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A Cross-Sectional Study of Lipids and ApoC Levels in Alzheimer's Patients With and Without Cardiovascular Disease

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Background. There is increasing evidence supporting the role of atherogenic phenomena in Alzheimer's disease (AD). The possible significance of specific plasma lipid levels in the pathogenesis of AD remains controversial. While lipids such as cholesterol or chaperons such as apolipoprotein (Apo) E2 to ApoE4 have been assessed in AD, ApoC2 and ApoC3 have not been studied before. The present study investigated possible differences in levels of these lipids in AD patients, with or without cardiovascular diseases or risk factors.

Methods. This is a cross-sectional study. The medical charts of patients diagnosed with probable AD were screened for the presence of cardiovascular disease and cardiovascular risk factors. Included in the study were 105 AD patients: 53 with cardiovascular risk factors (AD_{+CVD}) and 52 without risk factors (AD_{-CVD}). Blood samples were analyzed for lipoproteins, ApoC2, and ApoC3. We used *t* tests, chi-square tests, and regression analyses to identify significant differences and to compare the relationships of variables among the groups.

Results. ApoC2 levels (3.5 ± 0.3 and 3.4 ± 0.4 mg/dl, respectively), ApoC3 (13.7 ± 0.9 and 14.7 ± 1.1 mg/dl, respectively), and high-density lipoprotein (HDL)/non-HDL ApoC3 ratios (1.6 ± 0.2 and 1.3 ± 0.2, respectively) were similar for the AD patients with and without cardiovascular risk factors. Levels of total cholesterol, triglycerides, low-density lipoproteins (LDL), very LDLs, and HDLs were similar in the two groups. A substantial proportion of both AD_{+CVD} and AD_{-CVD} patients showed high levels of total cholesterol and LDL, as well as low levels of HDL, ApoC2, and ApoC3, compared to normative values. Surprisingly, patients treated by cognitive enhancers showed significantly higher cholesterol ($p = .002$) and triglyceride ($p = .015$) levels, independent of age, gender, and cognitive level.

Conclusions. There was no difference between AD patients, either with or without cardiovascular diseases or risk factors, with respect to plasma lipid profile, including ApoC2 and ApoC3. This could indicate that lipid metabolism may play a role in AD, whether with or without cardiovascular risk factors. The higher levels of some lipids, observed in a subset of patients treated by cognitive enhancers, deserves further investigation.

RECENT advances in the epidemiology and pathogenesis of Alzheimer's disease (AD) suggest a strong association between cardiovascular risk factors and diseases and AD (1,2). Moreover, cerebrovascular disease may increase the probability that individuals with typical Alzheimer's neurohistological lesions will express a dementia syndrome (3). At least three epidemiological studies have found that use of lipid-lowering agents reduced dementia risk and that it is not clear whether this effect depends on lipid-lowering effect (4–6).

Apolipoproteins (Apos) are crucial constituents of many biochemical processes and serve as enzyme cofactors. Those of the C family consist of three different fractions (C1, C2, C3), freely transferable between several different lipoproteins. ApoC1 and ApoC2 serve as activators for lecithin:cholesterol acyltrasferase and lipoprotein lipase (7), while ApoC3 takes part in processes involving lipoprotein lipase and hepatic triglyceride lipase (8). Changes in the levels and distribution of such Apos may serve as markers of various lipid metabolic disturbances (9). Some investigators have shown that the ratio between ApoC3 and ApoC2 is important in triglyceride metabolism (10,11) and that the distribution of ApoC3 in lipoproteins, in patients with different dyslipoproteinemias, is of crucial importance. In healthy people, 60–70% of ApoC3 is present in high-density lipoproteins (HDL), as compared with 15–20% in patients with severe hypertriglyceridemia (12,13).

