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Short Term Effects of Atorvastatin on Endothelial Functions and Oxidized LDL Levels in Patients with Type 2 Diabetes

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Abstract. Objective: This study was designed in order to investigate the short term effects of atorvastatin on endothelial function and oxidized LDL (oxLDL) levels and to evaluate the association of endothelial dysfunction to oxLDL levels and inflammatory markers in type 2 diabetic patients. Material and Methods: Thirty type 2 diabetic and 11 healthy subjects with LDL levels between 100–160 mg/dl. without a history of cardiovascular event were included in the study. Both groups were matched with respect to age, gender, body mass indices and lipid levels. Flow- mediated dilatation (endothelium dependent, FMD) and nitroglycerine-induced dilatation (endothelium independent, NID) were measured in the brachial artery using high-resolution ultrasound in all participants and carotid artery intima media thickness (IMT) were also evaluated. OxLDL levels, lipid parameters, blood glucose, C-peptide, HbA1c and inflammatory markers including C-reactive protein (CRP), fibrinogen, erythrocyte sedimentation rate (ESR) were studied. Type 2 diabetic patients received 10 mg. Atorvastatin for 6 weeks and FMD and NID were reevaluated and oxLDL levels and inflammatory markers remeasured. Results: Basal FMD, NID, IMT and oxLDL levels besides inflammatory markers were not significantly different between patients and controls. No correlation was found between inflammatory markers and FMD and NID. Only IMT correlated with fibrinogen levels obtained before treatment. In non-diabetics, IMT also correlated with oxLDL levels (p: 0.013). FMD and NID significantly improved after atorvastatin therapy ((7.62 ± 7.6 vs. 12.65 ± 7.8, $p<0.001$ and 18.22 ± 9.57 vs. 21.43 ± 9.6, p: 0.007, respectively). Atorvastatin significantly reduced oxLDL levels (57.85 ± 10.33 vs. 44.36 ± 6.34, $p<0.001$). Conclusion: Atorvastatin improves endothelial functions and reduces oxLDL levels in type 2 diabetics with average lipid levels in the short term and may have beneficial effects in the prevention of early atherosclerotic changes.

Key words: Atherosclerosis, Atorvastatin, Endothelial dysfunction, Oxidized LDL, Type 2 diabetes
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ENDOTHELIUM plays a central role on vessel wall functions by synthesizing and secreting different kinds of vasodilator and vasoconstrictor mediators [1]. Probably the most important mediator secreted from a healthy endothelium is nitric oxide (NO). Factors such as aging, dyslipidemia, diabetes, smoking and sedentary life style inhibits the secretion of NO thereby disrupting endothelial functions [2]. Endothelial dysfunction is an early finding in atherosclerotic vascular disease and also predicts cardiovascular outcome.

Patients with diabetes have a high risk for cardiovascular events. Endothelial dysfunction is shown in both type 1 and type 2 diabetic patients [3]. In diabetes, hyperglycemia activates protein kinase C and thus cause increased secretion of vasoconstrictor prostaglandins. Hyperglycemia can also decrease the activity of endothelial nitric oxide synthase and this in turn decrease the activity and expression of NO [4]. Dyslipidemia is another factor that may be responsible from endothelial dysfunction in diabetic patients. Recently, it has been shown that oxidized LDL (oxLDL) which is the most atherogenic form of LDL cholesterol (LDL-C) has been increased in diabetic patients and

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this may contribute to the increased atherogenesis in diabetes.

Statins are lipid lowering drugs and they are shown to improve endothelial functions and reduce the risk of cardiovascular disease in non-diabetic and diabetic patients [5]. Besides modifying LDL-C levels, statins have pleiotropic effects that protect the vascular endothelium and prevent the formation of atherosclerotic plaques. Statins improve endothelial functions in hyperlipidemic diabetic patients in the long term [6]. Restoring endothelial functions may prevent cardiovascular disease and may reduce the mortality from diabetes. Since studies investigating the effects of statins on endothelial functions are mostly performed on hyperlipidemic subjects, it is not clear whether this effect is independent of its lipid lowering effect. Moreover, despite normal lipid levels, oxidized LDL levels may be elevated in diabetic patients and this may account for the disordered endothelial functions.

In this study, we aimed to investigate the short term effects of atorvastatin on endothelial functions and oxLDL levels in diabetic patients. For evaluating endothelial dysfunction we conducted a study which took flow mediated dilatation of brachial artery as a base.

Material and Methods

Thirty type 2 diabetic patients and 11 healthy subjects with LDL-cholesterol levels between 100–160 mg/dl. and without a history of cardiovascular event were included in the study. Groups were matched with respect to age, gender, body mass indices and lipid levels. Endothelial functions were assessed after withholding all vasoactive medications for at least four half-lives and after 12 hours fasting. Subjects did not exercise, consume caffeine, vitamin C or tobacco for at least 6 hours before the study. Subjects underwent a complete history and physical examination and an ECG was obtained from all participants in order to exclude a cardiac ischemia.

