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 (χ^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 ε4 effect upon adjusted apo B and LDL-c levels was ≈+12% and the ε2 effect was ≈–15%; in northerners the ε4 and ε2 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 ε2 and ε4 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

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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 pecularity 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.[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[2] and factory workers.[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.[4] As to

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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.[4] 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) E[5–10] and lipoprotein(a) (Lp(a)).[11–15]

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–Médecine 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 γ-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 aprotinin (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), NaN_3 (10 mg/ml), Tritriplex (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 education, 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,[16] by means of six categories that are primarily based on occupation: three non-manual higher categories (classes I, II, IIIN; 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 (m^2). 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 $MgCl_2$/phosphotungstic acid and polyvinyl sulphate, respectively. Besides, LDL-c was estimated by the original Friedewald formula,[17] as well as by a modified Friedewald formula that corrects for the Lp(a)-cholesterol contribution.[6] 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). γ-Glutamyltransferase (GGT) activity was determined using γ-glutamyl-carboxynitroanilide and glycylglycine 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.[18,19] 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).[20] Apo E phenotyping was performed by a micro-method based on isoelectric focusing (pH 4–7) of delipidated serum samples, followed by immunoblotting on a nitrocellulose filter and use of polyclonal rabbit anti-apo E antiserum as the first antibody.[21] Serum lipoprotein(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 χ^2 test where appropriate. 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 contraceptive use, smoking, physical excercise, 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 σ^2_A of these effects attributable to genotypic differences were estimated according to the method of Sing and Davignon.[5] This σ^2_A is a component of the total genetic variance σ^2_G (5). Significance of each estimated α_i (i = 2,3,4) was also tested.

A χ^2-goodness-of-fit test was used to evaluate the genetic Hardy-Weinberg equilibrium for the apo E polymorphism. Apo E allele frequencies were estimated using the gene-counting method. The difference in apo E allele frequencies between the two regions was tested using a χ^2-association test. Differences in mean lipid and (apo)lipoprotein levels between apo E phenotypic 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 $\alpha = 0.05$ 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. Socioeconomic 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/l in Flemings and 75 mg/l 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). Analogously, 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 distributions 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 ε2, 0.767 for ε3 and 0.141 for ε4.

The average impact of the apo E polymorphism on apo B-containing lipoprotein levels appeared large in this population 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 polymorphism 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

