BJPS

Cholesterol oxides inhibit cholesterol esterification by lecithin:cholesterol acyl transferase

Eder de Carvalho Pincinato[2], Patricia Moriel[3], *Dulcinéia Saes Parra Abdalla[1]

[1]Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo, [2]Mackenzie Presbyterian University, [3]Department of Clinical Pathology, Faculty of Medical Sciences, State University of Campinas

Cholesterol oxides are atherogenic and can affect the activity of diverse important enzymes for the lipidic metabolism. The effect of 7β-hydroxycholesterol, 7-ketocholesterol, 25-hydroxycholesterol, cholestan-3β,5α,6β-triol,5,6β-epoxycholesterol, 5,6α-epoxycholesterol and 7α-hydroxycholesterol on esterification of cholesterol by lecithin:cholesterol acyl transferase (LCAT, EC 2.3.1.43) and the transfer of esters of cholesterol oxides from high density lipoprotein (HDL) to low density lipoproteins (LDL) and very low density lipoproteins (VLDL) by cholesteryl ester transfer protein (CETP) was investigated. HDL enriched with increasing concentrations of cholesterol oxides was incubated with fresh plasma as source of LCAT. Cholesterol and cholesterol oxides esterification was followed by measuring the consumption of respective free sterol and oxysterols. Measurements of cholesterol and cholesterol oxides were done by gas-chromatography. ^{14}C-cholesterol oxides were incorporated into HDL_2 and HDL_3 subfractions and then incubated with fresh plasma containing LCAT and CETP. The transfer of cholesterol oxide esters was followed by measuring the ^{14}C-cholesterol oxide-derived esters transferred to LDL and VLDL. All the cholesterol oxides studied were esterified by LCAT after incorporation into HDL particles, competing with cholesterol by LCAT. Cholesterol esterification by LCAT was inversely related to the cholesterol oxide concentration. The esterification of ^{14}C-cholesterol oxides was higher in HDL_3 and the transfer of the derived esters was greater from HDL_2 to LDL and VLDL. The results suggest that cholesterol esterification by LCAT is inhibited in cholesterol oxide-enriched HDL particles. Moreover, the cholesterol oxides-derived esters are efficiently transferred to LDL and VLDL. Therefore, we suggest that cholesterol oxides may exert part of their atherogenic effect by inhibiting cholesterol esterification on the HDL surface and thereby disturbing reverse cholesterol transport.

Uniterms: Cholesterol/oxides. Cholesterol/acyl transferase. Lecithin. Protein/cholesteryl ester transfer. Lipoprotein/high density. Cholesterol/reverse transport.

Os óxidos de colesterol são aterogênicos e podem afetar a atividade de diversas enzimas importantes para o metabolismo lipídico. Este estudo investigou o efeito dos óxidos 7β-hidroxicolesterol, 7-cetocolesterol, 25-hidroxicolesterol, colestan-3β,5α,6β-triol, 5,6β-epoxicolesterol, 5,6α-epoxicolesterol e 7α-hidroxicolesterol na esterificação do colesterol por ação da lecitina colesterol aciltransferase (LCAT, EC 2.3.1.43) e a posterior transferência dos óxidos esterificados da lipoproteína de alta densidade (HDL) para as lipoproteínas de baixa densidade (LDL) e muito baixa densidade (VLDL) mediada pela proteína de transporte de éster de colesterol (CETP). Para atingir os objetivos, HDL enriquecida com concentrações crescentes de óxidos de colesterol foi incubada com plasma fresco pobre em lipoproteínas, como fonte de LCAT; posteriormente a esterificação do colesterol e dos óxidos de colesterol foi medida pelo consumo do colesterol livre e dos óxidos livres presentes na HDL. As determinações de colesterol e dos óxidos de colesterol foram realizadas por cromatografia gasosa. ^{14}C-óxidos de colesterol foram incorporados nas subfrações HDL_2 e HDL_3 e posteriormente incubados com plasma fresco, contendo LCAT e CETP. A transferência dos ésteres de óxidos de colesterol foi medida e quantificada pela presença desses ésteres na LDL e VLDL. Todos os óxidos de colesterol estudados foram esterificados pela LCAT após incorporação nas partículas de HDL e competiram com a esterificação do colesterol nativo. A esterificação do colesterol pela LCAT foi inversamente relacionada à concentração de óxidos de