In all participants, by using high resolution ultrasound, flow mediated dilatation (FMD) (endothelium dependent) and nitroglycerine induced dilatation (NID) (endothelium independent) were measured in the brachial artery according to the guidelines described by Correttti *et al.* [7]. In addition, carotid artery intima-media thickness (IMT) were also evaluated. For the assessment of FMD, the right arm was placed in extension in the elbow and the hand was placed in supination. An optimal longitudinal image of the brachial artery just above the elbow with clear anterior and posterior intimal interfaces between the lumen and vessel wall was established and kept stable. A sphygmomanometric cuff was placed distally from the elbow. A baseline rest image was obtained, thereafter arterial occlusion was created by cuff inflation to 50 mmHg above the systolic blood pressure for 4 minutes. After deflation, the longitudinal image of the artery was recorded continously from 30 seconds before to 2 min after deflation and the lumen diameter was measured at about 60 seconds. Lumen diameter was defined as the distance between media-adventitia interfaces of the vessel wall. After 10 min resting interval, 0.4 mg. Nitroglycerine spray was administered sublingually and vascular relaxation was measured on the 5th minute. FMD was defined as the percent change in brachial artery diameter within 1 minute after ischemia compared to baseline. NID was defined as the percent change within 5 minutes after nitroglycerine administration.

High-resolution B-mode ultrasound was used to measure IMT of the carotid artery. The anterior, lateral and posterolateral projections were used to image longitudinally the right and left common carotid arteries. At each longitudinal projection, three determinations of IMT were made at 2 cm proximal to the bulb and at the site of greatest thickness. The values at each site were averaged, and the greatest value of the averaged IMT was used as the value for each subject.

Blood samples were collected after 12 hours fasting from all subjects and standart lipid parameters (total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol), blood glucose, C-peptide, HbA1c, inflammation markers including C-reactive protein (CRP), fibrinogen and erythrocyte sedimentation rate (ESR) levels were studied. CRP levels were measured nephelometrically (Beckman Image). Fibrinogen levels were measured coagulometrically with Stago-Compact analyzer. Fasting serum samples were also frozen at –70°C without delay and stored until all the samples are collected. Oxidized LDL levels were measured by use of commercially available Mercodia competetive ELISA kits (Mercodia, Sweden).

The purpose and the procedure of the tests were explained to the subjects and informed consent was obtained from each participant. The experimental pro-

tocol was designed and performed according to the principles of the Declaration of Helsinki and was approved by the Ethical Commitee of Eskisehir Osmangazi University Medical Faculty.

Type 2 diabetic patients received 10 mg. Atorvastatin daily for 6 weeks and FMD, NID were reevaluated and oxLDL levels and inflammation markers were remeasured.

All statistical analysis was performed using SPSS version 10. Comparisons between 2 different groups were assessed by independent t test and changes of variables within group were assessed by paired samples t test. Pearson correlation tests were used to test the relationship between variables. A p value<0.05 was accepted as indicating statistical significance. Results are mean ± SD.

Results

Clinical characteristics of the diabetic patients and the controls are shown in Table 1a and Table 1b. Baseline CRP, fibrinogen, ESR, oxLDL levels, FMD, NID and IMT of the diabetic patients were not significantly different from the controls. Pretreatment IMT of the diabetic patients were significantly correlated with baseline fibrinogen levels (r: 0.036, p<0.05). In nondiabetic subjects oxLDL levels were significantly correlated with IMT (r: 0.071, p: 0.013). No significant correlation was found between inflammatory markers and FMD and NID in diabetic patients. Total cholesterol, triglyceride and LDL-cholesterol levels significantly reduced after the treatment period (Table 2). Also, oxLDL levels significantly reduced after Atorvastatin therapy (57.85 ± 10.33 vs. 44.36 ± 6.34, p<0.001) (Table 2). The ratio of LDL-cholesterol to oxidized LDL-C did not change significantly after the treatment period (0.4 ± 0.09 vs. 0.49 ± 0.03, p = 0.216). CRP, fibrinogen and ESR levels reduced after the treatment but the difference was found to be significant only for ESR levels (Table 2). FMD and NID significantly improved after atorvastatin therapy (7.62% ± 7.6 vs. 12.65% ± 7.8, p<0.001 and 18.22% ± 9.57 vs. 21.43% ± 9.6, p: 0.007, respectively) (Table 3). However, there was no significant correlation between the delta values for FMD, NID, IMT and lipids, oxLDL and inflammatory markers (For delta FMD; triglyceride, r: 0.061, p: 0.75, total cholesterol, r: –0.07, p: 0.72, HDL, r: –0.16, p: 0.37, LDL-C, r: 0.035, p: 0.85, ESR, r: –0.01, p: 0.96, CRP, r: 0.34, p: 0.063, Fibrinogen, r: –0.11, p: 0.56, oxLDL, r: –0.21, p: 0.26, for delta NID; triglyceride, r: –0.8, p: 0.68, total cholesterol, r: –0.032, p: 0.86, HDL, r: –0.03, p: 0.89, ESR, r: 0.015, p: 0.93, CRP, r: 0.16, p: 0.39, Fibrinogen, r: –0.15, p: 0.43, oxLDL, r: –0.19, p: 0.30, for delta IMT, triglyceride, r: –0.09, p: 0.63, total cholesterol, r: –0.06, p: 0.72, HDL, r: –0.16, p: 0.37, LDL-C, r: 0.03, p: 0.85, ESR, r: –0.01, p: 0.96, CRP, r: –0.16, p: 0.06, fibrinogen, r: –0.11, p: 0.56, oxLDL, r: –0.34, p: 0.05, respectively).

