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Esterification of HDL cholesterol is Decreased in Diabetes Mellitus and CAD and Enhanced Following Treatment with Statins

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ABSTRACT

Background: The main goal of using statins is to reduce the level of plasma cholesterol, meanwhile they have a wide spectrum of actions. **Objectives:** To identify the effect of statins on fractional cholesterol esterification (FCE) as well as the complete profile of lipids and (apo)lipoproteins. **Design and methods:** In an age and sex matched case-control study, 400 subjects who were referred for coronary angiography were divided into two groups according using statins. **Results:** Total cholesterol was decreased significantly following treatment with statins (165.6 ± 38.0 mg/dL vs. 205.3 ± 48.4, p≤0.001). About 90% of the reduction was occurred in nonHDL and 10% in HDL fraction. Reduction of nonHDL cholesterol (125.2 ± 35.2 mg/dL vs. 162.8 ± 45.2, p≤0.001) occurred on both unesterified (52.4 ± 21.5 mg/dL vs. 65.2 ± 25.5, p≤0.001) and esterified cholesterol (74.7 ± 27.3 mg/dL vs. 96.6 ± 34.1, p≤0.001). But the decrease in HDL cholesterol (40.4 ± 10.0 mg/dL vs. 42.3 ± 9.9, p≤0.079) happened exclusively in unesterified fraction (10.9 ± 3.4 vs. 15.2 ± 5.1, p≤0.001) and was counterbalanced with a significant increase in esterified portion (29.5 ± 8.2 mg/dL vs. 27.2 ± 9.5, p≤0.020). The ratio of esterified- per total- cholesterol in HDL was 67.5 ± 8.1% in the control group and was decreased to 58.0 ± 14.9% (p≤0.01) in diabetes and CAD and increased to 73.5 ± 6.9 (p≤0.01) after using statins. **Conclusions:** The results suggest that the percent of esterified cholesterol in HDL fraction is decreased in diabetes and CAD patients and increased by using statins.

Keywords: Cholesterol, Esterification, HDLc, Statin, Unesterified.

1. INTRODUCTION

Statins have a wide spectrum of actions which contribute to their ability to decrease the risk for cardiovascular disease. These 'pleiotropic properties' include hypolipidemic, antiatherogenic, antithrombotic and direct effects on endothelial cells (1, 2). The most recognized action of statins is reduction of serum cholesterol level (3). All studies confirmed uniformly that statins inhibit the intracellular cholesterol synthesis and accumulation (4-7). Statins inhibit HMG-CoA reductase, the key regulatory enzyme involved in *de novo* synthesis of cholesterol (3). Statins also have a potent direct inhibitory effect on cellular ACAT and thereby inhibition of cholesterol esterification and accumulation in macrophages (4), enterocytes (5, 6) and hepatocytes (7, 8). These effects also influence indirectly the secretion of lipids from the cells (5-8).

The HDL fraction has a central role in collecting and transferring cholesterol from the peripheral tissue into the liver to excrete. In vascular space, unesterified (free) cholesterol is transported to HDL in the direction of the gradient of concentration (9). This process has been varied in diabetes mellitus (10-12), coronary artery disease (CAD) (11-14) and modified by statins treatment (15-22). In blood circulation, free cholesterol is esterified by LCAT (10). The activity of acyl transferase is found on both HDL (called as αLCAT) and nonHDL fractions (called as βLCAT), but the former is more active (10). There is inconsistent data on cholesterol esterification at different physiological and pathological states (11-22). In vitro experiments, the HDL fraction of patients serum is incubated with an emulsion of radiolabeled cholesterol. The results of such studies showed that the rate of transferring and esterification of cholesterol to HDL was higher in the patients with diabetes or CAD and was lower if statins were used (11-17). Opposite results were observed if cholesterol esterification was mea-

sured directly in serum of these patients (18-20). Finally cholesteryl esters (CE) are transferred to the lighter fractions by cholesteryl esters transfer protein (CETP). The activity of CETP has also changed at diabetes or CAD and following treatment with statins (20-23).

