Relation Among Lipoprotein Subfractions and Carotid Atherosclerosis in Alaskan Eskimos (From the GOCADAN study)

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INTRODUCTION

The Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) population can provide unique insight into studies of the determinants of atherosclerosis. Although many Alaska Eskimos maintain a traditional diet and lifestyle, rates of CVD are high[1,2,3,4] and systematic measures of carotid atherosclerosis indicate that plaque prevalence is > in other U.S. groups.[5] Smoking rates[6] and subclinical inflammation[7] are high; on the other hand LDL-C and triglyceride (TG) levels are not elevated and HDL-C levels are high.[8] The aim of this study was to evaluate the relations between lipoprotein subfraction particle concentrations and size and measures of carotid atherosclerosis in this population with high cardiovascular risk but little hyperlipidemia.

METHODS

The GOCADAN study population includes 1214 family members age 18 who are residents of 8 villages and the town of Nome in the Norton Sound Region of Alaska and were recruited in 2000–2004.[9,10] Each participant underwent a physical examination, personal interview, collection of biological specimens, and other diagnostic tests. Permission was granted by each community to conduct the study; written informed consent was obtained from all participants. Details of the study have been published.[9,10]

The current analysis focused on 796 GOCADAN participants, age 35. Those missing carotid ultrasound examination (n=61) and lipoprotein particle data (n=56) were excluded as well as participants who were diagnosed as having diabetes according to 1998 World Health Organization criteria[11] (n = 40) or were receiving hypolipidemic agents (n = 69), leaving 656 persons in the final dataset.

Anthropometric measurements, including height, weight, and waist circumference, were performed with participants fasting, according to standard procedures.[9] Weight was measured to the nearest tenth pound. Waist circumference was measured with an anthropometric tape applied at the level of the umbilicus, with the subject supine, and approximated to the nearest quarter inch. Sitting blood pressure was measured on the right brachial artery using a mercury sphygmomanometer after the participant rested for 5 minutes. Three readings were taken, with the mean of the second and third measurements used as the final measure. Smoking habits were evaluated via questionnaire and participants were categorized as current, former, and never-smokers.[9]

Samples of whole blood, plasma, serum, and urine were collected from each participant and stored at −80° C until used. All laboratory methods have been published.[9] Plasma lipids were analyzed by a conventional enzymatic chemistry analyzer (Virtos 950, Ortho-Clinical Diagnostics, Rochester, NY, USA) using a dry multilayered analytical element coated on a polyester support.[12,13] LDL-C was calculated by the Friedewald formula.[14] Apolipoprotein

values were 2-tailed, and values < 0.05 were considered statistically significant. Data were analyzed using SAS version 9.1.

RESULTS

Women comprised a greater proportion of the study population (Table 1) BMI, and systolic and diastolic blood pressures were mostly normal, with a low portion of participants receiving antihypertensive drugs. Plasma lipid concentrations were on average lower than those of the general U.S. population, with high HDL-C. A large percentage of the population smoked and almost half had at least 1 focal plaque.

Lipoprotein subfractions are reported in Table 2. The majority of the VLDL and HDL particles were small, whereas the LDL particles were equally distributed.

To evaluate the relationship between lipoprotein concentration and size vs. IMT and plaque score, the cohort was analyzed according to tertiles of IMT and plaque score groups (0, 1, and 2–7). The same was done for standard lipids, and all data were adjusted for age, gender, BMI, systolic blood pressure, and current smoking. Both IMT and plaque score increased with increasing LDL-C (Table 3). Conversely, plasma triglycerides decreased with increasing IMT and plaque score, reaching statistical significance for plaque score (Table 3). Apo B levels tended to increase with increasing IMT tertiles and plaque score, but without reaching statistical significance. No associations were found with HDL-C and apo A1 (Table 3).

