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The relationship between apolipoprotein AI-containing lipoprotein fractions and environmental factors: the prospective epidemiological study of myocardial infarction (PRIME study)

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Abstract

Apolipoprotein (apo) AI is distributed within high-density lipoproteins (HDL) between different types of particles, one containing both apoAI and apoAII (LpAI:AII), the other containing no apoAII (LpAI). We investigated the associations between LpAI and LpAI:AII with several factors such as body mass index (BMI), waist to hip ratio (WHR), alcohol intake, cigarette consumption and physical activity, in three French and one Northern Irish male populations included in a prospective study (PRIME study). LpAI and LpAI:AII were associated with variations in all environmental factors, except LpAI:AII, which was not associated with WHR. These relationships were unchanged after adjustment for other environmental factors, but slightly modified after adjustment for triglyceride levels. LpAI decreased when BMI, WHR and cigarette smoking increased, and increased with alcohol consumption and physical activity. LpAI:AII had a similar variation except for the absence of LpAI:AII modification associated with WHR variation. The associations between LpAI and BMI, alcohol consumption and cigarette smoking were largely dependent on HDL-cholesterol as indicated by the lack of any significance when the adjustment for HDL-cholesterol was made. Conversely, after adjustment for HDL-cholesterol, the significant association between LpAI:AII and BMI disappeared, while the associations between LpAI:AII and alcohol consumption, cigarette smoking and physical activity remained significant. These results suggest that the mechanisms of LpAI and LpAI:AII modulations differ according to each environmental factor, some dependent on the lipid content of lipoproteins and others not, but LpAI and LpAI:AII levels seem independent of triglyceride concentration. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Prospective study; Apolipoprotein AI; LpAI; Environmental factors

1. Introduction

A number of epidemiological studies have shown an inverse relationship between the incidence of coronary heart disease (CHD) and high-density lipoprotein (HDL)-cholesterol [1–3]. Furthermore, decreased levels

of apolipoprotein (apo) AI, the major protein in HDL, occur in angiographically documented CHD and in survivors of myocardial infarction and appear to be an independent risk factor for CHD [4,5]. ApoAI is an important structural protein for the biosynthesis of HDL and is an activator of LCAT [6,7]. Moreover, it interacts with receptors such as SR-BI [8] and thus facilitates reverse cholesterol transport from peripheral cells to the liver.

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It is now established that HDL particles exhibit considerable heterogeneity of size, density, flotation rate, and both lipid and apolipoprotein composition. Also, the cholesterol content of the entire HDL family is a complex function of the composition and particle number of several subclasses [9,10]. Specifically, HDL can be separated into several subclasses by different procedures. Evidence of heterogeneity has been provided by the analysis of hydrated density, molecular weight, particle size, chemical composition, hydrodynamic properties and apolipoprotein content [11,12]. It is now recognized that HDL contain two main types of apoAI-containing particles which may have different metabolic functions and clinical significance [13–15]. One species contains both apoAI and apoAII (LpAI:AII), while apoAII is absent from the other (LpAI). Physicochemical and biological properties of both these particles have been widely analyzed [16,17]. Moreover, a case-control study of myocardial infarction (ECTIM study) has shown a lower LpAI level in a region (Northern Ireland) with a high incidence of CHD compared to France where the incidence of CHD is three-fold lower [18]. This case-control study suggests but does not establish LpAI as a risk factor. The PRIME study was therefore partly set up to evaluate LpAI as a risk factor. Genetic factors largely contribute to the variation in apoAI and LpAI [19], but little evaluation has been carried out of the effect of factors such as weight, smoking, alcohol consumption and physical activity on LpAI and LpAI:AII. Smoking, alcohol consumption and physical activity are typically classified as environmental factors while other parameters analyzed in the present paper, such as body mass index (BMI) and waist to hip ratio (WHR), result from an interaction between environmental factors such as diet and physical training and genetic factors. Specific determinants of apoAI and LpAI variation such as excess weight and alcohol consumption, have been evaluated [20–22]. The variation of LpAI:AII has been analyzed along with LpAI only in subjects who had stopped smoking [23]. A large number of healthy subjects were included in PRIME so that the relationship between LpAI, LpAI:AII and environmental factors could be precisely determined to analyze their impact on apoAI containing lipoproteins.

