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Genetic and environmental covariance of serum cholesterol and blood pressure in female twins

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Summary

Blood pressure elevation is frequently associated with elevated cholesterol, triglyceride or low density lipoprotein (LDL-C) or low high density lipoprotein (HDL-C). The relative importance of genetic and environmental factors in these associations is unclear. We examined the relative contribution of genetic and environmental influences to the association between blood pressure and serum lipids in 75 pairs of female twins using path analysis and maximum-likelihood model fitting. Associations between systolic blood pressure and total cholesterol ($r = 0.44$, $P < 0.001$), and LDL-C ($r = 0.38$, $P < 0.001$), but not HDL-C ($r = 0.05$, N.S.), remained significant after age and body mass index adjustment. Univariate models suggested genetic effects contributed 60–70% to the variance of total cholesterol, LDL-C, HDL-C and systolic blood pressure. The remaining variance was explained by age and/or unique environmental influences. Using bivariate models, we demonstrated genetic ($P = 0.017$) and unique environmental covariance ($P = 0.011$) of cholesterol and systolic blood pressure. Significant genetic covariance ($P = 0.038$) was observed between LDL-C and systolic blood pressure. The association between blood pressure and total cholesterol in these twins results from shared genetic and similar unique environmental influences. The association between LDL-C and blood pressure is partly due to shared genetic influences. We conclude that both additive genetic and environmental factors unique to the individual are important determinants of the relationships between serum lipids and blood pressure.

Key words: Cholesterol; Lipoproteins; Blood pressure; Twins; Genetic

Introduction

There is considerable epidemiological evidence suggesting that a substantial proportion of hypertensive subjects have higher cholesterol levels than their normotensive counterparts [1–3]. Positive associations between serum cholesterol level and blood pressure have been repeatedly demonstrated in cross-sectional population studies [3–5], consistent with a relationship between these variables even within the 'normal' reference range. More recently, the term 'familial dyslipidaemic hypertension' has been ascribed to subjects with elevated blood pressures in the setting of either elevated cholesterol, low density lipoprotein cholesterol (LDL-C) or triglyceride or low high density lipoprotein cholesterol (HDL-C). This syndrome has been reported to have both genetic and environmental determinants on the basis of family studies [6] and observations in hypertensive male twins [7].

The association between lipids, lipoproteins and blood pressure assumes considerable importance in the context of those risk factors responsible for the development of premature atherosclerosis. Hypertension is a well recognized risk factor for coronary heart disease and has been shown to enhance atheroma formation in the presence of hyperlipidaemia in animal models [8,9]. In longitudinal epidemiological studies, it is clear that a synergism exists between hypertension and hypercholesterolaemia with regard to the relative risk from a subsequent coronary event [5]. Clinical trials [1,10,11] have demonstrated an unexpected lack of benefit of antihypertensive treatment on morbidity and mortality from coronary heart disease in man. This may partly reflect the underlying synergism between hypertension and hyperlipidaemia with inadequate treatment of coexistent hyperlipidaemia or possibly adverse effects of antihypertensive drugs on lipid and lipoprotein metabolism minimizing the effects of blood pressure reduction to reduce coronary artery plaque formation.

Twin studies have been widely used to investigate the genetic and environmental influences which determine individual differences in serum lipids and blood pressure. Genetic and environmental influences have been shown to significantly

influence the variability of total serum cholesterol and lipoproteins [12–16] and triglyceride levels [12–14,16]. Similar effects have been shown to influence blood pressure [17–20]. To date, no twin study has assessed the role of these genetic and environmental effects on the relationship between blood pressure and lipid phenotypes. In the present study, we assessed serum lipids and blood pressure in 75 pairs of healthy female twins. We used bivariate path analysis and maximum-likelihood model fitting to investigate the relative importance of additive genetic and environmental factors in determining the observed association between serum lipids and blood pressure. Such information is essential if optimal strategies for the prevention of atherosclerotic vascular disease are to be devised.

Methods

Subjects

Seventy-five pairs of monozygotic (MZ) and dizygotic (DZ) female twin pairs, aged between 17 and 65 years, were recruited through local community newspaper advertising and the Australian National Health & Medical Research Council Twin Registry as part of a previous study of platelet intracellular free calcium and blood pressure [20]. Subjects were screened to exclude any pairs in which one or both twins were receiving drug treatment for chronic diseases including hypertension, cardiac failure, angina pectoris or diabetes mellitus. Subjects with renal or liver impairment or a history of myocardial infarction were also excluded. The protocol for this study was approved by the University of Western Australia's Committee for Human Rights and informed consent was obtained from all participants.

