Objective—Levels of lipids and (apo)lipoproteins are known to increase after menopause, but it is unknown whether the genetic and environmental variability alters or whether lipids and (apo)lipoproteins are influenced by different genes before and after menopause.

Methods and Results—We studied 453 monozygotic and 1280 dizygotic pairs of female white twins recruited from the St. Thomas’ UK Adult Twin Registry and measured total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, lipoprotein(a) [Lp(a)], apolipoprotein A1 (apoA1), and apolipoprotein B (apoB). Variance components software was used to estimate genetic and environmental influences on serum lipid levels in premenopausal and postmenopausal women. Total variance was higher for triglycerides, HDL, and apoB after menopause. Postmenopausal women showed larger genetic variance for most lipids, apart from apoB and Lp(a). In premenopausal females, total cholesterol, LDL, HDL, apoA1, and apoB all showed an influence of the shared environment (22% to 34%), which, after menopause, decreased in HDL and completely disappeared in total cholesterol, LDL, and apoA1. Only for Lp(a), with a high heritability of 87%, did the same model fit premenopausal and postmenopausal women. Generally, there was no indication that different genes influence lipids before and after menopause.

Conclusions—These findings imply that genetic studies of lipids can pool results from premenopausal and postmenopausal women and that family-based interventions, such as changes in diet, are more likely to succeed in younger women, in whom the environmental influences are greater. (Arterioscler Thromb Vasc Biol. 2002;22:1142-1147.)

Keywords: lipids • genetics • twin study • menopause

Postmenopausal women are 2 to 3 times more likely to suffer from coronary heart disease than are premenopausal women. Part of this increased risk is likely to be due to adverse changes in lipids and (apo)lipoproteins after menopause. Levels of total cholesterol, LDL, triglycerides, and apoB have been reported to increase, probably (at least partly) because of a reduction in LDL receptor activity in response to the gradual decline in blood estrogen levels in the perimenopausal years. Levels of lipids, lipoproteins, and apolipoproteins, which have been established as important predictors of atherosclerotic coronary disease, vary considerably over a lifespan, and their phenotypic variance generally shows an increase with age. Such an increase in lipid and (apo)lipoprotein variance may be due to interindividual variation in the rise of lipid levels over time, and several studies now indicate that different genetic and/or environmental factors are likely to be involved at different ages. Superimposed on the global change across the whole lifespan, 4 specific time periods are associated with more dramatic changes. Menopause is one of these; the others are old age, adolescence, and the first years after birth.

We recently reviewed twin studies of lipids and (apo)lipoproteins and showed that most studies report heritability (h²) estimates between 40% and 80% for total cholesterol, LDL, HDL, triglycerides, apoA1, and apoB, with Lp(a) h² values of ≈90%. Only one study, which reported on HDL alone, focused on the effect of menopause and found that postmenopausal monozygotic (MZ) twins were more similar (r=0.79) than premenopausal MZ twins (r=0.61), whereas dizygotic (DZ) similarity did not change (r=0.31 and r=0.32, respectively). These results suggest that genetic mechanisms that affect individual variation in HDL level may differ in premenopausal and postmenopausal women. For the other lipids and (apo)lipoproteins, it is unknown whether an increase in phenotypic variance after menopause is due to genes, environment, or both or whether they may be influenced by different genes before and after menopause.
Therefore, the aim of this classic twin study was to quantify and compare the genetic and environmental sources of individual differences in lipids and (apo)lipoproteins in premenopausal and postmenopausal women. A secondary aim was to determine whether these traits are influenced to some degree by different genes before and after menopause, which may have an impact on the design of gene discovery studies. Quantitative genetic modeling techniques were used to analyze lipid data in a large sample of unselected female adult twins across a broad age range.

Methods

Subjects

The study cohort was composed of 1733 (453 MZ and 1280 DZ) female white twin pairs aged 18 to 79 years from the St. Thomas’ UK Adult Twin Registry. Twins from the registry were ascertained from the general population through national media campaigns in the United Kingdom. Participating twins were unaware of the specific hypotheses tested, and informed consent was obtained from all subjects. The study was approved by the St. Thomas’ Hospital Research Ethics Committee.