Variable	**20–29 years**			**30–39 years**		
Mean ± SD or %	Flanders	Wallonia	*P*-value	Flanders	Wallonia	*P*-value
MALE	N = 116	N = 41		N = 164	N = 91	
Age (years)	25.6 ± 2.7	26.2 ± 2.4	0.227	34.7 ± 2.7	35.0 ± 2.9	0.422
Height (cm)	178.2 ± 6.0	176.9 ± 5.8	0.246	177.5 ± 6.6	175.6 ± 6.1	0.029
Weight (kg)	72 3 ± 9.9	77.4 ± 12.3	0.008	75.7 ± 10.5	79.3 ±12.0	0.014
Body mass index (kg/m^2)	22.7 ± 2.7	24.7 ±3.6	0.002	24.0 ± 3.0	25.7 ± 3.7	<0.0005
Systolic blood pressure (mmHg)	123 ± 11	126 ± 12	0.101	123 ± 11	130 ± 13	<0.0005
Diastolic blood pressure (mmHg)	76 ± 9	76 ± 9	0.811	76±8	79 ± 10	0.034
GGT[a] (U/l)	11.6 ± 6.3	16.2 ± 18.8	0.433	16.0 ± 12.9	8.8 ± 3.5	0.105
Age at graduation (years)	21.3 ± 2.3	19.8 ± 2.5	0.001	21.3 ± 2.6	19.6 ± 2.8	<0.0005
Current smokers (%)	23.3	41 5	0.043	21.3	38.5	0.005
Alcohol users (%)	89.7	78.0	0.107	89.6	85.5	0.330
Socioeconomic class[16] (%)			<0.0005			<0.0005
I	13.9	12.2		18.9	4.4	
II	45.3	9.8		41.5	18.7	
IIIN	28.7	12.2		26.8	8.8	
IIIM	11.3	48.8		9.8	42.9	
IV	0.9	17.1		3.0	23.1	
V	–	–		–	2.2	
FEMALE	N = 211	N = 32		N = 196	N = 53	
Age (years)	25.6 ± 2.6	25 7 ± 2.7	0.891	34.6 ± 2.8	34.8 ± 3.0	0.724
Height (cm)	166.1 ± 6.0	164.0 ± 5.5	0.055	164.8 ± 5.6	161.8 ± 7.5	0.008
Weight (kg)	60.2 ± 9.4	60.9 ± 9.3	0.692	60.2 ± 7.9	61.1 ± 11.2	0.579
Body mass index (kg/m^2)	21.8 ± 3.0	22.6 ± 3.1	0.143	22.2 ± 2.7	23.4 ± 4.5	0.057
Systolic blood pressure (mmHg)	115 ± 10	119 ± 14	0.163	116 ± 11	120 ± 15	0.113
Diastolic blood pressure (mmHg)	73 ± 8	71 ± 8	0.333	74 ± 8	74 ± 11	0.717
GGT[a] (U/l)	8.3 ± 4.2	8.9 ± 7.0	0.791	8.8 ± 3.5	11.0 ± 6.9	0.164
Age at graduation (years)	20.5 ± 2.1	19.5 ± 1.9	0.014	19.6 ± 2.8	18.8 ± 2.6	0.067
Current smokers (%)	23.2	28.1	0.701	27.0	37.7	0.178
Alcohol users (%)	64.0	50.0	0.186	63.8	58.5	0.585
Oral contraceptive use (%)	67.3	65.6	1.000	39.3	50.9	0.171
Socioeconomic class[16] (%)			0.002			0.009
I	5.2	–		4.1	–	
II	42.2	28.1		36.7	28.3	
IIIN	35.5	25.0		31 1	20.8	
IIIM	10.4	31.3		7.7	20.8	
IV	6.2	15.6		16.3	20.8	
V	0.5	–		4.1	9.4	

[a] γ-glutamyltransferase activity.

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 (ln) 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 ε4 and ε2 effects upon adjusted

Table 2 Mean (± SD) serum lipid, lipoprotein and apolipoprotein levels in Belgians, by region, sex and age category