The zygosity of each twin pair was assessed by questionnaire administered independently to each twin [21]. This method has a likely error rate of approximately 3%. In a subset of 35 twin pairs examined by DNA fingerprinting [22], 1 pair classified as DZ by the questionnaire were found to be MZ by DNA fingerprinting indicating an error rate of 2.8%. The misclassified pair were reclassified to the correct zygosity group prior to the genetic analysis. There were 31 DZ and 44 MZ twin pairs.

Methods

Both members of a twin pair attended on the same day on two occasions, 7 days apart. Pairs were seen between 07:00 and 10:00 h after fasting overnight. A standard health and lifestyle questionnaire was administered to each twin [23]. Finally, a blood sample was obtained from each subject without stasis after 5 min recumbency.

On the second visit, subjects were measured for weight by calibrated beam balance and for height by stadiometer. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Blood pressure was measured at least 10 min after arrival at the clinic by semi-automatic, non-invasive sphygmomanometry using a 'Dinamap 845XT' (Critikon Inc, Tampa, FL) with readings taken at 2-min intervals for 20 min, subjects resting supine. After discarding the first reading for each subject, systolic and diastolic blood pressure were calculated as the mean of 10 supine measurements.

Fasting serum total cholesterol and triglyceride were determined enzymatically using Abbott reagents on a COVAS-MIRA analyser (Roche Diagnostic, Basle, Switzerland) by the Department of Biochemistry at the Royal Perth Hospital. HDL-C was similarly assayed after precipitation with heparin manganese chloride. LDL-C was calculated using the equation: $LDL-C = \text{total cholesterol} - HDL-C - (\text{triglyceride} \times 0.46)$.

Data analysis

The preliminary statistical work was carried out using the Statistical Package for the Social Sciences (SPSS Inc, USA). Prior to analysis, the frequency distribution of each variable was examined to determine if any deviations from Gaussian distributions occurred. The triglyceride distribution was skewed and was transformed to normality prior to analysis using the algorithm: $\log_{10} [(\text{triglyceride} - 0.2) \times 10]$. Two pairs of twins were excluded from the HDL-C analysis on the basis of being greater than 3 standard deviations from the sample mean.

Associations between phenotypes were analysed by Pearson product moment correlations and multiple linear regression. All *P* values were two-sided. While the data are from twin pairs, we have regarded the observations in this study as indepen-

dent in order to explore the possible relationships between our lipid and blood pressure phenotypes.

Using the health and lifestyle questionnaire, a number of 'lifestyle' variables were considered as potentially relevant to blood pressure and blood lipids. These included: level of physical activity (assessed from self-reported number of days per week on which a variety of vigorous physical activities were undertaken), marital status (single = 0, married or defacto = 1), level of secondary education (<3 years high school = 0, >3 years high school = 1), level of tertiary education (no tertiary education = 0, tertiary education = 1), smoking status (never or ex-smoker = 0, current smoker = 1), caffeine consumption (no regular coffee consumption = 0, 1 or more regular cups of coffee per week = 1). Alcohol consumption (calculated in ml ethanol/week from a retrospective diary of the number and volume of all drinks consumed in the preceding week) was classified into those subjects consuming no alcohol, <120 ml/week and >120 ml/week. A screening step which searched for associations between 'lifestyle' variables and the continuous dependent variables was performed using Spearman rank correlations. Each variable was considered for further examination if the correlation was significant at the 10% level. 'Lifestyle' variables selected from the univariate correlations, along with age and BMI, were entered in a stepwise multiple regression allowing SPSS to determine the order of entry and retaining only those terms significant at the 5% level.

The methodology for the genetic analysis has been described previously [20] and was based upon the standard biometric model described by Jinks and Fulker [24] and Eaves et al. [25]. Briefly, the raw data were summarized into covariance-variance matrices using the pre-processor package PRELIS 1.12 [25]. A series of models was specified and fitted to the observed covariance matrices using LISREL 7.16, a programme for linear structural equation modelling [27]. These models may include: only environmental variance specific, i.e. unique, to an individual and not shared by her twin (E); this E variance plus either familial factors due to shared, i.e. common, environment rather than shared genes (C) or genetic variation due to the additive effects of genes (A); or all three. Age,

a potential source of variation contributing to the difference between but not within pairs, was included in the models where appropriate [28].

The fit of each model was assessed by maximum-likelihood methods and resulted in a χ^2 goodness of fit index which tested the agreement between the observed and the predicted statistics. Comparisons of each model using the likelihood ratio χ^2 test led to a preferred, most parsimonious model. A more detailed explanation is given by Neale et al. [29]. The model of best fit provided estimates of the variance attributable to each parameter (A, C, E and age) and a test of the significance of each. The parameters are expressed as an estimated percentage of the total phenotypic variance. These percentages were calculated by dividing the square of the estimate of each parameter by the sum of the square of all the estimates.