When relations among ultrasound variables and lipoprotein subfractions concentration and size were assessed (Table 4), higher IMT tertile was associated with a significant increase in total LDL particle concentration. Both large and small LDL particles showed corresponding but non-significant increases with increasing IMT tertile. Higher plaque score was associated with higher levels of large LDL particles (Table 4). No relationship was found between IMT or plaque score and LDL particle size. With increasing plaque score there was a significant linear decrease in large VLDL concentration and a nonsignificant decrease in intermediate particles. Therefore, higher plaque score was associated with a smaller VLDL size. No relation was found between IMT tertiles and VLDL particle concentrations and size. IMT and plaque score were not associated with HDL particle concentrations or size (Table 4).

To evaluate the independent and/or combined effect of LDL-C and total LDL particle concentration on IMT, we divided the population into 4 groups based on their quartiles of LDL-C and LDL-P: 1) those with low LDL-C and low LDL particle concentration (group 1, n = 248); 2) those with high LDL-C and low LDL particle concentration (group 2, n = 80); 3) those with low LDL-C and high LDL particle concentration (group 3, n = 81); and 4) those with high LDL-C and high LDL particle concentration (group 4, n = 247) using a 2-way analysis of variance (Figure 1). The groups with only 1 lipid abnormality (high LDL-C or high LDL particle concentration, groups 2 and 3, respectively) had an intermediate value of IMT, not significantly different from group 1 (both normal LDL-C and LDL particle

concentration). On the contrary, participants with both high LDL-C and high LDL particle concentration (group 4) showed higher IMT values (p = 0.015 vs group 1).

The same analyses were performed for plaque score, using large LDL particle concentration instead of total LDL particle concentration (Figure 2). Again, only the group with both abnormalities showed a significantly higher plaque score compared with the group with no abnormality (p = 0.006, group 4 vs group 1).

DISCUSSION

This study, performed in a population with a high prevalence of CVD despite mostly favorable lipid and blood pressure patterns and a physically active lifestyle, showed several findings:

- LDL particle concentration was relatively low and LDL were large in this population.

- A high proportion of the GOCADAN participants had plaque.

- Higher IMT levels were significantly associated with higher levels of LDL-C and total LDL particle concentration, independently of other traditional cardiovascular risk factors (i.e., age, BMI, systolic blood pressure, and current smoking). These 2 lipid abnormalities appeared to be additive in their effects on IMT.

- Carotid plaque was associated with higher levels of LDL-C, higher levels of large LDL particles, higher concentrations of small VLDL, and smaller VLDL size. The effects of LDL-C and LDL particle size on plaque score were additive.

- IMT and plaque score were not associated with HDL-C or HDL subfraction concentrations.

Reports relating LDL particle concentration and/or size and IMT have varied: 2 studies found no relations with LDL size,[21,22] whereas another found a significant relation with LDL size in asymptomatic participants with familial combined hyperlipidemia.[23] Another study in a multiethnic population, examining both LDL size and particle concentrations, found a significant relation both with large and small LDL particle concentrations,[18] while the current analysis found a significant relation with total LDL particle concentration and trends with large and small particles when analyzed separately. The population studied by Mora et al.[18] was larger and differed from our population in that it included a large percentage of diabetic participants and had higher concentrations of small LDL particles.

An intriguing finding is the inverse relation of plaque score with plasma triglycerides. This association was accompanied by inverse associations with large VLDL and VLDL size. To our knowledge there have been no other reported analyses of the relations among lipoprotein particles and plaque score. However, small VLDL particles can more easily enter the subendothelial space, and some studies[24,25]

The current analysis indicated possible differences in lipoprotein determinants of IMT and occurrence and/or worsening of plaque. LDL particle concentration may be more closely related to IMT, whereas small VLDL and large LDL may be more closely related to plaque score. The relationship of plaque score with large LDL instead of total or small LDL is consistent with some studies, including that of Mora et al.,[18] suggesting that large LDL particles, not only small ones, may be implicated in the evolution of the atherosclerotic process and CVD. Furthermore, it must be emphasized that large LDL particle concentrations were significantly and inversely associated with VLDL size. Therefore, the association of large LDL particles with plaque score could be mediated through their association with small VLDL.

The lack of association of HDL-C, apo A1, or HDL particle concentration with carotid atherosclerosis is of interest because of the high levels of HDL-C characterizing our population and because CVD rates are high despite the high HDL-C levels. More studies are needed to understand HDL-C metabolism in this group.