2. Materials and methods

2.1. Subjects

Four cohorts, three in France, (Lille in the North, Strasbourg in the North-East and Toulouse in the South-West), and one in Northern Ireland (Belfast), comprising an approximately equal number of 50–59-year-old males were recruited between 1991 and 1993

for inclusion in a prospective epidemiological study of myocardial infarction (PRIME) [24]. Altogether 10 596 subjects were included. Because the present study focuses on HDL particles, subjects with diabetes, a cardiovascular disease, those with triglyceride levels above 400 mg/dl, or taking antihypertensive or hypolipidemic drugs were excluded. The analysis therefore included 2059 subjects in Toulouse, 2076 in Strasbourg, 1963 in Lille and 2259 in Belfast. Each cohort was recruited to broadly match the social class structure of the background population.

Questionnaires relating to demographic factors were completed at home by the participants and checked by the interviewers at the clinic. Additional questionnaires were completed at the clinic by a trained interviewer. These questionnaires enabled us to determine tobacco and alcohol consumption, physical activity and exclusion criteria for the present analysis. Alcohol consumption was assessed by adding together alcoholic beverages and taking into account different types of beverages such as wine, beer, spirits and their respective alcohol contents consumed over a week, and cigarette smoking was quantified by the number of cigarettes smoked. Physical activity was assessed using a standardized questionnaire [25]. In the present paper, physical activity was evaluated by its intensity during leisure time and was marked from 1 (no physical activity at least once a week) to 4 (intensive physical activity at least 20 min three times a week or more). Anthropometric measurements including weight, height, waist and hip circumferences were carried out using standardized procedures to minimize inter-center variation. BMI was calculated as weight (kg)/height (m²), and WHR as the waist measurement/hip circumference ratio. General features of the PRIME study and its recruitment and selection methods are described elsewhere [24].

2.2. Lipoprotein analysis

Blood was drawn after a 12-h fast into tubes containing EDTA. Plasma was separated by centrifugation at 4°C within 15 min at each clinic, kept at 4°C and sent weekly to the central laboratory at the Pasteur Institute in Lille, France. Cholesterol and triglycerides were determined by automated enzymatic procedures (Boehringer, Mannheim, Germany) adapted to a Hitachi 705 analyzer. Cholesterol was measured in the HDL-containing supernatant after phosphotungstate/magnesium chloride precipitation of apoB-containing lipoproteins (Boehringer, Mannheim, Germany). ApoAI was quantified using commercial reagent immunonephelometry (Behringwerke, Marburg, Germany). LpAI was measured using differential immunoelectrophoresis as previously described [18]. LpAI:AII level was calculated as its apoAI content by subtracting LpAI from total apoAI. The variation co-

efficients were 2 and 4% for apoAI and LpAI, respectively.

2.3. Statistical analysis

Statistical analysis was carried out using the statistical SAS package (SAS Institute, Cary, Indiana). Mean values and standard deviations were computed without adjustment. The differences between centers were evaluated by variance analysis. Evaluation of the effect of parameters such as BMI, WHR, alcohol consumption, cigarette smoking and physical activity was carried out by linear regression models (GLM procedure), each with apoAI, LpAI or LpAI:AII as the dependent variable. Several models were used: (model 1) regression analysis with adjustments for age and center; (model 2) regression using BMI, WHR, alcohol consumption, number of cigarettes, physical activity, age and center as independent variables; (model 3) regression including the variables of model 2 plus log-triglycerides; and (model 4) regression including the variables of model 2 plus log-triglycerides and HDL-cholesterol. Significance was accepted at the 1% level.