Results

Means, 95% confidence intervals and ranges for our variables are presented in Table 1. On average, the sample was non-obese, normotensive and normolipidaemic but included the expected range of blood pressures from low normal to mildly hypertensive, and a broad range of lipid values from low to high in terms of potential risk of coronary heart disease. Total cholesterol was positively associated with age, BMI and systolic and diastolic blood pressure (Table 2). After multiple regression analysis ($F_{3,143} = 15.24$, $P < 0.001$, $r^2 = 0.24$) which took into account the effects of age and

BMI, total cholesterol remained a significant predictor of supine systolic blood pressure (partial $r = 0.35$, $P < 0.001$) and supine diastolic blood pressure (partial $r = 0.20$, $P = 0.015$ and $F_{3,143} = 17.67$, $P < 0.001$, $r^2 = 0.27$).

Similarly, LDL-C was positively associated with age, BMI and systolic and diastolic blood pressure (Table 2). LDL-C remained a significant, independent predictor of systolic (partial $r = 0.30$, $P < 0.001$ and $F_{3,142} = 13.08$, $P < 0.001$, $r^2 = 0.22$) and diastolic blood pressure (partial $r = 0.16$, $P = 0.050$ and $F_{3,142} = 16.62$, $P < 0.001$, $r^2 = 0.30$). HDL-C was negatively correlated with BMI but was not significantly related to age or blood pressure (Table 2).

Serum triglyceride levels were also positively associated with age, BMI and systolic and diastolic blood pressure (Table 2). After adjusting for the effects of age and BMI, serum triglyceride levels remained a significant predictor of systolic (partial $r = 0.23$, $P = 0.006$ and $F_{3,143} = 10.68$, $P < 0.001$, $r^2 = 0.18$) and diastolic blood pressure (partial $r = 0.18$, $P = 0.030$ and $F_{3,143} = 17.14$, $P < 0.001$, $r^2 = 0.26$).

In stepwise multiple regression analysis including the 'lifestyle' variables, only age ($\beta = 0.36$) was a significant predictor of systolic blood pressure ($F_{1,145} = 21.93$, $P < 0.001$, $r^2 = 0.13$). Age ($\beta = 0.40$) and level of secondary education ($\beta = -0.17$) were significant predictors of diastolic blood pressure ($F_{2,144} = 23.68$, $P < 0.001$, $r^2 = 0.25$).

Total cholesterol was positively related to smoking ($r = 0.17$, $P = 0.037$) and marital status

TABLE 1

MEANS, 95% CONFIDENCE INTERVALS (CI) AND RANGES FOR THE ANTHROPOMETRIC, BLOOD PRESSURE AND SERUM LIPID VARIABLES

Variable	Mean	95% CI	Range	n
Age (years)	37.6	35.4–39.8	17–64	150
BMI (kg/m^2)	23.2	22.6–23.7	16.8–36.3	148
Systolic BP (mmHg)	116.4	114.9–117.9	96.3–144.8	150
Diastolic BP (mmHg)	69.2	68.0–70.4	53.7–92.4	150
Total cholesterol (mmol/l)	4.8	4.7–5.0	2.8–8.5	149
HDL-C (mmol/l)	1.44	1.40–1.48	0.86–2.09	144
LDL-C (mmol/l)	2.9	2.8–3.1	1.2–6.6	148
Triglyceride (mmol/l)	0.84	0.79–0.91	0.3–3.6	149

TABLE 2

PEARSON CORRELATIONS BETWEEN ALL VARIABLES^a

	BMI	Systolic BP	Diastolic BP	Cholesterol	HDL-C	LDL-C	Triglyceride
Age	0.44 $P < 0.001$	0.36 $P < 0.001$	0.48 $P < 0.001$	0.46 $P < 0.001$	0.12 (NS) ^b	0.39 $P < 0.001$	0.22 $P < 0.01$
BMI		0.26 $P = 0.001$	0.28 $P = 0.001$	0.26 $P < 0.001$	-0.21 $P < 0.05$	0.24 $P < 0.01$	0.37 $P < 0.001$
Systolic BP			0.66 $P < 0.001$	0.44 $P < 0.001$	0.04 (NS)	0.38 $P < 0.001$	0.30 $P < 0.001$
Diastolic BP				0.38 $P < 0.001$	0.05 (NS)	0.34 $P < 0.001$	0.28 $P < 0.001$
Cholesterol					0.07 (NS)	0.95 $P < 0.001$	0.49 $P < 0.001$
HDL-C						-0.12 (NS)	-0.34 $P < 0.001$
LDL-C							0.39 $P < 0.001$

^aNumber of subjects ranges from 142 to 150.^bNS denotes non-significant.

