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Lifestyle Factors in Monozygotic and Dizygotic Twins

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In examining genetic influences on biological variables using twins, it may be important to examine the distribution between and within twin pairs of demographic and lifestyle factors that may themselves affect the biological variable being studied. We explored the distribution of demographic and lifestyle factors that may affect blood lipid levels or ischaemic heart disease (IHD) risk among a sample of 106 monozygotic (MZ) and 94 like-sex dizygotic (DZ) twin pairs. In our sample, MZ twins were statistically significantly different from DZ twins only in marital status, cigarette smoking habits, and the ratio of polyunsaturated to saturated fat (P:S ratio) in their dietary intake. The latter variable was among many dietary variables examined (using 4-day weighed food diaries), and the size of the difference in intake was small. When comparisons were made of the similarities within twin pairs, we found members of MZ twin pairs to be statistically significantly closer than DZ twins in educational achievement, occupation, cigarette smoking, and exercise habits, and the number of days a week on which alcohol was consumed. These last three variables were consistently closer among twins with closer contact than among those with a smaller degree of current shared environment. For 12 of the 13 nutrients examined, the within-pair correlations were higher for MZ than for DZ twins, although our test for significant genetic variance showed statistical significance only for intake of complex carbohydrates. We conclude that MZ twins share demographic and lifestyle factors that might influence the risk of IHD and blood lipid levels to a greater degree than do DZ twins, although it is difficult to say if these similarities in lifestyle result from genetic influences or not. Nevertheless, ascribing differences between correlations

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in MZ and DZ twin pairs for lipid levels as being purely "genetic"—as implicit in conventional measures of heritability—is likely to overestimate the influence of genetic factors.

Key words: twins, blood lipids, environmental effects, heritability

INTRODUCTION

Many studies examining the genetic influence on biological variables such as lipid levels have used twins as subjects. Because monozygotic (MZ) twins share twice as many genes as dizygotic (DZ) twins, closer similarities observed in MZ than in DZ pair members have been taken to suggest a genetic effect. There are, however, a number of demographic and lifestyle factors that influence lipid levels and there may be similarities in these lifestyle factors within twin-pair members, and these similarities may be greater in MZ than in DZ pairs [Fabsitz et al., 1978a,b]. In addition, MZ twins as a group may adopt different types of lifestyles from those adopted by DZ twins or others, and, thus, comparisons between the two types of twins may measure more than just genetic differences. As part of a study to explore the genetic influences on lipid levels, we have examined the distribution of demographic and lifestyle factors that may influence lipid levels in MZ and DZ twin pairs. Our hypotheses were 1) that MZ twins differ from DZ twins in a number of lifestyle characteristics that may influence lipid levels or the risk of ischaemic heart disease (IHD) and 2) that MZ twin pairs will have closer similarities in lifestyle factors that may influence lipid levels and the risk of IHD than will DZ pairs.

Apart from the relevance of these questions to the understanding of possible genetic influences on lifestyle factors, they are of importance in planning analyses of twin studies where adjustment for closer similarities among MZ pairs for lifestyle factors that influence lipid levels may have to be made.

METHODS

Study Sample

A group of 200 adult twin pairs (106 MZ and 94 like-sex DZ) were assembled in Newcastle (98 pairs) and Sydney (102 pairs) in New South Wales, Australia. The majority of Sydney twins were recruited from a large twin register established and maintained by the NH & MRC [Clifford and Hopper, 1986]; the Newcastle twins were respondents to advertisements and personal contacts.

Measurements

Each subject was required to visit one of the study centres on two occasions, both members of the pair attending at the same time. On the first occasion, the twins completed a questionnaire that asked about age, marital status, educational attainment, occupation, frequency of current contact with his/her co-twin (answers could range from "almost every day," "at least once a week," and "once or twice a month" to "a few times a year" or "less often"), length of time they have lived apart, previous history of heart disease, and related risk factors such as elevated lipid levels and hypertension, lifestyle patterns (exercise, smoking, alcohol consumption), and oral contraceptive use (women only).

