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Serum Lecithin Cholesterol Acyltransferase Activity, HDL_2 and HDL_3 Amounts and Composition in Hypertensive Patients with Various High Atherosclerosis Risk

تقدير نشاط أنزيم اللستين كولسترول أسيل ترانسفرايز وكمية وتركيب الليبوبروتينات (HDL_2) و (HDL_3) لدى مرضى مصابين بارتفاع ضغط الدم وبالعديد من عوامل خطر الإصابة بداء تصلب الشرايين

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ABSTRACT: The effect of high risk atherosclerosis was investigated on HDL_2, HDL_3 amounts and composition and Lecithin cholesterol acyltransferase (LCAT) activity in 448 patients (M/W, 223/225) with high risk atherosclerosis. The mean age was 53 ± 4 years and the mean BMI was 26 ± 2. The subjects were divided into eight groups (GI- GVIII) and compared with 51 controls (M/W, 25/26) with a mean age of 50 ± 6 and BMI of 22 ± 1. Triacylglycerols concentrations were 1.8- to 2.3-fold higher in all groups than in the controls. Total cholesterol concentrations were 1.3- to 1.6-fold higher in all high risk atherosclerosis patients, than in controls. LCAT activity was decreased in patients of GI ($P<0.05$), GII, GIII, GIV ($P<0.01$), GV, GVI, GVII and GVIII ($P<0.001$), compared with the control values. A negative correlation was noted between LCAT activity and HDL_3-PL in atherosclerosis high risk patients ($r= - 0.69$, $P< 0.01$), a positive correlation between LCAT and HDL_2- CE ($r= 0.55$, $P< 0.01$). An inverse relationship was found between LCAT activity and hypertriglyceridemia ($r= - 0.76$, $P<0.01$) and between hypertriglyceridemia and HDL_2- CE ($r=- 0.63$, $P< 0.01$). A negative correlation was also reported between total serum cholesterol and HDL2- CE ($r= - 0.87$, $P<0.01$). A positive correlation was reported between total serum cholesterol and serum triacylglycerols ($r= 0.99$, $P<0.01$). The degree of alteration in the HDL_2 and HDL_3 amounts and composition, as well as LCAT activity decrease were proportional to the number of risk factors. According to the presence of one or several atherosclerosis risk factors, the alterations in HDL_2 and HDL_3 amounts and composition contribute to reduced efficacy of reverse cholesterol transport, which is another risk factor for cardiovascular disease.

Keywords: *Atherosclerosis, risk factors, LCAT, HDL_2, HDL_3.*

المستخلص : أجريت هذه الدراسة لمعرفة مدى تأثير خطر الإصابة بداء تصلب الشرايين على كمية وتركيب الليبوبروتينات (HDL_2) و (HDL_3) ونشاط أنزيم اللستين كولسترول أسيل ترانسفرايز (LCAT) لدي 448 مريضا (رجل/إمرأة، 225/223) أكثر عرضة للإصابة بداء تصلب الشرايين على عمر 53 ± 4 سنة ومؤشر لكتلة الجسم و/ط^2 26 ± 2 كلغ/م^2 تم تقسيمهم إلى ثمانية مجموعات. كما شملت الدراسة مجموعة من أشخاص أصحاء ظاهريا على عمر 50 ± 6 سنة ومؤشر لكتلة الجسم و/ط^2 22 ± 1 كلغ/م^2 اتخذت كمجموعة ضابطة. أظهرت النتائج بأن تركيز الجلسريدات الثلاثية لدى المرضى يفوق بحوالي 1.8 إلى 2.3 مرة قيمة هدا المؤشر لدى المجموعة الضابطة، وأن تركيز الكولسترول الكلي أكبر بحوالي 1.3 إلى 1.6 مرة لدى المرضى مقارنة بالمجموعة الضابطة. كما أظهرت النتائج انخفاضا معنويا لنشاط أنزيم (LCAT) وبدرجات متفاوتة أشدها لدى الفئات ف6، ف7 و ف8 ($P<0.001$) مقارنة مع المجموعة الضابطة. كما أشارت النتائج لدى المرضى إلى ارتباط سلبي بين نشاط أنزيم (LCAT) والفوسفوليبيدات (HDL_3-PL) ($r = -0.69$، $P<0.01$) وبين نشاط هدا الأنزيم وتركيز الجلسريدات الثلاثية ($r = -0.76$، $P<0.01$) وبين هذا الأخير وأسترات الكولسترول (HDL_3-CE) ($r = -0.63$، $P<0.01$) وبين الكولسترول الكلي وأسترات الكولسترول (HDL_2-CE) ($r = -0.87$، $P<0.01$) وارتباط إيجابي بين نشاط أنزيم (LCAT) وأسترات الكولسترول (HDL_2-CE) ($r = 0.55$، $P<0.01$) وبين الكولسترول الكلي والجلسريدات الثلاثيه ($r = 0.99$، $P<0.01$). يظهر من خلال هذه النتائج الإرتباط الوثيق الموجود بين درجة تغيرات كمية وتركيب الليبوبروتينات (HDL_2) و (HDL_3) وكذا انخفاض نشاط أنزيم (LCAT) وعدد عوامل خطر الإصابة بداء تصلب الشرايين بحيث كلما زاد عدد هذه العوامل كلما زاد انخفاض نشاط هذا الأنزيم يرافقه بالتوازي تغير في كمية وتركيب الليبوبروتينات (HDL_2) و (HDL_3) وهذا كله يؤدي إلى خلل في أيض الليبيدات وبالتالي خلل في أيض الكولسترول بزيادة تركيزه في الدم مما يساهم في ارتفاع احتمال الإصابة بداء تصلب الشرايين.