The role of cholesterol in lipoprotein fractions is well defined in the pathogenesis of atherosclerosis. Free cholesterol is biologically active form, whereas CE is for storage or transport. We proposed the hypothesis that the atherogenicity of LDL particles may be attributed partly to its unesterified cholesterol, whereas antiatherogenic activity of HDL may be due to its esterified cholesterol (24). In spite of the large number of studies on the role of cholesterol, there are limited data on esterified- and unesterified- cholesterol in lipoprotein fractions at different situations. In the current study, the effect of statin treatment was studied on fractional cholesterol esterification (FCE) as well as the complete profile of lipids and lipoproteins in a case-control study.

2. SUBJECTS AND METHODS

Experimental design and subjects

The research was an age and sex matched case-control study. The study population was 400 subjects who were referred regularly to check for cardiovascular risk factors. They had abnormal sport-test and introduced to have their first coronary angiography at Zahra Hospital of Mazandaran University. The exclusion criteria of the patients were as: recent history of acute myocardial infarction, percutaneous transluminal coronary angioplasty, infectious, liver or renal disease, neoplasm and hematologic disorders. The subjects were divided into two groups according using statins. The case patients were treated with atorvastatin or rosuvastatin (20-40 mg/day) at least for 12 weeks, the control subjects never have used antilipidemic drugs. The criterion to be CAD cases was significant narrowing (lesions ^{3}50%) at any coronary artery (25). The patients who had fasting glucose more than 125 mg/dL or consumed hypoglycemic agents were defined as diabetes mellitus. The anthropometrics were measured as described previously (25).

Biochemical and hematological measurements

The collection of blood samples and preparation of serums are described in reference 25. All measurements were done on fresh serum except that of apolipoproteins, LDLc and HDLc, which stored at -70°C before analysis for maximum of six months. The concentration of serum total cholesterol and triglycerides (TG) were analyzed enzymatically by the CHOD-PAP and GPO-PAP methods respectively. New homogeneous methods were used to determine LDL- and HDL- cholesterol directly (27). Unesterified total cholesterol and unesterified HDLc were measured by the same kits but without the enzyme of cholesterol esterase (Pars-Azmon Inc., Tehran). Esterified total cholesterol and esterified HDLc were computed by subtractions of unesterified fractions from total cholesterol and HDLc (24). Immuno-turbidometric assay was used to measure apoB100 and apoAI (26). Inter- and intra-assay coefficients of variance were less than 5% for all measurements. Routine laboratory methods were applied to measure all other biochemical and hematological parameters.

Statistical analysis

The results of variables with normal distribution are presented as means ± SD, whereas skewed variables are shown as median (25th-75th percentiles). Proportions and means (or median) were calculated for risk factors. The student's t-test and Mann-Whitney test were used to evaluate the significance of differences in proportions (or medians) and in means respectively using SPSS version-21. The p- values are two-tailed and the differences were considered significant when $p \leq 0.05$.

3. RESULTS

Characteristics of the sample study

Clinical characteristics of the study population are presented in table-1. Of the 400 participants, 187 (45.6%) were men, 247 (60.2%) were verified to have CAD and 125 (30.5%) individuals had diabetes mellitus. More than third of the patients consumed aspirin, nitrates, beta-blockers and statins.

Demographic and clinical parameters of case-control groups

There were no significant differences in age, sex and cigarette smoking between two groups (Table 2). Diabetes mellitus and hypertension was more prevalent in the case group relative to controls. The values of hemoglobin level and leukocytes counts are presented as the markers of dehydration and inflammation. The levels of serum glucose, creatinine and hemoglobin were not changed significantly between two groups. The subjects in the case group had higher levels of BUN and leukocytes counts than the control subjects due to more prevalence of diabetes in this group (25, 26).

