Regional serum cholesterol differences in Belgium: do genetically determined cardiovascular risk factors contribute?

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**Background** Differences in serum lipid distribution and mortality from ischaemic heart disease have repeatedly been reported between Belgian northerners and southerners. We investigated whether serum lipoprotein(a) (Lp(a)) and apolipoprotein (apo) E polymorphism were involved.

**Methods** Fasting serum lipids, apo A-I and B, and Lp(a) levels were examined in randomly selected, 20–39 year old Belgian males and females from the north (Flanders) and the south (Wallonia) of Belgium (N = 900). Apo E phenotype distribution was investigated in random subsamples from either region (N = 249).

**Results** Mean serum cholesterol, low density lipoprotein cholesterol (LDL-c), apo B and triglyceride levels were higher in Walloons compared to Flemings within each gender, the difference being significant in 30–39 year old males. Average high density lipoprotein cholesterol and apo A-I levels were significantly lower in 30–39 year old male southerners, compared to their northern counterparts. Median Lp(a) was 67 mg/l in northerners and 75 mg/l in southerners (NS). The apo E phenotype distribution was similar in both regions ($\chi^2 = 7.213$; d.f. = 5; $P = 0.2053$), whereas the average effects of the apo E alleles differed between the regions. In southerners the e4 effect upon adjusted apo B and LDL-c levels was $+12\%$ and the e2 effect was $-15\%$; in northerners the e4 and e2 effects were $+5\%$ and $-25\%$, respectively. The apo E polymorphism did not affect serum Lp(a) levels.

**Conclusions** Regional cholesterol differences between Flemings and Walloons cannot be explained by differences in serum Lp(a) or apo E phenotype distribution. The less favourable e2 and e4 effects in southerners compared to northerners reflect modulation of the apo E gene by particular environments.

**Keywords** Lipoprotein(a) levels, apolipoprotein E polymorphism, Flemings, Walloons, Belgium, cardiovascular risk factors

Accepted 21 November 1997

Regional differences in serum lipid distribution and mortality from ischaemic heart disease have repeatedly been described for Belgium, a small industrialized country of only 11 781 square miles with the peculiarity of two cultural communities, i.e. a Dutch-speaking community in the north (Flanders and Campine), and a French-speaking community in the south (Wallonia). The linguistic difference has created a cultural frontier, cutting horizontally through Belgium, hampering the transmission of information from one side to the other, and leading to newspapers, radio and television addressing themselves separately to one or other community.

It was first discovered at the end of the 1960s, during an epidemiological survey in the Belgian army, that important regional serum cholesterol differences existed in males in all 5-year age classes between the age of 15 and 55 years.\textsuperscript{1} The higher serum cholesterol in the southerners, coupled to a higher morbidity from ischaemic heart disease and peripheral vascular disease, was confirmed in the 1970s by surveys in male postal\textsuperscript{2} and factory workers.\textsuperscript{3} In the 1980s the Belgian Interuniversity Research on Nutrition and Health (BIRNH) documented similar cholesterol differences between Flemings and Walloons in all age categories, both in males and females, and found differences of 20% in coronary mortality between the two regions.\textsuperscript{4} As to
the origin of these serum cholesterol differences, the less favourable food pattern in Walloons in terms of saturated and polyunsaturated fat intake as well as nutritional cholesterol intake have been held responsible. Whether, besides differences in lifestyle factors, variations in genetically determined cardiovascular risk factors might contribute to the observed north-south differences in serum cholesterol has not been investigated so far. Two potential candidates, co-determining serum lipid and serum cholesterol levels, that might be involved are the type and blood levels of apolipoprotein (apo) E5-10 and lipoprotein(a) (Lp(a)).

This investigation was undertaken to determine whether the alleles governing apo E and apo(a) might contribute to regional cholesterol differences in Belgium. To this end, apo E polymorphism and serum Lp(a) levels were examined in randomly selected 20-39 year old males and females from either region, in relation to serum lipid and apolipoprotein levels. Besides, it was investigated whether apo E polymorphism affected serum Lp(a) levels.