كلمات مدخلية : *داء تصلب الشرايين، عوامل الخطر ، لستين كولسترول أسيل ترانسفرايز (LCAT)، HDL_2، HDL_3.*

INTRODUCTION

Lecithin Cholesterol Acyltransferase (LCAT, EC 2.3.1.43) is an enzyme present in plasma (Von Eckardstein, *et al.* 2001a; Jonas, 1991). It is a 63-kDa glycoprotein produced exclusively in the liver. This enzyme circulates associated with HDL, particularly HDL_3 and is activated by apolipoprotein A-I (apoA-I). LCAT catalyzes the etherification of cholesterol, including unesterified cholesterol (UC) released while triglyceride-rich lipoproteins are broken down (Von Eckardstein, *et al.* 2001a; Tall, 1998). LCAT transfers fatty acid from the 2-position of phosphatidylcholine to the 3-hydroxyl group of cholesterol, resulting in the formation of lysophosphatidylcholine and cholesteryl esters (Moulin and Berthezène, 1994). Cholesterol esterification in plasma contributes to the formation of larger HDL_2 from small HDL_3 particles. The plasma cholesterol esterification process is for maintaining the gradient of UC between cells and HDL, Which is necessary for reverse cholesterol transport, cholesterol efflux from cells and particularly from vascular epithelial cells to plasma (Tall, 1998). The Framingham Heart Study has found that low levels of HDL_2-cholesterol are strongly associated with high risk of atherosclerotic disease (Castelli, *et al.* 1992). Thus, cholesterol esterification by LCAT may play a key role in the prevention of heart disease, improving cholesterol exportation in biliary acids (Hovingh, *et al.* 2005; Von Eckardstein, *et al.* 2001a; Tall, 1998).

Arteriosclerosis, a disease of arteries is a primary cause of heart disease and stroke (Aviram, 1999) and is the cause of 50% of all deaths in industrialized countries (Fruchart and Duriez, 2001; Hilary, *et al.* 2001). Epidemiological studies have revealed several important environmental (smoking, style feeding) and genetic risk factors associated with atherosclerosis (Fruchart and Duriez, 2001). It is now demonstrated that atherosclerosis is not a simple chronic degenerescence of vascular wall, depending on age and other factors (dyslipidemia, smoking, diabetes)

but rather a chronic inflammatory disease, inducing plaque rupture and acute heart disease (myocardial infarction and stroke) (Després, *et al.* 2000; Fruchart and Duriez, 2001). Hypertension and atherosclerosis are strongly associated and are the major cause of cardiovascular deaths (Lusis, 2000). By its effects on the artery wall, hypertension acts like a promoter of lipid metabolic disturbance and the development of atherosclerosis, artery lesion is more pronounced when hypertensive patients presents others risk factors such as diabetes (Kanter, *et al.* 2008; Ishizaka, *et al.* 2003) or abdominal obesity (Bonora, *et al.* 2000).