Discussion

There are many studies about statins and their lipid lowering effects but the data about the short term effects of statins have not been discussed too much. In the present study, we have shown that treatment with 10 mg Atorvastatin daily for 6 weeks resulted in statistically significant improvement of endothelial functions and oxidized LDL levels in type 2 diabetic patients with average lipid levels.

Correcting the endothelial dysfunction can prevent cardiovascular diseases and can reduce the mortality from diabetes mellitus. Losing weight and having an optimum body mass index, controlling hypertension, maintaining a strict glycemic control and preventing dyslipidemia either by dietary or pharmalogically are the key stones of the treatment. For many years, statins have been used for controlling dyslipidemia. Previously, it had been thought that statins only controlled lipid levels but today it is known that they have many pleiotropic effects, such as protecting the endothelium, preventing the formation of atherosclerotic plaques or maintaining the stabilization of previously formed plaques. In addition, statins behave as antioxidant agents and have some effects such as decreasing superoxide generation in human macrophages and reducing the oxidation of LDL [8, 9]. Statins can also increase endothelial nitric oxide synthase expression and activity in blood vessels [10] and this can result with an increase in NO levels.

Although the effects of statins are known for many years, the efficacy of these drugs on endothelial functions is conflicting. There are many methods for evaluating endothelial dysfunction but the easier, cheapest and non invasive one is measuring flow mediated dilatation (FMD) of brachial artery. An impaired function

Table 1a. Clinical characteristics, lipid levels, inflammatory markers, caroid IMT, FMD and NID of the patients and the control group

	Diabetic patients n: 30	Controls n: 11	Significance
Age (y)	54.87 ± 8.00	55.91 ± 8.94	NS
M/F	15/15	5/6	NS
BMI (kg/m^2)	28.92 ± 4.38	28.35 ± 4.98	NS
Diabetes duration (y)	9.88 ± 7.56		
HbA1c %	9.06 ± 2.53	5.80 ± 0.73	P<0.001
TC (mg/dl)	221.53 ± 27.05	228.45 ± 36.63	NS
TG (mg/dl)	176.03 ± 78.63	181.00 ± 45.94	NS
HDL-C (mg/dl)	52.83 ± 16.49	47.82 ± 18.49	NS
LDL-C (mg/dl)	132.67 ± 21.72	144.09 ± 29.93	NS
oxLDL (mg/dl)	57.84 ± 10.33	55.73 ± 6.85	NS
CRP (mg/dl)	0.743 ± 0.73	0.587 ± 0.71	NS
ESR (mm/h)	29.13 ± 21.57	17.64 ± 9.81	NS
Fibrinogen (mg/dl)	419.26 ± 82.51	403.81 ± 64.42	NS
IMT (mm)	0.627 ± 0.17	0.718 ± 0.23	NS
FMD (%)	7.620 ± 7.60	10.770 ± 4.29	NS
NID (%)	18.227 ± 9.57	15.642 ± 8.01	NS

BMI; body mass index, TC; total cholesterol, TG; triglyceride, HDL-C; high density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol, oxLDL; oxidized LDL, CRP; C-reactive protein, ESR; erythrocyte sedimentation rate, IMT; intrimamedia thickness, FMD; flow mediated dilatation, NID; nitroglycerine induced dilatation
Data are mean ± SD.