The eating habits of the twins were estimated from a weighed diary that was kept over a 4-day period (Wednesday through Saturday) and in which participants recorded the amounts and types of all foods, liquids, and vitamin supplements consumed over that period. Digital scales were supplied to the twins, and detailed instructions on their use were given on the first visit. Telephone contact was maintained over the following week to ensure compliance in the diary keeping and to answer any problems about this procedure. The scales and diaries were returned at the second visit to the centre, and the diary was checked carefully for completeness. In some instances, one or both of the twins could not attend the second visit, so the scales and diaries were returned by mail.

Analysis of Food Diaries

The entries in the food diary were coded by a dietitian using the food tables of McCance and Widdowson and analyzed by a computer program (DIARYAN) [Baghurst and Record, 1984]. For each person, this program produced a daily summary of nutrient intake including total kilojoules, macronutrients (including alcohol) and micronutrients, and the average intake over the 4 days. From this, the average daily percentage contribution to total kilojoules from protein, fats, carbohydrates, and alcohol and the ratio of polyunsaturated to saturated fat intake (P:S ratio) were calculated.

Zygosity Determination

For the majority of the twin pairs, zygosity was determined on the basis of this question: "Non-identical twins are no more alike than ordinary brothers and sisters. Identical twins on the other hand have such a strong resemblance to each other in stature, colouring, features of the face, etc., that people often mistake one for the other. Having read this statement do you think you are an identical twin or a non-identical twin?" A number of authors have found excellent agreement between the responses to this or a similar question and zygosity as determined by blood testing [Martin and Martin, 1975]. In our study where there was some doubt about zygosity (nine sets), blood samples were taken for blood group analysis (antigens ABO, Rh, K, k, Fy^a, Fy^b, Jk^a, Jk^b, M, N, S, and s). HLA typing (*A* and *B* loci) was needed for six of the nine pairs. As a result of this blood typing, eight of the nine pairs were determined to be MZ.

Statistical Methods

Differences between MZ and DZ twins in demographic and lifestyle characteristics were assessed using the chi-square statistic (with continuity correction for binary characteristics). Nutrient intakes were compared in the two types of twins via Christian and Norton's [1977] t'-test on the means. Since the DZ twin group had proportionately more women, nutrient values for the two groups were adjusted using a method described by Kleinbaum and Kupper [1978]—these are referred to as the sex-adjusted values. In this method, women were chosen as the reference group and the nutrient values for men were adjusted to have the same mean and variance as for the women. In addition, $\log_e$ transforms were carried out on some of the nutrients to make the distributions approximately normal before tests of significance were performed.

Within twin-pair concordance on demographic and lifestyle characteristics was defined as the proportion of twin pairs in which the responses of both members were to the same category. An estimate of heritability (h^2) may be obtained as the ratio of the difference in the proportions in concordance in MZ and DZ twins over 1 minus the proportion in concordance in DZ twins [Jacquard, 1983].

Differences in concordance in MZ and DZ twins were tested for statistical significance via continuity corrected chi-square statistics. Intraclass correlation coefficients for nutrient intake were calculated separately for MZ and DZ twins from a components of variance model. Using $\log_e$ transformed values where appropriate, equality of variances was checked first by comparing the ratio of the sums of the among and within mean squares for MZ (A_{MZ} and W_{MZ}) and DZ (A_{DZ} and W_{DZ}) twins [$F' = (A_{MZ} + W_{MZ})/(A_{DZ} + W_{DZ})$] with an F distribution with degrees of freedom calculated according to Satterthwaite [see, for example, Haseman and Elston, 1970]. The corresponding tabled probability was doubled for a two-tailed test and a level of .2 was used to determine statistical significance [Christian et al., 1974]. When the test for the equality of variances failed to reach statistical significance, an estimate of genetic variance was obtained from the within mean squares as $G_W = W_{DZ} - W_{MZ}$. The significance of this estimate was tested by the F ratio W_{DZ}/W_{MZ} with ($n_{DZ} - 1$, $n_{MZ} - 1$) degrees of freedom. When there are significant differences in the variances, this estimate may be biased [Christian et al., 1987], and the among-component estimate [$G_{AC} = (A_{MZ} - A_{DZ} + W_{DZ} - W_{MZ})/2$] as proposed by Christian et al. [1974] was used. A test of significance for this estimate of genetic variance is given by $F' = (A_{MZ} + W_{DZ})/(A_{DZ} + W_{MZ})$, with degrees of freedom given by Cochran [1951].

