Achieving an LDL-Cholesterol Goal of <70 mg/dL but not a Goal of 70-100 mg/dL is Associated with Cognitive Performance in Diabetic Patients

Diyabetik Hastalarda LDL-Kolesterolün 70-100 mg/dL Arası Değil, 70 mg/dL'nin Altına Düşürülmesi Bilişsel Performansı Etkilemekte

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ABSTRACT Objective: To compare the effects of achieving an low-density lipoprotein (LDL) cholesterol level of <70 mg/dL versus the goal of 70-100 mg/dL using simvastatin or atorvastatin on cognitive functions. **Material and Methods:** In this open-label randomized controlled trial, diabetic patients were randomly assigned to simvastatin or atorvastatin 10 mg. If LDL-cholesterol levels, monitored at 30-day intervals, were >100 mg/dL, dose increase was allowed. Patients were stratified only according to achieved LDL-cholesterol goals, irrespective of the statin used, into two groups. Cognitive functions were assessed using neuropsychological tests and event-related potential P300 at baseline and after 3 months of therapy. **Results:** One hundred fourteen subjects completed the study. After 12 weeks of statin therapy, the mean LDL-cholesterol level was 57.9 mg/dL in the group with LDL-cholesterol <70 mg/dL compared with 87.1 mg/dL in the group with LDL cholesterol levels of 70 to 100 mg/dL. Lowering LDL-cholesterol to <70 mg/dL resulted in statistically significant worsening of learning subscore of the auditory verbal learning test and in borderline significant improvement in immediate memory subscore of the auditory verbal learning test (p=0.03, and p=0.046, respectivly); statin dose was negatively correlated with the changes in these parameters (r=-0.304, p<0.05, and r=-0.312, p<0.05, respectively). Alternatively, no significant changes in neuropsychological test scores were noted in the other group. **Conclusion:** Lowering LDL-cholesterol levels to between 70 to 100 mg/dL is not associated with cognitive performance. On the other hand, lowering LDL-cholesterol levels to <70 mg/dL may differentially affect verbal learning and short term verbal memory.

Key Words: Diabetes mellitus; cholesterol, LDL; simvastatin; atorvastatin; cognition

ÖZET Amaç: Düşük dansiteli lipoprotein (LDL) kolesterol düzeyinin simvastatin veya atorvastatin ile 70 mg/dL'nin altına düşürülmesinin, 70-100 mg/dL arasına düşürülmesi ile karşılaştırılarak bilişsel işlevler üzerindeki etkisinin değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntemler:** Bu açık-uçlu randomize kontrollü çalışmada, diyabetik hastalar simvastatin ya da atorvastatin 10 mg almak üzere 2 gruba ayrılmıştır. 30 günlük aralıklarla yapılan değerlendirmelerde LDL-kolesterol düzeyleri 100 mg/dL üzerinde saptandığında doz artışına gidilmiştir. Hastalar, kullanılan statinden bağımsız olarak, sadece elde edilen LDL-kolesterol hedeflerine göre iki gruba ayrılmıştır. Bilişsel işlevler tedaviden önce ve tedaviden 3 ay sonra nöropsikolojik testler ve olay-ilişkili potansiyel P300 kullanılarak değerlendirilmiştir. **Bulgular:** Çalışmayı 114 hasta tamamlamıştır. Statin tedavisinden 12 hafta sonra, ortalama LDL-kolesterol düzeyi <70 mg/dL olan grupta 57,9 mg/dL, LDL-kolesterol 70-100 mg/dL olan grupta ise 87,1 mg/dL olarak saptanmıştır. LDL-kolesterolün 70 mg/dL'nin altına düşürülmesi işitsel sözel öğrenme testinin öğrenme alt testi puanında istatistiksel olarak anlamlı düşüş (p=0,03), anlık bellek alt testi puanında ise sınırda iyileşme ile sonuçlanmıştır (p=0,046). Statin dozu ile bu parametrelerdeki değişiklikler arasında negatif korelasyon gözlenmiştir (öğrenme alt testi için r=-0,304, p<0,05; anlık bellek alt testi için r=-0,312, p<0,05). Buna karşılık, diğer grupta nöropsikolojik test puanlarında anlamlı değişiklik saptanmamıştır. **Sonuç:** LDL-kolesterol düzeylerinin 70-100 mg/dL arasına düşürülmesi bilişsel performans ile ilişkili değildir. Diğer taraftan LDL-kolesterolün 70 mg/dL'nin altına düşürülmesi özellikle sözel öğrenme ve sözel kısa süreli belleği etkileyebilir.

Anahtar Kelimeler: Diabetes mellitus; LDL-kolesterol; simvastatin; atorvastatin; idrak

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An LDL-cholesterol target of <70 mg/dL recommended by the American Diabetes Association for diabetic patients with a history of cardiovascular disease (CVD) have raised concerns about its safety.[1] Cholesterol is an essential component of the cell membranes of the brain, the spinal cord and the peripheral nerves. It is incorporated into the myelin sheaths that insulate the axons. Additionally, lipids account for half of the dry matter of the brain.[2,3] Thus, anything that affects the balance of cholesterol and lipid metabolism in the brain could affect its function. The finding that the LDL-cholesterol range is approximately 50 to 70 mg/dL in hunter/gatherer populations without evidence of atherosclerosis does not indicate that lowering cholesterol pharmacologically to these levels is safe.[4]

The association of high cholesterol, a proven risk factor for CVD, with cognition appears complicated. Some studies have shown high lipid levels to be a risk factor for cognitive dysfunction or dementia, whereas others either have found no association or a protective association.[5-10] In the case of lipoprotein fractions, elevated LDL cholesterol levels were an independent risk factor for the development of dementia with stroke in a prospective study.[11] On the other hand, in contrast to the cardiovascular benefits, the effects of statins on cognition and neuronal function are also still not well established. Evidence concerning the impact of statins on these functions is quite inconsistent with several studies reporting improvement in cognitive performance with statin treatment and other reporting decreased memory.[12-15] Furthermore, all of these studies focused on specific dose-related effects of statins on lipid parameters and cognition rather than neurocognitive effects of achieving a specific LDL-cholesterol goal.