Measures

Subjects were interviewed and studied by trained research nurses. Zygosity was determined by standardized questionnaire, and DNA fingerprinting was used for confirmation. Information on (gynecological) medical history, medication use, fasting duration, lifestyle, and demographic variables was obtained by a standardized nurse-administered questionnaire. Postmenopausal status was defined as the cessation of menstruation for at least 12 months. Fasting administered questionnaire. Postmenopausal status was defined as

Levels of all lipids were measured by using a Cobas Fara machine (Roche Diagnostics). Total cholesterol, HDL, and triglycerides were determined by a colorimetric enzymatic method. HDL cholesterol was determined after precipitation of larger particles (chylomicron, VLDL, and LDL) by magnesium and dextran sulfate. ApoA1, apoB, and Lp(a) were determined by an immunoturbidometric method. LDL levels were estimated by using the Friedewald equation. This formula was applied only if the triglyceride concentration of subjects did not exceed 4.52 mmol/L. Four subjects on lipid-lowering medication and 17 subjects with extremely high (or low) lipid values (>4 SD above or below the mean) were excluded from the analyses.

Statistical Analysis

Background for Twin Analysis

The classic twin study makes use of the fact that MZ twins share identical genotypes, whereas DZ twins are no more alike genetically than siblings, sharing, on average, 50% of their segregating genes. If MZ twins show a larger resemblance for a specific trait than do DZ twins, this is attributed to genetic factors. Genetic model fitting is based on the comparison of the variance-covariance matrices in MZ and DZ twin pairs and allows separation of the observed phenotypic variance into its genetic and environmental components: additive genetic variance, dominant genetic variance, shared (or common) environmental variance, and specific (or unique) environmental variance, which also contains measurement error. The term heritability can be defined as the ratio of additive genetic variance to total phenotypic variance.

Analytical Approach

The distributions of total cholesterol, triglycerides, LDL, and Lp(a) were skewed and transformed by natural logarithm to normalize distributions before analysis. Preliminary data analysis was performed, and intraclass correlations were calculated by using STATA. Each of the lipids and (apo)lipoproteins was adjusted for the effect of age, fasting, menopausal status, and use of hormone replacement therapy (HRT) by multiple regression, and trait residuals were saved. All model fitting was performed on these residuals.

The aims of our analysis were, first, to establish the genetic influence overall by estimating the genetic and environmental influences on all variables for the entire group. Second, we investigated the effect of menopause on changes in (1) total trait variance and (2) genetic and environmental sources of variance. We specified a path model in which the total sample was subdivided into 6 subsets: MZ pre/pre, DZ pre/pre, MZ pre/post, DZ pre/post, MZ post/post, and DZ post/post, where pre/pre indicates twins that are both premenopausal, pre/post indicates 1 premenopausal and 1 postmenopausal twin, and post/post indicates twins that are both postmenopausal) to estimate variance components in premenopausal and postmenopausal women separately. Using the principle of parsimony, we subsequently homed in on the best fitting model, ie, the simplest model (in terms of number of parameters estimated) that still described the data well. If traits showed equal total variance and the same model in premenopausal and postmenopausal women, we tested whether variance components could be set equal for premenopausal and postmenopausal women.

Finally, we examined whether in menopause-discordant pairs the correlation between the latent genetic factors (r_g) was lower than the
normal expected correlation of 1 in MZ pairs and 0.5 in DZ pairs, which would indicate that (partly) different genes influence the lipids and (apo)lipoproteins in premenopausal and postmenopausal women. Alternatively, it was tested whether discordant twins share their environment to a lesser extent than concordant pairs, ie, whether the shared environmental correlation \( r_c \) is lower than the expected correlation of 1 in MZ pairs and 0.5 in DZ pairs, which would indicate that (partly) different genes influence the lipids and (apo)lipoproteins in premenopausal and postmenopausal women.

**Results for the Entire Group**

The data in Table 1 show the general characteristics of MZ and DZ twins for the entire study population of 1733 twin pairs. Comparison of the groups revealed that MZ pairs were, on average, 3.5 years older than DZ pairs at the time of ascertainment and that a larger proportion were, consequently, postmenopausal. All other characteristics were very similar for MZ and DZ twins. The mean values for all lipids, lipoproteins, and apolipoproteins were similar for both groups and are shown in Table 2.

The intraclass correlations for the entire group of MZ and DZ twins for the different lipid variables are presented in the last column of Table 3. For each of the measures, MZ correlations were greater than DZ correlations, implying an important genetic influence, which was subsequently confirmed by model fitting to the entire group (Table 4). Dominant genetic effects did not contribute significantly to the explanation of the data for any of the lipid variables; ie, dominant genetic effects could be dropped from the model without a significant worsening of the fit. A model specifying additive genetic, common environmental, and unique environmental variance components gave the best balance between goodness of fit and parsimony. Estimates of variance components and their 95% CIs were obtained from the best fitting model.

