J Membrane Biol (2012) 245:697–705
DOI 10.1007/s00232-012-9441-7

The Effects of Atorvastatin Therapy on Rheological Characteristics of Erythrocyte Membrane, Serum Lipid Profile and Oxidative Status in Patients with Dyslipidemia

Hüseyin Avni Uydu · Sermet Yıldırmış · Cihan Örem · Mustafa Calapoglu · Ahmet Alver · Birgül Kural · Asım Örem

Received: 7 October 2011 / Accepted: 1 June 2012 / Published online: 16 June 2012
© Springer Science+Business Media, LLC 2012

Abstract The statins, most commonly used in the treatment of hyperlipidemia, have certain beneficial effects including improved endothelial function, plaque stability and decreased oxidative stress and inflammation, beyond their lipid-lowering effect in plasma. We evaluated the pleiotropic impact of atorvastatin on erythrocyte structural/mechanical properties and lipid peroxidation in dyslipidemics. The study group included 44 patients with dyslipidemia and was divided into subgroups according to triglyceride and cholesterol levels as hypercholesterolemic ($n = 29$) and mixed-type hyperlipidemic ($n = 15$). Subjects were given 10 mg atorvastatin per day for 12 weeks. Changes in serum lipid composition, lipid contents, Na^+/K^+-ATPase activity and osmotic fragility in erythrocytes and oxidative stress parameters of erythrocytes and plasma were studied. Atorvastatin therapy improved the serum lipid profile of both subgroups. This alteration was accompanied by a decreased level of cholesterol in erythrocyte membranes. Moreover, enhanced activity of Na^+/K^+-ATPase in erythrocytes reflected the improvements in membrane lipids of both subgroups. However, a significant change was observed in osmotic fragility values of the mixed-typed dyslipidemic group. This treatment lowered the lipid peroxidation in plasma and erythrocytes and increased plasma total antioxidant capacity in all groups. The present study shows that the use of atorvastatin reversed the structural and functional features of erythrocyte membranes in dyslipidemic subjects. Also, hypolipidemic therapy had a beneficial impact on a balance between oxidant and antioxidant systems.

Keywords Atorvastatin · Dyslipidemia · Erythrocyte lipid · Na^+/K^+-ATPase · Osmotic fragility · TBARS · TAS

H. A. Uydu (✉)
Department of Medical Biochemistry, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey
e-mail: hauydu@hotmail.com

S. Yıldırmış
Department of Chemistry, Faculty of Pharmacia, Karadeniz Technical University, Trabzon, Turkey

C. Örem
Faculty of Medicine, Department of Cardiology, Karadeniz Technical University, Trabzon, Turkey

M. Calapoglu
Faculty of Science, Department of Chemistry, Suleyman Demirel University, Isparta, Turkey

A. Alver · B. Kural · A. Örem
Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

Introduction

Dyslipidemia is one of the most common and important risk factors for developing of atherosclerosis and the resulting cardiovascular diseases. This lipid disorder, viewed as a lipid triad, is characterized by increased triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) (Austin et al. 2000; Orem et al. 2002). Hypercholesterolemia and mixed-type hyperlipidemia are the most common forms of atherogenic dyslipidemia. Numerous clinical trials have focused on the effect of atherogenic lipids on hemorheological factors associated with cardiovascular diseases (Kanakaraj and Singh 1989; Koter et al. 2004; Saklamaz et al. 2005; De Queiroz Mello et al. 2010). Cholesterol is an essential and functional

component of cell membranes, and any change in erythrocyte membrane cholesterol levels reflects substantial modification of the serum lipid profile because of not having de novo cholesterol synthesis in the cell (Dwight et al. 1996; Calişkan et al. 2000; Uyuklu et al. 2007). Cholesterol enrichment in erythrocytes results in decreased deformability and fluidity of these cells. This may cause impairment of functional properties including membrane-bound enzymes and rheological behaviors such as osmotic fragility that can promote atherosclerotic lesions (Ramanadham and Kaplay 1982; Kanakaraj and Singh 1989; Koter et al. 2004; Vayá et al. 2008). Besides the effect of hypercholesterolemia on erythrocyte rheological characteristics, recent studies have indicated that the free cholesterol in erythrocyte membrane could structurally contribute to plaque formation as well as serum lipoprotein fractions (Mason et al. 2006; Vayá et al. 2008). On the other hand, oxidative stress is the underlying cause of many diseases and closely associated with cardiovascular risk factor (Koter et al. 2004). Free radicals, the mediators of oxidative stress, primarily attack membrane lipids and lead to irreversible functional and structural disorders including membrane-bound ion transport proteins and other proteins involved in signal-transduction pathways (Koter et al. 2004; Mason et al. 2006). As a result of lipid peroxidation, the crystallized cholesterol formed in the extracellular milieu of the plasma membrane contributes directly to the pathogenesis of cardiovascular diseases by leading to cell death and inflammation. Free cholesterol exchange between erythrocytes and lipoprotein fractions can easily occur despite the crystallized form being insensitive to pharmacologic intervention or reverse cholesterol transport mechanisms (Mason et al. 2006).