The concentrations of triglyceride, atherogenic index (log(TG)/HDLc) and aopAI had not significant changes

Variable	
Age, year	58.0 ± 9.9
Gender, male%(n)	45.6 (187)
CAD, %(n)	60.2 (247)
BMI, kg/m^2	27.4 ± 4.3
Physical inactivity, %(n)	45.4 (186)
Smoking, %(n)	18.3 (75)
Diabetes mellitus, %(n)	30.5 (125)
Systolic pressure, mmHg	118.3 ± 21.3
Diastolic pressure, mmHg	67.2 ± 16.5
Hypertension, %(n)	55.1 (226)
Drugs:	
Hypoglycemic, %(n)	12.0 (49)
Statins, % (n)	30.7 (126)
Diuretics, %(n)	7.3 (30)
Nitrates, %(n)	31.2 (128)
Beta-blockers, %(n)	38.3 (157)
Calcium antagonists, %(n)	8.3 (34)
ACE-inhibitors, %(n)	9.5 (39)
Aspirin, % (n)	43.7 (179)

Table 1. Characteristics of the study sample. The continuous and dichotomous variables were presented as means ± SD and frequencies, respectively. The number in each group has shown in parentheses.

	Without Statins (n≈250)	With Statins (n≈125)	P
Clinical characteristics: Age, year	58.0 ± 10.1	58.1 ± 9.9	0.913
Gender, male%(n)	47.5 (121)	46.0 (58)	0.794
Smoking, %(n)	21.6 (55)	13.5 (17)	0.056
Diabetes mellitus, %(n)	26.7 (68)	41.3 (52)	0.003
Hypertension, %(n)	50.6 (129)	74.5 (94)	0.001
Biochemicals: Glucose, mg/dL	122.6 ± 56.2	130.5± 34.4	0.232
BUN, mg/dL	17.0 ± 5.5	18.6 ± 9.6	0.044
Creatinine, mg/dL	1.03 ± 0.48	1.05 ± 0.45	0.718
Hemoglobin, g/dL	13.0 ± 1.6	13.2 ± 1.6	0.278
Leukocyte counts (cells/nL)	8.0 ± 2.2	8.8 ± 2.1	0.001
Lipids profile:			
Triglycerides, mg/dL	156 (114–244)	159 (114–233)	0.912*
Log(TG)/HDLc	2.22 ± 0.23	2.22 ± 0.23	0.991
Total cholesterol, mg/dL	205.3 ± 48.4	165.6 ± 38.0	0.001
Unesterified	80.3 ± 26.7	63.2 ± 22.2	0.001
Esterified	124.1 ± 35.8	102.5 ± 30.3	0.001
HDLc, mg/dL	42.3 ± 9.9	40.4 ± 10.0	0.079
Unesterified	15.2 ± 5.1	10.9 ± 3.4	0.001
Esterified	27.2 ± 9.5	29.5 ± 8.2	0.020
LDLc, mg/dL	109.6 ± 32.8	84.9 ± 27.0	0.001
NonHDLc	162.8 ± 45.2	125.2 ± 35.2	0.001
Unesterified	65.2 ± 25.5	52.4 ± 21.5	0.001
Esterified	96.6 ± 34.1	74.7 ± 27.3	0.001
ApoAI, mg/dL	172.8 ± 46.0	172.6 ± 54.8	0.978
ApoB100, mg/dL	122.3 ± 37.6	103.9 ± 34.4	0.001

Table 2. Demographic and clinical characteristics in controls and statin treated patients. The continuous and categorical variables were compared by t- and X^2-tests, respectively. The number in each group has shown in parentheses. The results are presented as the means ± SD and median (25% and 75% interquartile range). Mann-Whitney test (*).

between two groups, but apoB100 decreased significantly in statin treated group (103.9 ± 34.4 mg/dL vs. 122.3 ± 37.6, p≤0.001). Total cholesterol was decreased significantly by about 20% by treatment with statins (165.6 ± 38.0 mg/dL vs. 205.3 ± 48.4, p≤0.001), and the reduction was occurred equally on both unesterified (63.2 ± 22.2 mg/dL vs. 80.3 ± 26.7, p≤0.001) and esterified cholesterol (102.5 ± 30.3 mg/dL vs. 124.1 ± 35.8, p≤0.001). About 90% of the reduction in total cholesterol was seen in nonHDL and 10% in HDL fractions. Reduction of non-HDL cholesterol (125.2 ± 35.2 mg/dL vs. 162.8 ± 45.2, p≤0.001) also occurred equally on both unesterified (52.4 ± 21.5 mg/dL vs. 65.2 ± 25.5, p≤0.001) and esterified cholesterol (74.7 ± 27.3 mg/dL vs. 96.6 ± 34.1, p≤0.001). But, the decrease in HDLc (40.4 ± 10.0 mg/dL vs. 42.3 ± 9.9, p≤0.079) was occurred exclusively in unesterified fraction (10.9 ± 3.4 vs. 15.2 ± 5.1, p≤0.001) and was compensated with a significant increase in esterified fraction (29.5 ± 8.2 mg/dL vs. 27.2 ± 9.5, p≤0.020).