Correspondence: D. S. P. Abdalla. Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, Cidade Universitária, 05508-900 - São Paulo – SP, Brasil. E-mail: dspa@usp.br

colesterol. A esterificação dos óxidos de colesterol foi maior na HDL_3 e a transferência desses ésteres foi maior a partir da HDL_2 para a LDL e VLDL. Estes resultados indicam que a esterificação do colesterol pela LCAT é inibida nas partículas de HDL enriquecidas com óxidos de colesterol e que os ésteres de óxidos de colesterol são eficientemente transferidos para a LDL e VLDL. Portanto, sugere-se que os óxidos de colesterol exercem parte de seu efeito aterogênico pela inibição da esterificação do colesterol na superfície da HDL, causando um distúrbio no transporte reverso do colesterol, além de aumentar o potencial aterogênico da LDL e VLDL.

Unitermos: Colesterol/óxidos. Colesterol/aciltransferase. Lecitina. Proteína/de transporte de éster de colesterol. Lipoproteína/de alta densidade. Colesterol/transporte reverso.

INTRODUCTION

High density lipoprotein (HDL) is the only anti-atherosclerotic lipoprotein in plasma (Kawano *et al.*, 2008) and plays an important role in the reverse cholesterol transport by removing cholesterol from peripheral cells to the liver and steroidogenic organs (Fielding and Fielding, 1995; Von Eckardstein, 1996; Oram and Yokoyama, 1996). There is a widespread view that HDL protects against the development of atherosclerosis and counteracts the effects of LDL by removing cholesterol from cells of the arterial intima, thus preventing the formation of foam cells (Fielding, Fielding, 1995). In a specific pathway preβ1-HDL (a HDL subfraction), interacts with cell membranes and takes up cholesterol via ATP-binding cassette (ABC) A1 expressed on the cell surface (Kawano *et al.*, 2008). The second major and critical step in reverse cholesterol transport is the esterification of cholesterol on the HDL surface in a reaction catalyzed by lecithin:cholesterol acyl transferase (LCAT; EC 2.3.1.43). LCAT catalyzes the transfer of sn-2 acyl group from phosphatidylcholine to cholesterol (Glomset, 1968; Aron *et al.,* 1978; Fielding, 1990), resulting in the movement of the formed cholesteryl esters to the center of the HDL, leaving the particle surface depleted of cholesterol. This creates a concentration gradient that promotes the transfer of cholesterol from cell membranes to HDL. An increased rate of LCAT-catalyzed cholesterol esterification will, therefore, enhance the rate of reverse cholesterol transport (Barter, Rye, 1996). This process is followed by the transfer of cholesteryl esters from HDL to apo B-containing lipoproteins in exchange for triglycerides mediated by the cholesterol ester transfer protein (CETP) (Iglesias *et al.*, 1996). This way, the cholesteryl esters formed in the HDL are mainly channeled to VLDL and LDL, and are finally removed primarily by the liver.

The interest on cholesterol oxides is mainly due to their various biological effects, such as, cytotoxicity, inhibition of HMG-CoA reductase activity, activation of ACAT, inhibition of LDL binding to cell receptors (Peng *et al*., 1991) as biomarker of oxidative stress (Ferderbar *et al*., 2007) and inhibition of cholesterol efflux from cells (Kilsdonk *et al*., 1995; Schroepfer, 2000).

Oxysterols are the 27-carbon products of cholesterol oxidation by both enzymatic and non-enzymatic mechanisms. Their roles on cholesterol homeostasis, as well as in diseases in which oxidative damage and lipid peroxidation are implicated (e.g. atherosclerosis), have been investigated (Van Reyk *et al.,* 2006). However, considering that the reverse cholesterol transport is a protective pathway of lipoprotein metabolism against cholesterol accumulation in the body, and considering that this mechanism would be interfered by cholesterol oxides, we investigated the effect of cholesterol oxides on cholesterol esterification by LCAT and the transfer of resulting cholesteryl esters formed in HDL to VLDL and LDL by CETP.