Statins are commonly used to lower high blood cholesterol levels. They are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (3-HMG-CoA) reductase, which catalyzes the conversion of HMG-CoA to mevalonate, the early rate-limiting step in the biosynthesis of cholesterol (Dwight et al. 1996; Calişkan et al. 2000; Koter et al. 2002). A number of clinical studies have demonstrated that treatment of dyslipidemic patients with HMG-CoA reductase inhibitors such as lovastatin and simvastatin caused a decrement in serum and cellular cholesterol levels, despite some reports showing no significance (Levy et al. 1992; Rabini et al. 1993; Martinez et al. 1996; Lijnen et al. 1996; Dwight et al. 1996; Uyuklu et al. 2007). Evidence concerning the hypolipidemic effect of statin-derived agents indicates that this therapy can lead to plaque stabilization, decreased platelet aggregation (Koter et al. 2002) and improvement in erythrocyte hemorheological action (Martinez et al. 1996) as well as antioxidant (Mason et al. 2006; Bełtowski et al. 2009; Chopra and Flanders 2009). Among statin-derived drugs, atorvastatin is a synthetic inhibitor of HMG-CoA and more effective than other statins. Experimental studies on the pleiotropic effects of atorvastatin have increased in recent years.

In the present study, the efficiency of atorvastatin therapy concerning the serum lipid profile and the structural and functional properties of erythrocyte membranes in individuals with dyslipidemia were researched. Also, the antioxidative potency of the therapy on erythrocytes and serum was examined.

Materials and Methods

Patients and Treatment Organization

The study involved 44 patients (23 men and 21 women, aged 30–76 years, average 54) with dyslipidemia. They were recruited via the Cardiology Department of the Medical Faculty at Karadeniz Technical University in Turkey. The research was approved by the local ethics committee at the Medical Faculty, and all patients gave informed consent before entering the study. Because of the strong interrelationships between elevated TG and LDL-C and reduced HDL-C, patients were classified into two groups according to their serum total cholesterol (TC) levels as 29 hypercholesterolemic (with cholesterol levels >240 mg/dl) and serum TC and TG levels as 15 mixed-type hyperlipidemic (with cholesterol and TG above 240 and 200 mg/dl, respectively). Patients were eligible to take part in the study if they met the criteria of the National Cholesterol Education Program-Adult Treatment Panel 2 (NCEP-ATP2) and were not receiving lipid-lowering therapy before entry into the study. All undertook a screening program consisting of medical history, examination and full hematologic and biochemical profiles. Patients with any of the following conditions were excluded: hypothyroidism, diabetes mellitus, nephrotic syndrome, renal insufficiency, hepatic dysfunction, malignant diseases, immune disorders, uncontrolled hypertension and coronary artery disease; also, cigarette smokers were excluded. The study lasted 16 weeks. Patients were started on atorvastatin (10 mg/day) and evaluated at 6–12 weeks to assess therapeutic efficacy. Patients were also assigned to a hypolipidemic diet regimen according to NCEP step 1 during the treatment. This diet limits cholesterol intake to <300 mg/day and total fats to 30 % of total calories, with <10 % of total calories from saturated fats, 10 % from polyunsaturated fats and 10–15 % from monounsaturated fats. All manual measurements were made in triplicate.

Measurement of Serum Lipids and Lipoproteins

Blood samples were drawn after an overnight fast of 12 h, and sera were separated by low-speed (2,500×*g*) centrifugation for 15 min. Levels of TC and TG were estimated with enzymatic methods using a Hitachi (Tokyo, Japan) 917 autoanalyzer with Boehringer Mannheim (Mannheim, Germany) original reagents. HDL-C was measured by the dextran sulfate-Mg^{2+} precipitation method. LDL-C was calculated by Friedewald's formula. Apolipoprotein A-I (Apo A-I) and apoprotein B (Apo B) levels were assessed by immunonephelometry using Date Behring (BN II, Marburg, Germany) and its original reagent. Results were expressed as milligrams per deciliter of serum.

Erythrocyte Preparation, Extraction and Measurement of Membrane Lipids

Erythrocytes were separated from blood plasma and leukocytes by means of centrifugation (3,000×*g*, 10 min) at 4 °C and washed three times with phosphate-buffered saline (PBS). Membrane lipids were extracted using the procedure of Rose and Oklander (1965), in which lipids are extracted from an aliquot of erythrocyte membrane suspension with chloroform-isopropanol (7:11, v/v) using a single extraction tube. Membrane cholesterol was assayed by the cholesterol oxidase method with a high-performance Monotest kit (Boehringer Mannheim) (Ott et al. 1982). Membrane total phospholipid phosphorus was estimated according to Findlay and Evans (1987). To prevent autooxidation, butylated hydroxytoluene (0.001 %, w/v) as antioxidant was added to all solvents.

Measurement of Na^+/K^+-ATPase Activity

For ATPase activity, erythrocyte membranes were prepared according to Dodge et al. (1963). The method described by Charalambous and Mir (1982) was used to assay Na^+/K^+-ATPase activity. It is based on the measurement of orthophosphate released from ATP during incubation of membrane suspensions with a medium containing (in mM) 2 ATP, 10 $MgCl_2$, 30 Tris-EGTA buffer (pH 7.4, 0.1 mM EGTA), 10 NaCN and 0.1 ouabain. Enzyme activity was expressed as the difference in the inorganic phosphate (P_i) released in the presence and absence of ouabain. Na^+/K^+-ATPase activity was expressed as micromoles P_i per hour per milligram membrane protein.