### Table 1. General Characteristics of Study Population

<table>
<thead>
<tr>
<th></th>
<th>MZ</th>
<th>DZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pairs</td>
<td>453</td>
<td>1280</td>
</tr>
<tr>
<td>Age, y</td>
<td>50.56</td>
<td>47.07</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>64.41</td>
<td>66.06</td>
</tr>
<tr>
<td>Height, cm</td>
<td>162.35</td>
<td>162.51</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.44</td>
<td>25.01</td>
</tr>
<tr>
<td>Postmenopausal, %</td>
<td>61.92</td>
<td>46.95</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>19.07</td>
<td>21.98</td>
</tr>
<tr>
<td>Current HRT user, %</td>
<td>14.09</td>
<td>18.20</td>
</tr>
<tr>
<td>Current OCP user, %</td>
<td>12.37</td>
<td>10.93</td>
</tr>
<tr>
<td>Hysterectomy, %</td>
<td>16.26</td>
<td>15.68</td>
</tr>
<tr>
<td>Bilateral oophorectomy, %</td>
<td>4.03</td>
<td>4.43</td>
</tr>
</tbody>
</table>

Mean (SD) is shown, unless stated otherwise. BMI indicates body mass index; OCP, oral contraceptive pill.

### Model-Fitting Procedure

Models were fitted to the raw data by using normal theory maximum likelihood. This method allowed us to use the information provided by unpaired observations, which contribute to the estimation of variance (but not covariance).

The significance of additive genetic variance, common environmental variance, and dominant genetic variance was tested by removing each sequentially in specific submodels. Variance components were dropped from the model if they did not give a significant contribution (ie, \( P > 0.05 \)). Submodels were compared with the full model by hierarchic \( \chi^2 \) tests. In comparing non-nested models, the \( \chi^2 \) test had the best balance between goodness of fit and parsimony. Estimates of variance components and their 95% CIs were obtained from the best fitting model. All quantitative genetic model fitting was carried out by statistical modeling using Mx.

### Results

#### Results for the Entire Group

The data in Table 1 show the general characteristics of MZ and DZ twins for the entire study population of 1733 twin pairs. Comparison of the groups revealed that MZ pairs were, on average, 3.5 years older than DZ pairs at the time of ascertainment and that a larger proportion were, consequently, postmenopausal. All other characteristics were very similar for MZ and DZ twins. The mean values for all lipids, lipoproteins, and apolipoproteins were similar for both groups and are shown in Table 2.

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#### Table 3. Intraclass Correlation Coefficients (Number of Complete Twin Pairs) for MZ and DZ Twins by Menopausal Status

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Pre/Pre</th>
<th>Pre/Post</th>
<th>Post/Post</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ</td>
<td>0.59</td>
<td>0.18</td>
<td>0.63</td>
<td>0.61</td>
</tr>
<tr>
<td>DZ</td>
<td>0.42</td>
<td>0.37</td>
<td>0.33</td>
<td>0.38</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.59</td>
<td>0.88</td>
<td>0.64</td>
<td>0.63</td>
</tr>
<tr>
<td>ApoA1</td>
<td>0.29</td>
<td>0.20</td>
<td>0.40</td>
<td>0.33</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.76</td>
<td>0.58</td>
<td>0.63</td>
<td>0.68</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.85</td>
<td>0.93</td>
<td>0.88</td>
<td>0.87</td>
</tr>
</tbody>
</table>

### Table 4. Variance Component Estimates of Lipid Traits of Best Models Fitted to the Entire Twin Sample

<table>
<thead>
<tr>
<th>Lipid</th>
<th>( h^2 ) (95% CI)</th>
<th>( c^2 ) (95% CI)</th>
<th>( e^2 ) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.42 (0.28, 0.56)</td>
<td>0.17 (0.07, 0.28)</td>
<td>0.40 (0.35, 0.46)</td>
</tr>
<tr>
<td>LDL</td>
<td>0.46 (0.32, 0.59)</td>
<td>0.17 (0.07, 0.28)</td>
<td>0.37 (0.32, 0.42)</td>
</tr>
<tr>
<td>HDL</td>
<td>0.42 (0.30, 0.54)</td>
<td>0.24 (0.15,0.33)</td>
<td>0.34 (0.29, 0.39)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.63 (0.58, 0.67)</td>
<td>0.37 (0.33, 0.42)</td>
<td></td>
</tr>
<tr>
<td>ApoA1</td>
<td>0.36 (0.20, 0.51)</td>
<td>0.18 (0.07, 0.30)</td>
<td>0.45 (0.40, 0.52)</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.39 (0.26, 0.51)</td>
<td>0.26 (0.16, 0.36)</td>
<td>0.35 (0.30, 0.40)</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.87 (0.85, 0.89)</td>
<td>0.13 (0.11, 0.15)</td>
<td></td>
</tr>
</tbody>
</table>