Methods
Population description and sample collection
Sera were obtained from healthy, unrelated, 20-39 year old employees from Flanders (N = 683) and Wallonia (N = 217). Hitherto, employees working at companies spread throughout the two regions (32 companies in Flanders; 15 companies in Wallonia) were, prior to a scheduled medical check-up, invited to participate in this study. Previous selection and subsequent invitation, and medical check-up were conducted by the Flemish IDEWE and the Wallonian CeSI, two 'Centres de Services Inter-enterprises— Médicène du Travail'. Informed consent was obtained from all participants.

Blood was collected in the morning at the point of medical check-up into whole blood tubes after overnight fasting (including abstinence from alcohol). The same day, the whole blood tubes were transported to the Central Laboratory of the Leuven University Hospital, Belgium. No special precautions were taken. Serum was harvested on the day of sample collection by centrifugation at 1500 g for 10 min at room temperature. Subsequently, the serum was divided into five aliquots: (1) for determination of serum lipids and y-glutamyltransferase activity (GGT); (2) for apo A-I and B analyses; (3) for Lp(a) analyses; (4) for apo E phenotyping and (5) a left-over serum aliquot for long-term storage at −70°C. Serum lipids and GGT were determined freshly, whereas aliquots for apo A-I and B, and Lp(a) analyses were stored frozen at −20°C for a maximum of 2 months. Before freezing, aliquots for apo E phenotyping (250 µl) were preserved with an anti-proteolytic cocktail (5 µl) containing aprotonin (1 mg/ml), lima bean trypsin inhibitor (2 mg/ml), soybean trypsin inhibitor (2 mg/ml), benzamidin (3.132 mg/ml), glutathione (20 mg/ml), D-phenylalanyl-L-propyl-L-arginine chloromethyl keton (50 µg/ml), NaF3 (10 mg/ml), Tritripex (0.65 mg/ml), streptomycin sulfate (8 mg/ml), and sodium benzylpenicillin (8 mg/ml).

Questionnaire
Questionnaires were distributed at the time of the medical check-up. Participants were invited to fill in their identity, home address and postcode, nationality, ethnicity (Caucasian/Asian/African), date of birth, type of profession, and age at graduation. Moreover, participants were questioned about the current use (and type) of hormonal contraceptives and possible pregnancies or hysterectomies (for females), about smoking, drinking, and sporting habits, and about the use of lipid-lowering medication. Hitherto, the number and type of alcoholic beverages (wine, beer, liqueur) drunk per day and per week were scored, as well as the number of cigarettes, cigars or pipes smoked per day, and the hours of physical exercise taken per week.

The filled-in questionnaires were checked for completeness by the physicians who conducted the medical check-up. Additional questions were raised whenever appropriate. Non-Caucasians, pregnant and hysterectomized women, and individuals taking any lipid-lowering medication were excluded from the study at this stage.

Socioeconomic status
Socioeconomic status was essentially scored as described by Black, by means of six categories that are primarily based on occupation: three non-manual higher categories (classes I, II, III N; categorized as 1, 2, and 3 respectively) and three manual lower categories (IIIM, IV, V; categorized as 4, 5, and 6 respectively). For classification, type of education and age at graduation were also taken into account. Scoring was done by two independent observers, who were blinded to all study results. Inconsistent scores were verified; in case of remaining disagreement definitive categorization was done by a third observer.

Anthropometric and blood pressure measurements
Body height was measured to the nearest 0.5 cm. Body weight was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by height (m²). Systolic (SBP) and diastolic (DBP) blood pressure were measured to the nearest mmHg. All measurements were performed by the physicians in charge of the medical check-up.

Serum GGT, serum lipid and apolipoprotein analyses
Cholesterol was determined enzymatically, using CHOD-PAP reagent. High density and low density cholesterol (HDL-c, LDL-c) were determined with CHOD-PAP reagents, after precipitation with MgCl₂/phosphotungstic acid and polyvinyl sulphate, respectively. Besides, LDL-c was estimated by the original Friedewald formula, as well as by a modified Friedewald formula that corrects for the Lp(a)-cholesterol contribution. Total triglycerides were determined using a GPO-PAP reagent; no free glycerol correction was made. Apolipoprotein A-I and B were determined by immunoturbidimetry (reference values: 0.80–1.50 g/l for apo A-I and 0.60–0.95 g/l for apo B). y-Glutamyltransferase activity was determined using γ-glutamyl-carboxynitroanilide and glycyglycine substrates (reference values: 5–28 IU/l in males; 4–18 IU/l in females). All reagents were purchased from Boehringer (Boehringer Mannheim, Mannheim, Germany).