Estimates of heritability may be obtained as the ratio of the genetic variance to the total variance. Appropriate numerators are twice the estimate of G_W or G_{AC}, with the average of the total variances for MZ and DZ twins given by $(A_{MZ} + W_{MZ} + A_{DZ} + W_{DZ})/4$ in the denominator.

Another estimate of heritability is given by $2(r_{MZ} - r_{DZ})$, where r_{MZ} and r_{DZ} are the intraclass correlation coefficients for MZ and DZ twins, respectively. If there is insufficient evidence to suggest inequality of variances in MZ and DZ twins, the estimate of heritability based on G_W is given with the P-value from the F-test; otherwise the heritability estimate based on G_{AC} and the P-value from the F'-test are reported. The estimate $h^2 = 2(r_{MZ} - r_{DZ})$ is reported even though it may be biased in some situations for comparability with other studies.

RESULTS

Of 203 twin pairs initially recruited, three pairs were excluded because of pregnancy (one pair) or an inability to complete the study (two pairs). Three more pairs were unable to keep adequate food diaries and have been excluded from some analyses. Thus, this report concerns 200 twin pairs—106 were MZ (39 male and 67 female) and 94 were like-sex DZ (25 male and 69 female). Ages ranged from 17 to 66 years with the mean ages of MZ and DZ twins being 36.9 (SD = 13.2) and 35.6 (SD = 11.5), respectively.

DZ female twins were more than twice as likely to be separated, widowed, or divorced than were MZ female twins, leading to a statistically significant difference between the marital status of female MZ and DZ twins ($P < .05$) (Table I). There

TABLE I. Demographic Characteristics of Twins (numbers are percentages of each group of twins)

	Male		Female		
	MZ (N = 78)	DZ (N = 50)	MZ (N = 134)	DZ (N = 138)	
Marital status					
Never married	33.3	40.0	26.1	22.5	*
Currently married	62.8	56.0	65.7	59.4	
Widowed/separated/ divorced	3.9	4.0	8.2	18.1	
Born in Australia	92.3	88.0	85.1	92.8	
Highest education achieved					
Primary	2.6	4.0	2.2	1.5	
Some secondary	19.2	14.0	24.6	28.3	
Finished secondary	26.9	34.0	48.5	49.3	
Tertiary	51.3	48.0	24.6	21.0	
Current occupation					
Professional/executive	33.3	36.0	23.9	16.7	
Sales/clerk	11.5	8.0	26.9	31.2	
Other paid employment	35.9	48.0	14.2	15.9	
No paid employment	19.2	8.0	35.1	36.2	

*Statistically significant differences between MZ and DZ twins, $P < .05$, chi-square test.

TABLE II. Distribution of Cardiovascular Risk Variables From Questionnaire Replies in Twins (numbers are percentages of each group of twins)

	Male			Female		
	MZ (N = 78)	DZ (N = 50)		MZ (N = 134)	DZ (N = 138)	
Smoking status						
Never smoked	66.7	46.0	*	61.9	50.7	*
Ex-smoker	21.8	14.0		13.4	24.6	
Current smoker	11.5	40.0		24.6	24.6	
Frequency of alcohol consumption						
Don't drink	20.5	22.0		23.1	18.1	
< 1 day per week	32.1	26.0		34.3	39.9	
1–2 days per week	26.9	20.0		24.6	26.8	
3+ days per week	20.5	32.0		18.0	15.3	
Engaged in vigorous exercise in past 2 weeks	50.0	36.0		47.8	41.3	
Oral contraceptive use						
Ever used	—	—		76.9	85.5	
Current user	—	—		29.1	30.0	
History of hypertension	16.7	16.0		19.4	20.3	
History of high cholesterol	10.3	14.0		3.0	5.1	

*Statistically significant difference between MZ and DZ twins, $P < .05$, chi-square test.