\( h^2 \) indicates heritability; \( c^2 \), shared environmental variance component; \( e^2 \), unique environmental variance component.
most parsimonious explanation of the data for all of the variables, with the exception of Lp(a) and triglycerides, for which shared environmental effects did not contribute significantly to the variation. Values of $h^2$ ranged from 0.36 for apoA1 to 0.87 for Lp(a) (Table 4).

**Effect of Menopause**

No significant differences for mean lipid values were found between premenopausal and postmenopausal twins within (age-identical) menopause-discordant twin pairs (data not shown). However, multiple regression using all the twin data showed significantly higher mean levels of total cholesterol ($P=0.006$), triglycerides ($P=0.002$), and apoB ($P=0.005$) in postmenopausal women after adjustment for age, fasting status, and HRT use.

Intraclass correlations for each of the 6 zygosity-by-menopause groups are shown in Table 3. Total variance was higher after menopause for HDL, triglycerides, and apoB and did not differ significantly between premenopausal and postmenopausal women for the other lipids (Figures 2 and 3). Genetic and environmental variance components estimates for premenopausal and postmenopausal women of the best fitting models are presented in Table 5 and Figures 2 and 3. Most lipids showed larger genetic variance in postmenopausal women, except for apoB and Lp(a), which showed no change. In the best fitting model for Lp(a), genetic and environmental variance components were identical for premenopausal and postmenopausal women ($h^2=87\%$).

**Different Genes or Shared Environment Before and After Menopause**

With the exception of HDL, there was no evidence that lipids or apolipoproteins are influenced by different genes or that the extent of environmental sharing differs before and after menopause. For most lipid variables, the genetic correlations ($r_g$ values) were estimated at or very close to 1 and 0.5 for menopause-discordant MZ and DZ twins, respectively. The shared environmental correlations ($r_c$ values) were estimated very close to 1 for both zygosities. For HDL, a model that specified less environmental sharing between discordant pairs ($r_c=0.28$) fitted slightly better than a model specifying different genetic influences before and after menopause ($r_g=0.13$), but variance components estimates were virtually identical for the 2 models. Both models were significantly better than a simpler model in which $r_c$ and $r_g$ were fixed to their usual values.

**Discussion**

The present study investigated whether the magnitude of genetic or environmental influences on lipids and (apo)lipoproteins changes with menopause and whether lipids and (apo)lipoproteins may be influenced by different genes before and after menopause. We observed that genetic variance in postmenopausal women was larger for most lipids and (apo)lipoproteins, except for apoB and Lp(a), which showed no change. Shared
null
Several practical implications follow from our findings. The importance of shared environmental influences on lipids and (apo)lipoproteins during early adulthood, as shown in the present study, may point to the likely success of family-based lifestyle interventions. Diet will most likely be an important component of such an intervention, because a recent study in young adult twins demonstrated that the association between diet on one hand and total cholesterol, LDL, and HDL on the other is due to environmental factors. The fact that no evidence was found for different genes influencing lipids before and after menopause implies that genome scan studies aiming to detect quantitative trait loci for lipids and (apo)lipoproteins can pool data from premenopausal and postmenopausal women. However, they may want to focus on the latter group, in which genetic variance seems to be larger. Future studies will need to determine the specific genes responsible for the increase in genetic variance after menopause. Polymorphisms underlying LDL receptor function and hepatic lipase activity are likely candidates because they are influenced by estrogen and progestin action.

In conclusion, we have demonstrated that genetic influences on lipid and (apo)lipoprotein levels, except for apoB and Lp(a), are larger after menopause, whereas at the same time, shared environmental influences tend to be smaller or even disappear. No evidence was found that lipids are influenced by different genes before and after menopause. The importance of shared environmental influences on lipids and (apo)lipoproteins during early adulthood points to the potential for family-based lifestyle interventions. Human genetic studies aiming to optimize power for detection of quantitative trait loci underlying the genetic lipid variance, on the other hand, may want to focus on older individuals in which the genetic variance seems to be larger.

Acknowledgments

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References

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