The present study attempts to compare the effects of achieving LDL-cholesterol levels <70 mg/dL versus achieving LDL-cholesterol levels of 70 to 100 mg/dL using statins on cognitive functions in high-risk diabetic patients.

MATERIAL AND METHODS

This open-label, randomized, controlled trial was conducted at the endocrinology clinic of Baskent University Hospital. The study protocol was approved by the University Human Research Ethics Committee. All participants provided written, informed consent before study enrollment.

PATIENTS

Details of the trial design have been reported previously.[16] In brief, 140 patients were enrolled in this study. To be included in the study, a patient with controlled type 2 diabetes mellitus had to have overt CVD, or to be over the age of 40 years, without overt CVD, but with one or more major cardiovascular risk factors. All patients hadn't to be on any lipid-lowering therapy for at least 6 weeks. Prior hypoglycemic treatment should not be altered at least 6 months before enrollment. In addition to men, a study population of postmenopausal females were chosen because significant fluctuations in plasma LDL-cholesterol levels may occur during physiological menstrual cycle in fertile women.[17] Patients were advised to continue their usual level of physical activity and not to embark upon any new physical exercise program while participating in this study.

Major exclusion criteria were as follows: severe hypertriglyceridemia; uncontrolled hypertension; evidence of active liver disease or hepatic dysfunction defined as a level of liver transaminases >2 times the upper limit of normal; uncontrolled myocardial ischemia; congestive heart failure (New York Heart Association classification IIIb or IV); hemodynamically important valvular disease; history of cerebrovascular disease within 3 months; peripheral neuropathy; major neuropsychiatric conditions; physically or mentally unable to complete tests; secondary hyperlipidemia; gastrointestinal disease or surgery that might limit drug absorption; myopathy or rhabdomyolysis; a known hypersensitivity to statins; any psychotropic medications, steroids, opiate analgesics, androgens, estrogens, fish oil preparations, quinidine, theophylline, barbiturates, antacids containing aluminum salts (regular use), other investigational drugs or anticoagulants; plasma creatine kinase levels >50% above the upper limit of normal; excessive ethanol consumption; and LDL-

cholesterol levels >100 mg/dL despite the maximum statin doses.

Eligible patients were randomly assigned to simvastatin 10 mg or atorvastatin 10 mg on an open-label basis. The allocation sequence was independent of the research team and was randomized in accordance with repeated measures design. Allocations were placed in sequentially numbered sealed opaque envelopes. Staff working in the department used the next envelope in the sequence to allocate participants. LDL-cholesterol levels were monitored at 30-day intervals. If LDL-cholesterol was >100 mg/dL, the doses of both agents were increased. The study protocol allowed the doses of simvastatin and atorvastatin to be increased up to a maximum of 40 and 80 mg, respectively. The sample size was chosen based on the study feasibility. Power analyses demonstrated that the sample size was adequate to achieve a greater than 80% power for all statistical tests. Patients were stratified only by achieved LDL-cholesterol levels, irrespective of the statin used, into two groups. Thereafter, they were followed for 12 weeks. All subjects underwent a complete clinical examination as well as laboratory, neuropsychological and neurophysiologic tests at baseline and at the end of the 12-week period. Furthermore, the patients were instructed to report any side effect of the drugs. Compliance was tested by pill counts and LDL-cholesterol levels.

LABORATORY ANALYSIS

After a minimum 8-hour overnight fast, venous blood samples were drawn between 8 AM and 9 AM after approximately 30 minutes of supine rest. Lipid analysis was done immediately. Blood samples for DHEA-S were centrifuged and stored at -70°C until measurement. Serum total cholesterol and triglyceride were assayed using enzymatic colorimetric tests (Roche Diagnostics, Mannheim, Germany). Serum LDL-cholesterol and HDL-cholesterol were determined by homogeneous enzymatic colorimetric methods (Roche Diagnostics, Mannheim, Germany). HbA1c level was measured using high-performance liquid chromatography (DIAMAT, Bio-Rad Laboratories, Milan, Italy; normal value, 4.2%-6.2%), with intra and interassay coefficients of variation of <2%. DHEA-S was assayed by solid-phase, competitive chemiluminescent enzyme immunoassays (Diagnostic Product Corporation, Los Angeles, CA).

COGNITIVE TESTING

Details of the tests used have been reported previously.[9]

1. Neuropsychological Tests

The battery consisted of standardized neuropsychological tests with published normative data which were used either in international research on cognitive function or in the context of routine neuropsychological assessment.[18-24] Alternative forms were used for tests in which recall of previous test materials might affect performance of subsequent testing and the order of these forms was randomly assigned. Tests used in the examination were listed below with a brief description:

Facial recognition test

This test is used to examine face recognition abilities without a memory component (complex visual discrimination). Normative data for Facial Recognition Test (FRT) in the Turkish population are established (Keskinkilic C, unpublished dissertation [Turkish], 1988).

Judgment of line orientation test

The Judgment of Line Orientation Test (JLOT) assesses visuospatial perception and orientation ability. The JLOT was standardized for the Turkish population.[25]

Visual aural digit span test-B

The Visual Aural Digit Span Test-B (VADST–B) is designed as a diagnostic tool for assessing short-term memory, concentration, and attention span. It is a forward digital span test with 4 subtests: aural-verbal, visual-verbal, aural-written, and visual-written. The VADST-B was standardized for Turkish adults.[26,27]

Clock drawing test

The Clock Drawing Test (CDT) provides a quick and simple screen for multiple areas of cognitive

function including executive functions (e.g., selective attention, auditory comprehension, visual memory, motor programming, numerical knowledge, planning, response inhibition, concentration, abstract thinking, and monitoring) as well as visuospatial organization, visuoconstructive abilities, psychomotor coordination. Norms for this test as well as a systematic scoring procedure were established for the Turkish population.[28]

Auditory verbal learning test

This test measures immediate memory span, verbal learning, susceptibility to retroactive, and proactive interference, delayed recall, and recognition memory. Normative data for Auditory Verbal Learning Test (AVLT) in Turkish population have been established (Genc-Acikgoz D, unpublished dissertation [Turkish], 1995).