Accuracy of the routine cholesterol and HDL-c assay methods used was checked retrospectively by the Lipid Reference Laboratory of the University Hospital, Rotterdam, the Netherlands, versus the Abell-Kendall Reference Method and the CDC HDL-c Designated Comparison Method, respectively. Throughout the study period (i.e. at five checkpoints) average
biases of all six samples analysed per checkpoint were ≤3% for
total cholesterol and ≤5% for HDL-c, fulfilling the recommended
1998 NCEP performance guidelines for these analytes.18,19

**Serum lipoprotein(a) quantification and
apo E phenotyping**

Lipoprotein(a) was determined using an anti-apo(a) polyclonal
capture ELISA from Biopool (TintElize lipoprotein(a), Cat. No.
610220; Biopool AB, Umeå, Sweden). Apo E phenotyping
was performed by a micro-method based on isoelectric focusing
(pH 4–7) of delipidated serum samples, followed by immuno-
blotting on a nitrocellulose filter and use of polyclonal rabbit
anti-apo E antiserum as the first antibody. Serum lipopro-
tein(a) mass measurements were performed in all volunteers
(N = 900), whereas apo E phenotyping was done in a subgroup
(N = 249) of Flemings (N = 125) and Walloons (N = 124).

After laboratory analysis, lipid and lipoprotein data were
reported by letter to each participant, together with reference
and/or consensus values of the investigated parameters and an
interpretation of the lipid profile (normal/abnormal).

**Statistical analysis**

Regional differences between baseline characteristics and serum
lipid and (apo)lipoprotein data were determined with Students’
T-test, Mann-Whitney rank-sum test, or χ² test where appro-
priate. The Lp(a) data were logarithmically transformed (natural
logarithm), due to extreme skewness of its distribution.

Multiple linear regression models controlling for a number of
covariables (ANCOVA) were used for examining the differences
in serum lipid and (apo)lipoprotein levels between Flanders and
Wallonia. To this end, cholesterol, LDL-c, apo B and Lp(a) data
were logarithmically transformed, whereas this was not
required for HDL-c and apo A-I. A first model controlled for
the covariables age, sex, and BMI. A second model controlled for
age, sex, BMI, SBP, DBP, γ-glutamyltransferase activity, oral con-
traceptive use, smoking, physical exercise, and socioeconomic
status. A third model controlled for apo E phenotypes, in
addition to the variables controlled for in model 2. The average
α_i (i = 2,3,4) effects of the apo E alleles on the adjusted serum
lipid and (apo)lipoprotein concentrations and the variance σ²_A
of these effects attributable to genotypic differences were
estimated according to the method of Sing and Davignon.5 This
σ²_A is a component of the total genetic variance σ²_G (5). Sig-
nificance of each estimated α_i (i = 2,3,4) was also tested.

A χ² goodness-of-fit test was used to evaluate the genetic
Hardy-Weinberg equilibrium for the apo E polymorphism. Apo E
alleles frequencies were estimated using the gene-counting
method. The difference in apo E allele frequencies between the
two regions was tested using a χ²-association test. Differences in
mean lipid and (apo)lipoprotein levels between apo E pheno-
typic groups were tested parametrically using one-way analysis
of variance with the Student-Newman-Keuls multiple range
test or Kruskal-Wallis non-parametric test. A significance level
of α = 0.05 was adopted throughout.

**Results**

**Description of the studied population sample**

Anthropometric and other characteristics of the Belgian
population sample, stratified by region, sex and 10-year age
classes, are presented in Table 1. Mean BMI was significantly
higher in French-speaking males than in Dutch-speaking males,
in both age classes. The BMI also tended to be higher in French-
speaking females than in Dutch-speaking females. Socio-
economic status and the age at graduation was generally lower
in Walloon participants compared to Flemish participants,
reaching statistical significance in nearly all strata.