Some information linking ApoC2 lipoproteins with amyloid formation has been published recently. A genetic association has been reported between a restriction fragment length polymorphism allele of the ApoC2 gene (on chromosome 19) and familial AD (14,15). Another study of allele frequencies for polymorphism in the ApoC2 gene (16), in subjects with late-onset AD, could not detect an association with AD. Moreover, human ApoC2 self-associates under certain conditions to form aggregates with the characteristics of amyloid. The increase in beta structure accompanying ApoC2 fibril formation has been interpreted as pointing to an alternative folding pathway and in vitro system to explore the general tendency of Apos to form the amyloid of AD (17).

To the best of our knowledge, there are no data regarding

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the possible significance of the plasma ApoC2 and ApoC3 levels as risk factors in the pathogenesis of AD, and we therefore attempted to investigate this issue.

Methods

Study Population

The study included community-dwelling and institutionalized patients of both genders with probable AD who were 65 years and older. Patients with atrial fibrillation or other significant conduction problems were excluded, as well as patients known to suffer from stroke, brain trauma, or tumors. Patients with recent significant acute illness and patients using lipid-lowering drugs were also excluded from the study. The study took place in the Geriatric Service of the Sheba Medical Center and in the Outpatient Clinic for Memory Disorders. It was conducted in accordance with the guidelines of the Helsinki Declaration, and informed consent was obtained from all patients and/or their first-degree relatives. Refusal rate was less than 1% (one patient).

Clinical Measures

The diagnosis of probable AD was established according to *DSM-4* (18). AD patients were divided in two groups: those with cardiovascular disease and/or significant cardiovascular risk factors (AD_{+CVD}) and those without known cardiovascular features (AD_{-CVD}). The presence of a single cardiovascular risk factor was sufficient to make one qualify for the AD_{+CVD} group. The medical charts of all participants were reviewed for age, gender, smoking habits, and current use of medications, as well as for the presence of diabetes mellitus, hypertension, coronary artery disease, peripheral vascular disease, and carotid artery disease.

Plasma levels of cholesterol, triglycerides, and HDL cholesterol were determined by the routine colometric enzymatic procedures, using the Cobas-Mira Plus Autoanalyzer. Low-density lipoproteins (LDL) and very LDLs (VLDLs) were calculated according to the Friedwald equation, whenever triglyceride levels were below 400 mg/dl. Serum ApoC2 and ApoC3 were determined by the turbidimetric immunoassay procedure (19), using reagent kits K-ASSAY ApoC2 and K-ASSAY ApoC3 (Kamiya Biomedical Company, Seattle, WA). Determination of lipids and apolipoproteins was carried out in the Clinical Laboratory of the Institute of Lipid and Atherosclerosis Research.

Statistical Analysis

This was a cross-sectional study. Two-tailed nonpaired *t* test was used to identify the significant differences among the means. The chi-square test was used to compare discrete variables among the groups. Regression analyses (logistic and linear) were used to identify the relationship between the various lipids and age, gender, or use of cognitive enhancers. The confidential probability (reliability) of the inference was chosen as conventional 95% for all statistical tests. The accepted level of significance was set at $p < .05$. We used the MATLAB statistical package in the Windows environment (Version 5.3, MathWorks, Inc., Natick, MA).

Results

A total of 183 patients with AD were screened for the study. Seventy-eight (42.6%) patients were excluded from the analysis, mostly due to inability to obtain informed consent from patients, families, and guardians. The clinical characteristics and lipid profiles of the remaining 105 patients are presented in Table 1.

Fifty-three suffered from AD_{+CVD}, and 52 had AD_{-CVD}. Mean age was 81.7 ± 14.0 and 78.9 ± 15.9, respectively. The two groups did not differ in terms of gender or ethnic distribution. Blood samples from all 105 patients were analyzed for plasma levels of lipids, lipoproteins, ApoC2, and ApoC3. ApoC2 levels (3.5 ± 0.3 and 3.4 ± 0.4 mg/dl, respectively), ApoC3 (13.7 ± 0.9 and 14.7 ± 1.1 mg/dl, respectively), and HDL/non-HDL ApoC3 ratios (1.6 ± 0.2 and 1.3 ± 0.2, respectively), were similar for the AD patients with and without cardiovascular risk factors. None of the studied parameters showed a statistical difference when AD_{+CVD} and AD_{-CVD} groups were compared. Moreover, there was no correlation between lipid profile and patients' age or gender.