The strengths of this study include the population-based sampling of a relatively homogeneous group, standardization of carotid measures, and availability of lipoprotein subfraction data in a large percentage of the participants. Also, the high prevalence of carotid disease in this cohort is not confounded by hyperlipidemia, thus allowing evaluation of the possible role of different lipoprotein particles and sizes as additional cardiovascular risk factors in a population with average plasma lipids within the normal range. The multiple carotid measures allowed evaluation of different aspects of the vessel wall and the atherosclerotic process. On the other hand, our data may not be applicable to other populations. Another weakness is the cross-sectional nature of the analysis, which precludes establishment of cause and effect. Furthermore, although the analyses have been adjusted for gender, a separate analysis for men and women was not possible due to the sample size.

Acknowledgments

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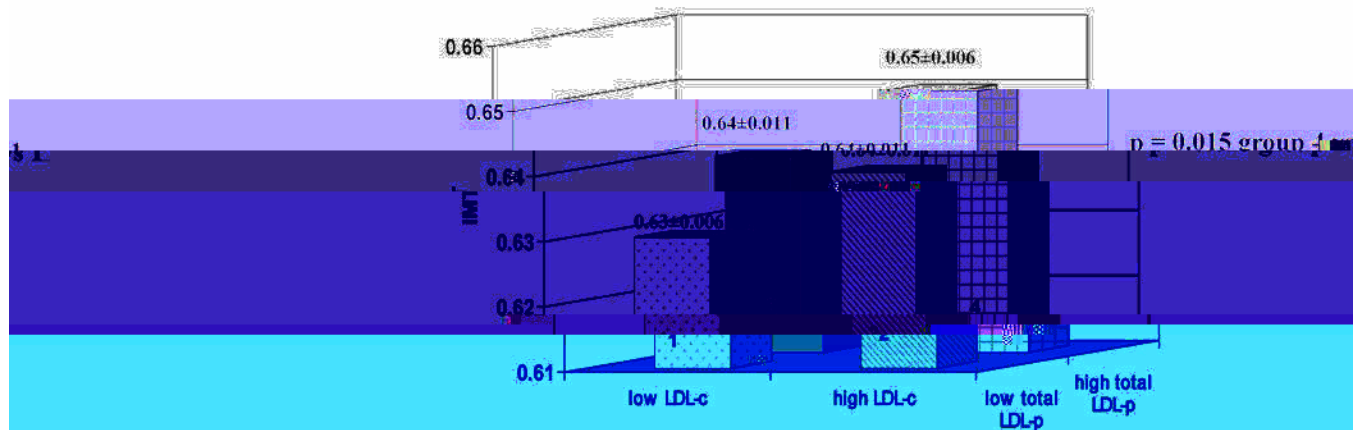
Figure 1.
Additive role of total LDL cholesterol and LDL particle concentration in early atherosclerosis as measured by intimal medial thickness

Group 1 = low LDL cholesterol and low LDL particle concentration, n = 248.

Group 2 = high LDL cholesterol and low LDL particle concentration, n = 80.

Group 3 = low LDL cholesterol and high LDL particle concentration, n = 81.

Group 4 = high LDL cholesterol and high LDL particle concentration, n = 247.