MATERIAL AND METHODS

Material

Cholesterol oxides (7β-hydroxyholesterol, 7-keto-cholesterol, 25-hydroxycholesterol, cholestan-3β,5α,6β-triol, 5,6β-epoxycholesterol, 5,6α-epoxycholesterol and 7α-hydroxycholesterol) were purchased from Steraloids, Inc. (Wilton NH, USA); ^{14}C-cholesterol was obtained from Amersham, Inc (Arlington Heights, IL); methanol (99 %), ethanol (99 %), chloroform (99 %), *bis*(trimethylsilyl)-trifluoracetamide (BSTFA-98 %), *N*-hexane, acid acetic diethyl ether and *N,N*,dimethylphormamide were purchased from Merck (Darmstadt, Germany); linoleic acid, lipoxidase from *Glycine max* (soybean), Na_2 EDTA and NaCl were obtained from Sigma Aldrich (St. Louis, USA).

Lipoprotein isolation

Venous blood was obtained from overnight-fasted adult human volunteers. Blood was collected into 10 mL Vacutainer tubes containing EDTA (1 mg/mL blood). VLDL (d<1.0021) LDL (1.021<d>1.065), total HDL (1.065<d>1.210) and lipoprotein deficient serum (LPDS;

d>1.210) were separated by preparative sequential ultracentrifugation with a Beckman 40T rotor (Redgrave, 1975). HDL_2 (1.065<d>1.125) and HDL_3 (1.125<d>1.210) were separated from total HDL by saline gradient ultracentrifugation in a Hitachi 4 ST rotor (Redgrave, 1975). All the lipoproteins and the LPDS were exhaustively dialyzed at 4 °C against 0.15 M NaCl/1 mM-Na_2EDTA, pH 7.4.

^{14}C-cholesterol oxides formation

To obtain ^{14}C-cholesterol oxides, a solution containing 1.89 µmol of ^{14}C-cholesterol and 9.45 µmol of linoleic acid in ether was prepared. Borate buffer (0.2 M, pH 9.0) was added to the solution and mixed to form micelles. After the solvent evaporation, lipoxidase (EC 1.13.11.12 - 300.000 U) was added (Fruebis *et al.*, 1992) and conjugated dienes kinetic formation was monitored at 234 nm (spectrophotometer UV-Vis U3000, Hitachi, Japan), to evaluate the lag phase of lipid peroxidation. When the lag phase was finished, more 300.000 U lipoxidase were added to the system to ensure the total cholesterol oxidation (Esterbauer *et al.*, 1992). The solution was mixed overnight and the cholesterol oxides were isolated from cholesterol and other lipids as described previously (Sevanian, Mcleod, 1987). In this procedure 68 % of ^{14}C-cholesterol was oxidized. The pool of ^{14}C-cholesterol oxides yielded from ^{14}C-cholesterol oxidation was used in all experiments.

^{14}C-cholesterol oxides and cholesterol oxides incorporation into HDL

Non-labeled cholesterol oxide standards or the pool of ^{14}C-cholesterol oxides (0.15 µCi/mL) were added to glass vials and the solvent was evaporated under nitrogen. These cholesterol oxides were re-suspended in ethanol and a 2 mL solution of HDL (35 mg/dL cholesterol) was added. The mixture was incubated at 37 °C for 3 h in a shaking water bath. An aliquot of red blood cells suspension was added as described (Oda *et al.*, 1990) to adsorb the cholesterol and cholesterol oxides not incorporated into HDL.

Esterification of cholesterol oxides (standards and radiolabeled)

Cholesterol oxide-enriched HDL particles (along with radiolabeled cholesterol or cholesterol oxide standards) were incubated with lipoprotein deficient serum (LPDS, 60 µg/mL protein, source of LCAT) at 37 °C, for 1h, in a shaking water bath. Immediately afterwards, the reaction was stopped by placing the tubes in ice. Aliquots

were collected at the moment of LPDS addition and at the end of specified incubation times and analyzed by GC-FID (Hewlett Packard, HP-6890) to measure cholesterol oxides and by cintilography to evaluate ^{14}C-cholesterol oxides. For the chromatographic analyzes samples were derivatized to trimethylsilyl (TMS) ethers and 4 µL aliquots were injected into the gas chromatograph equipped with flame ionization detector as described elsewhere (Sevanian *et al.*, 1994).