($r = 0.21$, $P = 0.011$) and negatively associated with tertiary education ($r = -0.28$, $P < 0.001$) in univariate analysis. After stepwise regression ($F_{2,140} = 29.60$, $P < 0.001$, $r^2 = 0.30$), only age ($\beta = 0.48$) and education ($\beta = -0.22$) were significant predictors of total cholesterol level.

In univariate analysis, HDL-C levels were positively associated with any alcohol consumption ($r = 0.32$, $P < 0.001$), drinking more than 120 ml ethanol equivalent per week ($r = 0.21$, $P = 0.011$), exercise ($r = 0.17$, $P = 0.042$) and coffee

consumption ($r = 0.22$, $P = 0.006$) and were negatively related to smoking ($r = -0.25$, $P = 0.002$). After stepwise regression ($F_{6,134} = 9.78$, $P < 0.001$, $r^2 = 0.30$), HDL-C remained related to BMI ($\beta = -0.28$), smoking ($\beta = -0.28$), age ($\beta = 0.24$), any alcohol consumption ($\beta = 0.20$), drinking more than 120 ml per week ($\beta = 0.18$) and coffee intake ($\beta = 0.19$).

LDL-C levels were positively associated with smoking ($r = 0.22$, $P = 0.009$) and marital status ($r = 0.14$, $P = 0.093$) and negatively related to ter-

TABLE 3

THE MODEL OF BEST FIT, χ^2 GOODNESS OF FIT INDEX, PERCENTAGE OF TOTAL VARIANCE ATTRIBUTABLE TO EACH PARAMETER IN THE MODEL AND LIKELIHOOD RATIO TEST OF SIGNIFICANCE OF EACH PARAMETER

Variable	Model of best fit	χ^2	d.f. ^a	P	% of total variance		
					Age	A	E
Total cholesterol	Age + A + E ^b	7.77	8	0.457	22***	64**	14***
HDL-C	A + E	4.36	4	0.359	—	71*	29***
LDL-C	Age + A + E	6.18	8	0.627	16***	67*	17***
Systolic BP	Age + A + E	5.58	8	0.694	14***	57*	29***
Diastolic BP	Age + A + E	6.10	8	0.636	23***	43	34***

^ad.f., degrees of freedom.^bAbbreviations: A, additive genetic variance; E, unique environmental variance.Tests of significance: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

tiary education ($r = -0.24$, $P = 0.004$). Age ($\beta = 0.43$) and smoking status ($\beta = 0.19$) were associated with LDL-C in stepwise regression ($F_{2,139} = 18.55$, $P < 0.001$, $r^2 = 0.21$).

Genetic analysis

One pair of twins was excluded from the cholesterol genetic analyses and two pairs from the lipoprotein analyses as data was missing on one twin member. A significant difference in means was observed between MZ and DZ twins for serum triglyceride levels (0.92 (0.84, 1.01), 0.78 (0.70–0.87), $P = 0.034$, respectively). This may indicate non-random sampling thereby invalidating one of the underlying assumptions of the classical twin study. Therefore, a genetic analysis of the serum triglyceride data was inappropriate. There was no significant difference in means or variances between MZ and DZ pairs for the other variables. There was a significant correlation between the absolute MZ intrapair differences and the intrapair sums for total cholesterol. This interaction could be attributed to the choice of scale since it was removed by logarithmic transformation of the data. Therefore, the genetic analysis for total cholesterol was performed on the transformed data. There were no similar problems with the other variables.

Cholesterol. Age contributed significantly to the variance of total cholesterol ($\chi^2 = 19.92$, 1 d.f.; $P < 0.001$) and was accordingly included as a parameter in each model. The full model, which included age, A, C and E effects provided a satisfactory fit to the data ($\chi^2 = 6.34$, 7 d.f.; $P = 0.500$). The reduced model from which the A effects were excluded had a significant loss of fit compared with the full model ($\chi^2 = 7.53$, 1 d.f.; $P = 0.006$) indicating that additive genetic effects contribute substantially to the variance of total cholesterol. However, when C effects were excluded from the model, the loss of fit was not significant ($\chi^2 = 1.43$, 1 d.f.; $P = 0.232$) suggesting that familial experiences common to both twins do not significantly contribute to the variance of total cholesterol. Consequently, the model which included age, A and E effects best explained the data (Table 3). Additive genetic effects accounted for 64% of the variance of total cholesterol, 22% was due to age and the remaining 14% was attributed to unique environmental influences (Table 3).