Abbreviations. IMT = intimal medial thickness; LDL-C = low-density lipoprotein cholesterol; LDL-P = low-density lipoprotein particle number.
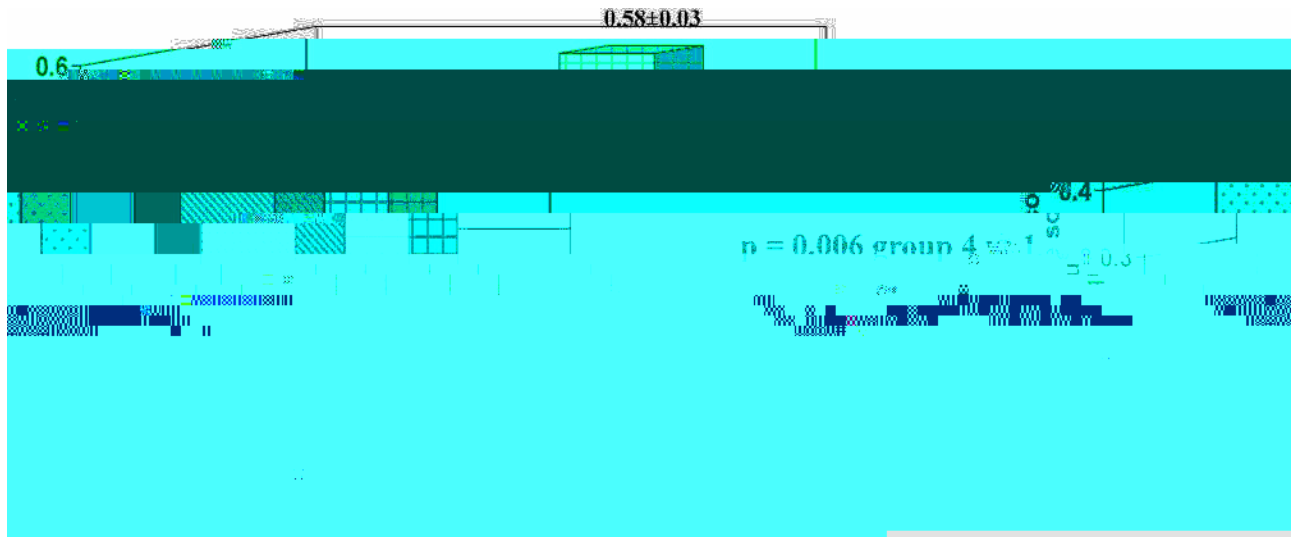
Figure 2.
Additive role of large LDL cholesterol and LDL particle concentration in later atherosclerosis as measured by plaque score

Table 1

General characteristics of the study population (n = 656)

Variable	Mean	Range
Age (years)	50	35–92
Women	55%	
Body mass index (kg/m^2)	27.4	16.7–52.7
Waist circumference (cm)	88.1	50.8–139.7
Systolic blood pressure (mmHg)	120	84–175
Diastolic blood pressure (mmHg)	77	52–107
Fasting plasma glucose (mmol/l)	5.1	3.9–6.9
(mg/dL)	92.7	71–124
Plasma cholesterol (mmol/l)	5.5	3.2–10.1
(mg/dL)	213.7	122–389
Plasma triglyceride (mmol/l)	1.4	0.36–10.1
(mg/dL)	126.4	32–891
LDL cholesterol (mmol/l)	3.3	1.2–7.1
(mg/dL)	125.6	48–275
HDL cholesterol (mmol/l)	1.6	0.6–4.4
(mg/dL)	63.3	24–170
Apolipoprotein A1 (mg/dL)	163.3	57–296
Apolipoprotein B (mg/dL)	104.0	35–196
Current smoker	58.4%	
Use of antihypertensive drugs	13.4%	
Intimal medial thickness (mm)	0.64	0.34–1.10

Table 3

Standard plasma lipids and apolipoproteins according to tertiles of intimal medial thickness and plaque score groups

	Triglycerides (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	Apolipoprotein A1 (mg/dL)	Apolipoprotein B (mg/dL)
Intimal medial thickness					
Tertile 1	114.7±1.0	121.6±2.6	62.9±1.3	162.7±2.20	101.1±1.77
Tertile 2	113.7±1.0	124.3±2.4	64.8±1.2	163.2±2.00	104.7±1.61
Tertile 3	108.0±1.0	130.9±2.8 *	62.1±1.4	164.1±2.36	106.4±1.90
P for trend	0.26	0.03	0.69	0.70	0.07
Plaque score					
Group 1	116.1±1.0	120.82.1	62.9±1.0	162.6±1.75	101.6±1.40
Group 2	114.5±1.0	131.13.2 **	62.3±1.6	161.5±2.72	108.1±2.19 *
Group 3	103.1±1.0 *§	131.2±3.0 **	64.6±1.5	164.7±2.55	106.2±2.05
P for trend	0.02	0.01	0.42	0.66	0.09