3. Results

Mean values of plasma lipids and parameters related to HDL are shown for each center in Table 1. As already observed in a case-control study set up in the same geographical regions (ECTIM study) [18], cholesterol and triglycerides were higher in Belfast than in France. However, cholesterol and triglycerides were slightly higher in the North and the East of France (Lille, Strasbourg) than in the South (Toulouse). Parameters related to HDL showed clear differences between centers. HDL-cholesterol was lower in Belfast than in the three French centers. ApoAI levels in Lille and Strasbourg were similar and higher than in Toulouse and Belfast. The similar apoAI levels in Strasbourg and Lille were due to comparable LpAI:AII levels and a significant but very small difference in LpAI between these centers. In contrast, the similar apoAI levels in Belfast and Toulouse were the consequence of different proportions of LpAI and LpAI:AII levels in these two regions, LpAI being significantly lower in Belfast than in Toulouse and the reverse for LpAI:AII.

Table 1

Mean values and standard deviations (S.D.) of plasma cholesterol, triglycerides, HDL-cholesterol (HDL-C), apoAI and apoAI-containing lipoproteins (LpAI and LpAI:AII) in the subjects at the four centers and in all population excluding those with CHD, those with triglyceride levels above 400 mg/dl and those taking antihypertensive or hypolipidemic drugs

Center	Cholesterol (mg/dl)	Triglycerides* (mg/dl)	HDL-C (mg/dl)	apoAI (mg/dl)	LpAI (mg/dl)	LpAI:AII (mg/dl)
Toulouse	214 (34)	128 (83)	49 (12) ^a	147 (22) ^a	47 (11) ^a	101 (18)
Strasbourg	224 (39)	148 (109)	49 (13) ^a	152 (24) ^b	47 (11) ^a	105 (19) ^a
Lille	222 (38)	132 (82)	52 (13)	153 (25) ^b	48 (12)	105 (19) ^a
Belfast	227 (39)	170 (98)	46 (13)	146 (24) ^a	42 (10)	104 (19)
All	222 (38)**	145 (95)**	49 (13)**	149 (24)**	46 (11)**	104 (19)**

* Statistical analysis was carried out after log-transformed triglycerides whereas means and S.D. were calculated on non-transformed triglycerides.

** Mean values were statistically different ($P < 0.001$) between the four centers (ANOVA). For each parameter, all values were significantly different between two centers with the exception of those that are noted by the same letter (a or b) which are not different between them.

Table 2

Mean values (standard deviation) of BMI, WHR, alcohol consumption, number of cigarettes smoked and physical activity of the subjects of the four centers and in all population excluding those with CHD, those triglyceride levels above 400 mg/dl and those taking antihypertensive or hypolipidemic drugs

Center	BMI (kg/m ²)	WHR ^a	Alcohol (g/day)	Cigarettes (/day)	Physical activity
Toulouse	26.1(3.1)	0.96(0.06)	36.7(34.2)	3.0(7.7) ^b	2.4(0.8)
Strasbourg	27.0(3.4) ^a	0.99(0.06)	42.3(34.4) ^b	3.6(8.6) ^c	2.1(0.8) ^b
Lille	26.2(3.4)	0.95(0.06)	43.7(38.3) ^b	3.3(7.8) ^{b,c}	2.3(0.9)
Belfast	26.1(3.4)	0.94(0.05)	28.4(43.5)	4.9(9.8)	2.1(0.5) ^b
All	26.3(3.3)*	0.96(0.06)*	37.7(38.7)*	3.7(8.6)*	2.2(0.76)*

^a Different from other centres ($P < 0.01$).

^b Not different between them ($P > 0.01$).

^c Not different between them ($P > 0.01$).

* $P < 0.01$ between the four centers.

Table 3

Changes in apoAI, LpAI and LpAI:AII (mg/dl) associated with specified change in characteristics using four regression models which adjust for other variables, PRIME study