were no other significant differences in demographic characteristics. Table II shows that DZ and MZ twins differ in their cigarette smoking habits. Among both men and women, DZ twins were more likely to have ever smoked: male DZ twins were more likely to be current smokers and female DZ twins were more likely to be ex-smokers. MZ twins were more likely to have engaged in vigorous exercise in the previous 2 weeks, but the differences were not statistically significant; this was consistent in men and women. There were no consistent differences in patterns of alcohol consumption, oral contraceptive use in women, or history of hypertension and high cholesterol. Table III shows that for most nutrients the mean daily intake calculated from the 4-day food record was similar in MZ and DZ twins. MZ twins had a statistically significantly lower P:S ratio than DZ twins, but the size of the absolute difference in intake was small.

Thus, in terms of demographic and behavioural characteristics, MZ twins as a group were similar to DZ twins with the exceptions that female MZ twins were more likely to be separated, widowed, or divorced, cigarette smoking habits were different among both men and women between MZ and DZ twins, and there was a small but significant difference in the P:S ratio in their diets.

Although there was no difference in the length of time the twins had been living apart, MZ twins tended to contact, see each other, and eat together more regularly than did the members of DZ twin pairs (Table IV). The differences were more marked in women, but consistent in both male and female twins.

The next two tables examine the similarities *within* twin pairs. Table V shows that there was statistically significantly higher concordance within MZ than within DZ pairs for occupation, educational attainment, current cigarette smoking status,

TABLE III. Mean Daily Nutrient Intake (SD) in Identical and Non-Identical Twins From 4-Day Weighed Food Diaries

	MZ (N = 206)	DZ (N = 188)	Difference in sex-adjusted means[a] (MZ − DZ)
Kilojoules	8922 (2871)	8505 (2522)	90
Protein (% of energy)[b]	15.4 (3.2)	15.1 (3.1)	0.4
Carbohydrate (% of energy)			
Total	42.3 (7.1)	42.8 (7.3)	−0.5
Simple	20.4 (6.0)	20.5 (6.9)	0
Complex	21.9 (5.3)	22.2 (5.4)	−0.4
Fat (% of energy)			
Total	37.6 (5.7)	37.4 (6.5)	0.3
Saturated	14.8 (2.8)	14.3 (3.0)	0.6
Monounsaturated	12.7 (2.4)	12.4 (2.5)	0.3
Polyunsaturated[b]	6.6 (2.5)	7.1 (2.9)	−0.5
P:S ratio[b]	0.47 (.21)	0.52 (.24)	−0.05*
Dietary cholesterol (mg)[b]	298.2 (166.3)	271.4 (138.3)	12.9
Fibre (g)[b]	19.0 (8.7)	19.4 (8.3)	−1.1
$\log_e$ (alcohol) (g) in drinkers[c]	2.75 (0.95)	2.65 (1.15)	0.05

[a]t'-tests were performed on sex-adjusted values.
[b]Data were $\log_e$ transformed before performing tests of significance.
[c]39.3% of MZ and 42.0% of DZ twins did not drink alcohol on the days the food diary was kept.
$^*P < .05$.

TABLE IV. Contact Between Members of Twin Pairs

	Male (%)		Female (%)	
	MZ (N = 39)[a]	DZ (N = 25)	MZ (N = 67)	DZ (N = 69)
At least once per week				
Contact each other	82.1	60.0	91.0	63.8***
See each other	64.1	36.0*	76.1	42.0***
Eat together	33.3	24.0	53.7	26.1**
Lived apart more than 15 years	61.5	60.0	47.8	46.4

[a]N is number of twin pairs.
*$P < .05$, **$P < .01$, ***$P < .001$ from Fisher's Exact Test.