The fraction of ApoC3 on HDL-C was slightly lower in patients of both AD_{+CVD} and AD_{-CVD} groups (57% and 55%, respectively), compared to a 60–70% range observed in our laboratory in an age-matched population. We have also calculated the ratio of ApoC3 in HDL-C to ApoC3 in non-HDL-C due to normal ApoC3 content on HDL-C. This ratio was 1.6 ± 0.2 in the AD_{+CVD} group and 1.3 ± 0.2 in the AD_{-CVD} group. Both ratios are lower than expected in the population without CVD risks (>1.7).

Though not statistically significant, we found that a substantial number of AD patients showed higher than normal levels of total cholesterol and LDL-C. Also, most patients tended to have low levels of HDL-C, ApoC2, and ApoC3 (Figure 1).

Table 2 shows the correlation between lipid levels and cognition (by the Mini-Mental State Examination) as continuous measures. None of these correlations were statistically significant when using lipid levels as continuous or as categorical (normal or abnormal).

Table 1. Clinical Characteristics and Lipids Profiles of the Study Population ($N = 105$)

Gender (Male/Female)	40/65
Age	80.3 ± 7.5
Mini-Mental State Examination score	16.0 ± 8.4
Cognitive enhancers (yes/no)	33/72
Cholesterol (mg%)	186.6 ± 41.2 (80–220)
Triglycerides (mg%)	114.4 ± 54.7 (30–200)
VLDL (mg%)	22.9 ± 10.9 (4–60)
LDL (mg%)	119.8 ± 36.8 (30–160)
HDL (mg%)	43.9 ± 14.9 (35–80)
ApoC2 (mg%)	3.4 ± 1.7 (3–7)
ApoC3 (mg%)	14.2 ± 3.9 (13–19)
ApoC3 on HDL (mg%)	7.8 ± 1.7 (7.8–13.3)
ApoC3 on (LDL + VLDL) (mg%)	6.4 ± 2.3 (4.5–6.7)
Ratio[†]	1.49 (>1.7)

Notes: Data are mean ± *SD*, normal values in parentheses. VLDL = very low-density lipoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein; Apo = apolipoprotein.

[†]Ratio of ApoC3 on HDL/ApoC3 on (LDL + VLDL).