Transfer of ^{14}C-cholesteryl oxide esters from HDL to apoB-containing lipoproteins

^{14}C-cholesterol-HDL subfractions (HDL_2 and HDL_3, previously incubated with LCAT) were incubated with LDL (140 mg/dl cholesterol) and VLDL (25 mg/dL cholesterol) for 6 hours at 37 °C. The reactions were stopped on ice and lipoproteins reisolated by sequential ultracentrifugation. Aliquots were extracted with chloroform:methanol (2:1) and the radioactivity analyzed in a liquid scintillation counter (Packard 1900 TR-liquid scintillation analyzer, Meriden, CT 06450). 5,5-Dithiobis-(2-nitrobenzoic acid (DTNB) was added in the transfer experiments to inhibit LCAT and *p*-chloromercuriphenylsulfonic acid (pCMPS) was added for CETP inhibition.

Free and esterified ^{14}C-cholesterol oxide analysis

Free and esterified ^{14}C-cholesterol oxides were separated by thin layer chromatography using a solvent mixture consisting of n-hexane/diethyl ether/acetic acid/methanol (85:20:1:1; v/v/v/v) and the chromatographic plates were visualized using iodine vapor. The spots corresponding to free cholesterol and cholesteryl esters were scraped from the plates and re-suspended in scintillation solution (POPOP - 0,15 g; PPO - 5,0g; Triton - 500 mL; toluene q.s.p 1000 mL) to measure the radioactivity.

Statistical Analysis

Data are reported as means ± SEM. Statistical analysis was performed by the Tukey test as a post-test after ANOVA, with p < 0.05 considered to be significant (Sigma Stat software).

RESULTS AND DISCUSSION

Cholesterol oxides incorporation into HDL is shown at Table I. The percentage of incorporation of cholesterol oxides into HDL ranged from 11 to 60% with the highest incorporation found for the 7-ketocholesterol and the lowest for cholestan-3b,5a,6b-triol (triol), the lowest incorporation

of triol may be related to its higher hydrophilicity. All the cholesterol oxides incorporated into HDL were esterified by LCAT from human plasma (Figure 1). This is in accordance with data previously described for LCAT catalyzed esterification of oxysterols in discoidal bilayer particles (Nishida *et al*., 1993). However, the esterification yield varied among the different cholesterol oxides with the lowest esterification taking place with triol. This suggests that triol is not an efficient substrate for LCAT, possibly due to its higher hydrophilicity compared to the other cholesterol oxides, and/or steric hindrance from the 5,6-hydroxyl moieties, which may reduce interaction with the hydrophobic catalytic site of LCAT (Peelman *et al*., 1998)

**TABLE I -** Incorporation of cholesterol oxides into HDL

	incorporation into HDL (%)
cholesterol	-
cholestan-3β,5α,6β-triol	11
25-hydroxycholesterol	53
cholesterylβ-epoxide	54
7α-hydroxycholesterol	43
cholesterylα-epoxide	56
7β-hydroxycholesterol	40
7-ketocholesterol	60
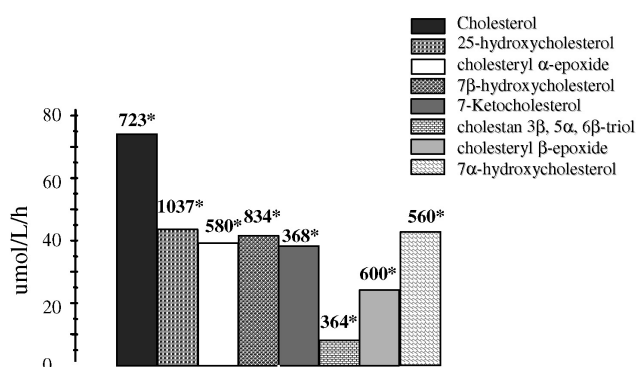
FIGURE 1 - Esterification rate of cholesterol oxides incorporated into HDL, in relation to an HDL particle containing no cholesterol oxides. *Initial concentrations of cholesterol oxides (uM) incorporated into HDL.