**Serum lipids and (apo)lipoprotein parameters**

Average serum lipid, Lp(a) and apo A-I and B levels by region,
sex and age class are presented in Table 2. Cholesterol and LDL-
c levels were higher in Walloons, irrespective of gender and age
class. The difference was significant in 30–39 year old males.
Analogously, the apo B level was significantly higher in 30–39
year old male Walloons compared to their Flemish counterparts.
Other adverse characteristics of the lipid profile in Walloon
males were the significantly lower HDL-c and apo A-I levels in
30–39 year old participants.

Serum Lp(a) levels (Table 3) were similar in Walloons and
Flemings, median Lp(a) being 67 mg/1 in Flemings and 75 mg/1
in Walloons. The overall median Lp(a) level in the 900 Belgians
was 68 mg/l.

**Factors influencing lipid, apolipoprotein and
Lp(a) parameters**

**Oral contraceptives**

Oral contraceptives increased average serum triglyceride and
apo A-I levels in all strata (P < 0.05; data not shown). Ana-
logously, HDL-c levels showed a tendency to higher levels
in females on oral contraceptives. The Lp(a) levels were not
affected by oral contraceptive use, nor by smoking or physical
exercise.

**Apo E polymorphism**

The apo E phenotype distribution, as determined in a subgroup
(N = 249) that was matched for region, is presented in Table 4.
The observed phenotype distribution was in Hardy-Weinberg
equilibrium within each region. The apo E phenotype distribu-
tions were similar in Flemings and Walloons, consequently, the
data were pooled. The overall relative apo E allele frequencies
in the studied sample were 0.092 for e2, 0.767 for e3 and 0.141
for e4.

The average impact of the apo E polymorphism on apo
B-containing lipoprotein levels appeared large in this popula-
tion sample, cholesterol, LDL-c and apo B levels being lowest in
E2-carriers and highest in E4-carriers. The trend was obvious,
already by univariate analysis using unadjusted values (Table 5).
On the contrary, no significant effect of the apo E polymor-
phism could be demonstrated upon serum apo A-I, HDL-c,
triglyceride and Lp(a) levels.

**Multivariable models explaining phenotypic variance**

The first and simplest MLR model (data not shown), controlling
for age, gender and BMI, explained 4–6.4% of the variances in
cholesterol, LDL-c and apo B levels, but 29.7% and 33.6% of
the apo A-I and HDL-c variances, respectively. Model 2, which
additionally adjusted for multiple lifestyle factors, explained
14.1–18.8% of the variances in cholesterol, LDL-c and apo B
levels, and 31.9, 33.9 and 39.9% of triglyceride, apo A-I and
HDL-c variances, respectively. The third MLR model controlling
Table 1 Anthropometric and other characteristics in Flemings and Walloons, as stratified by sex and age category

<table>
<thead>
<tr>
<th>Variable</th>
<th>20-29 years</th>
<th>30-39 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flanders</td>
<td>Wallonia</td>
</tr>
<tr>
<td><strong>MALE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.6 ± 2.7</td>
<td>34.7 ± 2.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.0 ± 5.5</td>
<td>164.0 ± 5.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.9 ± 9.3</td>
<td>60.9 ± 9.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.6 ± 3.1</td>
<td>22.6 ± 3.1</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110 ± 10</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74 ± 8</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>GGT(U/l)</td>
<td>11.6 ± 6.3</td>
<td>16.0 ± 12.9</td>
</tr>
<tr>
<td>Age at graduation (years)</td>
<td>21.3 ± 2.3</td>
<td>21.3 ± 2.3</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>23.3</td>
<td>21.3</td>
</tr>
<tr>
<td>Alcohol users (%)</td>
<td>89.7</td>
<td>89.6</td>
</tr>
<tr>
<td>Socioeconomic class(I) (%)</td>
<td>13.9</td>
<td>13.9</td>
</tr>
<tr>
<td>II</td>
<td>45.3</td>
<td>41.5</td>
</tr>
<tr>
<td>IIIN</td>
<td>28.7</td>
<td>26.8</td>
</tr>
<tr>
<td>IIIM</td>
<td>11.3</td>
<td>9.8</td>
</tr>
<tr>
<td>IV</td>
<td>0.9</td>
<td>3.0</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FEMALE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>26.2 ± 2.7</td>
<td>34.6 ± 2.8</td>
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<tr>
<td>GGT(U/l)</td>
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<td>8.8 ± 3.5</td>
</tr>
<tr>
<td>Age at graduation (years)</td>
<td>20.5 ± 2.1</td>
<td>19.5 ± 1.9</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>23.2</td>
<td>27.0</td>
</tr>
<tr>
<td>Alcohol users (%)</td>
<td>64.0</td>
<td>63.8</td>
</tr>
<tr>
<td>Oral contraceptive use (%)</td>
<td>67.3</td>
<td>39.3</td>
</tr>
<tr>
<td>Socioeconomic class(I) (%)</td>
<td>5.2</td>
<td>4.1</td>
</tr>
<tr>
<td>II</td>
<td>42.2</td>
<td>36.7</td>
</tr>
<tr>
<td>IIIN</td>
<td>35.5</td>
<td>31.1</td>
</tr>
<tr>
<td>IIIM</td>
<td>10.4</td>
<td>7.7</td>
</tr>
<tr>
<td>IV</td>
<td>6.2</td>
<td>16.3</td>
</tr>
<tr>
<td>V</td>
<td>0.5</td>
<td>4.1</td>
</tr>
</tbody>
</table>