Measurement of Osmotic Fragility

The test was performed by the method of Parpart et al. (1947). Calibration for percentage hemolysis was performed with the same groups by mixing 0.1 % saline solution with 0.02 ml packed cells. This reading was treated as 100 % hemolysis, and distilled water was used as a blank for calibration.

Measurements of Lipid Peroxidation in Plasma and Erythrocytes

Plasma concentrations of thiobarbituric acid reactive substances (TBARS) were measured according to method of Yagi (1994). Lipids and proteins were precipitated using 10 % phosphotungstic acid and 0.04 M sulfuric acid (w/v). The sediment was resuspended in distilled water, and thiobarbituric acid (TBA) was added to the mixture. The reaction mixture was heated to 95 °C for 60 min. TBARS were extracted with *n*-butanol. After centrifugation, the butanol layer was taken for fluorometric measurements at 515 nm excitation and 553 nm emission.

Erythrocyte TBARS was estimated according to the method described by Stocks and Dormandy (1971). Cell suspension (3 ml) was added to trichloroacetic acid-arsenite solution (2 ml). Supernatant (3 ml) was transferred to TBA solution (1 ml) following centrifugation of the mixture for 10 min. An air condenser was fitted to the tube, and the mixture was incubated for exactly 15 min in a boiling water bath. The tube was cooled. The absorption spectrum of the mixture between 500 and 600 nm was plotted by a recording spectrophotometer. The formula ($OD_{532} - OD_{600}$) × 900 gave the TBARS concentration in nanomoles per gram Hb. Mean hemoglobin concentration was measured by a Coulter (Hialeah, FL) analyzer.

Measurement of Total Antioxidant Status in Plasma

Total antioxidant status (TAS) was assayed using the method of Miller et al (1993). In this method, metmyoglobin reacts with hydrogen peroxide to form ferrylmyoglobin, a free radical species. All reagent and calibrator/control materials were supplied by Randox Laboratories (Antrim, UK). The within-run coefficient of variance (CV) for the control was 4.8 % ($n = 10$).

Statistical Analysis

All results are expressed as means ± SD. Comparisons for serum lipids, lipoproteins, membrane lipids, ATPase activity, osmotic fragility, TBARS and TAS levels before and after lipid-lowering therapy were performed with the paired *t*-test for normally distributed data or Wilcoxon's signed-rank test for non-normally distributed data. The unpaired *t*-test or Mann–Whitney *U*-test was used to compare parameters between subgroups. Relationships among variables were assessed by Spearman's rank or

Pearson's correlation coefficient. $p < 0.05$ was considered significantly different. The SPSS 16.0 software package (SPSS, Inc., Chicago, IL) was used in all statistical analyses.

Results

The demographic, clinical and treatment data of the dyslipidemic groups are given in Table 1. In our study population 29 (66 %) were hypercholesterolemic and 15 (34 %) were mixed-type hyperlipidemic subjects. Of the participants, 21 (48 %) were hypertensive, 13 (30 %) were current smokers, 10 (23 %) had obesity and 16 (36 %) had a family history of dyslipidemia. There was no statistically significant difference among experimental groups in demographic features (Table 1). Higher TG and lower LDL-C and HDL-C levels of mixed-type hyperlipidemics compared to hypercholesterolemics were found in the pretreatment period, but no difference was observed in other parameters. The dyslipidemic subgroups showed similar responses to atorvastatin treatment concerning the studied parameters. As expected, atherogenic lipid components (TC, LDL-C and TG) and Apo B level significantly decreased, while HDL-C and Apo A-I levels increased in both groups after treatment. No significant change was found in Lp (a) level in all groups after the therapy. Table 2 presents membrane cholesterol, phospholipids levels, C/P ratio, Na^+/K^+-ATPase activity and osmotic fragility before and after treatment with atorvastatin in the two groups. Erythrocyte membranes from the atorvastatin-treated group contained lower concentrations of cholesterol and a lower C/P ratio than those of the untreated group ($p < 0.001$, $p < 0.01$ in hypercholesterolemic group and $p < 0.05$, $p < 0.05$ in mixed-type dyslipidemic group, respectively). Following the therapy, there was a significant increase in phospholipid level in erythrocytes of subjects with dyslipidemia. Erythrocyte Na^+/K^+-ATPase activity was also enhanced during the lipid-lowering therapy in hypercholesterolemics and mixed-type dyslipidemics ($p < 0.001$ and $p < 0.05$, respectively). Following the treatment, the osmotic fragility of erythrocytes showed a significant increasing trend in the mixed-type hyperlipidemic group, while there was an insignificant rise in the hypercholesterolemic group. Our finding showed that the balance between the oxidant and antioxidant

Table 1 Distribution of demographic features in patients with hypercholesterolemia and mixed-type hyperlipidemia and lipid profiles in the dyslipidemic groups before and after lipid-lowering therapy