Figure 2 shows the esterification intensity of both cholesterol and cholesterol oxides in the same HDL particle. It is noteworthy that the esterification of cholesterol is affected by the presence of cholesterol oxides in HDL. Most of the cholesterol oxides, except the triol and 7α-hydroxycholesterol, showed a trend for greater esterification compared to

cholesterol. However, significant differences were observed only for 7-ketocholesterol and 5,6β-epoxycholesterol. The most pronounced inhibition of cholesterol esterification by LCAT was induced by 7-ketocholesterol.
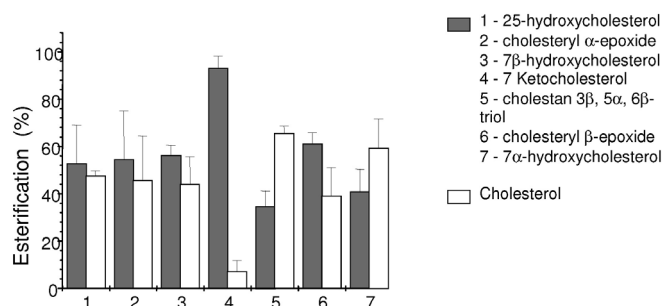
FIGURE 2 - Esterification (%) of cholesterol and cholesterol oxides both in the same HDL particle. The relative esterification was calculated considering a sum of the esters formed from cholesterol and cholesterol oxides as 100% of esterification.

The esterification of oxysterols catalyzed by LCAT was also affected when examined in HDL subfractions. ^{14}C-cholesterol oxide-enriched HDL subfractions showed different degrees of esterification efficiency. The ^{14}C-cholesterol oxides esterification was higher in the HDL_3 subfraction (2.6 ± 0.5 nCi, n=6) as compared to HDL_2 (1.4 ± 0.3 nCi, n=6), as occurs for cholesterol esterification by LCAT. Accordingly, Fielding *et al*. (1989) proposed that the efflux of radiolabeled cholesterol from fibroblasts promoted by HDL, and its further esterification on this lipoprotein by LCAT, differed among HDL subfractions. The enhancement of LCAT activity was directly related with the increase of HDL_3 and inversely correlated with HDL_2 (Dobiasova *et al*., 1991, 1992).

The investigation of the influence of cholesterol oxides concentration on cholesterol esterification showed that the inhibition of cholesterol esterification by LCAT was strongly dependent on cholesterol oxides concentration. As it can be seen at Table II, a concentration-dependent inhibition of cholesterol esterification was found, as the cholesterol oxide concentration increases in HDL particle.

Another important step for reverse cholesterol transport is the transfer of cholesteryl esters formed in HDL to the apo-B containing lipoproteins. Figures 3 and 4 show the transfer of labeled cholesterol oxides present in HDL subfractions to LDL and VLDL, respectively. ^{14}C-cholesterol oxides were first esterified in HDL enriched particle in the presence or absence of DTNB (5,5-Dithio-*bis*-(2-nitrobenzoic acid), a LCAT inhibitor. Later, these cholesterol oxides-enriched HDL particles were separately incubated with LDL and VLDL and LPDS, as source of

TABLE II - Dependence of cholesterol and cholesterol oxide esterification rate on cholesterol oxide concentration