The effects of diabetes, CAD and statins on FCE

The study population of 400 subjects was divided into four groups according to have diabetes and statin consumption. Fractional cholesterol esterification (FCE) was computed as the ratio of esterified- to total- cholesterol in each fraction of lipoproteins. Fig-1 shows that 61.0 ± 9.7 % of total cholesterol is esterified in the control group and was reduced in diabetes cases slightly and enhanced in statins treated groups. Cholesterol esterification was changed totally and significantly in HDL but not in non-HDL fraction. Cholesterol esterification in HDL was decreased in diabetes group (58.0 ± 14.9 % vs. 65.3 ± 11.9, p≤0.01) and increased significantly following treatment with statins (73.5 ± 6.9 % vs. 58.0 ± 14.9, p≤0.01). The percent of cholesterol esterification did not change significantly in nonHDL fractions (59.3 ± 9.4 % vs. 59.4 ± 12.4, p≤0.153).

We also divided the study population into four groups according to have CAD and statin consumption. Figure 2 shows a similar pattern, so that cholesterol esterification in HDL fraction was diminished in CAD patients (60.7 ± 14.9 % vs. 67.5 ± 8.1, p≤0.01) and enhanced by treatment with statins (72.4 ± 7.8 % vs. 60.7 ± 14.9, p≤0.01).

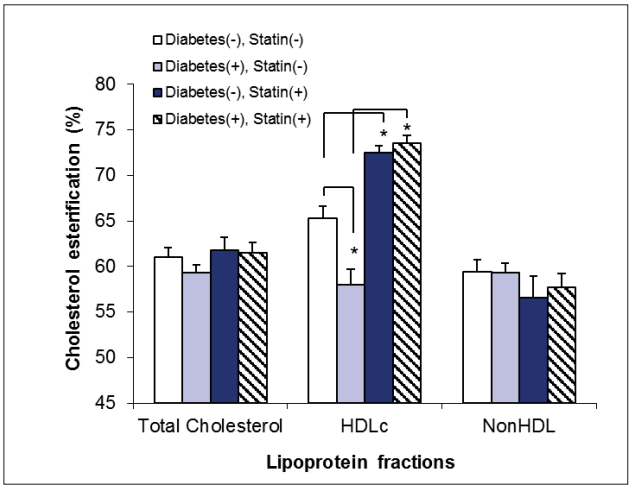

Figure 1. The effects of diabetes and statin treatment on FCE. The study population was stratified into four groups according to have diabetes mellitus and statin consumption. The number of subjects in each group was 187, 82, 70 and 69 respectively. * Indicates p<0.01.

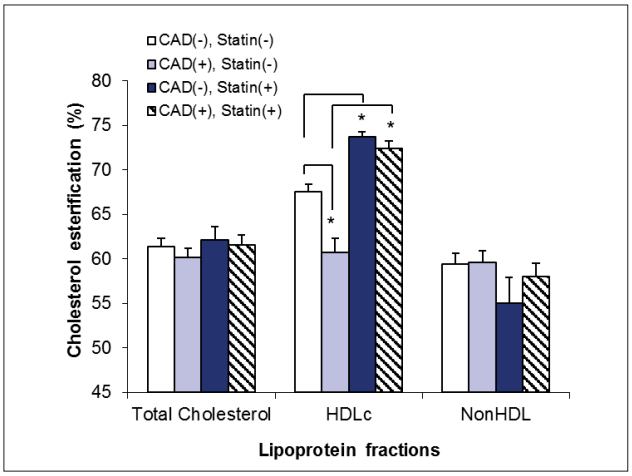

Figure 2. The effects of CAD and statin treatment on FCE. The study population was stratified into four groups according to have CAD and statin consumption. The number of subjects in each group was 93, 60,150 and 80 respectively. * Indicates p<0.01.