Trail making test (Parts A and B)

The Trail Making Test (TMT) provides information on visual search or scanning, visuospatial sequencing, cognitive-set shifting abilities, perceptual-motor speed of processing, mental flexibility, and executive functions. The TMT was standardized for Turkish adults.[29]

2. Neurophysiologic Examination

The subjects were requested to keep their eyes closed, with minimum movement, and to remain as relaxed as possible. Event-related P300 potential was evoked by the standard auditory odd-ba paradigm using a Synergy machine (Medelec, Oxford Instruments, Surrey, UK) with a sensitivity of 2 to 5 μV per division, and a sweep speed of 100 milliseconds per division. The settings for the highpass filter and a low-pass filter were 0.1 and 30 Hz, respectively. Tones with a duration of 100 milliseconds and intensity above the individual hearing threshold were generated at 1000 Hz (frequent nontarget stimuli) and 2000 Hz (rare target stimuli). The rare target tones (occurrence probability of 20%) and the frequent nontarget tones (occurrence probability of 80%) were presented binaurally in a random sequence with a minimum interstimulus interval of 1010 milliseconds. All subjects were instructed to ignore nontarget stimuli and to count the target stimuli first aloud, then mentally. Trials were rejected when patients miscounted the number of the target stimuli. The event-related potentials were recorded from frontal (Fz), vertex (Cz), left (C3), and right (C4) central scalp sites, according to international 10/20 system using Ag/AgCl electrodes. Linked mastoid electrodes served as a reference and the forehead was used as the ground. Interelectrode impedance was maintained below 2000ω. Trials were visually checked by an expert, and those contaminated with artifacts were removed from the analysis. One hundred artifact-free potentials after the target stimuli were averaged separately for each subject. The P300 component was regarded the maximum positive deflection of the trace following stimulus onset in a segment between 250 and 600 milliseconds. Latency of P300 was in milliseconds. Amplitudes were measured from peak to baseline.

STATISTICAL ANALYSIS

Normality of distribution of the variables was analyzed using Shapiro-Wilk test, and Levene test was used to assess the homogeneity of variances in the different groups. Demographic and clinical characteristics showing normal distribution and homogeneous variance were analyzed by the Student's t test. Variables such as plasma lipid levels measured before and after treatment were analyzed by a three-factor repeated measures analysis of variance. The results were expressed as the number of observations (n) and the mean ± the standard deviation (). Parametric test assumptions were not available for some variables; thus, the comparisons were performed with the Mann-Whitney *U* test for independent groups and the Wilcoxon signed rank test for dependent groups. The results of nonparametric tests were expressed as the number of observations (n), the mean ± the standard deviation, the median and minimum-maximum values (, M(min-max)). The Pearson product-moment correlation coefficient was used to show the correlations between normally distributed variables. The Spearman rho correlation coefficient was used to evaluate the correlations between nonnormally

distributed variables. Data analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 13.0, SSPS Inc, Chicago, IL). A $p< 0.05$ was considered statistically significant.

RESULTS

PATIENTS

Of the 140 subjects initially enrolled, 114 completed the study. All "dropouts" were due to personal/administrative reasons. The demographic and clinical features of the study population were shown in Table 1. All groups were well balanced with respect to the demographic characteristics.

TABLE 1: Demographic and clinical characteristics of the study population.

Characteristic	LDL<70 mg/dL $\bar{x}= s_x$ M (min-max)	LDL<100 mg/dL $\bar{x}= s_x$ M (min-max)	p
No of patients completing the study	58	56	NS
Female/Male	39/19	32/24	
Age (years)	60.4±7.4	61.7±7.7	NS
Diabetes duration (years)	4.5±7.6	4.1±5.0	NS
	3 (0-40)	2 (0-20)	
Dose (mg/day)	36.5±7.6	33.6±12.3	NS
	40 (20-40)	40 (10-60)	

The results of nonparametric tests were expressed as the mean±the standard deviation, the median and minimum-maximum values [$\bar{x}= s_x$, M(min-max)]. The baseline values were not significantly different between the groups. M, Median; max, maximum; min, minimum; NS, not significant.

Biochemical and Hormonal Parameters

The mean values and standard deviations of lipid parameters for both groups were given in Table 2. Baseline lipid parameters were comparable in both groups as well as in males and females within and between both groups (data not shown). After 12 weeks of statin therapy, the mean LDL-cholesterol level was 57.9 mg/dL in the group with LDL-cholesterol <70 mg/dL compared with 87.1 mg/dL in the group with LDL-cholesterol levels of 70 to 100 mg/dL; both within-group changes from baseline in LDL-cholesterol and between-group differences were statistically significant. However, we found no significant differences between males and females within and between both groups (data not shown).

Serum triglyceride levels decreased significantly at week 12 in both groups, there was no significant between-group difference. In contrast, mean HDL-cholesterol levels increased significantly in the group with LDL-cholesterol levels of

TABLE 2: Statistical analysis of serum lipids, HbA1c and DHEA-S before and after treatment.

	LDL<70 mg/dL $\bar{x}= s_x$ M (min-max)			LDL<100 mg/dL $\bar{x}= s_x$ M (min-max)		
	Baseline	12th week	p	Baseline	12th week	p
Total cholesterol (mg/dL)	211.9±32.5	134.0±20.2*	<0.001	224.3±30.3	168.5±16.6	<0.001
HDL-cholesterol (mg/dL)	49.3±11.2	50.1±12.0	NS	51.2±10.7	54.5±12.1	<0.01
LDL-cholesterol (mg/dL)	129.4±22.0	57.9±9.5*	<0.001	138.3±20.2	87.1±8.7	<0.001
	152.4±64.8	117.8±51.0	<0.001	148.1±60.6	128.9±49.2	
Triglycerides (mg/dL)	138.5	109		133	124	<0.01
	(50-348)	(23-298)		(59-337)	(41-316)	
HbA1c (%)	6.4±0.8	6.5±0.8	NS	6.4±0.8	6.6±0.8	NS
	69.2±47.8	0.9±42.8**		68.7±44.1	68.9±48.5	
DHEA-S (µg/dL)	61.5	48.2	<0.001	59.3	56.5	NS
	(15-306)	(15-269)		(15-210)	(15-275)	

The results of nonparametric tests were expressed as the mean±the standard deviation, the median and minimum-maximum values [$\bar{x}= s_x$, M(min-max)]. The baseline values were not significantly different between the groups. M, Median; max, maximum; min, minimum; NS, not significant.