	Model 1	Model 2	Model 3	Model 4
<i>Body mass index (1 kg/m²)</i>				
apoAI	-1.14	-1.17	-0.90	-0.13 ^a
LpAI	-0.62	-0.47	-0.37	-0.07 ^a
LpAI:AII	-0.52	-0.69	-0.52	-0.07 ^a
<i>Waist to hip ratio (0.1)</i>				
apoAI	-0.41	-1.82	-1.00 ^a	-0.16 ^a
LpAI	-0.34	-1.33	-1.01	-0.68
LpAI:AII	-0.07 ^a	-0.49 ^a	0.02 ^a	0.52 ^a
<i>Alcohol consumption (10 g/day)</i>				
apoAI	1.39	1.63	1.68	0.42
LpAI	0.21	0.41	0.43	-0.07 ^a
LpAI: AII	1.21	1.22	1.25	0.49
<i>Cigarette number (10/day)</i>				
apoAI	-0.97	-2.59	-2.18	-0.86
LpAI	-0.19 ^a	-0.59	-0.43	0.08 ^a
LpAI:AII	-0.78	-2.00	-1.75	-0.95
<i>Physical activity (1)</i>				
apoAI	2.39	1.92	1.66	0.91
LpAI	0.79	0.60	0.50	0.20 ^a
LpAI:AII	1.58	1.31	1.15	0.70

^a Not statistically significant ($P > 0.01$). Model 1: analysis after adjustment for age and center; model 2: analysis after adjustment for age, center and the other environmental factors; model 3: analysis after adjustment and triglycerides; and model 4: analysis after adjustment for variables included in model 3, triglycerides and HDL-cholesterol.

Data on BMI, WHR, alcohol consumption, cigarette smoking and physical activity are presented in Table 2. Clear differences appear in anthropometric measurements. Indeed, BMI and WHR were higher in Strasbourg than in other centers and WHR because significantly higher from Belfast at the bottom, followed by Lille, then Toulouse and finally Strasbourg. Alcohol consumption was higher in France particularly in the North (Lille) and East (Strasbourg) than in Northern Ireland. In contrast, cigarette smoking was significantly more elevated in Northern Ireland than in France. Tobacco use was relatively similar in the three French centers. Physical activity was moderately higher in Toulouse and Lille than in the two other centers.

Associations of apoAI, LpAI and LpAI:AII with BMI, WHR, alcohol consumption, cigarette smoking and physical activity were evaluated by linear regression. Table 3 shows the difference in apoAI, LpAI and LpAI:AII associated with specified variations of each parameter as estimated from the models. As non-interaction was displayed between factors and centers, subjects of all centers were pooled. Significantly higher BMI was associated with lower apoAI in variance analysis (model 1). A similar association

remained after simultaneous adjustment for WHR, alcohol consumption, cigarette smoking and physical activity (model 2), and was to some extent independent of triglycerides (model 3), but became insignificant after adjustment for HDL-cholesterol (model 4). The change in apoAI associated with a change in BMI was the consequence of changes in both LpAI and LpAI:AII which were, like the change in apoAI, to some extent independent of triglycerides and insignificant when HDL-cholesterol was added to the model.

Higher WHR is associated with lower apoAI. A 0.1 increase in WHR (corresponding for example to an increase in waist circumference of 10 cm in a man with a hip circumference of 1 m) is associated with 4 mg/dl lower apoAI. The change in apoAI associated with that of WHR was essentially due to a change in LpAI. Adjustment for other parameters (model 2) did not modify the results, although regression coefficients between WHR and apoAI, LpAI and LpAI:AII were higher than those calculated in model 1. The introduction of triglycerides in the model abolished any significance of the relationship between apoAI and WHR whereas LpAI remained significantly associated with WHR. The same result was observed after adjustment for HDL-cholesterol.

Moderate alcohol consumption (10 g/day) increased apoAI, LpAI and LpAI:AII by 1.39, 0.21 and 1.21 mg/dl, respectively. This effect remained virtually unchanged by simultaneous adjustment for BMI, WHR, cigarette smoking, physical activity and triglycerides (models 2 and 3). Moreover, the effect of alcohol on apoAI and LpAI:AII remained significantly positive, if attenuated after adjustment for HDL-cholesterol. Conversely, the relationship between LpAI and alcohol consumption disappeared after adjustment for HDL-cholesterol.

Cigarette smoking had a significant effect on apoAI according to model 1, because of the effect of smoking on LpAI:AII. However, cigarette smoking was associated with a significant, negative effect on both LpAI and LpAI:AII after simultaneous adjustment for BMI, WHR, alcohol consumption, physical activity (model 2) and triglycerides (model 3). This association remained significant for apoAI and LpAI:AII but not for LpAI after adjustment for HDL-cholesterol.