Numerous clinical and epidemiological studies have demonstrated the inverse and independent association between HDL-cholesterol and the risk of coronary heart disease (CHD) events (Hailing, *et al.* 2002). Moreover, HDL exerts various potentially antiatherogenic properties: for example, HDL mediates the efflux of cholesterol from lipid-loaded cells and delivers it to liver and steroidogenic organs (Von Eckardstein, *et al.* 2001a). HDL inhibits the oxidation of LDL, for example, through the action of the enzymes paraoxonase and PAF-AH (Von Eckardstein, *et al.* 2001b; Bonora, *et al.* 2000; Després, *et al.* 2000; Aviram, 1999; Mackness, *et al.* 1998). Patients with atherosclerotic high risk had, in general, a reduction of HDL_2 (Hailing, *et al.* 2002; Von Eckardstein, *et al.* 2001a; Bonnefont-Rousselot, *et al.* 1998; Moulin and Berthezène, 1994). The protective role of HDL from cardiovascular diseases has been assigned to HDL_2 (Rey, *et al.* 1999; Barrans, *et al.* 1996; Fumeron, *et al.* 1991). LpA-I is an HDL that contains apo A-I but not apo A-II. This lipoprotein is capable to promote cholesterol efflux (Fidge, 1999; Fielding and Fielding, 1995; Moulin and Berthezène, 1994). Moreover, HDL is in strong relation with the metabolism of VLDL- triacylglycerols by the lipoprotein lipase (LPL) (Von Eckardstein, *et al.* 2001a; Widhalm and Pakosta, 1991). Indeed, LPL and LCAT participate in HDL conversion process (Von Eckardstein, *et al*, 2001a; Rey, *et al.* 1999). In addition to its role in the metabolism of VLDL and chylomicrons, LPL plays a role in HDL metabolism, since the action of this enzyme permits to provide phospholipids (PL) and unesterified cholesterol as well as apolipoproteins (apo E and C) to HDL_3 that will be tranformed in HDL_2 by the action of LCAT (Rader and Jaye, 2000; Krieger, 1999). Therefore, we studied the effect of hypertension with or without other atherosclerosis risk factors such as obesity, diabetes and coronopathy on HDL_2, HDL_3 amounts and composition and Lecithin Cholesterol Acyltransferase (LCAT) activity in a sample of hypertensive patients.

MATERIAL AND METHODS

Participants

Fifty one control subjects were recruited among apparently healthy, normolipidemic Algerian blood donors. There were 25 men and 26 women with a mean age of 50 ± 6 years and mean body mass index BMI, kg/m^2 of 22 ± 1. Four hundred and forty eight hypertensive patients with or without other atherosclerosis risk factors such as diabetes, obesity and coronopathy (previous myocardial infarction or effort angina) were recruited at the Cardiology Ward of the Hospital of Tiaret (west of Algeria).The participants' blood pressure levels were retrieved from their medical records. Hypertensive people are those who had blood pressure levels > 140/90 mmHg or used antihypertensive medications. There were 223 men (M) and 225 women (W) with a mean age of 53 ± 4 years. All men were current smokers but not women. No physical activity was practised by all patients (men and women). Patients were divided into eight groups: Group I (GI), (n=54, Men/Women 27 /27) with hypertension alone (mean age 52±6 years, BMI 23±1); Group II (GII), (n=57, 28/29) with hypertension and diabetes (mean age 49±4 years and BMI 23±1); Group III (GIII), (n=54, 26/28) with hypertension and obesity (mean age 54±4 years and BMI 28±1); Group IV (GIV), (n=57, 28/29) with hypertension, diabetes and obesity (mean age 51±6 and BMI 29±2); Group V (GV), (n=57, 28/29) with hypertension and

coronopathy (mean age 50±7 years and BMI 22±2); Group VI (GVI), (n=57, 30/27) with hypertension, diabetes and coronopathy (mean age 54 ± 5 years and BMI 23±1); Group VII (GVII), (n=55, 27/28) with hypertension, obesity and coronopathy (mean age 55±5 years and BMI 29±2) and Group VIII (GVIII), (n=57, 29/28) with hypertension, coronopathy, diabetes and obesity (mean age 56 ± 3 years and BMI 32±4). The purpose of this study was explained to the subjects and the investigation was carried out with their consent. The experimental protocol was approved by Tiaret Hospital Committee for research on human subjects.

Measurements

Blood samples were drawn after a 12-h overnight fast by antecubital venipuncture in all subjects. Serum samples were collected by low speed centrifugation at 3000 x g at 5 °C, for 15 min and were preserved with 0.1% Na_2EDTA and 0.02% sodium azide.

Triacylglycerols (TG) and total cholesterol (TC) were determined in all subjects (controls and patients) by enzymatic methods (Boehringer Kits, Mannheim, Germany).