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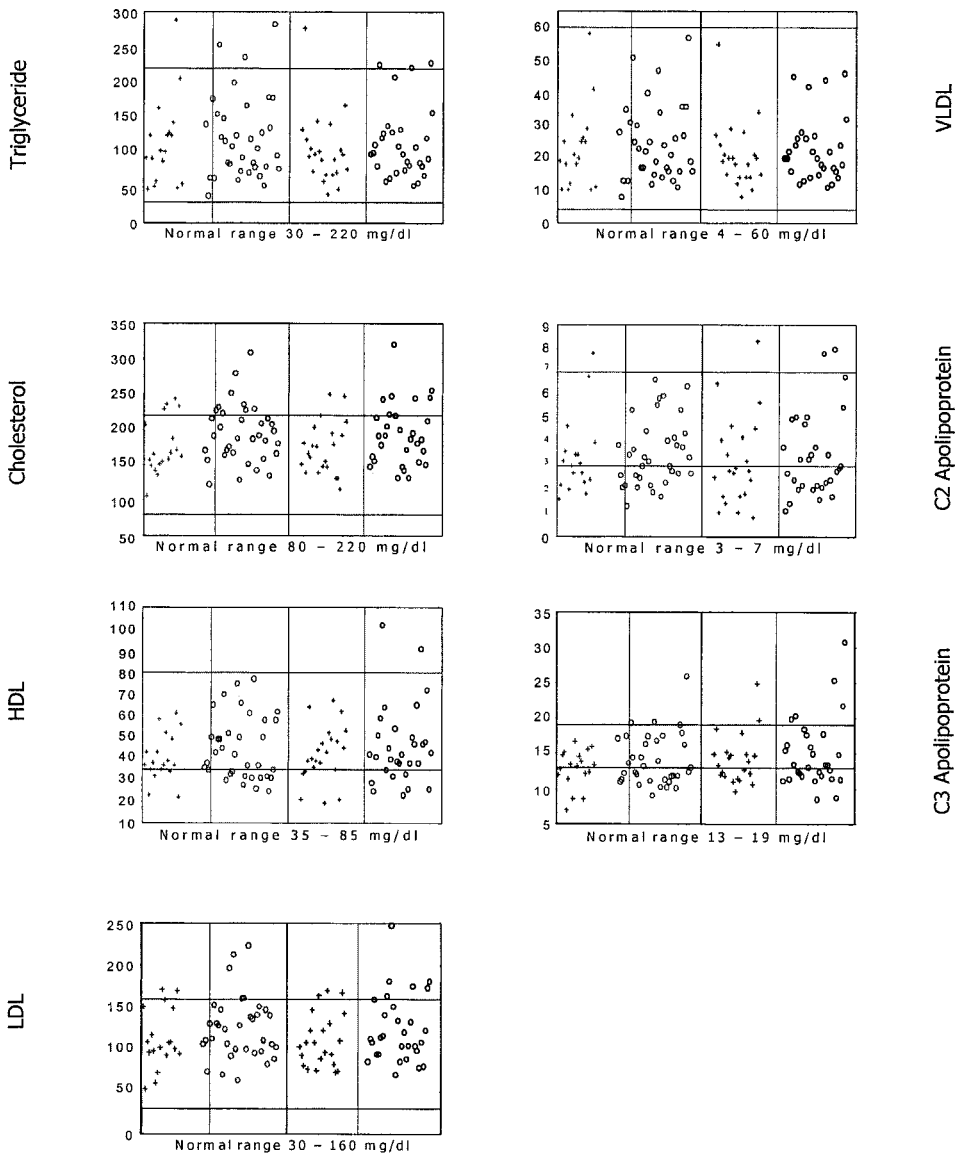

Figure 1. Distribution of lipid levels of the study population ($N = 105$). + = Men; o = Women. Left side of each scatter plot contains data for AD_{+CVD}, right side for AD_{-CVD}. HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.

However, using a linear regression model that incorporates age, gender, and use of cognitive enhancers, versus each of the lipids as an independent variable, we found a significant correlation between levels of cholesterol, triglycerides, VLDL and LDL, and current use of cognitive enhancers (aricept [Donepezil]). A logistic regression incorporating the same parameters versus lipids levels (normal or abnormal) proved significant for cholesterol and triglycerides only (Table 3).

Discussion

The present study investigated plasma levels of ApoC2 and ApoC3 and their distribution on lipoproteins, in AD patients with and without cardiovascular disease and/or risk factors. The lipid parameters that were investigated were within normal limits in both groups. However, we observed a trend toward a more atherogenic lipid profile in both groups of AD patients, reflected by a relatively higher percentage of patients with high total cholesterol and LDL, as well as a high percentage of patients with low HDL, ApoC2, and C3 levels (Figure 1).

Our results regarding C2 and C3 levels are the first to be reported in AD patients. Results of two previous studies (20,21) comprising study groups of healthy men were controversial. One study concluded that there is a modest increase in serum levels of ApoC2 and ApoC3 in the age group of 60–70 years, while a second study found no difference in serum levels between men of different ages. In our study, ApoC2 and ApoC3 levels were similar in both AD_{+CVD} and AD_{-CVD} as total levels, and as HDL/non-HDL ratio. In both groups, the results of HDL/non-HDL ApoC3 levels are similar to those observed in cardiovascular patients, thus indi-

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Table 2. Pearson† and Unpaired *t* test‡ Correlations Between Lipid Levels and Mini-Mental State Examination Score ($N = 105$)