Physical activity, assessed by its intensity during leisure time, increased both LpAI and LpAI:AII fractions and as a consequence apoAI in model 1 analysis. The relationship remained significant when the adjustment for other environmental factors and triglycerides was included in the model (models 2 and 3), but with the inclusion of HDL-cholesterol in the model (model 4), LpAI:AII remained significantly associated with physical activity, whereas LpAI did not.

4. Discussion

It is agreed that HDL is the plasma lipoprotein fraction with a protective role in CHD. ApoAI is the major apolipoprotein in HDL. However HDL is heterogeneous and apoAI is present on the surface of two fractions, LpAI and LpAI:AII, which have different biological and metabolic properties. An evaluation was therefore carried out, in a cohort of healthy males, of the effect of factors such as alcohol consumption, cigarette smoking and physical activity, which are largely self-determined, and those with strong interactions between genetic and environmental factors such as BMI and WHR on apoAI, LpAI and LpAI:AII levels. Each of these factors in the PRIME study had a significant negative (BMI, WHR, cigarette smoking) or positive (alcohol consumption, physical activity) effect on LpAI and LpAI:AII, except for WHR on LpAI:AII (Table 3, model 2). The association of all factors except WHR with LpAI appeared to be related to the cholesterol content of HDL, as is shown by the disappearance of the significance when HDL-cholesterol was added into the model. The same finding was noted for the association between BMI and LpAI:AII but on the other hand, alcohol consumption, cigarette smoking and physical activity were associated with LpAI:AII partly independently of HDL-cholesterol. Our results also show that triglycerides have little effect per se on apoAI, LpAI and LpAI:AII (Table 3, model 3).

Adiposity is consistently associated with reduced apoAI [26–28], with rare exceptions [29,30]. The reduced apoAI associated with the increase in BMI in the PRIME study was due to the decrease in both LpAI and LpAI:AII. The association of LpAI and LpAI:AII with BMI was little influenced by triglyceride levels, and so these results after adjustment for this last variable were barely modified. Conversely, associations between LpAI and LpAI:AII, and BMI are nearly abolished after controlling for HDL-cholesterol. That could signify that obesity affects the cholesterol content of HDL. The structural modification of HDL induced by obesity could alter the metabolism of those particles and thus of their plasma levels. Indeed, the influence of HDL structure on metabolism is suggested by kinetic studies *in vivo* [31].

The index of upper-body and abdominal fat represented by WHR is associated with LpAI but not with LpAI:AII (Table 3). This finding has already been noted in a group of overweight subjects [30] and is coherent with the significant correlation between WHR and HDL₂ and the absence of any significant correlation between WHR and HDL₃ [32]. Indeed, LpAI is mainly in HDL₂ and LpAI:AII in HDL₃. Although WHR has been inversely related to triglyceride levels [28], the adjustment for triglycerides has little influence on the relation between WHR and LpAI. The two

variables related to fat mass, BMI and WHR, have therefore different associations with LpAI and LpAI:AII. WHR, which is highly correlated to the proportion of visceral fat [33], is more closely related to an atherogenic lipid profile, i.e. high triglyceride and low HDL-cholesterol levels, than BMI [26]. The inverse relation between WHR and LpAI is coherent with a lower LpAI level, which represents a higher risk. The different effects of fat distribution on lipoprotein metabolism, particularly apoAI-containing lipoproteins, are not yet understood. Low HDL-cholesterol would be the consequence of a low LpAI level, a hypothesis coherent with lower LpAI in survivors of myocardial infarction [18].