LCAT activity was measured in all subjects (controls and patients) by the non-radioactive endogenous cholesterol esterification method (Albers, *et al.* 1986), in which the decrease in serum unesterified cholesterol with incubation at 37° C was determined. Unesterified cholesterol concentration was determined at time t_0 by enzymatic colorimetric method (COD-PAP, Wako Chemicals, GmbH-Germany), and at time 4-h at 37°C t_4 with 40 µl of serum. LCAT activity was determined according to the following:

$$\text{LCAT activity (nmol EC/ ml. serum/ h)} = \frac{UC\ t_0 - UC\ t_4}{4} \cdot 10^{-6}$$

Serum lipoprotein fractions (VLDL, LDL, HDL_2 and HDL_3) were isolated among all subjects (controls and patients) according to the method of Burstein, *et al.* (Burstein, *et al.* 1989). Precipitant agents differed according to the density of each lipoprotein fraction. VLDL ($1.006 < d < 1.019$ g/ml) and LDL ($1.019 < d < 1.063$ g/ml) were precipitated using phosphotungstate (Prolabo, Paris, France) + $MgCl_2$ 2M (Merck). HDL_2 ($1.063 < d < 1.120$ g/ml), HDL_3 ($1.120 < d < 1.210$ g/ml) using dextran sulfate wt 500 000 (Sigma Chemical Co, St. Louis, (MO) + $MgCl_2$. All centrifugations were made at 4000 x g for 30 mn at ambient temperature.

Protein contents were determined for each patient by the method of Lowry (Lowry, *et al.* 1951) using bovine serum albumin (Sigma Chemical Co, St. Louis, MO) as standard. Triacylglycerols and total cholesterol assays were performed by Boehringer reagent Kits (Manheim, Germany). Unesterified cholesterol (UC) and phospholipids (PL) were determined by enzymatic colorimetric methods (COD-PAP, Wako Chemicals, GmbH- Germany). Esterified cholesterol amounts were obtained by difference between total cholesterol (TC) and unesterified cholesterol (UC) values. Cholesteryl esters (CE) amounts were estimated as 1.67 multiplied by the esterified cholesterol amount. This factor took into account the average molecular weight of cholesterol-esterifying fatty acid.

Statistical Analyses

Data are presented as mean values ± standard deviation and were initially analysed by Anova. Statistical significance was evaluated using Kruskal Wallis 1- way Anova procedure for comparisons between groups without and with one, two, three and four atherosclerosis risk factors. A probability value of < 0.05 was considered significant. There was no significant difference between values among men, compared with women in this study. Linear regression analysis was used to determine correlation coefficients between LCAT activity and HDL_2, HDL_3 amounts and composition. All statistical calculations were performed using SPSS version 14.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Serum Triacylglycerols and Total Cholesterol

Significant increase in Serum triacylglycerols and total cholesterol concentrations (Table 1) was observed in the eight groups of high risk atherosclerosis patients, compared with controls.

Triacylglycerol concentrations were 1.8- to 2.3-fold higher in all groups than in controls. However, there was no significant difference between the eight high risk atherosclerosis groups. According to the risk factors number, total cholesterol concentrations were proportionally 1.3- to 1.6-fold higher in all high risk atherosclerosis patients, than in controls. Compared with hypertensive patients (GI), total cholesterol concentrations were 1.2-fold higher ($P<0.05$) in patients with three risk factors (GVI, GVII) and four risk factors (GVIII).

Table 1. Serum Triacylglycerols and Total Cholesterol in High Risk Atherosclerosis Patients and Controls.

	Triacylglycerols (mmol/L)	Total cholesterol (mmol/L)
GI	1.57 ± 0.45 ***	6.13 ± 0.77 **
GII	1.69 ± 0.44 ***	6.49 ± 0.60 **
GIII	1.66 ± 0.47 ***	6.39 ± 0.63 **
GIV	1.71 ± 0.40 ***	6.60 ± 0.32 ***
GV	1.79 ± 0.55 ***	#6.87 ± 0.39 ***
GVI	1.91 ± 0.54 ***	#7.34 ± 0.31 ***
GVII	1.88 ± 0.57 ***	#7.22 ± 0.29 ***
GVIII	1.96 ± 0.69 ***	#7.39 ± 0.39 ***
Controls	0.85 ± 0.36	4.59 ± 0.87