HDL-C. Age did not contribute significantly to the variance of HDL-C ($\chi^2 = 1.24$, 1 d.f.; $P = 0.266$). The full model, excluding age, was fitted to the data ($\chi^2 = 4.31$, 3 d.f.; $P = 0.230$). There was a loss of fit when A ($\chi^2 = 4.81$, 1 d.f.; $P = 0.028$), but not C ($\chi^2 = 0.05$, 1 d.f.; $P = 0.823$), effects were excluded leading to the model of best fit in Table 3. From this model, 71% of the variance of HDL-C was due to additive genetic effects and the remainder was explained by unique environmental effects (Table 3).

LDL-C. Age contributed to the variance of LDL-C ($\chi^2 = 14.51$, 1 d.f.; $P < 0.001$). The model containing age, A, C and E influences was fitted to the data ($\chi^2 = 5.23$, 7 d.f.; $P = 0.633$). Excluding A effects led to a significant loss of fit ($\chi^2 = 6.13$, 1 d.f.; $P = 0.013$) while excluding the C effects did not lead to a significant loss of fit ($\chi^2 = 0.95$, 1 d.f.; $P = 0.330$). Therefore, the model of best fit for LDL-C included age, A and E effects with the variance partitioned into 16% due to age, 67% due to additive genetic effects and 17% due to unique environmental influences (Table 3).

Systolic blood pressure. Age contributed significantly to the variance of systolic blood pressure ($\chi^2 = 14.12$, 1 d.f.; $P < 0.001$) and the full model provided a satisfactory fit to the data ($\chi^2 = 5.56$, 7 d.f.; $P = 0.592$). While excluding A led to a significant loss of fit of the model ($\chi^2 = 3.91$, 1 d.f.; $P = 0.048$), exclusion of the C effects did not significantly alter the fit of the model ($\chi^2 = 0.02$, 1 d.f.; $P = 0.888$). From the model of best fit (Table 3), 14% of the total variance of systolic blood pressure was attributable to age, 57% was due to additive genetic effects and 29% was due to the environment unique to each individual (Table 3).

Diastolic blood pressure. The full model explained the diastolic blood pressure data well ($\chi^2 = 2.87$, 7 d.f.; $P = 0.897$). Excluding either the A or the C effects did not result in a significant loss of fit ($\chi^2 = 1.07$, 1 d.f.; $P = 0.301$ and $\chi^2 = 0.38$, 1 d.f.; $P = 0.538$, respectively). However, excluding both parameters led to a loss of fit ($\chi^2 = 20.83$, 2 d.f.; $P < 0.001$). This indicates that there is a significant family contribution but there is insufficient power to ascribe the variance to either A or C effects. Since excluding the additive genetic influence gave a slightly greater χ^2 , the model preferred by us included significant age and

E effects and a non-significant A effect (Table 3). Using this model age, genetic and unique environmental effects accounted for 23%, 43% and 34% of the variance of diastolic blood pressure in this population, respectively.

Bivariate analysis of serum lipid-blood pressure relationships. The methods used to estimate the relative genetic and environmental effects of a trait may be used to determine whether some or all of the factors that contribute to the variance of one trait could also contribute to the variance of another trait [20,30]. We wished to test for either genetic or environmental covariance between total cholesterol and systolic blood pressure and between LDL-C and systolic blood pressure. A bivariate analysis of the HDL-C and systolic blood pressure data was not performed since no significant correlation was observed between these variables. The models specified in the present bivariate analysis included age, A and E influences for both variables.

Table 4 shows the bivariate models fitted to the total cholesterol and systolic blood pressure data. The simple bivariate factor model explained the data well. There was a significant loss of fit when both the genetic and the unique environmental correlations were excluded from the model (set $h_B = e_B = 0$) confirming covariation of our

TABLE 4

BIVARIATE FACTOR MODELS FOR TOTAL CHOLESTEROL AND SYSTOLIC BLOOD PRESSURE^a

Models	χ^2	d.f.	P
1. Simple bivariate	18.82	21	0.597
2. Set $h_B = 0$ †	24.48	22	0.323
3. Set $e_B = 0$ ‡	25.31	22	0.282
4. Set $h_B = e_B = 0$	35.76	23	0.044

^aThis Table includes the χ^2 goodness of fit index and the specific submodels to test for covariation, genetic covariation and unique environmental covariation. † There was a significant loss of fit when model 1 was compared with model 2 ($\chi^2 = 5.66$, 1 d.f.; $P = 0.017$) which excluded the genetic correlation (set $h_B = 0$). ‡ There was a significant loss of fit when model 1 was compared with model 3 ($\chi^2 = 6.49$, 1 d.f.; $P = 0.011$) which excluded the unique environmental correlation (set $e_B = 0$). Therefore, it was concluded that both genetic and unique environmental influences contribute to the association between systolic blood pressure and total cholesterol.