*γ-glutamyltransferase activity.

Estimates and significances of the average effects of the common apo E alleles on adjusted serum lipid, apolipoprotein and lipoprotein(a) levels are presented in Table 6. Striking are the regional differences in apo E allelic effects on adjusted serum lipid levels: in southerners the e4 and e2 effects upon adjusted

for apo E phenotypes in addition to the parameters controlled for in model 2, is obviously the best, explaining 25–41% of the observed phenotypic variation of all lipid parameters, except for Lp(a). Common predictors of total cholesterol, LDL-c and apo B levels were smoking, linear age, GGT (In) and apo E2-containing phenotypes. None of the models predicted Lp(a) variances (probability F statistics: NS).

Applying the second best MLR model to the entire Belgian population sample (N = 900) smoking and BMI were major predictors associated with all lipoprotein and apolipoprotein levels, except with Lp(a) (data not shown). The activity of γ-glutamyltransferase, an indicator of alcohol (ab)use, was positively associated with triglycerides, total and LDL-c, and apo B levels. As expected, apo A-I and HDL-c were associated positively with use of contraceptive hormones, and negatively with smoking and BMI.

Average effects of the three common apo E alleles on adjusted serum lipid and (apo)lipoprotein levels

Estimates and significances of the average effects of the common apo E alleles on adjusted serum lipid, apolipoprotein and lipoprotein(a) levels are presented in Table 6. Striking are the regional differences in apo E allelic effects on adjusted serum lipid levels: in southerners the e4 and e2 effects upon adjusted
apo B and LDL-c levels were approximately +12% and −15% respectively (P < 0.05), whereas they were halved (+5%; NS) and doubled (+25%; P < 0.05) respectively in northerners.

Overall, presence of the e2 allele reduced serum cholesterol levels on average by 11%, and LDL-c and apo B levels by 20 and 19%, respectively (P < 0.05). Presence of the e4 allele increased cholesterol levels by 5% (NS), and LDL-c and apo B levels by 11% (P < 0.05). In case of the e3 allele serum triglyceride levels were significantly lower (P < 0.05).

### Contribution of the apo E locus to the total variability of serum lipids and (apo)lipoproteins in the Belgian population sample

Using the third MLR model, the apo E locus explained 8.8% of the total cholesterol variance, 13.5% of the LDL-c variance and 16.9% of the apo B variance. Less than 4% of the HDL-c, apo A-I, triglyceride and Lp(a) variances could be attributed to the apo E locus (data not shown).

### Discussion

It has been established that besides lifestyle factors, heredity contributes to about 50% of interindividual serum cholesterol variation. The involvement of many candidate genes in determining phenotypic variation in serum cholesterol and other serum lipids is supported by research reviewed by e.g. Ferrell. In general, polymorphic variation in several genes influences variation in a particular trait, e.g. serum cholesterol, and each gene influences variation in more than one trait. As a rule, a particular gene explains only a fraction of variation in a particular trait. The apo E polymorphism e.g. explains 1% of the variation in total serum cholesterol in males, but 10% in females.
sites located in the northern and southern regions of Belgium respectively because no special exclusion criteria were used—with the exception of the exclusion of non-Caucasians, and pregnant or hysterectomized women—and because participants were recruited from 32 worksites located in the northern part of Belgium, and 15 worksites in the southern part of Belgium. Third, the study population contains both males and females at a reproductive age (20–39 years), excluding effects of menopause on serum lipid profiles.