*Values are means ± SD. GI, hypertension alone; GII, hypertension + diabetes; GIII, hypertension + obesity; GIV, hypertension + diabetes + obesity; GV, hypertension + coronopathy; GVI, hypertension + diabetes + coronopathy; GVII, hypertension + obesity + coronopathy; GVIII, hypertension +diabetes + obesity + coronopathy. Comparisons of the means was performed by Kruskal Wallis 1- way Anova procedure. *: High Risk Atherosclerosis Groups vs. Controls. #: GI vs. (GII, GIII, GIV, GV, GVI, GVII and GVIII). * # P<0.05; ** ## P<0.01; *** ### P<0.001.*

LCAT Activity and Serum Apolipoprotein A-I

LCAT activity, expressed in nmoles of esterified cholesterol/h/ml.serum (Table 2), was decreased in patients of GI ($P<0.05$), GII, GIII, GIV ($P<0.01$), GV, GVI, GVII and GVIII ($P<0.001$), compared with control values. A reduction by 16% in hypertensive patients GI, about 33% in patients with diabetes GII, obesity GIII, or both (two risk factors), 59% in patients with coronopathy GV, about 72% in patients with coronopathy and diabetes GVI or obesity GVII, and by 77% in GVIII (four risk factors), respectively, compared with controls. LCAT activity was 1.2-fold higher in GII, GIII (two risk factors), GVI (three risk factors), 2-fold in patients with coronopathy GV, 3-fold in patients of GVI and VII (three risk factors) and 3.6-fold in patients with four risk factors GVIII, than in hypertensive patients. A progressive decrease was noted in serum apolipoprotein (apo) A-I according to risk factors number. Indeed, a decrease by 28% in hypertensive patients GI, 51% in patients with diabetes, obesity, or both (three risk factors), 70% in patients with coronopathy GV, about 84% in patients with coronopathy associated with diabetes GVI or obesity GVII and 87% in patients with four risk factors GVIII. No significant differences were noted between diabetes and obesity action on LCAT activity and serum Apo A-I concentrations in GII, compared with GIII and in GVI, compared with GVII.

Table 2. Serum Apo A-I and LCAT Activity in Atherosclerosis High Risk Patients and Controls.

	Apo-AI (g/l)	LCAT (nmol EC/ ml.serum/h)
GI	1.08 ± 0.06 *	64.92 ± 9.62 *
GII	0.72 ± 0.05 *** #	51.88 ± 8.32 ** #
GIII	0.74 ± 0.11 *** #	52.38 ± 6.53 ** #
GIV	0.71 ± 0.06 *** #	50.78 ± 5.58 ** #
GV	0.45 ± 0.06 *** ##	31.89 ± 7.76 *** #
GVI	0.23 ± 0.05 *** ###	21.08 ± 5.10 *** ##
GVII	0.25 ± 0.04 *** ###	22.13 ± 3.63 *** ###
GVIII	0.19 ± 0.04 *** ###	17.86 ± 3.96 *** ###
Controls	1.49 ± 0.25	77.40 ± 17.3

*Values are means ± SD. GI, hypertension alone; GII, hypertension + diabetes; GIII, hypertension + obesity; GIV, hypertension + diabetes + obesity; GV, hypertension + coronopathy; GVI, hypertension + diabetes + coronopathy; GVII, hypertension + obesity + coronopathy; GVIII, hypertension +diabetes + obesity + coronopathy. Comparisons of the means was performed by Kruskal Wallis 1- way Anova procedure. *: High Risk Atherosclerosis Groups vs. Controls. #: GI vs. (GII, GIII, GIV, GV, GVI, GVII and GVIII). * # P<0.05; ** ## P<0.01; *** ### P<0.001.*

HDL_2 and HDL_3 Amounts and Composition

HDL_2 and HDL_3 amounts, expressed in g/L.serum, were determined by adding their apolipoprotein and lipid components (apolipoproteins+triacylglycerols+phospholipids + unesterified cholesterol + cholesteryl esters).

Serum HDL_2 amounts (Fig. 1) were significantly different in the eight high risk atherosclerosis groups and controls. According