Alcohol intake is associated with higher LpAI and LpAI:AII, and this effect remains for LpAI:AII after adjustment for HDL-cholesterol while the association between alcohol and LpAI or LpAI:AII is unaffected by triglyceride adjustment. Marquesvidal et al. [34] observed an effect of the alcohol dose on apoAI and on levels of its subfractions LpAI and LpAI:AII in the ECTIM study. However, in the PRIME study, the increase in apoAI of about 1.4 mg/dl for 10 g of alcohol per day was less than in the ECTIM study (4 mg/dl for 10 g/day) or in a Finnish study (2.5 mg/dl) [22]. As in the Finnish study, most of the apoAI variation in the PRIME study was due to that of LpAI:AII. The Finnish study indeed showed that two-thirds of the apoAI variation was accounted for by the variation in LpAI:AII. In the PRIME study, the increments of LpAI and LpAI:AII accounted for 25 and 75%, respectively, of the entire increase in apoAI.

The adjustment for HDL-cholesterol cancelled out the increase in LpAI and partially decreased the relationship between LpAI:AII and alcohol consumption. The adjustment for triglycerides did not significantly modify the relationship between apoAI-containing lipoproteins and alcohol consumption. However, the absence of any effect of triglyceride adjustment may be explained by the absence of any increase in triglycerides in drinkers [22,34,35], or a limited relationship between triglycerides and alcohol consumption as in the PRIME study (data not shown). The partially independent HDL-cholesterol increase in LpAI:AII can be accounted for by a direct effect of alcohol on apoAII synthesis as we have recently shown [36]. Total (LpAI) or partial (LpAI:AII) dependency on HDL-cholesterol content could be linked to modification in the catabolism of HDL particles due to a modulation of cholesteryl ester transfer protein (CETP) and/or phospholipid transfer protein (PLTP) activities due to alcohol [37].

In this study, cigarette smoking was associated with a decrease in apoAI, essentially due to the decrease in LpAI:AII, independently of triglycerides and HDL-cholesterol. However, this analysis is limited, as it does

not take into account the possible differences in nutritional habits related to smoking. Adjustment for variables such as the effect of changes in nutritional status after giving up smoking [38] shows that the modification of LpAI:AII is at least partly dependent on smoking [23]. Furthermore, smokers usually have a lower BMI than non-smokers, suggesting a long-term effect of smoking on weight. The adjustment for BMI to account for this confounding factor shows the independent effect of smoking (Table 3, model 2). The effect of cigarette smoking on apoAI-containing particles was previously only analyzed longitudinally in a group of smokers who had stopped smoking [23]. After adjustment for BMI and a few nutritional parameter changes, it was found that smoking cessation led to an increase in LpAI:AII and no modification of LpAI, a finding coherent with ours. Additionally, smoking which was correlated with HDL₃, the LpAI:AII-rich fraction, and not correlated with HDL₂, a LpAI rich fraction [27] adds coherence to this finding.

Physical activity was significantly associated with LpAI and LpAI:AII after controlling for other environmental factors and triglycerides. This association persisted for LpAI:AII but disappeared for LpAI when the adjustment for HDL-cholesterol was used. The positive association between LpAI and physical activity is consistent with the increase in HDL₂-cholesterol in subjects completing a period of physical training [39,40], because most LpAI is in the HDL₂ fraction. On the other hand, LpAI:AII is very positively associated with physical activity. The association between apoAII and physical activity observed in the Northern Irish population supposes an increase in LpAI:AII [41] with almost all the apoAII being included in LpAI:AII, a finding coherent with our results. HDL₃-cholesterol increase related to physical activity [42], is also coherent with our results. LpAI:AII is essentially included in the HDL₃ fraction, but HDL₃-cholesterol decrease with physical activity [40] appears discordant with these findings. This discordance could be explained by a differential modification in the apolipoprotein and cholesterol content of HDL with regard to physical activity, as was observed in Northern Irish men [41]. Therefore, distribution and structure of HDL subfractions appeared to be modified in physically fit as compared to unfit subjects [43]. Consequently, the relative proportions of LpAI and LpAI:AII in HDL₂ and HDL₃ may not be similar in unfit and fit people. A relation between HDL₃ and LpAI:AII may not therefore be so clear in unfit as in fit individuals.

In conclusion, this study shows the effect of various factors on HDL fractions defined by their apolipoprotein content. The associations between HDL parameters and environmental factors are small and cannot thus be applied on the individual level. However, this study gives evidence that these associations

exist and they could have a significant clinical effect on the population level.

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