the genetic effects determining 55% of the variation in cholesterol cannot be inferred from this study, except to say that there must be several of importance. The nature of the shared environmental factors is also of importance, as they are more likely to be susceptible to modification; the possibility that E_2 variance may be more important in women seems to point to dietary factors since a pair of female twins may be more likely to retain family dietary habits than a pair of male twins.

If there is assortative mating (the tendency of like to marry like) for cholesterol levels then the extra additive genetic variance this generates will be included with E_2 estimates in twin studies. However, only modest marital correlations (< 0.2) have been reported for serum cholesterol [16] so assortative mating is unlikely to be a major contribution to E_2.

The incidence of increased cholesterol in this population is high; 14% of men and 10% of women had a total cholesterol greater than 7.0 mmol/l (270 mg/100 ml) and the mean value was 5.5 mmol/l, which many authorities would argue [17] should be reduced by changes to the diet. On a simple view, diet could be considered an E_1 or E_2 factor, and if so only a quarter or half of the variance is due to these, but this ignores possible genetic influences on dietary preferences [18].

HDL cholesterol

Two studies on HDL have appeared recently which measured the proteins apo A-I and apo A-II rather than HDL cholesterol [19,20]. Their results are not in complete agreement, especially for apo A-I where one study found a significant heritability at 0.55 while the other found none. Both studies were consistent with a heritability of about a third for apo A-II, but this did not reach statistical significance. The Brazilian study [13] found heritability of 0.52 for the electrophoretically separated α-lipoprotein. One of these studies [20] looked for but did not detect E_2 effects but with the small sample size used (40 pairs) the probability of detecting a small E_2 effect is low.

We found a low but still significant heritability for HDL cholesterol, $h^2 = 0.24$, and a shared environmental component of 47% for the two sexes combined. Once again there is the possibility that, although a model producing common estimates for the two sexes cannot be rejected, shared environment is more important in women and genetic factors in men. The concentration of HDL cholesterol in plasma can be influenced by exercise, smoking, and alcohol intake, and the sex difference in means is thought to be due to an oestrogen effect in women, but it is difficult to assign the E_2 effect to any of these.

Triglycerides

Heritabilities found by others for triglycerides have included 0.68 for raw data or 0.39 after $\log_{10}$ transformation [21], and 0.80 after $\log_e$ transformation [13]. We consider it essential to perform transformation for triglycerides, and this has resulted in a satisfactory fit when sex differences are taken into account. The triglycerides show the interesting feature that, while the overall population variance and its E_1 and V_A components are about twice as great in men as in women, the heritability estimate and the genetic effects controlling plasma triglycerides appear to be the same.

As with the other variables, the nature of the twin study method is not such as to identify the genes influencing triglycerides to different extents in men and women. Amongst the possible environmental contributions, the factor which comes to mind is alcohol consumption. A significant correlation ($r = 0.22$, $P < 0.001$) between log weekly alcohol consumption and log triglyceride was found in the men but not in the women (in whom the range of alcohol intake was less), but even in men the correlation only accounts for about 5% of the total variance in triglycerides. There are indications that alcohol consumption is influenced by genetic factors ([22] and Martin, in preparation), so it would be necessary to partition even this 5% between the observed E_1 and V_A variances in triglycerides. Although it might be possible in theory to trace the causal path of these correlations, the low correlation and the limit to the number of subjects make this impractical.

A further factor influencing E_1 contributions may be marker type at the MN and Jk blood group loci. Magnus et al. [23] have reported markedly higher intrapair variances for total cholesterol in MZ twins who are M^- or Jk^{a+} and we have found evidence to support their hypothesis both for total cholesterol and its fractions and for triglyceride [24].

Conclusion

All the lipid variables we have studied show significant heritability and all except triglycerides show an effect of shared environment in this comparatively young sample. It seems likely that the contribution of lipids to the CHD risk of an individual within 'Western' populations is determined partly at the time of conception and partly in the first two or three decades of life.

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