TABLE 5

BIVARIATE FACTOR MODELS FOR LDL-C AND SYSTOLIC BLOOD PRESSURE^a

Models	χ^2	d.f.	P
1. Simple bivariate	13.17	21	0.902
2. Set $h_B = 0$ †	17.86	22	0.714
3. Set $e_B = 0$ ‡	15.95	22	0.819
4. Set $h_B = e_B = 0$	23.71	23	0.420

^aThis Table includes the χ^2 goodness of fit index and the specific submodels to test for covariation, genetic covariation and unique environmental covariation.

† As there was a significant loss of fit when model 1 was compared with model 2 ($\chi^2 = 4.69$, 1 d.f.; $P = 0.030$) which excluded the genetic correlation (set $h_B = 0$), it was concluded that genetic factors contribute to the association between systolic blood pressure and LDL-C.

‡ When model 1 was compared with model 3 ($\chi^2 = 2.78$, 1 d.f.; $P = 0.095$) which excluded the unique environmental correlation (set $e_B = 0$), there was a trend towards a loss of fit suggesting that these effects may also influence the relationship.

phenotypes. When the genetic correlation was excluded from the model (set $h_B = 0$), there was a significant loss of fit ($\chi^2 = 5.66$, 1 d.f.; $P = 0.017$) indicating genetic covariation between our two phenotypes. Additionally, excluding the unique environmental correlation (set $e_B = 0$) led to a significant loss of fit ($\chi^2 = 6.49$, 1 d.f.; $P = 0.011$) indicating the presence of unique environmental covariation between total cholesterol and systolic blood pressure. From the simple bivariate factor model, the estimated genetic and environmental correlations were moderate ($r_g = 0.31$ and $r_e = 0.37$, respectively). Direction of causation modelling can further test whether one variable has a direct effect upon the other, vice versa, both, or whether a third factor is influencing their covariation. However, while each of these models fitted the data well (data not shown), we were unable to discriminate between the factor and causality models due to insufficient resolving power.

Table 5 shows the bivariate models fitted to the LDL-C and systolic blood pressure data. The simple bivariate model explained the data. There was significant evidence for genetic covariation ($\chi^2 = 4.69$, 1 d.f.; $P = 0.030$) with the estimated genetic correlation at $r_g = 0.48$. In addition, there was a tendency towards a loss of fit when the uni-

que environmental correlation was excluded ($\chi^2 = 2.78$, 1 d.f.; $P = 0.095$) suggesting that unique environmental covariation may also exist between our phenotypes. The environmental correlation was estimated at $r_e = 0.24$. Each of the direction of causation models fitted the data well (data not shown) but we were unable to discriminate between the factor and direction of causation models.

Discussion

The present study in healthy female twin pairs confirms that additive genetic and non-shared or unique environmental effects contribute to the variability of total cholesterol, LDL-C, HDL-C and systolic blood pressure. In addition, we confirm previous observations of a positive and independent association between blood pressure and total cholesterol, LDL-C and triglyceride but not HDL-C. The present study is the first to investigate the association between blood pressure and serum lipids in twins using bivariate genetic analysis. We report that common additive genetic and similar unique environmental factors contribute substantially to the co-variance of total cholesterol and systolic blood pressure. Furthermore, we present evidence that common additive genetic effects influence the relationship between LDL-C and systolic blood pressure in this sample.

We began our investigation by partitioning the genetic and environmental contributions of the variance of total cholesterol, lipoproteins and blood pressure. Our finding that additive genetic effects contributed 60–70%, of the variability of our lipid phenotypes is consistent with previous studies which have generally shown a large genetic component [12–16]. However, a wide range of genetic estimates have been observed for cholesterol, LDL-C and HDL-C including reports of little or no genetic variability [31], and this may be partly accounted for by differences in the populations, the age range of the participants, the study design and the estimation methods employed. In addition, a number of different procedures have been suggested to correct for the effects of age [32]. This study incorporated the age effects in the linear model using the technique described by Neale and Martin [28] and suggests

that age determines 22% and 16% of the variance of serum cholesterol and LDL-C in these women, respectively.

The present study would suggest that shared environmental experiences are not significant determinants of the variation in adult cholesterol, LDL-C or HDL-C levels but that an individual's specific environmental experiences are dictating some of their variability. The biggest source of shared environment in studies such as this often results from twins being bled and assayed on the same day in the same batch as each other but different from other pairs. In the present study, samples were treated as such but did not appear to generate any pseudo-common environment. In contrast, Whitfield and Martin [12], in a study of 205 twin pairs, attributed approximately 25% of the variability of both total cholesterol and LDL-C and 47% of the variability of HDL-C to environmental effects shared by members of a twin pair. The disparity between their results and ours may reflect differences in the power of each study given the difference in the number of twin pairs [33]. In the present study, the lack of inclusion of a common environment effect in our best fitting model does not necessarily imply that the effect was zero but rather that it could not be significantly distinguished from zero. In support of the present study, shared environmental effects were not shown to contribute significantly to the variation of total cholesterol, LDL-C or HDL-C in 233 pairs of 11 year old twins [14].