Parameters	Patients with hypercholesterolemia ($n = 29$, mean ± SD)	Patients with mixed-typed hyperlipidemia ($n = 15$, mean ± SD)
Gender, male, *n* (%)	18 (62)	5 (33)
Hypertension, *n* (%)	13 (45)	8 (53)
Smoking, *n* (%)	10 (35)	3 (20)
Obesity, *n* (%)	6 (21)	4 (27)
Familiy history, *n* (%)	12 (41)	4 (27)
Age (years)	54 ± 12	54 ± 9
BMI (kg/m^2)	29 ± 5	27 ± 3
Treatment dosage (mg/day)	10	10

Parameters	Patients with hypercholesterolemia ($n = 29$, mean ± SD)		Patients with mixed-typed hyperlipidemia ($n = 15$, mean ± SD)	
	Before treatment	After treatment	Before treatment	After treatment
TG (mg/dl)	132 ± 39	112 ± 39[a,***]	311 ± 84[c,***]	199 ± 79[a,***]
TC (mg/dl)	282 ± 33	205 ± 37[a,***]	290 ± 26	209 ± 36[a,***]
LDL-C (mg/dl)	210 ± 30	134 ± 34[a,***]	190 ± 33[c,*]	129 ± 39[a,***]
HDL-C (mg/dl)	43 ± 8	47 ± 9[a,***]	38 ± 5[c,*]	41 ± 6[a,**]
Apo A-I (mg/dl)	122 ± 20	136 ± 17[a,***]	123 ± 11	134 ± 14[a,**]
Apo B (mg/dl)	132 ± 16	101 ± 12[b,***]	142 ± 27	108 ± 21[b,***]
Lp (a) (mg/dl)	21 ± 31	19 ± 29	21 ± 22	17 ± 24

TC total cholesterol, *TG* triglyceride, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *Apo* apoprotein

[a,b] According to paired *t*-test[a] and Wilcoxon test[b] for comparison of plasma lipid parameters before and after treatment with atorvastatin

[c] According to paired *t*-test for comparison of plasma lipid parameters between hypercholesterolemic and mixed-type hyperlipidemic groups

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2 Na^+/K^+-ATPase activity, osmotic fragility and levels of plasma membrane lipid in patients with hypercholesterolemia and mixed-type hyperlipidemia before and after lipid-lowering therapy

Parameters	Patients with hypercholesterolemia ($n = 29$, mean ± SD)		Patients with mixed-typed hyperlipidemia ($n = 15$, mean ± SD)	
	Before treatment	After treatment	Before treatment	After treatment
Cholesterol (μmol/mg Prt)	0.33 ± 0.12	0.22 ± 0.06***	0.31 ± 0.12	0.24 ± 0.05*
Phospholipid (μmol/mg Prt)	0.55 ± 0.17	0.75 ± 0.30*	0.58 ± 0.21	0.83 ± 0.33*
Cholesterol/phospholipid	0.64 ± 0.33	0.34 ± 0.13**	0.62 ± 0.37	0.32 ± 0.10*
Na^+/K^+-ATPase activity (μmol P_i/h/mg Prt)	0.79 ± 0.30	0.93 ± 0.36***	0.61 ± 0.18	0.81 ± 0.14*
Osmotic fragility[a] (g/l)	4.03 ± 0.27	4.14 ± 0.34	3.96 ± 0.18	4.26 ± 0.26*

[a] NaCl concentration giving 50 % hemolysis

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, according to paired *t*-test

Table 3 Lipid peroxidation intensity of erythrocyte and plasma and plasma TAS values in patients with hypercholesterolemia and mixed-type hyperlipidemia before and after lipid-lowering therapy

Parameters	Patients with hypercholesterolemia ($n = 29$, mean ± SD)		Patients with mixed-typed hyperlipidemia ($n = 15$, mean ± SD)	
	Before treatment	After treatment	Before treatment	After treatment
TBARS in the whole erythrocyte (nmol/g hemoglobin)	276 ± 94	233 ± 82*	265 ± 79	191 ± 42**
TBARS in plasma (nmol/ml)	5.63 ± 1.81	3.69 ± 0.80***	6.64 ± 3.32	4.02 ± 1.28***
TAS (mmol/l)	1.41 ± 0.09	1.51 ± 0.09***	1.38 ± 0.11	1.49 ± 0.09**

TBARS thiobarbituric acid reactive substances, *TAS* total antioxidant status

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, according to paired *t*-test

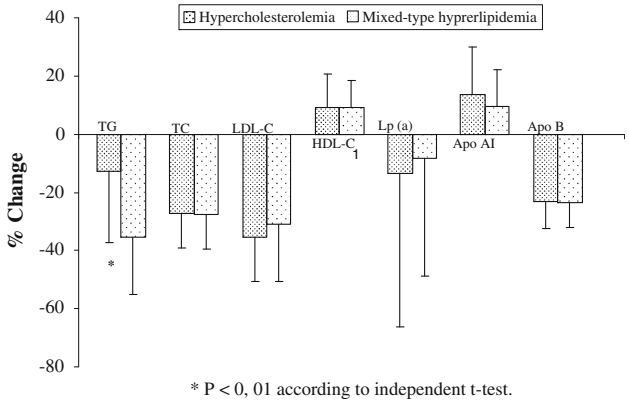

Fig. 1 Comparison of percent changes in plasma lipid profile in patients with hypercholesterolemia and mixed-type hyperlipidemia after 12-week atorvastatin therapy