Parameter	20–29 years			30–39 years		
mean ± SD	Flanders	Wallonia	*P*-value	Flanders	Wallonia	*P*-value
MALE	N = 116	N = 41		N = 164	N = 91	
Triglycerides (mmol/l)	1.032 ± 0.438	1.213 ± 0.711	0 408	1.381 ± 1.212	2.088 ± 2.105	0.002
Cholesterol (mmol/l)	4.834 ± 0.767	5.087 ± 0.815	0.076	5.297 ± 0.892	5.900 ± 1.455	<0.001
LDL-cholesterol (mmol/l) (PVS)[a]	3.126 ± 0.837	3.248 ± 0.733	0 410	3.533 ± 0.898	3.936 ± 1.260	0.009
LDL-cholesterol (mmol/l) (Friedewald)[b]	3.034 ± 0.737	3.243 ± 0.639	0.108	3.400 ± 0.836	3.786 ± 1.247	0.009
LDL-cholesterol (mmol/l) (Friedewald, corrected for Lp(a)-cholesterol)[c]	2.937 ± 0.725	3.097 ± 0.607	0.207	3.276 ± 0.800	3.651 ± 1.241	0.010
HDL-cholesterol (mmol/l)	1.326 ± 0.310	1.292 ± 0.282	0.539	1.284 ± 0.290	1.164 ± 0.290	0.002
Lp(a) (mg/l)	125 ± 169	188 ± 207	–	165 ± 204	174 ± 221	–
Ln Lp(a)[d] (Ln mg/l)	4.082 ± 1.340	4.300 ± 1.672	0.406	4.312 ± 1.419	4.354 ± 1.402	0.822
Apolipoprotein A-I (g/l)	1.24 ± 0.21	1.21 ± 0.20	0.495	1.27 ± 0.22	1.16 ± 0.21	<0.001
Apolipoprotein B (g/l)	0.70 ± 0.16	0.72 ± 0.16	0.523	0.79 ± 0.18	0.87 ± 0.25	0.010
FEMALE	N = 112	N = 32		N = 196	N = 53	
Triglycerides (mmol/l)	1.090 ± 0.404	1.109 ± 0.465	0.725	1.005 ± 0.464	1.148 ± 0.579	0.136
Cholesterol (mmol/l)	5.111 ± 0.960	5.438 ± 1.100	0.080	5.045 ± 0.897	5.212 ± 1.160	0.333
LDL-cholesterol (mmol/l) (PVS)[a]	3.101 ± 1.015	3.424 ± 1.124	0.099	3.057 ± 0.946	3.179 ± 1.188	0.490
LDL-cholesterol (mmol/l) (Friedewald)[b]	2.963 ± 0.894	3.284 ± 1.016	0.064	2.954 ± 0.874	3.107 ± 1.100	0.352
LDL-cholesterol (mmol/l) (Friedewald, corrected for Lp(a)-cholesterol)[c]	2.856 ± 0.885	3.136 ± 0.964	0.101	2.841 ± 0.862	3.001 ± 1.050	0.253
HDL-cholesterol (mmol/l)	1.653 ± 0.366	1.649 ± 0.392	0.961	1.634 ± 0.343	1.583 ± 0.357	0.340
Lp(a) (mg/l)	136 ± 177	192 ± 269	–	146 ± 166	137 ± 183	–
Ln Lp(a)[d] (Ln mg/l)	4 188 ± 1.291	4.144 ± 1.708	0.888	4.283 ± 1.295	4.013 ± 1.524	0.197
Apolipoprotein A-I (g/l)	1.56 ± 0.32	1.52 ± 0.29	0.537	1.55 ± 0.30	1.50 ± 0.31	0.221
Apolipoprotein B (g/l)	0.71 ± 0.19	0.73 ± 0.18	0.726	0.70 ± 0.18	0.70 ± 0.23	0.926

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

[b] LDL-cholesterol as estimated by the Friedewald formula, i.e. LDL-cholesterol (mmol/l) = total cholesterol – high density cholesterol (HDL)-cholesterol – triglycerides/2.2.

[c] LDL-cholesterol as estimated by the Friedewald formula, corrected for Lp(a)-cholesterol, i e. LDL-cholesterol (mmol/l) = total cholesterol – HDL-cholesterol – triglycerides/2.2 – (0.3*Lp(a)/386 7) [6]

[d] Ln Lp(a) = naturally logarithmically transformed Lp(a).

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 ε2 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 ε4 allele increased cholesterol levels by 5% (NS), and LDL-c and apo B levels by 11% ($P < 0.05$). In case of the ε3 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, heridity contributes to about 50% of interindividual serum cholesterol variation.[22] 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.[22] 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.[23] The

Table 3 Serum lipoprotein (Lp(a)) levels in 900 randomly recruited Belgian employees, by region, sex and age class

Region	Age category (years)	Sex	No. of subjects	Lp(a) mean (mg/l)	Lp(a) percentiles (mg/l)						
					5th	10th	25th	50th	75th	90th	95th
Flanders	20–29	Male	115	125	6	10	31	64	131	355	497
		Female	210	136	8	11	30	63	171	366	530
	30–39	Male	162	165	8	11	26	77	243	475	549
		Female	196	146	8	13	27	72	213	437	539
	Pooled		683	144	8	12	30	67	186	403	536
Wallonia	20–29	Male	41	188	3	4	24	81	351	508	634
		Female	32	192	4	4	15	70	251	689	834
	30–39	Male	91	174	5	13	34	69	240	496	698
		Female	53	137	4	6	21	61	141	415	571
	Pooled		217	170	4	8	26	75	247	513	664
Overall			900	150	6	11	28	68	192	426	561

After natural log transformation, average Lp(a) was not significantly different between Walloons and Flemings ($P = 0.983$).