Fourth, information was collected on various factors that relate to lifestyle, such as smoking, physical exercise, oral contraceptive use, alcohol use and GGT activity, or that reflect both environmental and genetic factors, such as BMI. Fifth, fasting sera were collected, enabling valid determinations of HDL-c, LDL-c and triglycerides.

Major outcomes of this study were the following. First, the more adverse serum lipid profile in southerners compared to northerners (Table 2) was reconfirmed in this study, the difference being significant in 30–39 year old males. This finding is in accordance with previous publications examining north-south differences in cardiovascular risk factor distribution, and implies that regional differences in serum lipid distribution in Belgium, first observed in the 1960s, still persist in the 1990s.

Second, similar median and log mean Lp(a) levels were found in Belgian northerners and southerners (Table 3). Accordingly, mean estimated LDL-c levels remained 5–11% lower in Flemings compared to Walloons within each age and gender class, also after correcting for Lp(a)-cholesterol content (Table 2). After all, the average contribution of Lp(a)-cholesterol to estimated LDL-c was similar in both regions and amounted maximally 4.5% within each category. Consequently Lp(a), and hence Lp(a)-cholesterol, do not contribute to the observed regional cholesterol differences.

Third, the apo E phenotype distribution was found to be similar in both regions (Table 4). Besides, the overall apo E allele frequencies counted in this study were similar to the apo E allele frequencies reported by Braeckman et al. in 30–59 year old Flemish males (f_E2, f_E3 and f_E4: 0.092, 0.767 and 0.141 (this study) versus 0.072, 0.765 and 0.163)26. Also, the associations of apo E polymorphism with the lipids and apolipoproteins analysed (Tables 5 and 6) were consistent with the well identified effects of apo E, the average effect of the e2 allele being to lower LDL-c and apo B, and of the e4 allele being to increase LDL-c and apo B.5,6,9,23 Whereas the e effect on serum LDL-c and apo B levels is consistent across most population studies, there are controversial results about the e effect on triglycerides, since these vary widely within and among individuals, masking a clear effect of apo E phenotype. In this study e2 and e4 alleles
Table 5 Unadjusted serum lipid, apolipoprotein and lipoprotein (Lp(a)) levels by apolipoprotein (apo) E phenotype in 249 apparently healthy Belgian males and females

<table>
<thead>
<tr>
<th>Parameter</th>
<th>E2/2</th>
<th>E2/3</th>
<th>E3/3</th>
<th>E4/2</th>
<th>E4/3</th>
<th>E4/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.48 ± 1.001</td>
<td>5.454 ± 1.063</td>
<td>5.767 ± 1.061</td>
<td>6.533 ± 1.198</td>
<td>6.709 ± 1.981</td>
<td>7.85 ± 2.07</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.267 ± 0.885</td>
<td>3.573 ± 1.100</td>
<td>3.713 ± 1.105</td>
<td>3.816 ± 1.143</td>
<td>4.310 ± 1.571</td>
<td>5.124 ± 1.900</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.19 ± 0.397</td>
<td>1.431 ± 0.372</td>
<td>1.360 ± 0.499</td>
<td>1.370 ± 0.426</td>
<td>1.304 ± 0.503</td>
<td>1.85 ± 0.71</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/l)</td>
<td>1.15 ± 0.26</td>
<td>1.27 ± 0.24</td>
<td>1.24 ± 0.23</td>
<td>1.24 ± 0.23</td>
<td>1.25 ± 0.45</td>
<td>1.43 ± 0.71</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>0.32 ± 0.22</td>
<td>0.80 ± 0.21</td>
<td>0.86 ± 0.17</td>
<td>0.86 ± 0.24</td>
<td>0.97 ± 0.30</td>
<td>1.12 ± 0.43</td>
</tr>
<tr>
<td>Lp(a) (mg/l)</td>
<td>4.852 ± 1.602</td>
<td>4.397 ± 1.419</td>
<td>5.109 ± 1.161</td>
<td>4.215 ± 1.646</td>
<td>3.565 ± 0.558</td>
<td>7.50 ± 1.90</td>
</tr>
<tr>
<td>Median Ln Lp(a) (Ln mg/l)</td>
<td>3.714</td>
<td>4.477</td>
<td>5.124</td>
<td>4.331</td>
<td>3.526</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Table 6 Estimates and significances of the average effect of the three common apo E alleles on adjusted serum lipid, apolipoprotein and lipoprotein(a) levels in a random sample of unrelated, healthy Flemings and Walloons (N = 249)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Flanders</th>
<th>Walloonia</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.48 ± 1.001</td>
<td>5.454 ± 1.063</td>
<td>5.767 ± 1.061</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.267 ± 0.885</td>
<td>3.573 ± 1.100</td>
<td>3.713 ± 1.105</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/l)</td>
<td>1.15 ± 0.26</td>
<td>1.27 ± 0.24</td>
<td>1.24 ± 0.23</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>0.32 ± 0.22</td>
<td>0.80 ± 0.21</td>
<td>0.86 ± 0.17</td>
</tr>
<tr>
<td>Lp(a) (mg/l)</td>
<td>4.852 ± 1.602</td>
<td>4.397 ± 1.419</td>
<td>5.109 ± 1.161</td>
</tr>
<tr>
<td>Median Ln Lp(a) (Ln mg/l)</td>
<td>3.714</td>
<td>4.477</td>
<td>5.124</td>
</tr>
</tbody>
</table>