TABLE V. Within Twin Pair Concordance on Demographic and Lifestyle Characteristics

	Percentage of twin pairs in agreement		
	MZ (N = 106)	DZ (N = 94)	[a]h^2
Marital status	79.2	68.1	0.35
Highest education achieved	80.2	66.0*	0.42
Occupation	62.3	43.6*	0.33
History of			
Hypertension	78.3	75.5	0.11
High cholesterol	88.7	80.9	0.41
Oral contraceptive use (Women only)			
Ever	77.6	85.5	−0.54
Current	74.6	62.3	0.33
Current smoking status	72.6	56.4*	0.37
Vigorous exercise in past 2 weeks	70.8	52.1**	0.39
Number of days alcohol consumed	61.3	37.2***	0.38

[a]h^2 = heritability (see Methods section).
*$P < .05$, **$P < .01$, ***$P < .001$ from Yates' Continuity Corrected Chi-square test.

having engaged in vigorous exercise in the past 2 weeks, and the number of days in a week alcohol is consumed. Marital status was also more similar in MZ than in DZ twin pairs ($P < .10$). The measure of heritability used (h^2) indicated a high degree of heritability for most of these demographic and lifestyle characteristics (Table V). Table VI examines the similarities in smoking habits, alcohol intake, and exercise patterns according to the degree of shared environment. For each of the three variables, and in both MZ and DZ twin pairs, there were consistent patterns of closer similarities with greater degrees of shared environment.

Table VII shows that within twin-pair correlations were greater for MZ than for DZ pairs for 12 of the 13 nutrients examined. Only for complex carbohydrates was the degree of genetic variance statistically significantly different from zero, but a number of the estimates of "heritability" were high. There were no consistent similarities in dietary preference as measured by nutrient intake according to frequency of contact between twin pairs.

DISCUSSION

This study is preliminary to an examination of the relationship between the genetic influences on blood lipid levels taking the effects of dietary intake and other

TABLE VI. Concordance in Smoking Behaviour, Frequency of Alcohol Consumption and Exercise in Identical and Non-Identical Twins by Degree of Shared Environment

	Percentage of Twin Pairs in Agreement			
	Frequency of contact			
	Almost daily		Less often	
	MZ (59)[a]	DZ (24)	MZ (47)	DZ (70)
Smoking	78.0	62.5	66.0	54.3
Alcohol	64.4	45.8	57.4	34.3*
Exercise	76.3	62.5	63.8	48.6
	Frequency of seeing each other			
	At least once per week		Less often	
	MZ (76)	DZ (38)	MZ (30)	DZ (56)
Smoking	73.7	68.4	70.0	48.2
Alcohol	59.2	44.7	66.7	32.1*
Exercise	75.0	63.2	60.0	44.6
	Frequency of eating together			
	At least once per week		Less often	
	MZ (49)	DZ (24)	MZ (57)	DZ (70)
Smoking	77.6	70.8	68.4	51.4*
Alcohol	65.3	45.8	57.9	34.3
Exercise	75.5	58.3	66.7	50.0
	Number of years lived apart			
	≤15 years		>15 years	
	MZ (56)	DZ (47)	MZ (50)	DZ (47)
Smoking	69.6	61.7	76.0	51.1*
Alcohol	62.5	38.3*	60.0	36.2*
Exercise	71.4	55.3	70.0	48.9

[a]Numbers of twin pairs in parentheses.
*$P < 0.05$ for test of difference between MZ and DZ twins (Yates' Continuity Corrected Chi-Square).

environmental factors into account [O'Connell et al., 1988]. The results show a number of ways in which there may be differences between the behaviour patterns of MZ and DZ twins in a way that could influence a variety of biological variables including lipid levels.

The twins are volunteers drawn from a variety of sources and may not be representative of all twins. Particular efforts were made to recruit DZ twins who are usually underrepresented in such studies. This means that differences between MZ and DZ twins as detailed in Tables I–IV may not reflect differences between these types of twins in general; however, attention to these differences is needed in the analysis of any twin study.