Correlation Coefficient	Cholesterol	Triglycerides	VLDL	LDL	HDL	ApoC2	ApoC3	ApoC3-HDL	Ratio
Pearson									
r	.04	.04	.04	−.16	.12	.05	.06	.13	.12
p	(.69)	(.71)	(.70)	(.87)	(.22)	(.58)	(.50)	(.19)	(.22)
Unpaired *t* test									
t	.90	.30	—§	−.9	−.3	.11	.04	.08	.10
p	(.70)	(.66)	—	(.58)	(.98)	(.92)	(.97)	(.78)	(.45)

Note: VLDL = very low-density lipoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein; Apo = apolipoprotein.
†Lipids calculated as continuous measures, *p* in parentheses.
‡Lipids calculated as normal versus abnormal, *p* in parentheses.
§Due to all values within normal limits.

cating that this may be a risk factor, or a marker, for AD even without cardiovascular background. In contrast, our results regarding total cholesterol and LDL are somewhat surprising, considering a previous study (22) showing no correlation of total cholesterol levels with AD in old people. These authors proposed that elevated levels of LDL were associated with the risk for vascular dementia in elderly patients, but not with AD. However, our data support a recent publication (23) showing a correlation between total cholesterol and LDL in AD patients, which remained significant after adjusting for ApoE genotype (known to associate with atherosclerosis) and for other risk factors. In terms of making this interpretation of our results, it is useful to know how many patients had lower, or higher, than the normal range for total cholesterol and LDL. For example, what seems to be a very modest increase of mean total cholesterol and LDL may practically reflect low levels in many patients, probably due to a low nutritional status, which is so frequent in AD patients. Otherwise, we would have seen much higher levels. Indeed, about 30% of our patients demonstrated total cholesterol levels below 160 mg/dl. These results are somewhat different compared with the results of the Cardiovascular Health Study (24), which included men and women 65–100 years old. That study has demonstrated total cholesterol levels of 160 mg/dl in only 11.6% of men and 3.2% of women. Similarly, the increased percentage of patients with lower HDL in both AD_{+CVD} and AD_{-CVD} patients, which is consistent with previous studies (25,26), clearly supports an atherogenic profile in AD patients.

There are a number of limitations to this study, such as the lack of a nondemented healthy, control elderly group and the hazards of making indirect comparisons with completely different samples of patients. This is particularly relevant because normal limits for ApoC2 and ApoC3 are still not well established. Moreover, possible changes in lipid profile could not be related to severity of AD disease as reflected by functional, behavioral, and nutritional statuses, rather than cognitive status.

We conclude that AD patients, either with or without cardiovascular diseases or risk factors, present similar lipid and ApoC profiles. This could indicate that lipid metabolism may play a role in AD, whether with or without a cardiovascular background. Higher levels of some lipids were observed in a subset of patients treated by cognitive enhancers.

Table 3. Regression Analysis of Lipid Factors Potentially Associated With Gender and Use of Cognitive Enhancers (Data are *p* Values)

	Linear Regression†		Logistic Regression‡	
Dependent Variable	Gender	Cognitive Enhancers	Gender	Cognitive Enhancers
Cholesterol	.002	.002	.13	.016
	(24.2)	(28.6)	(2.3)	(3.7)
Triglycerides	.25	.015	.44	.019
	(12.5)	(31.5)	(1.8)	(6.0)
HDL	.192	(.86)	.28	.69
	(4.0)	(.63)	(1.6)	(.82)
VLDL	.27	.014	—§	—
	(2.4)	(6.3)		
LDL	.012	.009	.36	.26
	(17.7)	(21.7)	(1.7)	(1.9)

Note: HDL = high-density lipoprotein; VLDL = very low-density lipoprotein; LDL = low-density lipoprotein.
†B values, in parentheses, for linear regression.
‡Odds ratios, in parentheses, for logistic regression.
§Due to all values within normal limits.

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