*The phenotype distribution is in Hardy-Weinberg equilibrium (Goodness-of-fit test; χ² = 2.334; d.f. = 5; P = 0.801).

*P-value indicating the significance level of the difference among phenotypic means of the lipid traits, calculated by one-way ANOVA and using a Student-Newman-Keuls (SNK) parametric test or a Kruskal-Wallis (KW) non-parametric test.

NS: not significant at α = 0.05.

Low density lipoprotein (LDL)-cholesterol as determined after polyvinyl sulfate (PVS) precipitation of LDL and Lp(a), by subtracting supernatant cholesterol from total cholesterol.

Ln Lp(a): naturally logarithmically transformed Lp(a).

Serum lipid, apolipoprotein and Lp(a) data were adjusted for age, sex, body mass index, systolic and diastolic blood pressure, smoking, physical exercise, use of oral contraceptives, γ-glutamyltransferase activity and socioeconomic status. Apo E allelic effects were calculated according to the method of Singh and Davignon.

Apo E allelic effects significant at α = 0.05.

Table 6 shows a lowering effect of e2 on cholesterol levels, double to fourfold the e4 increasing effect (Flemings), whereas similar effects of both have been reported elsewhere (Walloons). Eco-genetic interactions have been held responsible, at least in homogeneous population samples, whereas confounding might have occurred in case of heterogeneous samples due to different apo E allele frequencies. As to the origin of the regional differences in e effects between Belgian northerners and southerners, the less favourable apo E allelic effects in southerners compared to northerners cause the more adverse lipid profile in southerners. Although the association of e2 with lower LDL-c and apo B, and of e4 with higher LDL-c and apo B is well established in the general population, considerable heterogeneity with respect to the magnitude of the allelic effects estimated in different populations has been reported. Several studies have shown a lowering effect of e2 on cholesterol levels, double to fourfold the e4 increasing effect (Flemings), whereas similar effects of both have been reported elsewhere (Walloons). Eco-genetic interactions have been held responsible, at least in homogeneous population samples, whereas confounding might have occurred in case of heterogeneous samples due to different apo E allele frequencies.
a gene-environment interaction could cause different allelic effects under different environmental conditions in the same population. In view of the well-documented higher intake of saturated fat and dietary cholesterol in the south of Belgium compared to the north,\textsuperscript{3,4} we hypothesize that the less favourable saturated fat intake in southerners explains the differences in \(e\) effects. Support is given by a recent meta-analysis, showing that apo \(E\) genotype effects are modulated via alterations of amount and type of dietary fat,\textsuperscript{30} and by others.\textsuperscript{31} In contrast to dietary saturated fat, the apo \(E\) gene loci do not have a major effect on the response of lipid levels to increased dietary cholesterol.\textsuperscript{32}