Table 4 Apolipoprotein (apo) E phenotype distribution in 249 apparently healthy Belgians, stratified by region

Apo E phenotype	Flanders[a]	Wallonia[a]	Pooled
E2/2	1	0	1
E2/3	18	21	39
E3/3	77	69	146
E4/2	1	4	5
E4/3	27	24	51
E4/4	1	6	7
All phenotypes	125	124	249

[a] The apo E phenotype distribution is similar in Flemings and Walloons ($\chi^2 = 7.213$; d.f. = 5; $P = 0.2053$).

gene coding for the apo(a) molecule is an exception, allelic variation in the apo(a) gene determining as much as 90% of the interindividual variation in Lp(a) mass, and hence Lp(a)-cholesterol, in the population at large.[24]

Although, with respect to the Belgian regional cholesterol differences, intake of a diet rich in saturated fat has been frequently invoked to explain the more adverse serum lipid distribution and higher mortality rate due to cardiovascular disease in Wallonia than in Flanders,[3,4] we aimed to examine whether differences in genetic stock between Belgian northerners and southerners might exist and contribute to the observed regional cholesterol differences. Hitherto, serum Lp(a) levels and apo E polymorphism, two genetically determined cardiovascular risk factors that affect serum cholesterol, were assessed.

The study was designed using an apparently healthy working population from the northern (Flanders) and the southern (Wallonia) region of Belgium. The study population has several advantages. First, the population is homogeneous with regard to ethnic origin as all participants were Caucasians. Second, the population is considered to be representative for 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.[18,19,25]

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.[1–4]

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 ($f_{\varepsilon2}$, $f_{\varepsilon3}$ and $f_{\varepsilon4}$: 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 ε2 allele being to lower LDL-c and apo B, and of the ε4 allele being to increase LDL-c and apo B.[5,6,9,23] Whereas the ε effect on serum LDL-c and apo B levels is consistent across most population studies, there are controversial results about the ε effect on triglycerides, since these vary widely within and among individuals, masking a clear effect of apo E phenotype. In this study ε2 and ε4 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

Mean ± SD	Apo E phenotype E2/2	E2/3	E3/3	E4/2	E4/3	E4/4	One-way ANOVA (SNK - KW)[b] Probability F ratio
No.	1	39	146	5	51	7	–
Relative frequency[a] (%)	0.4	15.7	58.6	2.0	20.5	2.8	–
Variable							
Triglycerides (mmol/l)	1.184	1.552 ± 1.226	1.191 ± 0.829	1.431 ± 0.408	1.499 ± 1.888	2.993 ± 4.317	NS[c]
Cholesterol (mmol/l)	3.501	4.878 ± 1.001	5.454 ± 1.063	5.767 ± 1.061	5.633 ± 1.198	6.709 ± 1.981	0.0011
LDL-cholesterol (mmol/l) (PVS)[d]	1.821	2.867 ± 0.885	3.573 ± 1.100	3.713 ± 1.105	3 816 ± 1.143	4.310 ± 1.571	0.0001
HDL-cholesterol (mmol/l)	1.19	1.382 ± 0.397	1.431 ± 0.372	1.360 ± 0.499	1.370 ± 0.426	1.304 ± 0.503	NS
Apolipoprotein A-I (g/l)	1.15	1.27 ± 0.26	1.27 ± 0.24	1.24 ± 0.23	1.24 ± 0.23	1.25 ± 0.45	NS
Apolipoprotein B (g/l)	0.32	0.67 ± 0.22	0.80 ± 0.21	0.86 ± 0.17	0.86 ± 0.24	0.97 ± 0.30	<0.0001
Ln Lp(a)[e] (Ln mg/l)	4.852	3.965 ± 1.602	4.397 ± 1.419	5.109 ± 1.161	4.215 ± 1.646	3.565 ± 0.558	NS
Median Ln Lp(a) (Ln mg/l)	–	3.714	4.477	5.124	4.331	3.526	–

[a] The phenotype distribution is in Hardy-Weinberg equilibrium (Goodness-of-fit test; $\chi^2 = 2\ 334$; d.f. = 5; $P = 0.801$).

[b] 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.

[c] NS: not significant at $\alpha = 0.05$.