Table 1b. Clinical characteristics and treatment types of the patients and the control group and late diabetic complication of the patients

	Diabetic patients n: 30	Controls n: 11	Significance
Hypertension	10/30	3/11	NS
Treatment			
OAD	15/30		
Insulin	15/30		
ACEI/ARB	8/10	2/3	NS
Ca ch blocker	2/10	1/3	NS
Late complications			
Retinopathy	4/30		
Neuropathy	2/30		
Nephropathy	0/30		
Macrovascular disease	0/30		

OAD; oral anti diabetic, ACEI/ARB; angiotensin converting enzyme inhibitors/angiotension receptor blocker, Ca ch Blockers; calcium channel blocker

of brachial artery can be correlated with an impaired coronary arterial function. Beishuizen *et al.* did not find an effect of 20 mg simvastatin on FMD after a 2-year period [11]. Similarly, van Venrooji *et al.* did not find an effect of 30 weeks of atorvastatin versus placebo on FMD [12]. On the contrary, Sheu *et al.* used simvastatin 20–40 mg in type 2 diabetic patients and showed that statin therapy have beneficial effects on endothelial functions [13]. Recently, a new study made by Pretnar-Oblak *et al.* showed that atorvastatin had improved endothelial functions not only on peripheral arterial system but also on central nervous

Table 2. Serum lipid levels and inflammation markers and HbA1c before and after atorvastatin therapy

	Pretreatment (n: 30)	Post treatment (n: 30)	Significance
TG (mg/dl)	176.03 ± 78.63	146.60 ± 49.42	P: 0.001**
TC (mg/dl)	221.53 ± 27.05	183.40 ± 29.73	P<0.001***
HDL-C (mg/dl)	52.83 ± 16.49	54.27 ± 15.32	NS
LDL-C (mg/dl)	132.67 ± 21.72	96.29 ± 27.68	P<0.001***
oxLDL (mg/dl)	57.84 ± 10.33	44.36 ± 6.34	P<0.001***
CRP (mg/dl)	0.760 ± 0.74	0.60 ± 0.68	NS
Fibrinogen (mg/dl)	420.41 ± 83.72	382.03 ± 105.41	NS
ESR (mm/h)	30.00 ± 21	23.79 ± 14.39	P: 0.016*
HbA1c (%)	9.06 ± 2.53	8.74 ± 1.36	NS

TC; total cholesterol, HDL-C; high density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol, oxLDL; oxidized LDL, CRP; C-reactive protein, ESR; erythrocyte sedimentation rate, NS; non significant
Data are means ± SD

Table 3. Endothelial functions of the diabetic patients before and after atorvastatin therapy

	Pretreatment (n: 30)	Post treatment (n: 30)	Significance
FMD (%)	7.62 ± 7.60	12.65 ± 7.81	P<0.001***
NID (%)	18.22 ± 9.57	21.43 ± 9.60	P: 0.007**

FMD; flow mediated dilatation, NID; nitroglycerin induced dilatation
Data are means ± SD

system [14]. The results of our study have shown that treatment with 10 mg atorvastatin daily for a six week period resulted in a statisticaly significant improvement of flow mediated and nitroglycerin induced dilatation in type 2 diabetic patients with average lipid levels. However, there was no statistically significant difference between endothelial functions of the diabetics and the control group at the onset of the sudy. We set up a control group with similar lipid levels and tried to select the diabetic patients without advanced long term complications and an average glycemic control. The absence of significant difference between the diabetics and the control group may be related with our patient selection. This suggests that impaired endothelial functions observed in diabetics in prior studies might be related to microvascular damage seen in long term complications or disturbed lipid levels. Although not significantly disturbed endothelial functions are furher improved by statin therapy in our diabetic population.

In addition to hyperglycemia and dyslipidemia, levels of CRP, a marker of systemic inflammation, are elevated in patients with type 2 diabetes [15]. This is because of the induction of acute phase response by ongoing intra arterial inflammation. Pasceri *et al.* have shown that CRP has a direct proinflammatory effect on human endothelial cells and affects endothelial function [16]. Similarly, Panayiotis *et al.* have shown that treatment with 20 mg atorvastatin daily for a 3 month period reduced the CRP and TNFα levels in subjects at risk of developing diabetes [17]. Ridker *et al.* found among 785 patients with primary hypercholesterolemia that cerivastatin reduced CRP levels in a relatively short period of time [18]. Like the latter study we found that the inflammatory markers, such as, ESR, CRP and fibrinogen decreased after a six-week period of atorvastatin therapy, but these results was not statistically significant except for ESR levels. It is possible statins to improve endothelial functions before its effects on inflammation.

Our study has its limitations. First of all, this is a small study and must be carried on large patient and control groups. Secondly, like Taneva *et al.* [19] after showing the changes on the endothelial functions, the effects of withdrawal of atorvastatin on endothelial functions might have been done.

In conclusion, in the present study, we have shown that atorvastatin improves endothelial functions on a

short term in diabetic patients with average lipid levels significantly. This finding suggests that atorvastatin may have additional vascular protective effects in diabetics and may slow the progression of atherosclerotic process. However, these effects may not be thoroughly independent from their lipid lowering properties.

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