Overall, the apo \(E\) locus contributed substantially to the variances of serum total cholesterol, LDL-c and apo \(B\) (8.8, 13.5 and 16.9\% respectively), far exceeding the 0.9\% and 2.8\% reported for total cholesterol respectively apo \(B\) by Braeckman \textit{et al.} in Flemish males.\textsuperscript{26} In view of the reported differences between men and women with regard to the impact of the apo \(E\) genotype on both the means and variances of the distributions of serum lipids and apolipoproteins,\textsuperscript{36} the large contribution of the apo \(E\) locus to the variances of cholesterol, LDL-c and apo \(B\) is probably related to the inclusion of women in the sample, and especially of women taking exogenous hormones (57\% of the studied females). After all, the apo \(E\) polymorphism is reported to explain up to 10\% of the adjusted interindividual serum cholesterol variation in females, compared to only 1\% in males.\textsuperscript{33}

Furthermore, if women are included the increasing effect of \(e4\) is smaller and the decreasing effect of \(e2\) is larger, as one can expect from the protective effect of oestrogen through upregulation of the hepatic LDL receptor.\textsuperscript{35} Also, women having an \(e4\) allele and taking exogenous hormones showed a greater cholesterol-elevating effect of this allele than women not taking hormones.\textsuperscript{36} In accordance with others,\textsuperscript{5,6,23,27} the contribution of the apo \(E\) locus to the triglyceride, apo \(A-I\) and HDL-c variances was minor (3.5, 1.0 and 2.0\% respectively).

Fourth, the apo \(E\) polymorphism did not affect serum Lp(a) levels, supporting the contention that the LDL-receptor is not a major contributor to the Lp(a) catabolism.\textsuperscript{12,24} The lack of relationship between apo \(E\) phenotype and Lp(a) was also observed by Schaefer \textit{et al.} in the Framingham Offspring Study\textsuperscript{33} and Muros \textit{et al.} in a Spanish working population of Tenerife,\textsuperscript{34} but is dissimilar to the findings of Tiret \textit{et al.} in the EARS study,\textsuperscript{9} where \(e2\) had a lowering effect on Lp(a), and to the findings of de Knijff \textit{et al.},\textsuperscript{35} who showed in a Dutch population sample a 25\% increasing effect on serum Lp(a) level by the \(e4\) allele, equal and opposite to the \(e2\) effect.

To the best of our knowledge, this study is the first examining the contribution of Lp(a) and apo \(E\) polymorphism to regional cholesterol differences in Flanders and Wallonia. Drawbacks of this study may be related to the limited sample size and the fact that apo \(E\) polymorphism was checked in a subgroup only. Second, no dietary survey was performed, and hence the hypothesis regarding a diet-apo \(E\) genotype interaction being responsible for the regional differences in \(e\) effects, remains to be proven. Third, other candidate gene loci which have not been investigated here might also be involved and co-determine responsiveness of serum lipids to dietary fat alterations.\textsuperscript{30}

In conclusion, the more adverse lipid profile in Belgian southerners compared to northerners is reconfirmed in this study. As to the origin of the regional cholesterol differences, no differences could be demonstrated in serum Lp(a) or apo \(E\) phenotype distribution between northerners and southerners, supporting a similar genetic background. On the contrary, the average effects of the apo \(E\) alleles upon adjusted LDL-c and apo \(B\) levels differed between the regions, the adverse \(e4\) effect being doubled and the protective \(e2\) effect being halved in southerners compared to northerners. These findings suggest that similar genetic information variably affects intermediate traits in Flanders and Wallonia in particular environments.

Acknowledgements

The authors are indebted to Prof. H Kesteloot (University Hospital Leuven, Belgium), Director P Jacques (Interbedrijfsgeneeskundig dienst voor werkgereken (IDEWE), Belgium) and Director J Brouwers (Centre de Services Interentreprises (CESI), Belgium) for giving the opportunity to carry out this study. The logistic assistance of B Vanhellemont (IDEWE) and E Den Hond (University Hospital Leuven, Belgium) is gratefully acknowledged. Financial support was given by the Belgian Cardiologic LIGA, Belgium. Partial sponsoring of serum Lp(a) analyses was obtained from DistriLabo, Belgium.

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