Cholesterol oxide	Concentration (uM)	Cholesterol esterification rate (umol/L/h)	Cholesterol oxide esterification rate (umol/L/h)
25-hydroxy-cholesterol	35	23.2	2.9
	52	13.4	3.0
	74.4	10.3	3.9
	163	1.6	6.9
cholestan 3β, 5α, 6β-triol	24.6	52.0	2.0
	93	46.6	6.9
	232	45.4	15.5
	444	17.4	16.1
7α-hydroxy-cholesterol	405	52.8	67.5
	544	43.9	77.9
	620	38.2	82.5
	744	36.1	86.8
5,6 β-epoxy-cholesterol	152	38.4	5.1
	348	35.6	17.6
	487	36.6	65.6
	1491	32.2	164.5
7-ketocholesterol	187	38.4	54.2
	343	35.7	100.0
	603	24.8	148.6
	1100	13.9	184.2
7β-hydroxy-cholesterol	91	54.2	13.4
	98	53.1	14.0
	209	45.1	27.9
	397	35.1	39.5
5,6 α-epoxy-cholesterol	64.5	165.0	20.9
	497	165.9	162.3
	1512	104.8	312.1
	3338	92.7	609.8

CETP, in the presence or absence of pCMPS (*p*-chloro-mercuriphenylsulfonic acid) which inhibits CETP. The results show that DTNB efficiently inhibited esterification by LCAT, although pCMPS inhibited only 50% of CETP activity. The transfer of labeled cholesterol oxide esters from HDL to LDL and VLDL was similar for both particles (approximately 50% of the labeled substrates being transferred in each case), which were incubated separately with HDL. The transfer of radiolabeled cholesterol oxide esters was higher in HDL_2 subfraction (approximately 50%) as compared with HDL_3 subfraction (approximately 13%). This may be due either to the HDL_2 subfraction containing more cholesteryl esters or by a major interaction of this particle with CETP, as proposed by Jonas (1991) for cholesterol transfer. The major esterification of cholesterol and cholesterol oxides in HDL_3 dynamically modifies this subfraction to the form HDL_2, which is a better substrate for CETP that transforms the HDL_2 again into HDL_3.
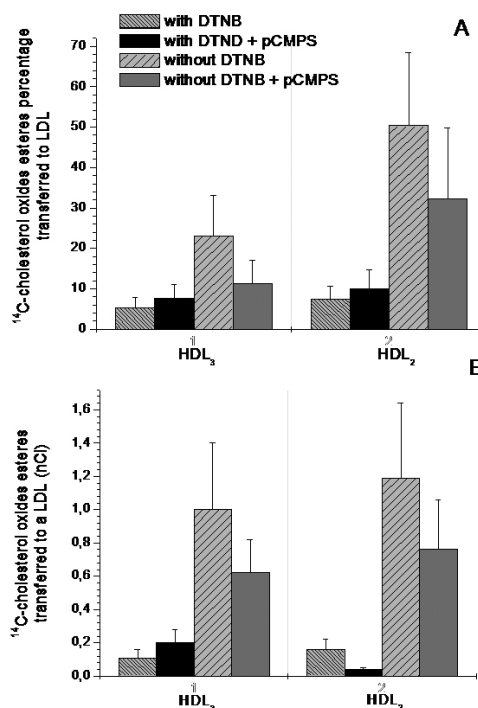
FIGURE 3 - Transfer of ^{14}C-cholesterol oxide esters from HDL_3 and HDL_2 to LDL, (A) in % of ^{14}C-cholesterol transferred and (B) in nCi. Both with DTNB (LCAT inhibitor), with DTNB plus pCMPS (CETP inhibitor) , without DTNB and, without DTNB plus pCMPS.
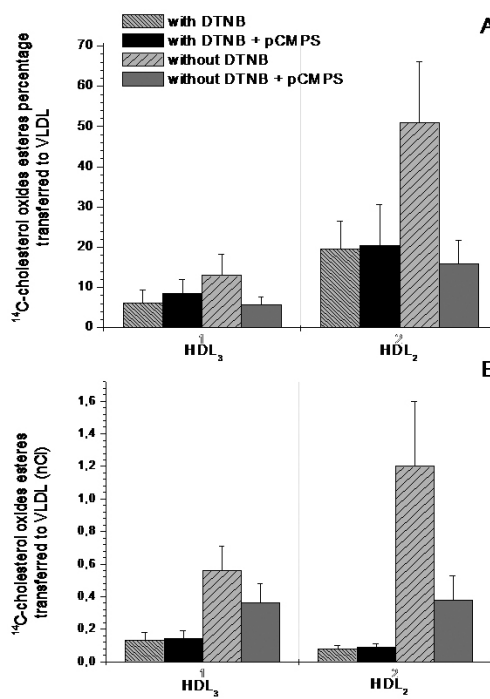
FIGURE 4 - Transfer of ^{14}C-cholesterol oxide esters from HDL_3 and HDL_2 to VLDL, (A) in % of ^{14}C-cholesterol transferred and (B) in nCi. Both with DTNB, with DTNB plus pCMPS , without DTNB and without DTNB plus pCMPS.