In this study, female MZ twins were less likely than DZ twins to be separated, widowed, or divorced and there were differences in cigarette smoking patterns between MZ and DZ twins in both men and women. There were also consistent differences between MZ and DZ twins in exercise patterns for males and females, although these were not statistically significant. Each of these makes MZ twins less

TABLE VII. Within Twin Pair Correlations and Heritability Estimates for Nutrient Intake†

	r_{MZ} (N = 103)	r_{DZ} (N = 94)	P_{EV}	$h^2{}_*$	h^2	P_*
Kilojoules	0.45	0.26	⩾.2	0.27	0.38	0.15
Protein[a]	0.43	0.38	⩾.2	−0.13	0.08	0.70
Carbohydrate						
Total	0.47	0.31	⩾.2	0.36	0.31	0.07
Simple	0.28	0.18	<.2	0.08	0.20	0.40
Complex	0.43	0.16	⩾.2	0.56	0.55	0.02
Fat						
Total	0.23	0.11	<.2	0.16	0.24	0.30
Saturated	0.15	0.09	⩾.2	0.27	0.10	0.22
Monounsaturated	0.27	0.11	⩾.2	0.41	0.33	0.10
Polyunsaturated[a]	0.21	0.19	<.2	−0.05	0.03	0.57
P:S ratio[a]	0.21	0.19	⩾.2	0.12	0.03	0.36
Dietary cholesterol[a]	0.17	0.26	⩾.2	−0.20	−0.17	0.73
Fibre[a]	0.32	0.29	⩾.2	0.03	0.05	0.46
Alcohol in drinkers[a]	0.34	0.23	<.2	0.02	0.22	0.49

†Analysis performed on sex-adjusted values.
[a]Data $\log_e$ transformed before analysis.
$h^2{}_*$—heritability estimate based on G_W or G_{AC}.
h^2—2 ($r_{MZ} - r_{DZ}$).
P_{EV}—significance level for test for equality of variances.
P_*—P-value for significance test of genetic variance.

likely as a group to be at risk of IHD [Koskenvuo et al., 1980; Cook et al., 1986; Siscovick et al., 1985]. The other lifestyle characteristics examined were similar in MZ and DZ twins as was dietary intake (with the exception of a statistically significant but small difference in the P:S ratio in their diets).

Despite similarities in the number of years lived apart, there were considerable differences in frequency of current contact among twin pairs (Table IV). This suggests that adjusting twin analyses for years of cohabitation [Clifford et al., 1984] may be insufficient to take account of current shared lifestyles, and that questions such as the one quoted by Fabsitz et al. [1978a,b], “how frequently do you and your twin get together now?”, or the ones used here should be used.

Of greater importance for twin study analyses is the observation that MZ pairs resemble each other more closely than do DZ pairs for a number of lifestyle variables. A recent study of female twins in the United States showed closer similarities among MZ than among DZ pairs for education (years at school), cigarette smoking (current packs per day), and alcohol intake (drinks per day) [Austin et al., 1987].

Other studies have also demonstrated closer similarities in dietary preferences among MZ than among DZ twin pairs [Fabsitz et al., 1978b; Wade et al., 1981]. However, in each case, the exact dietary constituents in question differ. The findings in our study, using weighed dietary diaries and a large number of twin pairs, is in general agreement with others and gives further support for the importance of genetic influence on lifestyle. Although the dietary diary is probably the most accurate of the various methods of assessing dietary intake, it is still subject to error and variation [Baghurst and Record, 1984]. In view of this, it may be unwise to speculate on whether our observations reflect genetic or environmental influence on food preference. The larger similarities in MZ than in DZ twin pairs in alcohol intake agree with other assessments [Austin et al., 1987; Clifford et al., 1984], although our study appears to be the only one using the accuracy of a weighed diary method.

The findings of differences within MZ and DZ twin pairs in dietary intake and a number of lifestyle factors may reflect genetic differences in choice of behaviour patterns or just the similarity in choice of people who have been exposed to a number of similar influences over the years. In any case, twin studies must take into account the apparently large differences in shared lifestyle factors between MZ and DZ twin pairs. Some have suggested methods of analysing twin studies that take account of differences in environmental factors in twin pairs [Clifford et al., 1984; Austin et al., 1987], and the importance of this is clearly demonstrated from our findings. Ascribing differences between the correlations in MZ and DZ pairs in biological variables as being purely "genetic" in nature—as implicit in conventional measures of heritability—is likely to overestimate the genetic influence on the variables that are affected by lifestyle. Complex multivariate modelling procedures are likely to be necessary to explore fully the interplay among genetic, environmental, and genetic-environmental correlations.

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