*p<0,001 effect of the treatment

**p<0,05 effect of the treatment

70 to 100 mg/dL. No significant change was observed in the HDL-cholesterol levels in the the group with LDL-cholesterol <70 mg/dL. The difference between both groups was not statistically significant. We found no significant differences between males and females within and between both groups (data not shown).

At the end of the study, HbA1c increased significantly in both groups; there was no significant between-group difference. Notwithstanding the slight increase in HbA1c, patients maintained good glycemic control throughout the study. Thus, the lipid levels were not influenced by the degree of glycemic control.

All groups were similar with regard to basal DHEA-S values. Nevertheless, basal levels of DHEA-S were significantly higher in men compared with women within each group (data not shown). A significant reduction in DHEA-S levels was observed in subjects with LDL-cholesterol <70 mg/dl (males, females as well as males and females combined) at 12 weeks. DHEA-S fell by 26.5%. Conversely, no significant effect was observed in the other group. The between-group difference was statistically significant.

Neuropsychological Findings

The neuropsychological findings are summarized in Table 3. Two patients refused to undergo neuropsychological tests. All groups were similar with regard to neuropsychological test results at baseline. Lowering LDL-cholesterol to <70 mg/dL resulted in statistically significant worsening of learning subscore of the AVLT (p=0.03) and in borderline significant improvement in immediate memory subscore of the AVLT (p=0.046) (Figure 1). Although the same trend was observed in the group with LDL-cholesterol levels between 70 to 100 mg/dL**,** the changes in the above-mentioned neuropsychological test scores did not reach statistical significance.

Neurophysiologic Findings

The neurophysiologic findings were summarized in Table 4. Two patients declined to undergo neurophysiologic examination. Both groups were similar with regard to P300 latency and amplitude at baseline. A significant reduction in P300 latency at all recording sites was noted in both groups after 12 weeks. However, there was no significant between-group difference. The amplitude of P300 waves at Fz declined significantly in both groups after 12 weeks of treatment, but not at other recording sites. Similarly, there was no significant between-group difference.

Correlations After Interventions

In the group with an LDL cholesterol goal of <70 mg/dL, statin doses were negatively correlated with the changes in learning subscore of the AVLT (r=-0.304, p<0.05) and in immediate memory subscore of the AVLT (r=-0.312, p<0.05). There was no significant correlation between the statin doses and the changes in P300 potentials in the group with LDL-cholesterol levels between 70 to100 mg/dL.

There was no significant correlation between the changes in immediate memory and learning subscores of the AVLT and LDL-cholesterol levels in the group with an LDL cholesterol goal of <70 mg/dL. There was no significant correlation between the reductions in LDL-cholesterol levels and P300 potentials in both groups.

We found no correlation between the changes in HbA1c and the P300 potentials and neuropsychological test results in both groups. In the group with an LDL cholesterol goal of <70 mg/dL, the reduction in DHEA-S levels was negatively correlated with the reduction in P300 latency at Fz (r=-0.311, p<0.05), Cz (r=-0.318, p<0.05), C3 (r=-0.298, p<0.05), and C4 (r=-0.311, p<0.05).

DISCUSSION

We studied the effects of achieving two different therapeutic LDL-cholesterol goals with statins on a broad range of cognitive skills in high-risk diabetic patients. The P300 latency, a temporal measure of the neural activity underlying the processes of attention allocation and immediate memory, decreased significantly at all electrodes in both groups after 12 weeks but there was no significant between-group differences. P300 amplitude is thought

TABLE 3: Neuropsychological test performance before and after ıntervention.

	LDL<70 mg/dL $\bar{x}= s_x$ M (min-max)			LDL<100 mg/dL $\bar{x}= s_x$ M (min-max)		
	Baseline	12th week	p	Baseline	12th week	p
Educational level						
Primary School	20			17		NS
Secondary School	22			17		
University	16			20		
Dominant hand (right/left)	57/1			53/1		
Facial Recognition	42.1±4.8	41.3±5.3	NS	41.5±5.3	40.5±6.1	NS
	42 (31-51)	40.5 (29-55)		41 (26-53)	41 (20-49)	
Judgement of Line Orientation	18.9±5.1	18.5±7.1	NS	18.0±5.5	16.6±7.4	NS
	19 (8-30)	19.5 (1-29)		18 (1-29)	14.5 (5-28)	
Visual Aural Digit Span B						
Aural-Verbal	5.3±1.2	5.3±1.2	NS	5.3±0.9	5.2±1.2	NS
	5 (3-9)	5 (3-9)		5 (3-8)	5 (2-8)	
Visual-Verbal	4.8±1.0	5.0±1.3	NS	4.8±1.0	5.1±1.2	NS
	5 (3-7)	5 (3-9)		5 (3-7)	5 (3-8)	
Aural-Written	5.1±1.1	4.9±1.1	NS	5.1±0.9	5.3±1.0	NS
	5 (3-8)	5 (2-8)		5 (3-8)	5 (4-8)	
Visual-Written	4.8±1.2	4.8±1.3	NS	4.8±1.3	5.1±1.2	NS
	5 (3-8)	5 (2-9)		5 (3-7)	5 (3-8)	
Total	20.1±3.6	20.1±3.8	NS	20.0±3.2	20.8±3.8	NS
	20 (14-30)	21 (14-30)		20 (14-29)	20 (13-31)	
Clock Drawing	3.1±1.1	2.8±1.2	NS	3.2±0.9	2.9±1.4	NS
	4 (1-4)	3 (1-4)		3 (1-4)	3 (1-7)	
Auditory Verbal Learning						
Immediate Recall	4.8±1.8	5.3±2.0	<0.05	5.0±1.7	5.2±1.7	NS
	5 (0-10)	5 (0-10)		5 (0-9)	5 (0-9)	
Learning	9.4±2.6	8.6±2.5	<0.05	9.5±2.4	9.1±2.6	NS
	9 (2-14)	8 (3-14)		10 (3-13)	9 (4-15)	
Immediate Recall after	7.7±2.4	7.8±2.7	NS	7.5±2.3	8.0±2.2	NS
Interference	8 (3-13)	8 (0-14)		7 (3-12)	8 (2-12)	
Long-term Memory	6.7±3.1	7.1±2.4	NS	7.0±2.3	7.3±2.4	NS
	7 (0-13)	7 (2-12)		7 (3-13)	7 (2-14)	
Total	38.1±9.2	37.0±9.1	NS	39.4±9.2	38.7±8.9	NS
	38.5 (19-62)	36.5 (17-57)		41 (17-54)	39.5 (21-57)	
Trail Making						
Part A	69.3±25.0	66.7±16.8	NS	65.3±20.0	69.6±23.2	NS
	60 (55-180)	60 (50-120)		60 (42-180)	60 (50-180)	
Part B	132.5±29.8	138.7±46.4	NS	126.9±31.6	131.1±53.2	NS
	122(98-240)	120.5(92-334)		121(94-300)	120(60-360)	