CONCLUSIONS

In conclusion, this study shows that cholesterol oxides are esterified by LCAT and that the efficiency of esterification differs among the different cholesterol oxides present in HDL. Cholesterol oxides diminished the LCAT-catalyzed cholesterol esterification and this may have an important effect on reverse cholesterol transport. Thus, these data indicate that the effect of cholesterol oxides on cholesterol esterification in HDL and on transfer of these cholesteryl esters to LDL and VLDL may contribute to the atherogenicity of these oxysterols.

AKNOWLEDGMENTS

REFERENCES

ARON, L.; JONES, S.; FIELDING, C.J. Human plasma lecithin-cholesterol acyltransferase. Characterization of cofactor-dependent phospholipase activity. *J. Biol. Chem.* v.253, n.20, p.7220-7226, 1978.

BARTER, P.J.; RYE, K.A. Molecular mechanisms of reverse cholesterol transport. *Curr. Opin. Lipidol.*, v.7, n.2, p.82-87, 1996.

DOBIASOVA, M.; STRIBRNA, J.; PRITCHARD, P.H.; FROHLICH, J.J. Cholesterol esterification rate in plasma depleted of very low and low density lipoproteins is controlled by the proportion of HDL2 and HDL3 subclasses: study in hypertensive and normal middle-aged and septuagenarian men. *J. Lipid Res.*, v.33, n.10, p.1411-1418, 1992.

DOBIASOVA, M.; STRIBRNA, J.; SPARKS, D.L.; PRITCHARD, P.H.; FROHLICH, J.J. Cholesterol esterification rates in very low density lipoprotein- and low density lipoprotein-depleted plasma. Relation to high density lipoprotein subspecies, sex, hyperlipidemia, and coronary artery disease. *Arterioscler. Thromb.*, v.11, n.1, p.64-70, 1991.

ESTERBAUER, H., GEBICKI, J., PUHL, H., JURGENS, G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biol. Med.*, v.13, n.4, p.341-390, 1992.

FERDERBAR, S.; PEREIRA, E.C.; APOLINÁRIO, E.; BERTOLAMI, M.C.; FALUDI, A.; MONTE, O.; CALLIARI, L.E.; SALES, J.E.; GAGLIARDI, A.R.; XAVIER, H.T.; ABDALLA, D.S. Cholesterol oxides as biomarkers of oxidative stress in type 1 and type 2 diabetes mellitus. *Diabetes Metab. Res. Rev.*, v.23, n.1, p.35-42, 2007.

FIELDING, C. J. Lecithin: cholesterol acyltransferase. In: ESFAHANI, M.; SWANEY, J. B. (Eds.). *Advances in cholesterol research*. New Jersey: Telford Press, 1990. p. 271-314.

FIELDING, C.J.; FIELDING, P.E. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* v.36, n.2, p.211-228, 1995.

FIELDING, P. E.; ISHIKAWA, Y.; FIELDING, C. J. Apolipoprotein E mediates binding of normal very low density lipoprotein to heparin but is not required for high affinity receptor binding. *J. Biol. Chem.*, v.264, n.21, p.12462-6, 1989.

FRUEBIS, J., PARTHASARATHY, S., STEINBERG, D. Evidence for a concerted reaction between lipid hydroperoxides and polypeptides. *Proc. Natl. Acad. Sci. USA*, v.89, n.22, p.10588-10592, 1992.

GLOMSET, J.A. The plasma lecithins:cholesterol acyltransferase reaction. *J. Lipid Res.* v.9, n.2, p.155-167, 1968.