4. DISCUSSION

In the current study, the effects of treatment with statins were investigated on serum cholesterol esterification and the profile of lipids and lipoproteins. The results showed that statins causes total cholesterol to decrease by about 20% and the reduction was occurred equally on both esterified and unesterified cholesterol. About 90% of total cholesterol reduction was done in nonHDL and 10% in HDL fractions. Reduction of nonHDL cholesterol also occurred equally on both esterified and unesterified cholesterol. But reduction in HDLc occurred exclusively in unesterified fraction and was accompanied with a significant increase in esterified cholesterol. The results also show that, cholesterol esterification in HDL was decreased significantly in diabetes and CAD patients and enhanced by using statins (Figure 1 and 2).

There are consistent reports about the effects of statins on the intracellular metabolism of cholesterol (3-5). Treatment by statins causes coordinate inhibition of HMG-CoA reductase, ACAT and LDL-receptors. These actions generally diminish *de novo* synthesis of cholesterol, cholesterol esterification and accumulation and the influx of exogenous cholesterol (4-5).

In the other hand, the reports of statin treatment are contradicted on the details of the process of cholesterol revers transport (11-22). In general, two types of experiments have been applied to study this process. *In vitro* experiments, an emulsion of radiolabeled cholesterol is incubated with the HDL fraction isolated from plasma of the patients. These studies uniformly showed that, cholesterol esterification is increased in diabetes (10-12), and CAD (11-14) and treatment with statins causes to diminish the transfer of unesterified cholesterol to HDL, esterification of cholesterol (catalyzed by LCAT) and transfer of CE to chylomicron and VLDL remnant (catalyzed by CETP) (15-22). The researchers making this finding may have been misled by the fact that statins inhibit intracellular esterification of cholesterol via ACAT (3-5). The results of experiments using radio-labeled tracers also may be affected profoundly by the phenomena named as isotopic dilution and isotopic exchanging (3). So that, as radio-labeled glycerolipids was added to the incubation mixture, the labeled fatty acid will be appeared rapidly on cholesteryl esters by lipolysis/esterification cycle (3).

In vivo case-control studies, the rate of cholesterol esterification and transferring are evaluated directly by measuring esterified cholesterol or indirectly by the assay of LCAT and CETP activities respectively (18-23). All these assays indicated uniformly that cholesterol esterification is reduced in diabetes or CAD and treatment with statins enhances both the LCAT and CETP activities. In the current study, cholesterol esterification was evaluated directly by measuring the percentage of esterified- to total- cholesterol. These results are exactly in contrast with the *in vitro* experiments (11-23). The findings of the present study in accordance with the other *in vivo* case-control studies confirmed that cholesterol esterification in HDL fraction is decreased significantly in diabetes and CAD patients. However, as our best knowledge, the current results show for the first time that statin treatment reduces unesterified cholesterol in non-HDL fraction while enhances cholesteryl esters in HDL fraction.

5. CONCLUSION

The results suggest that cholesterol esterification in HDL fraction was decreased in diabetes and CAD patients and enhanced following treatment with statins.

- **Competing interests:** All authors declare that they have no conflict of interest.
- **Authors' contributions:** Prof Rasouli designed and conducted the study, analyzed the data, interpreted the findings and wrote the manuscript. Prof Bagheri performed coronary angiography and analyzed its results. Alikhani, and Mokhtari were PhD students and performed the experiment.
- **Acknowledgments:** The authors thank Mal Haysom, Australia for proof-reading this manuscript.
- **Abbreviations:** CAD; Coronary Artery Disease, CE; Cholesteryl Esters, CETP; Cholesteryl Esters Transfer Protein, HDL; High Density Lipoproteins, LDL; Low Density Lipoproteins, LCAT; Lecethin:Cholesterol Acyl Transferase, VLDL; Very Low Density Lipoproteins.

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