The results of nonparametric tests were expressed as the mean±the standard deviation, the median and minimum-maximum values [$\bar{x}= s_x$, M(min-max)]. The baseline values were not significantly different between the groups. M, Median; max, maximum; min, minimum; NS, not significant.

to index attentional resource allocation and to update environmental context.[30-33] Literature generally supports the argumentative statement that elderly adults exhibit larger P300 amplitude over frontal sites than over central sites.[34,35] Relevantly, the more marked potentials at Fz in the present study might

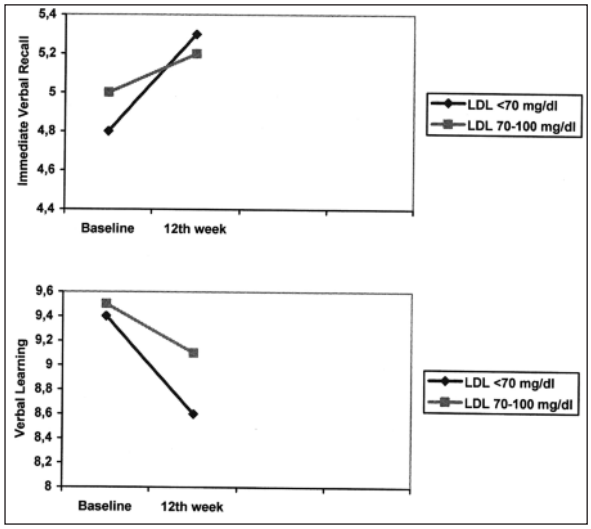

FIGURE 1: Changes in immediate recall and learning subscores of the AVLT after lowering LDL-cholesterol levels to <70 mg/dL. AVLT, Auditory Verbal Learning Test; LDL, low-density lipoprotein.

be attributed to the mean age of the study population. On the other hand, electrooocular artifactual signals have large amplitude in frontal electrodes and contaminate mostly the frontal electroencephalographic channels. Therefore, the absence of electrooculographic screening in the present study might further contribute to the more marked potentials at Fz. After 12 weeks of treatment, the amplitude of P300 waves at Fz declined significantly in both groups but not at other recording sites. We interpreted the observed reduction in stimulus evaluation time along with the concurrent decrease in P300 amplitude in both groups as being result of a progressive automation of target stimulus processing, implying that subjects learnt to update the context information after identifying the target and of familiarization with repeated stimuli after 12 weeks. The fact that the changes in P300 amplitude were prominent only in the frontal lobe could be because the challenge task required the frontal lobe activity, affecting the P300 recorded subsequently. Additionally, methodological problems and factors as food consumption, hydration, or sleep deprivation have to be kept in mind when interpreting the data.

TABLE 4: Neurophysiologic findings before and after ınterventıon.

	LDL<70 mg/dL $\bar{x}= s_x$ M (min-max)			LDL<100 mg/dL $\bar{x}= s_x$ M (min-max)		
Wave	**Baseline**	**12th week**	**p**	**Baseline**	**12th week**	**p**
Amplitudes (μV)						
Fz	9.1±4.5	7.6±3.3	0.01	9.8±6.2	7.3±2.9	0.001
	8.3(2.2-26.8)	7.3(2.5-15.6)		7.4(2.6-33.6)	6.8(3.2-16.2)	
Cz	8.8±4.1	8.1±3.3	NS	9.2±5.7	8.1±3.2	NS
	7.7(2.0-22.6)	8.0(1.9-18.7)		6.9(1.8-28.7)	7.5(3.0-16.2)	
C3	7.6±3.6	7.9±3.2	NS	8.5±5.6	7.8±3.4	NS
	7.1(1.2-19.7)	8.0(2.3-16.7)		5.9(1.6-33.1)	7.2(2.4--16.9	
C4	7.4±3.5	7.3±2.9	NS	8.0±5.5	7.2±3.0	NS
	6.6(1.8-17.7)	7.4(2.0-15.5)		6.3(2.0-30.1)	6.3(2.7-15.1)	
Latencies (msec)						
Fz	390.6±57.3	379.5±57.6	<0.001	385.1±49.7	372.9±48.7	<0.001
Cz	392.3±55.7	380.4±56.1	<0.001	387.6±49.6	373.1±48.3	<0.001
C3	392.8±57.5	381.4±57.1	<0.001	389.8±50.0	373.3±48.1	<0.001
C4	394.4±58.4	381.3±57.2	<0.001	385.9±53.3	374.3±48.3	0.001
Response						
Yes	55 (94.8%)	58 (100.0%)	NS	52 (96.3%)	54 (96.4%)	NS
No	3 (5.2%)	0 (0%)		2 (3.7%)	0 (0%)	

The results of nonparametric tests were expressed as the mean±the standard deviation, the median and minimum-maximum values [$\bar{x}= s_x$, M(min-max)]. The baseline values were not significantly different between the groups. M, Median; max, maximum; min, minimum; NS, not significant.