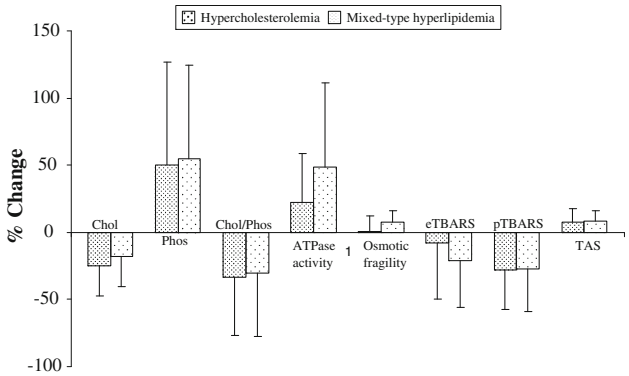

Fig. 2 Comparison of percent changes in erythrocyte membrane lipid composition and in lipid peroxidation products in erythrocyte and plasma of patients with hypercholesterolemia and mixed-type hyperlipidemia after 12-week atorvastatin therapy. *eTBARS* and *pTBARS* Thiobarbituric acid reactive substances in erythrocyte and plasma, respectively

systems was affected by atorvastatin therapy. In all study groups, the lower TBARS levels in erythrocytes and plasma were obviously estimated after this treatment. Also, plasma TAS values of the two subgroups compared to baseline were higher after the therapy (Table 3). The percentage changes in serum lipid profile and in erythrocyte lipid composition, Na^+/K^+-ATPase activity, osmotic fragility and components of the oxidant and antioxidant systems are given in Figs. 1 and 2. As can be seen in these figures, the degree of change in all of these parameters, except for triglyceride, was similar in both subgroups. In addition, Fig. 3 indicates the relationship between the percentage changes of C/P ratio and Na^+/K^+-ATPase activity in erythrocytes of hypercholesterolemics ($r = -0.786$, $p < 0.02$). The enzyme activity of erythrocyte membranes was enhanced according to the decrease in

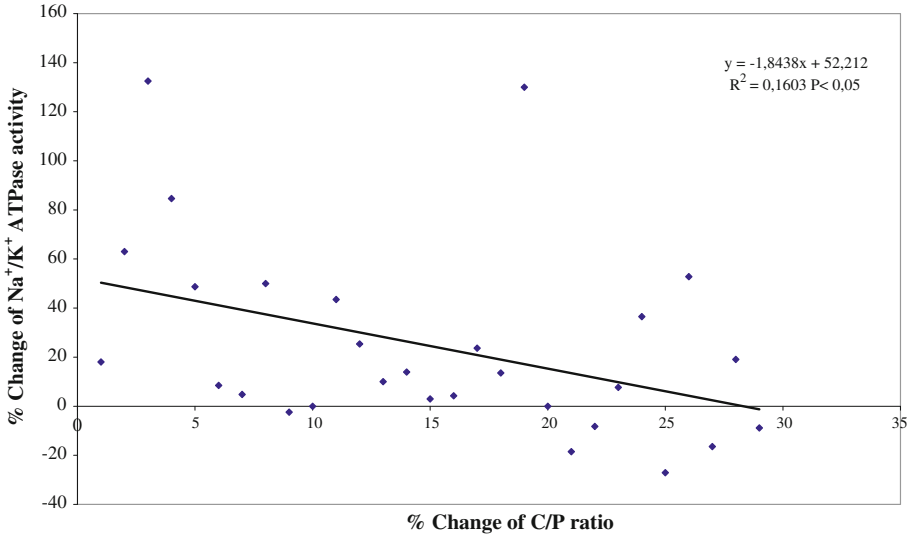

Fig. 3 Relationship between percent changes of Na^+/K^+-ATPase activity and C/P ratio of erythrocytes in subjects with dyslipidemia

membrane C/P ratio of both groups. Also, it was estimated that there was a negative correlation between the percentage change of erythrocyte TBARS amount and the C/P ratio and plasma TAS level in subjects with dyslipidemia ($r = -0.714$, $p < 0.047$ and $r = -0.424$, $p < 0.022$, respectively).