We confirmed a positive association between total cholesterol and blood pressure in this population of women. This relationship was maintained after adjustment for the effects of BMI and age. A positive association between total cholesterol and blood pressure has been observed in a number of previous studies [3–5]. Most recently, investigators from the Tromso Study [4] observed a positive association between blood pressure and total cholesterol in a population of 8081 men and 7663 women. While BMI modified this relationship, smoking, physical activity and alcohol consumption had little influence. Bonaa and Thelle [4] showed that increases in serum lipids with blood pressure tended to be greater in overweight than lean subjects indicating that adiposity may modify the association between total

cholesterol and blood pressure. However, it may be that more subtle differences in adiposity such as body fat composition and distribution may be the more important issues as an abdominal fat distribution has been shown to be a greater predictor of both blood pressure and dyslipidaemia [34,35] than total body fat.

Since total cholesterol is made up of HDL-C, LDL-C and very low density lipoprotein cholesterol (VLDL-C), its association with blood pressure could be due to any one of these lipoproteins. A subgroup analysis in the Tromso Study [4] suggested that the association was due to both VLDL-C and LDL-C. While we observed an association between LDL-C, but not HDL-C, and blood pressure, results from other groups have not shown this to be consistent [1]. The possibility of confounding effects of triglycerides and HDL-C in various populations may partially explain these inconsistencies.

Bivariate path analysis has been used previously with family or twin studies to assess the relationships between such variables as blood pressure and weight [36] and left ventricular mass and weight [37]. The increased risk of coronary heart disease in subjects with both dyslipidaemia and hyperten-

sion compared with either condition alone, emphasizes the need to understand why these risk factors are associated. While there is considerable evidence to suggest familial aggregation of these phenotypes, less is known regarding whether this is due to genetic or common environmental influences. Our primary aim was to investigate whether the genes or environment that influence blood pressure are specific to that trait or whether they share influence on other risk factors for coronary artery disease. By studying a sample of 'healthy' adult twins we were able to avoid the complicating effects of diet or drug treatment on established hypertension or dyslipidaemia. Even in our relatively healthy population there was a large range of blood pressure and lipid values indicating a widely varying risk of coronary artery disease. The bivariate method employed in this study is an extension of the univariate models and allows us to draw inferences regarding the association between two traits. In the present case, we wished to test for the presence of genetic and/or environmental covariation between systolic blood pressure and both total cholesterol and LDL-C. This study demonstrated that common additive genetic and similar non-shared environmental factors influ-

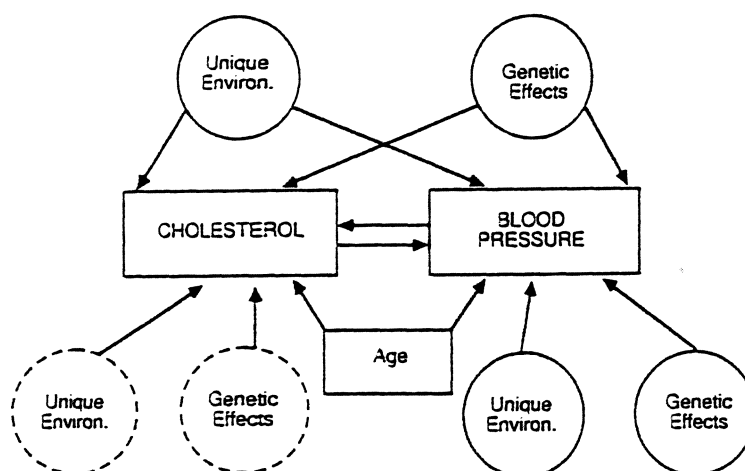


Fig. 1. Schematic diagram of proposed hypothesis relating genetic and environmental influences to the relationship between blood pressure and cholesterol. In this hypothesis we propose that the genetic and unique environmental influences that control the variability of blood pressure include those that are specific for blood pressure and those common to blood pressure and cholesterol. Similarly, the genetic and unique environmental influences that control the variability of cholesterol include those that are specific for cholesterol and those in common with blood pressure. This hypothesis combines models from both the univariate (dashed lines) and bivariate analyses.

ence the relationship between total cholesterol and systolic blood pressure. Additionally, we report that there was genetic covariation between LDL-C and systolic blood pressure with a trend towards unique environmental covariation. Importantly, these results help us to understand the consistently reproducible association between cholesterol and blood pressure. As seen in the schematic diagram in Fig. 1, we propose that the genetic and unique environmental influences that control the variability of blood pressure include those that are specific to blood pressure and those common to blood pressure and cholesterol.