The neuropsychological assessment showed a statistically significant increase in the immediate recall subscore of the AVLT but a significant decline in verbal learning score in the group with LDL-cholesterol <70 mg/dL after 12 weeks. The factors or mechanisms mediating these opposite results are unclear. These results could firstly reflect low statistical power. Furthermore, changes in immediate verbal memory were only of borderline significance. With respect to the neuropsychological tests, test repetition would more likely cause an improvement in test performance. However, parallel alternative forms were used to bring out a learning or practice effect. On the other hand, the neuropsychological tests are prone to response bias. Patients often find neuropsychological testing stressful and fatiguing, and these factors can negatively influence performance. That the observed impairment in learning was secondary to changes in mood was also a possibility. Some authors reported increase in depression symptoms after simvastatin treatment.[36] As there was no measure of participant's mood at either testing session in the present study, it remains unclear whether there is such a relationship. Furthermore, this is unlikely to be an adequate explanation of the present findings.

Differential effects of simvastatin and atorvastatin on the brain cells might be another explanation for the observed results. It is possible that these statins cause different changes of functional connectivity of prefrontal cortex, temporo-cortical regions and the hippocampus, which have important roles in verbal learning and short term verbal memory. We have previously observed impairment in verbal learning after lowering LDL-cholesterol to <70 mg/dL with atorvastatin but not with simvastatin.[16] On the other hand, lowering LDL-cholesterol to <70 mg/dL using simvastatin but not atorvastatin resulted in minor improvement of immediate recall. An *in vitro* study demonstrated that simvastatin and pravastatin could have profoundly different effects on brain cells-both beneficial and detrimental.[37] These statin differentially affected expression of genes involved in neurodegenerative processes, and that statin-dependent gene expression regulation is cell-type specific. Such effects might be valid for simvastatin and atorvastatin.

It was unclear why cognitive functions are affected after lowering LDL-cholesterol to <70 mg/dL. There was no significant correlation between the changes in immediate memory and learning subscores of the AVLT and LDL-cholesterol levels in the group with an LDL cholesterol goal of <70 mg/dL. Statin doses were negatively correlated with the changes in learning and immediate memory subscores of the AVLT in this group. This finding suggested that statin doses could be responsible for the decreased performance on some subtests of the AVLT. However, it is clear that the minor improvement in immediate memory was related to factors othet than dose. With respect to the doses, although the doses of statins used for reaching an LDL-cholesterol goal of<70 mg/dL were a bit higher than those for achieving levels between 70 to 100 mg/dL, there was no significant between-group difference. Yet the starting LDL-cholesterol values were not different. This finding might, indeed, lead to the conclusion that good responders to statins were selected from poor responders. Notwithstanding the slight increase in HbA1c at 12 weeks, patients maintained good glycemic control throughout the study. In addition, there was no correlation between the changes in HbA1c and the P300 potentials and neuropsychological test results in both groups. Additionally, the reduction in DHEA-S levels was negatively correlated with the reduction in P300 latencies. These factors are unlikely to account for the changes in cognitive functions.

Evidence concerning the impact of statins on cognitive functions is inconsistent. Several studies have suggested that statins have potential to promote brain health while other studies have reported harmful effects of statins on the central nervous system.[12,13,15,38] It is difficult to compare the present study with other trials because of the heterogeneity of the population, variable age groups, different study designs, use of a variety of neurocognitive tests, different follow-up periods, various statins used and variable treatment end points.

There are possible mechanisms by which statins may affect cognition. The neuroprotection could be attributed to their antioxidant, anti-inflammatory, immunomodulatory, antiatherosclerotic actions and the ability to decrease β-amyloid formation.[39-46] Statins may also increase endothelial nitric oxide synthase and reduce endothelin-1, thereby increase cerebral blood flow.[40-44] The lipophilic simvastatin might alter dopaminergic functions in the prefrontal cortex possibly via a central mechanism (a nitric oxide mechanism involving endothelial nitric oxide synthase). The upregulation of dopamine receptors probably requires exposure to high dosages.[47] Possible mechanisms responsible for detrimental effects of statins on the brain could be activation of both pro-inflammatory pathways, increased cell death and higher susceptibility to oxidative damage in the brain tissue or brain cells exposed to statins.[48-52] A recent study has shown that statins induce significant DNA damage in neuronal cells *in vivo*.[53] Furthermore, HMG-CoA reductase inhibition affects neurite outgrowth and neuronal cell survival in vitro.[54-58] Changes of other isoprenoid products could also be involved.[55-58] Some authors found that atorvastatin caused neurite loss by interfering with geranylgeranyl pyrophosphate synthesis. They excluded that a lack of cholesterol mediated the observed effects of statins on neurites.[59]

Possible limitations of our study are the limited sample size and the relatively short follow-up period. The lack of a placebo-controlled arm is another limitation. However, we decided that it was unethical to treat the subjects with statins after the 3-month hypocholesterolemic diet period because our patients were at very high risk of CVD. Although there were some attempts to reduce bias while randomizing our subjects, this study was not conducted in a blind manner. Our study was not designed to determine whether the response to statin therapy affected the cholesterol metabolism in the brain. Levels of 24S-hydroxycholesterol in the circulation as well as the ratio between 24S-hydroxycholesterol and cholesterol were not measured. Therefore, the effects of achieving specific LDL-cholesterol goals using simvastatin and atorvastatin on cholesterol metabolism in the brain could not be determined.

CONCLUSION

In summary, the present study suggests that lowering LDL-cholesterol levels to between 70 to 100 mg/dL is not associated with cognitive performance. On the other hand, achieving LDL-lowering LDL-cholesterol levels to <70 mg/dL differentially affects verbal learning and short term memory as measured by AVLT. Achieving this specific LDL-cholesterol goal using statins, which form the cornerstone in the preventive therapy, may have special meaning because cognitive function, especially verbal memory and complex information, are affected in patients with diabetes. This may have an impact on daily functioning and may be a potential for developing strategy related to cognitive functions. As with coronary disease, 1 to 2 years of lowering LDL-cholesterol levels to <70 mg/dL may be necessary to demonstrate whether cognitive side effects of statins outweigh their potential cognitive benefits or have any long-term sequelae.