Discussion

Our findings show that short-term therapy with low-dose atorvastatin in subjects with dyslipidemia was efficient at reducing the serum concentration of TC, LDL-C, TG and Apo B and at increasing HDL-C and Apo AI, while the Lp (a) level was not changed. All these changes were independent of possible cofounders such as gender, family history, obesity, smoking and hypertension. The changes in these parameters were similar to the results obtained by many studies (Koter et al. 2002; Uyuklu et al. 2007; Koh et al. 2010; Jones et al. 2010, Arsenault et al. 2011; Thongtang et al. 2011; Athyros et al. 2011). Also, we demonstrated that there was no statistical difference between baseline lipid and apolipoprotein characteristics of the dyslipidemic groups except for TG and HDL-C. This indicated that the study groups had the proper demographic characteristics. The effects of statins on HDL-C levels have been controversial. Some studies have shown a marked elevation in levels of serum HDL-C during atorvastatin treatment (Barter et al. 2010; Arsenault et al. 2011) and others no change (Rabini et al. 1993; Lijnen et al. 1996; Koter et al. 2002; Arsenault et al. 2011). The lipid-lowering effect of atorvastatin, as for other statins on serum lipid, is due to a competitive inhibition of the rate-limiting enzyme 3-HMG CoA reductase in the intracellular biosynthesis of cholesterol, subsequently leading to induction of receptor production and catabolic rate of LDL-C (Rubenfire et al. 1991; Lijnen et al. 1996). In both dyslipidemic groups, there were close correlations between reduced Apo B levels, increased Apo A-I levels and plasma levels of LDL-C and HDL-C. In general, an unchanged serum Lp (a) level has been reported during statin-derived drug treatment of hypercholesterolemic patients (Miller et al. 1993; Bełtowski et al. 2009). A number of studies have shown that alterations in the serum lipid composition were accompanied by changes in erythrocyte lipid content (Lijnen et al. 1996; Martinez et al. 1996; Koter et al. 2002), but not all agree (Dwight et al. 1996; Tziakas et al. 2008). In the present study, a significant decrement in erythrocyte membrane cholesterol in subjects with hypercholesterolemia and mixed-type hyperlipidemia after atorvastatin therapy was clearly observed. After a 12-week therapy, serum concentrations of TC and LDL-C in the subgroups were similarly lowered. Also, the percent change of erythrocyte C/P ratio was positively correlated with that of serum LDL-C. There are some reports that conflict with our finding. Of these, Dwight et al. (1996) indicated that 8-week treatment with simvastatin in low doses altered membrane acyl chain composition and serum cholesterol but had no effect on erythrocyte cholesterol content. In contrast to the mentioned studies, there are many more studies showing that the short- and long-term statin therapies resulted in decreased erythrocyte cholesterol and C/P ratio (Rabini et al. 1993; Martinez et al. 1996; Koter et al. 2004; Vayá et al. 2008). The lipid-lowering impact of atorvastatin as well other statins was thought to result from an influence on cholesterol exchange by binding to the plasma membrane and displacing cholesterol between the lipoproteins and the erythrocytes in which the rate of

cholesterol synthesis is relatively insignificant (Garnier et al. 1988; Lijnen et al. 1996; Martinez et al. 1996). One of the most important determinants of erythrocyte function and survival throughout the blood circulation is its deformability property, which is determined by some factors: membrane viscoelasticity, intracellular viscosity and area to volume ratio of the cell surface causing the membrane C/P ratio and a younger cell population. Na^+/K^+ pump activity and osmotic fragility are perfect indices of cell age (Ramanadham and Kaplay 1982). The reduction in C/P ratio of the erythrocyte membrane was associated with a significant enhancement of erythrocyte membrane Na^+/K^+ pump activity. This was obviously shown by our study. Recently, many reports have demonstrated that erythrocyte Na^+/K^+ pump activity is inversely related to its membrane contents of cholesterol, polyunsaturated fatty acid and particularly phospholipid subclasses (Uyuklu et al. 2007). Increased Na^+/K^+-ATPase activity in both dyslipidemic groups may be evident only in the presence of an elevation in membrane fluidity, depending on the reduction of erythrocyte C/P ratio after atorvastatin treatment. This is consistent with previous reports suggesting that the improved membrane fluidity by hypolipidemic therapy enhanced pump activity in subjects with dyslipidemia (Rabini et al. 1993; Lijnen et al. 1996; Martinez et al. 1996; Koter et al. 2004).

Serum lipid composition could change the osmotic fragility of erythrocytes by disrupting phospholipid compounds and the cholesterol content of cell membranes (Kanakaraj and Singh 1989). Even though the erythrocyte Na^+/K^+ pump activity and C/P ratio altered in both groups after treatment, increased osmotic resistance to hypotonic hemolysis was significant only in the mixed-type hyperlipidemic group. This behavior in the mixed-type group only may be a consequence of the small size of the population. There have been a number of studies showing that the erythrocytes of hypercholesterolemics were osmotically more resistant to lysis in a hypotonic medium (Kanakaraj and Singh 1989; Özdemirler et al. 2001). In the present study, the unaltered osmotic fragility values of the hypercholesterolemic group after treatment are, however, consistent with the finding that Dwight and colleagues (1996) obtained in 2-month simvastatin therapy of individuals with a clinical history of atherosclerosis. After atorvastatin treatment, the increment in osmotic fragility may be due to lost cholesterol or acquired phospholipid content of erythrocytes, particularly phosphatidylcholine.

On the basis of the literature data, clinical trials have shown that dyslipidemia contributes to the development of cardiovascular diseases and that this course is amplified by oxidative stress, resulting in the formation of atheromatous plaque (Koter et al. 2004; De Freitas et al. 2010; Barter et al. 2010). Erythrocytes are more susceptible to the free radicals which cause structural and functional disorders in the cell membrane, including reduced enzyme activities, essential fatty acid content and membrane rigidity, thus decreasing the cell's ability to deform (Koter et al. 2004). As a measurement of oxidative stress, TBARS level in the erythrocyte and plasma of the study groups was estimated during pre- and posttreatment periods. There was a highly significant decrease in TBARS values in both erythrocyte and plasma of subgroups compared to baseline. Our results were confirmed by the report of Koter et al. (2004), showing that TBARS concentrations in erythrocyte and plasma of hypercholesterolemic subjects were notably lowered after 12 weeks of atorvastatin treatment. Given the fact that statins indicate pleiotropic effects, their antioxidant activity may be to bind to the phospholipids of erythrocyte membranes and lipoprotein fractions, thereby preventing the diffusion of free radicals generated under oxidative stress (Bellosta et al. 2000). In addition, Mason and colleagues (2006) propounded that when comparing other statins the higher antioxidant potency of atorvastatin resulted from the *o*-hydroxy metabolite form, about 70 % presented in serum. The metabolite protects the lipid moiety against free radical attack. This protective mechanism is due to electron donation and proton stabilization associated with its phenoxy group located in the membrane lipid core. Correlatively, total plasma antioxidant status, an indication of antioxidant capacity, was higher in both dyslipidemic groups after treatment; and all these data clearly confirm the antioxidative action of this antilipidemic agent.