IGLESIAS, A.; ARRANZ, M.; ALVAREZ, J.J.; PERALES, J.; VILLAR, J.; HERRERA, E.; LASUNCIÓN, M.A. Cholesteryl ester transfer activity in liver disease and cholestasis, and its relation with fatty acid composition of lipoprotein lipids. *Clin. Chim. Acta.*, v.248, n.2, p.157-174, 1996.

JONAS, A. Lecithin-cholesterol acyltransferase in the metabolism of high-density lipoproteins. *Biochim. Biophys. Acta.*, v.1084, n.3, p.205-220, 1991.

KAWANO, M.; NAGASAKA, S.; YAGYU, H.; ISHIBASHI, S. Pitavastatin decreases plasma prebeta1-HDL concentration and might promote its disappearance rate in hypercholesterolemic patients. *J. Atheroscler. Thromb.*, v.15, n.1, p. 41-46, 2008.

KILSDONK, E.P.; MOREL, D.W.; JOHNSON, W.J.; ROTHBLAT, G.H. Inhibition of cellular cholesterol efflux by 25-hydroxycholesterol. *J. Lipid Res.*, v.36, n.3, p.505-516, 1995.

NISHIDA, H.I.; ARAI, H.; NISHIDA, T. Cholesterol ester transfer mediated by lipid transfer protein as influenced by changes in the charge characteristics of plasma lipoproteins. *J. Biol. Chem.,* v.268, n.22, p.16352-16360, 1993.

ODA, H.; YAMASHITA, H.; KOSAHARA, K.; KUROKI, S.; NAKAYAMA, F. Esterified and total 7 alpha-hydroxycholesterol in human serum as an indicator for hepatic bile acid synthesis. *J. Lipid Res.*, v.31, n.12, p.2209-2218, 1990.

ORAM, J.F.; YOKOYAMA, S. Apolipoprotein-mediated removal of cellular cholesterol and phospholipids. *J. Lipid Res.* v.37, n.12, p.2473-2491, 1996.

PEELMAN, F.; VINAIMONT, N.; VERHEE, A.; VANLOO, B.; VERSCHELDE, J.L.; LABEUR, C.; SEGURET-MACE, S.; DUVERGER, N.; HUTCHINSON, G.; VANDEKERCKHOVE, J.; TAVERNIER, J.; ROSSENEU, M. A proposed architecture for lecithin cholesterol acyl transferase (LCAT): identification of the catalytic triad and molecular modeling. *Protein Sci.,* v.7, n.3, p.587-599, 1998.

PENG, S.K.; HU, B.; MORIN, R.J. Angiotoxicity and atherogenicity of cholesterol oxides. *J. Clin. Lab. Anal.*, v.5, n.2, p.144-152, 1991.;

REDGRAVE, T.G.; ROBERTS, D.C.; WEST, C.E. Separation of plasma lipoproteins by density-gradient ultracentrifugation. *Anal. Biochem.*, v.65, n.1-2, p.42-49, 1975.

SCHROEPFER, G.J. JR. Oxysterols: modulators of cholesterol metabolism and other processes *Physiol. Rev.,* v.80, n.1, p.361-554, 2000.

SEVANIAN, A.; MCLEOD, L.L. Cholesterol autoxidation in phospholipid membrane bilayers. *Lipids,* v.22, n.9, p.627-636, 1987.

SEVANIAN, A.; SERAGLIA, R.; TRALDI, P.; ROSSATO, P.; URSINI, F.; HODIS, H. Analysis of plasma cholesterol oxidation products using gas- and high-performance liquid chromatography/mass spectrometry. *Free Radic. Biol. Med.*, v.17, n.5, p.397-409, 1994.

VAN REYK, D.M.; BROWN, A.J.; HULT'EN, L.M.; DEAN, R.T.; JESSUP, W. Oxysterols in biological systems: sources, metabolism and pathophysiological relevance. *Redox Rep.,* v.11, n.6, p.255-262, 2006.

VON ECKARDSTEIN, A. Cholesterol efflux from macrophages and other cells. *Curr. Opin. Lipidol.* v.7, n.5, p.308-319, 1996.