Acknowledgments

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REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes--2006. Diabetes Care 2006;29(Suppl 1):S4-42.
2. Benton D. Do low cholesterol levels slow mental processing? Psychosom Med 1995;57(1): 50-3.
3. Dietschy JM, Turley SD. Cholesterol metabolism in the brain. Curr Opin Lipidol 2001;12(2): 105-12.
4. O'Keefe JH Jr, Cordain L, Harris WH, Moe RM, Vogel R. Optimal low-density lipoprotein is 50 to 70 mg/dl: lower is better and physiologically normal. J Am Coll Cardiol 2004; 43(11):2142-6.
5. Yaffe K, Barrett-Connor E, Lin F, Grady D. Serum lipoprotein levels, statin use, and cognitive function in older women. Arch Neurol 2002;59(3):378-84.

6. Stewart R, White LR, Xue QL, Launer LJ. Twenty-six-year change in total cholesterol levels and incident dementia: the Honolulu-Asia Aging Study. Arch Neurol 2007;64(1): 103-7.
7. Reitz C, Tang MX, Luchsinger J, Mayeux R. Relation of plasma lipids to Alzheimer disease and vascular dementia. Arch Neurol 2004; 61(5):705-14.
8. Reitz C, Luchsinger J, Tang MX, Manly J, Mayeux R. Impact of plasma lipids and time on memory performance in healthy elderly without dementia. Neurology 2005;64(8): 1378-83.
9. Romas SN, Tang MX, Berglund L, Mayeux R. APOE genotype, plasma lipids, lipoproteins, and AD in community elderly. Neurology 1999;53(3):517-21.
10. Elias PK, Elias MF, D'Agostino RB, Sullivan LM, Wolf PA. Serum cholesterol and cognitive performance in the Framingham Heart Study. Psychosom Med 2005;67(1):24-30.
11. Moroney JT, Tang MX, Berglund L, Small S, Merchant C, Bell K, et al. Low-density lipoprotein cholesterol and the risk of dementia with stroke. JAMA 1999;282(3):254-60.
12. Muldoon MF, Barger SD, Ryan CM, Flory JD, Lehoczky JP, Matthews KA, et al. Effects of lovastatin on cognitive function and psychological well-being. Am J Med 2000;108(7):538-46.
13. Etminan M, Gill S, Samii A. The role of lipid-lowering drugs in cognitive function: a meta-analysis of observational studies. Pharmacotherapy 2003;23(6):726-30.
14. King DS, Wilburn AJ, Wofford MR, Harrell TK, Lindley BJ, Jones DW. Cognitive impairment associated with atorvastatin and simvastatin. Pharmacotherapy 200;23(12):1663-7.
15. Muldoon MF, Ryan CM, Sereika SM, Flory JD, Manuck SB. Randomized trial of the effects of simvastatin on cognitive functioning in hypercholesterolemic adults. Am J Med 2004; 117(11): 823-9.
16. Berberoglu Z, Guvener N, Cangoz B, Asik M, Yazici AC, Karatas M. Effects of achieving an LDL-cholesterol level of <70 mg/dL compared with the goal of <100 mg/dL using simvastatin or atorvastatin on cognitive processes in high-risk diabetic patients. Endocrinologist 2009; 19(6):271-9.
17. Tonolo G, Ciccarese M, Brizzi P, Milia S, Dessole S, Puddu L, et al. Cyclical variation of plasma lipids, apolipoproteins, and lipoprotein(a) during menstrual cycle of normal women. Am J Physiol 1995;269(6 Pt 1):E1101-5.
18. Reitan RM. Validity of the trail making test as an indicator of organic brain damage. Percept Mot Skills 1958;8(3):271–6.
19. Koppitz EM. Bender gestalt test, visual aural digit span test and reading achievement. J Learn Disabil 1975;8(3):154-8.
20. Benton AL, Varney NR, Hamsher KD. Visuospatial judgment. A clinical test. Arch Neurol 1978;35(6):364-7.
21. Benton AL. Facial recognition test. In: Benton AL, Sivan AB, deS. Hamsher K, Varney N, Spreen O, eds. Contributions to Neuropsychological Assessment: A clinical Manual. 2nd ed. New York: Oxford University Press; 1994. p.35-52.
22. Benton AL. Judgment of line orientation. In: Benton AL, Sivan AB, deS. Hamsher K, Varney N, Spreen O, eds. Contributions to Neuropsychological Assessment: A Clinical Manual. 2nd ed. New York: Oxford University Press; 1994. p.53-64.
23. Goodglass H. Diagnosis of conduction aphasia. In: Kohn SE, ed. Conduction Aphasia. 1st ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1992. p.39-49.
24. Lezak MD. Memory II: Batteries, paired memory tests, and questionnaires. Neuropsychological Assessment. 3rd ed. New York: Oxford University Press; 1995. p.499-517.
25. Karakaş S, Başer E. [Standardization of neuropsychological tests in Turkish population according different age and educational level]. Kriz Dergisi 1995;3(1-2):177-84.
26. Karakas S, Yalın A. [Normative study of visual aural Digit Span test Form-B on Turkish adolescents and adults (ages 13–54)]. Turk J Psychology 1995;10(34):20-31.
27. Karakaş S, Er N, Tavat B. [Standardization study of Visual Aural Digit Span Test Form B on adults and elderly people]. Topsever Y, Göregenli M, editörler. 8. Ulusal Psikoloji Kongresi Bilimsel Çalışmalar. İzmir: Türk Psikologlar Derneği Yayını; 1996. p.79-87.
28. Cangoz B, Karakoc E, Selekler K. [The norm determination and validityreliability studies of Clock Drawing Test on Turkish adults and elderly (ages 50 and over)]. Turk J Geriatr 2006;9(3):136–42.
29. Cangoz B, Karakoc E, Selekler K. [Standardization on study of "Trail Making Test" for Turkish adults and elderly people (ages 50 and over)]. Turk J Geriatr 2007;10(2):73-82.
30. Romero R, Polich J. P3(00) habituation from auditory and visual stimuli. Physiol Behav 1996;59(3):517-22.
31. Donchin E, Coles MGH. Is the P300 component a manifestation of context updating? Behav Brain Sci 1988;11(3):357-74.
32. Picton TW. The P300 wave of the human event-related potential. J Clin Neurophysiol 1992;9(4):456-79.
33. Polich J, Kok A. Cognitive and biological determinants of P300: an integrative review. Biol Psychol 1995;41(2):103-46.
34. Anderer P, Saletu B, Semlitsch HV, Pascual-Marqui RD. Non-invasive localization of P300 sources in normal aging and age-associated memory impairment. Neurobiol Aging 2003; 24(3):463-79.
35. Yamaguchi S, Knight RT. Age effects on the P300 to novel somatosensory stimuli. Electroencephalogr Clin Neurophysiol 1991;78(4): 297-301.
36. Hyyppä MT, Kronholm E, Virtanen A, Leino A, Jula A. Does simvastatin affect mood and steroid hormone levels in hypercholesterolemic men? A randomized double-blind trial. Psychoneuroendocrinology 2003;28(2): 181-94.
37. Dong W, Vuletic S, Albers JJ. Differential effects of simvastatin and pravastatin on expression of Alzheimer's disease-related genes in human astrocytes and neuronal cells. J Lipid Res 2009;50(10):2095-102.
38. Wagstaff LR, Mitton MW, Arvik BM, Doraiswamy PM. Statin-associated memory loss: analysis of 60 case reports and review of the literature. Pharmacotherapy 2003;23(7):871-80.
39. Grobbee DE, Bots ML. Statin treatment and progression of atherosclerotic plaque burden. Drugs 2003;63(9):893-911.
40. Laufs U. Beyond lipid-lowering: effects of statins on endothelial nitric oxide. Eur J Clin Pharmacol 2003;58(11):719-31.
41. Nangle MR, Cotter MA, Cameron NE. Effects of rosuvastatin on nitric oxide-dependent function in aorta and corpus cavernosum of diabetic mice: relationship to cholesterol biosynthesis pathway inhibition and lipid lowering. Diabetes 2003;52(9):2396-402.
42. Raggatt LJ, Partridge NC. HMG-CoA reductase inhibitors as immunomodulators: potential use in transplant rejection. Drugs 2002;62(15): 2185-91.
43. Stalker TJ, Lefer AM, Scalia R. A new HMG-CoA reductase inhibitor, rosuvastatin, exerts anti-inflammatory effects on the microvascular endothelium: the role of mevalonic acid. Br J Pharmacol 2001;133(3):406-12.
44. Turkel Y, Terzi M. [Role of statins in the practice of clinic neurology]. J Exp Clin Med 2009; 26(2):55-61.
45. Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, et al. Simvastatin strongly reduces levels of Alzheimer's disease beta -amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. Proc Natl Acad Sci U S A 2001;98(10):5856-61.
46. Kojro E, Gimpl G, Lammich S, Marz W, Fahrenholz F. Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha -secretase ADAM 10. Proc Natl Acad Sci U S A 2001;98(10):5815-20.
47. Wang Q, Ting WL, Yang H, Wong PT. High doses of simvastatin upregulate dopamine D1 and D2 receptor expression in the rat prefrontal cortex: possible involvement of endothelial nitric oxide synthase. Br J Pharmacol 2005; 144(7):933-9.