In the present research study, we have shown that treating dyslipidemic patients with atorvastatin not only causes favorable changes in serum, erythrocyte lipid profile and some aspects of cell deformability related to membrane components but also leads to an antioxidative impact against oxidative stress beyond cholesterol lowering. However, further studies are needed to elucidate the molecular mechanisms underlying the changes in lipid composition of erythrocyte membrane and the balance between oxidant and antioxidant status changed by atorvastatin therapy.

Acknowledgements This work was supported by Grants from the Karadeniz Technical University Research Foundation.

References

Arsenault BJ, Barter P, DeMicco DA, Bao W, Preston GM, LaRosa JC, Grundy SM, Deedwania P, Greten H, Wenger NK, Shepherd J, Waters DD, Kastelein JJ, TNT Study Investigators (2011) Prediction of cardiovascular events in statin-treated stable coronary patients by lipid and nonlipid biomarkers. J Am Coll Cardiol 57(1):63–69

Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP (2011) Dyslipidaemia of obesity, metabolic syndrome and type 2

diabetes mellitus: the case for residual risk reduction after statin treatment. Open Cardiovasc Med J 5:24–34

Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AG (2000) Cardiovascular disease mortality in familial forms of hypertriglyceridemia: a 20-year prospective study. Circulation 101(24):2777–2782

Barter PJ, Brandrup-Wognsen G, Palmer MK, Nicholls SJ (2010) Effect of statins on HDL-C: a complex process unrelated to changes in LDL-C: analysis of the VOYAGER database. J Lipid Res 51(6):1546–1553

Bellosta S, Ferri N, Bernini F, Paoletti R, Corsini A (2000) Non-lipid-related effects of statins. Ann Med 32(3):164–176

Bełtowski J, Wójcicka G, Jamroz-Wiśniewska A (2009) Adverse effects of statins: mechanisms and consequences. Curr Drug Saf 4(3):209–228

Calişkan S, Calişkan M, Kuralay F, Onvural B (2000) Effect of simvastatin therapy on blood and tissue ATP levels and erythrocyte membrane lipid composition. Res Exp Med (Berl) 199(4):189–194

Charalambous BM, Mir MA (1982) An improved procedure for the preparation and measurement of (Na^+/K^+)-ATPase in human erythrocytes. Biochim Biophys Acta 691:71–82

Chopra V, Flanders SA (2009) Does statin use improve pneumonia outcomes? Chest 136(5):1381–1388

De Freitas MV, de Oliveira MR, dos Santos DF, de Cássia Mascarenhas Netto R, Fenelon SB, Penha-Silva N (2010) Influence of the use of statin on the stability of erythrocyte membranes in multiple sclerosis. J Membr Biol 233(1–3): 127–134

De Queiroz Mello AP, da Silva IT, Oliveira AS, Nunes VS, Abdalla DS, Gidlund M, Damasceno NR (2010) Electronegative low-density lipoprotein is associated with dense low-density lipoprotein in subjects with different levels of cardiovascular risk. Lipids 45(7):619–625

Dodge JT, Mitchell C, Hanahan DJ (1963) The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch Biochem Biophys 100:119–130

Dwight JF, Mendes Ribeiro AC, Hendry BM (1996) Effects of HMG-CoA reductase inhibition on erythrocyte membrane cholesterol and acyl chain composition. Clin Chim Acta 256(1):53–63

Findlay JB, Evans WH (1987) Biological membranes. Oxford University Press, New York, pp 119–121

Garnier M, Perret G, Pilardeau P, Vaysse J, Rolland Y, Uzzan B, Vassy R (1988) Effect of diosmin upon red blood cell deformability and osmotic fragility. Relationship with lipid content. Methods Find Exp Clin Pharmacol 10(4):259–262

Jones PH, Goldberg AC, Knapp HR, Kelly MT, Setze CM, Stolzenbach JC, Sleep DJ (2010) Efficacy and safety of fenofibric acid in combination with atorvastatin and ezetimibe in patients with mixed dyslipidemia. Am Heart J 160(4):7 59–766

Kanakaraj P, Singh M (1989) Influence of hypercholesterolemia on morphological and rheological characteristics of erythrocytes. Atherosclerosis 76(2–3):209–218

Koh KK, Quon MJ, Han SH, Lee Y, Kim SJ, Shin EK (2010) Atorvastatin causes insulin resistance and increases ambient glycemia in hypercholesterolemic patients. J Am Coll Cardiol 55(12):1209–1216