In 1988, Williams et al. [6] suggested the presence of a specific syndrome entitled 'familial dyslipidaemic hypertension' based on data from individuals identified as having familial hypertension. Further to this, Selby et al. [7] identified 60 cases of so called 'dyslipidaemic hypertension', classified as a blood pressure greater than 160/90 mmHg and one extreme lipid value, from 1028 male twin pairs. The majority of these extreme lipid values were low HDL-C levels with fewer raised VLDL-C. While the prevalence of this 'disorder' was similar by zygosity, proband concordance was 3 times greater in MZ than DZ twins suggesting the presence of familial, i.e. genetic or shared environmental, influences. Further examination 10 years later of the same data set reported that MZ concordance levels were lower while DZ concordance rates were increased. This was primarily due to the failure of affected MZ twins to return for the second examination and highlights the difficulties involved in this type of analysis. Twins concordant for lipid abnormalities in the study by Selby et al. [7] were more likely to be obese and have evidence of impaired glucose tolerance suggesting that a genetic link may be mediated via obesity. Given that hypertension and dyslipidaemia are the extreme conditions that characterise the continuous and normal distribution of blood pressure and serum lipids, our data from healthy women would tend to support the hypothesis that these traits have a multifactorial inheritance and that common additive genetic effects and similar environmental factors specific to each individual determine the association between hypertension and dyslipidaemia.

The greatest criticism of the twin method is its

dependence on the assumption that environmental effects within families act equally on MZ and DZ pairs [38]. This limitation has been well reviewed recently [30]. It has been suggested that an overestimation of additive genetic effects may occur due to the closer similarity of shared 'lifestyle' factors in MZ than in DZ twins, and a number of studies have adjusted for some of these effects prior to genetic analysis [15,16]. This additional similarity may arise because twins select or create their environments and this may be partly influenced by genetics [39]. However, if parents or others treat the twins on the basis of their zygosity, i.e. when the MZ twins are passive recipients of more similar environments than DZ pairs, potential problems may arise. For most environmental variables associated with cardiovascular risk such as smoking, alcohol consumption and exercise, there are no a priori grounds for assuming that twins are passive recipients of their environment [30]. Furthermore, adiposity has been shown to be under the dual influence of genetic and environmental factors [40] and there are indications that dietary preferences [41] and alcohol consumption [42] are also influenced by both genetic and environmental influences. Therefore, adjusting for these 'lifestyle' factors prior to the analysis will not necessarily correctly account for a supposed overestimation of the genetic effects. Using the data obtained from our health and lifestyle questionnaire, we have confirmed the findings from previous studies [43–46] that alcohol consumption, smoking and coffee intake are variously associated with lipid and lipoprotein levels. Our data were not corrected for various 'lifestyle' factors such as alcohol consumption, smoking habit or obesity before the genetic analysis was performed. The ideal situation would have been to extend our models to multivariate models including these 'lifestyle' factors. However, the relatively low correlations observed between the phenotypes of interest and the 'lifestyle' variables in this study and our relatively small number of subjects made this option impractical.

The majority of twin studies investigating serum lipids have been in either male subjects or have considered males and females together. It is accepted that men and women differ in the levels and variability of serum lipids possibly due to basic hormonal differences between the sexes. Given

this, it would be unwise to over-extrapolate the results observed in the present female sample to the general male population. However, Whitfield and Martin [12] presented no significant evidence for sex heterogeneity in their predominately European sample and so used a combined model including both men and women in their analysis of total cholesterol, HDL-C and non HDL-C. In the recent Medical College of Virginia Twin Study [14], different magnitudes of genetic effects were seen for total cholesterol in 11-year-old boys and girls, results which the authors suggested may be consistent with the girls being at a slightly more advanced stage of sexual maturity. It is known that lipoprotein levels are affected by the oral contraceptive pill, depending on the formulation, and hormone replacement therapy [47,48]. In the present sample of women, it was difficult to detect relationships between these factors and our lipid phenotypes since only 9% reported current use of the oral contraceptive pill and only 1 pair were on oestrogen replacement therapy.

In summary, this study indicates that the observed association between blood pressure and total cholesterol is due to shared additive genetic and similar unique environmental influences. We also report that shared additive genetic factors contribute to the relationship between LDL-C and blood pressure. Although genetic factors are important in determining the variance of lipid and blood pressure levels between individuals, comparisons between populations and of migrants suggest that absolute levels of lipids and blood pressure are strongly influenced by environmental factors, particularly diet. Our observations emphasize the necessity for screening and treating these risk factors simultaneously rather than considering them in isolation. It also highlights the need for early identification of individuals from families already known to have dyslipidaemia, hypertension or both in populations with a high incidence of coronary heart disease.

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