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

[e] Ln Lp(a): naturally logarithmically transformed Lp(a).

Table 6 Estimates and significancies 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)

	Estimated average effect of the apo E alleles								
	Flanders			Wallonia			Pooled		
Parameter	α_2	α_3	α_4	α_2	α_3	α_4	α_2	α_3	α_4
Cholesterol (mmol/l)	× 0.85*	× 1.02	× 1.02	× 0.92*	× 1.00	× 1.06	× 0.89*	× 1.00	× 1.05
LDL-cholesterol (PVS)[a] (mmol/l)	× 0.73*	× 1.03*	× 1.04	× 0.85*	× 1.00	× 1.12*	× 0.80	× 1.01	× 1.11*
Apolipoprotein B (g/l)	× 0.77*	× 1.02	× 1.06	× 0.85*	× 1.00	× 1.13*	× 0.81*	× 1.01	× 1.11*
Triglycerides (mmol/l)	× 1.12	× 0.98	× 1.05	× 1.07	× 0.97	× 1.12	× 1.14	× 0.97*	× 1 11
Lp(a) (mg/l)	× 0.75	× 1.01	× 1.13	× 1.05	× 1.07	× 0.68	× 0.96	× 1.03	× 0.86
HDL-cholesterol (mmol/l)	–0.046	+0.001	+0.023	+0.003	+0.016	–0.091	–0.036	+0.014	–0.051
Apolipoprotein A-I (g/l)	+0.026	–0.004	+0.002	+0.006	+0.007	–0.040	+0.010	+0.004	–0.027

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 Sing and Davignon.[5]

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

* Apo E allelic effects significant at $\alpha = 0.05$

tended to increase the level of triglycerides (NS), in accordance with the meta-analysis of Dallongeville *et al.*,[27] and the EARS study results.[9]

Strikingly, the magnitudes of the apo E allelic effects upon adjusted LDL-c and apo B levels differed between Belgian northerners and southerners (Table 6). In northerners, the LDL-c and apo B increasing effect attributable to ε4 was minor and insignificant (≈+5%), whereas the decreasing effect by ε2 was huge and significant (≈–25%); in southerners however, ε4 increased adjusted LDL-c and apo B levels by ≈+12% ($P < 0.05$), while ε2 decreased these lipid parameters to a similar extent ($P < 0.05$). The less favourable apo E allelic effects in southerners compared to northerners cause the more adverse lipid profile in southerners. Although the association of ε2 with lower LDL-c and apo B, and of ε4 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.[6] Several studies have shown a lowering effect of ε2 on cholesterol levels, double to fourfold the ε4 increasing effect (Flemings), whereas similar effects of both have been reported elsewhere (Walloons[28,29]). Eco-genetic interactions have been held responsible, at least in homogeneous population samples,[30] whereas confounding might have occurred in case of heterogeneous samples due to different apo E allele frequencies.[29] As to the origin of the regional differences in ε effects between Belgian northerners and southerners,

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,[3,4] we hypothesize that the less favourable saturated fat intake in southerners explains the differences in ε 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,[30] and by others.[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.[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 *et al.* in Flemish males.[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,[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.[23] Furthermore, if women are included the increasing effect of ε4 is smaller and the decreasing effect of ε2 is larger, as one can expect from the protective effect of oestrogen through upregulation of the hepatic LDL receptor.[33] Also, women having an ε4 allele and taking exogenous hormones showed a greater cholesterol-elevating effect of this allele than women not taking hormones.[36] In accordance with others[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.[12,24] The lack of relationship between apo E phenotype and Lp(a) was also observed by Schaefer *et al.* in the Framingham Offspring Study[33] and Muros *et al.* in a Spanish working population of Tenerife,[34] but is dissimilar to the findings of Tiret *et al.* in the EARS study,[9] where ε2 had a lowering effect on Lp(a), and to the findings of de Knijff *et al.*,[35] who showed in a Dutch population sample a 25% increasing effect on serum Lp(a) level by the ε4 allele, equal and opposite to the ε2 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 ε 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.[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 ε4 effect being doubled and the protective ε2 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.

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