Koter M, Broncel M, Chojnowska-Jezierska J, Klikczynska K, Franiak I (2002) The effect of atorvastatin on erythrocyte membranes and serum lipids in patients with type-2 hypercholesterolemia. Eur J Clin Pharmacol 58(8):501–506

Koter M, Franiak I, Strychalska K, Broncel M, Chojnowska-Jezierska J (2004) Damage to the structure of erythrocyte plasma membranes in patients with type-2 hypercholesterolemia. Int J Biochem Cell Biol 36(2):205–215

Levy Y, Leibowitz R, Aviram M, Brook JG, Cogan U (1992) Reduction of plasma cholesterol by lovastatin normalizes erythrocyte membrane fluidity in patients with severe hypercholesterolaemia. Br J Clin Pharmacol 34(5):427–430

Lijnen P, Echevaría-Vázquez D, Petrov V (1996) Influence of cholesterol-lowering on plasma membrane lipids and function. Methods Find Exp Clin Pharmacol 18(2):123–136

Martinez M, Vaya A, Marti R, Gil L, Lluch I, Carmena R, Aznar J (1996) Effect of HMG-CoA reductase inhibitors on red blood cell membrane lipids and haemorheological parameters, in patients affected by familial hypercholesterolemia. Haemostasis 26(Suppl 4):171–176

Mason RP, Walter MF, Day CA, Jacob RF (2006) Active metabolite of atorvastatin inhibits membrane cholesterol domain formation by an antioxidant mechanism. J Biol Chem 281(14):9337–9345

Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A (1993) A novel method for measuring antioxidant capacity and its application to monitoring antioxidant status in premature neonates. Clin Sci 34:407–412

Orem C, Orem A, Uydu HA, Celik S, Erdöl C, Kural BV (2002) The effects of lipid-lowering therapy on low-density lipoprotein autoantibodies: relationship with low-density lipoprotein oxidation and plasma total antioxidant status. Coron Artery Dis 13(1):65–71

Ott P, Binggeli Y, Brodbeck U (1982) Rapid and sensitive assay for determination of cholesterol in membrane lipid extracts. BBA Biomembr 685:211–213

Özdemirler G, Küçük S, Orhan Y, Aykaç-Toker G, Uysal M (2001) Lipid and protein oxidation in erythrocyte membranes of hypercholesterolemic subjects. Clin Biochem 34(4):335–339

Parpart AK, Lorenz PB, Parpart ER, Gregg JR, Chase AM (1947) The osmotic resistance (fragility) of human red cells. J Clin Invest 26(4):636–640

Rabini RA, Polenta M, Staffolani R, Tocchini M, Signore R, Testa I, Mazzanti L (1993) Effect of hydroxymethylglutaryl-CoA reductase inhibitors on the functional properties of erythrocyte membranes. Exp Mol Pathol 59(1):51–57

Ramanadham M, Kaplay SS (1982) Erythrocyte osmotic fragility in protein-energy malnutrition: cholesterol, phospholipid, and Ca^{2+}, Mg^{2+} adenosine triphosphatase. Biochem Med 27: 226–231

Rose HG, Oklander M (1965) Improved procedure for the extraction of lipid from human erythrocytes. J Lipid Res 6:428–431

Rubenfire M, Maciejko JJ, Blevins RD, Orringer C, Kobylak L, Rosman H (1991) The effect of pravastatin on plasma lipoprotein and apolipoprotein levels in primary hypercholesterolemia. The Southeastern Michigan Collaborative Group. Arch Intern Med 151(11):2234–2240

Saklamaz A, Comlekci A, Temiz A, Caliskan S, Ceylan C, Alacacioglu A, Yesil S (2005) The beneficial effects of lipid-lowering drugs beyond lipid-lowering effects: a comparative study with pravastatin, atorvastatin, and fenofibrate in patients with type IIa and type IIb hyperlipidemia. Metabolism 54(5): 677–681

Stocks J, Dormandy TL (1971) The autoxidation of human red cell lipids induced by hydrogen peroxide. Br J Haematol 20:95–111

Thongtang N, Ai M, Otokozawa S, Himbergen TV, Asztalos BF, Nakajima K, Stein E, Jones PH, Schaefer EJ (2011) Effects of maximal atorvastatin and rosuvastatin treatment on markers of glucose homeostasis and inflammation. Am J Cardiol 107(3):387–392

Tziakas DN, Chalikias GK, Stakos D, Tentes IK, Chatzikyriakou SV, Mitrousi K, Kortsaris AX, Boudoulas H, Kaski JC (2008) Cholesterol composition of erythrocyte membranes and its association with clinical presentation of coronary artery disease. Coron Artery Dis 19(8):583–590

Uyuklu M, Meiselman HJ, Baskurt OK (2007) Effect of decreased plasma cholesterol by atorvastatin treatment on erythrocyte mechanical properties. Clin Hemorheol Microcirc 36(1):25–33

Vayá A, Martínez Triguero M, Réganon E, Vila V, Martínez Sales V, Solá E, Hernández Mijares A, Ricart A (2008) Erythrocyte membrane composition in patients with primary hypercholesterolemia. Clin Hemorheol Microcirc 40(2):289–294

Yagi K (1994) Lipid peroxides and related radicals in clinical medicine. In: Free radicals in diagnostic medicine. Pleneum Press, New York, pp 1–15