48. Konat GW, Krasowska-Zoladek A, Kraszpulski M. Statins enhance toll-like receptor 4-mediated cytokine gene expression in astrocytes: implication of Rho proteins in negative feedback regulation. J Neurosci Res 2008;86(3): 603-9.

49. Tsai HI, Tsai LH, Chen MY, Chou YC. Cholesterol deficiency perturbs actin signaling and glutamate homeostasis in hippocampal astrocytes. Brain Res 2006;1104(1):27-38.

50. Pannu R, Barbosa E, Singh AK, Singh I. Attenuation of acute inflammatory response by atorvastatin after spinal cord injury in rats. J Neurosci Res 2005;79(3):340-50.

51. Pahan K, Sheikh FG, Namboodiri AM, Singh I. Lovastatin and phenylacetate inhibit the induction of nitric oxide synthase and cytokines in rat primary astrocytes, microglia, and macrophages. J Clin Invest 1997;100(11):2671-9.

52. Pavlov OV, Bobryshev YuV, Balabanov YuV, Ashwell K. An in vitro study of the effects of lovastatin on human fetal brain cells. Neurotoxicol Teratol 1995;17(1):31-9.

53. Coetsee TN, Pretorius PJ, Terre'blanche G, Bergh JJ. Investigating the potential neuroprotective effects of statins on DNA damage in mouse striatum. Food Chem Toxicol 2008; 46(9):3186-92.

54. Sato-Suzuki I, Murota S. Simvastatin inhibits the division and induces neurite-like outgrowth in PC12 cells. Neurosci Lett 1996; 220(1):21-4.

55. Michikawa M, Yanagisawa K. Inhibition of cholesterol production but not of nonsterol isoprenoid products induces neuronal cell death. J Neurochem 1999;72(6):2278-85.

56. Tanaka T, Tatsuno I, Uchida D, Moroo I, Morio H, Nakamura S, et al. Geranylgeranyl-pyrophosphate, an isoprenoid of mevalonate cascade, is a critical compound for rat primary cultured cortical neurons to protect the cell death induced by 3-hydroxy-3-methylglutaryl-CoA reductase inhibition. J Neurosci 2000; 20(8):2852-9.

57. Fan QW, Yu W, Gong JS, Zou K, Sawamura N, Senda T, et al. Cholesterol-dependent modulation of dendrite outgrowth and microtubule stability in cultured neurons. J Neurochem 2002;80(1):178-90.

58. Meske V, Albert F, Richter D, Schwarze J, Ohm TG. Blockade of HMG-CoA reductase activity causes changes in microtubule-stabilizing protein tau via suppression of geranylgeranylpyrophosphate formation: implications for Alzheimer's disease. Eur J Neurosci 2003; 17(1):93-102.

59. Schulz JG, Bösel J, Stoeckel M, Megow D, Dirnagl U, Endres M. HMG-CoA reductase inhibition causes neurite loss by interfering with geranylgeranylpyrophosphate synthesis. J Neurochem 2004;89(1):24-32.