to atherosclerosis risk factors number, the most decreased serum HDL_2 amounts was noted in GVIII (four risk factors), GVI, GVII (three risk factors), compared with GI ($P<0.01$). A decrease by about 47% was observed in GVI, GVII (three risk factors) and GVIII (four risk factors), compared with hypertensive patients GI. Progressive decrease from 20 to 74% in HDL_2-apolipoproteins were observed according to risk factor atherosclerosis number, compared with controls. HDL_2-apo concentrations were 3-fold lower in GVII (four risk factors), GVI and GVII (three risk factors), 1.6-fold in patients with coronopathy GV, and 1.2-fold in GII, GIII and GIV, than in hypertensive patients GI. According to risk factors atherosclerosis number, HDL_2-TG were 1.2- to 2-fold higher in patients of GII ($P<0.05$), GIV ($P<0.01$), GV, GVI, GVII and GVIII ($P<0.001$), than in controls. HDL_2-TG were 1.6-fold higher in GV ($P<0.001$), 1.9-fold in GVI, GVII and GVIII ($P<0.001$), 1.4-fold in GIV and 1.2-fold in GII than in hypertensive patients GI. An increase in HDL_2-PL by 12 to 74% was noted in high risk atherosclerosis patients, compared with controls. According to risk factors atherosclerosis number, HDL_2-PL amounts were significantly increased by about 27% in GII and GIII ($P<0.05$), GIV, GV, GVI and GVII ($P<0.01$) and in patients with four risk factors GVIII ($P<0.001$), respectively, compared with hypertensive patients GI. An increase in HDL_2-UC by 36% was notes in GII ($P<0.05$), 50% in GIV and GV ($P<0.05$), about 57% in GVI ($P<0.05$), 64% in GVI and GVIII ($P<0.01$), compared with controls values. According to risk factors atherosclerosis number, HDL_2-UC values were 1.2- to 1.5-fold higher in GII, GIII, GIV, GV, GVI, GVII and GVIII, than in GI (one risk factor). HDL_2-CE were significantly lower in all patients groups, compared with controls ($P<0.001$). Compared with hypertensive patients GI, a progressive decrease by 43% was noted in patients with diabetes GII ($P<0.01$), 30% in patients with obesity GIII ($P<0.05$), 51% in patients with diabetes and obesity GIV ($P<0.01$), 68% in patients with coronopathy GV ($P<0.001$), about 89% in patients with coronopathy associated with diabetes or obesity ($P<0.001$) and 97% in patients with the four risk factors GVIII ($P<0.001$). A significant difference was noted between diabetes and obesity action on HDL_2-CE in GII compared with GIII ($P<0.01$).

Serum HDL_3-amounts (Fig. 2) were significantly decreased in the eight high risk atherosclerosis groups compared with controls ($P<0.001$). According to risk factors atherosclerosis number, a progressive reduction of HDL_3-amounts by about 33% in patients of GII, GIII and GIV ($P<0.05$), 37% in patients with coronopathy GV ($P< 0.05$) and by about 50% in patients with coronopathy associated with diabetes GVI or obesity GVII or the both GVIII ($P<0.01$), compared with hypertensive patients GI. HDL_3-apo were significantly reduced in patients of GI ($P<0.01$), GII, GIII, GIV, GV, GVI, GVII and GVIII ($P<0.001$), compared with controls. According to risk factors atherosclerosis number, a decrease in HDL_3-apo by 38 to 70% were noted in GII, GIII, GIV, GV, GVI, GVII and GVIII, compared with GI. A significant increase in HDL_3-TG was noted in high risk atherosclerosis groups, compared with controls ($P<0.001$). According to risk factors atherosclerosis number, HDL_3-TG were 1.2-fold higher in GII, GIII, GIV and GV ($P<0.05$), 1.3- to 1.5-fold in GVI, GVII and GVIII ($P<0.01$), than in hypertensive patients GI. HDL_3-PL were significantly decreased in the eight high risk atherosclerosis groups compared with controls ($P<0.001$). According to risk factors atherosclerosis number, HDL_3-PL were 1.5-fold higher in GIV ($P<0.05$), about 1.9-fold in GV, GVI, GVII ($P<0.01$) and 2.2-fold in patients with four risk factors GVIII ($P<0.001$), than in hypertensive patients GI. A significant increase in HDL_3-UC was noted in all high risk atherosclerosis groups, compared with controls ($P<0.001$). According to risk factors atherosclerosis number, HDL_3-UC values were 1.2-fold higher in GIV and GV ($P< 0.05$), 1.4-fold in GVI and GVII ($P<0.01$) and 1.6-fold in patients with four risk factors GVIII, than in hypertensive patients GI ($P<0.001$). HDL_3-CE values were decreased in all high risk atherosclerosis groups, compared with controls ($P<0.001$). According to risk factors atherosclerosis number, a progressive decrease in HDL_3-CE by 45 to 95% was noted in GII, GIII and GIV ($P<0.05$), GV ($P<0.01$), GVI, GVI and GVIII ($P<0.001$), compared with hypertensive patients GI.

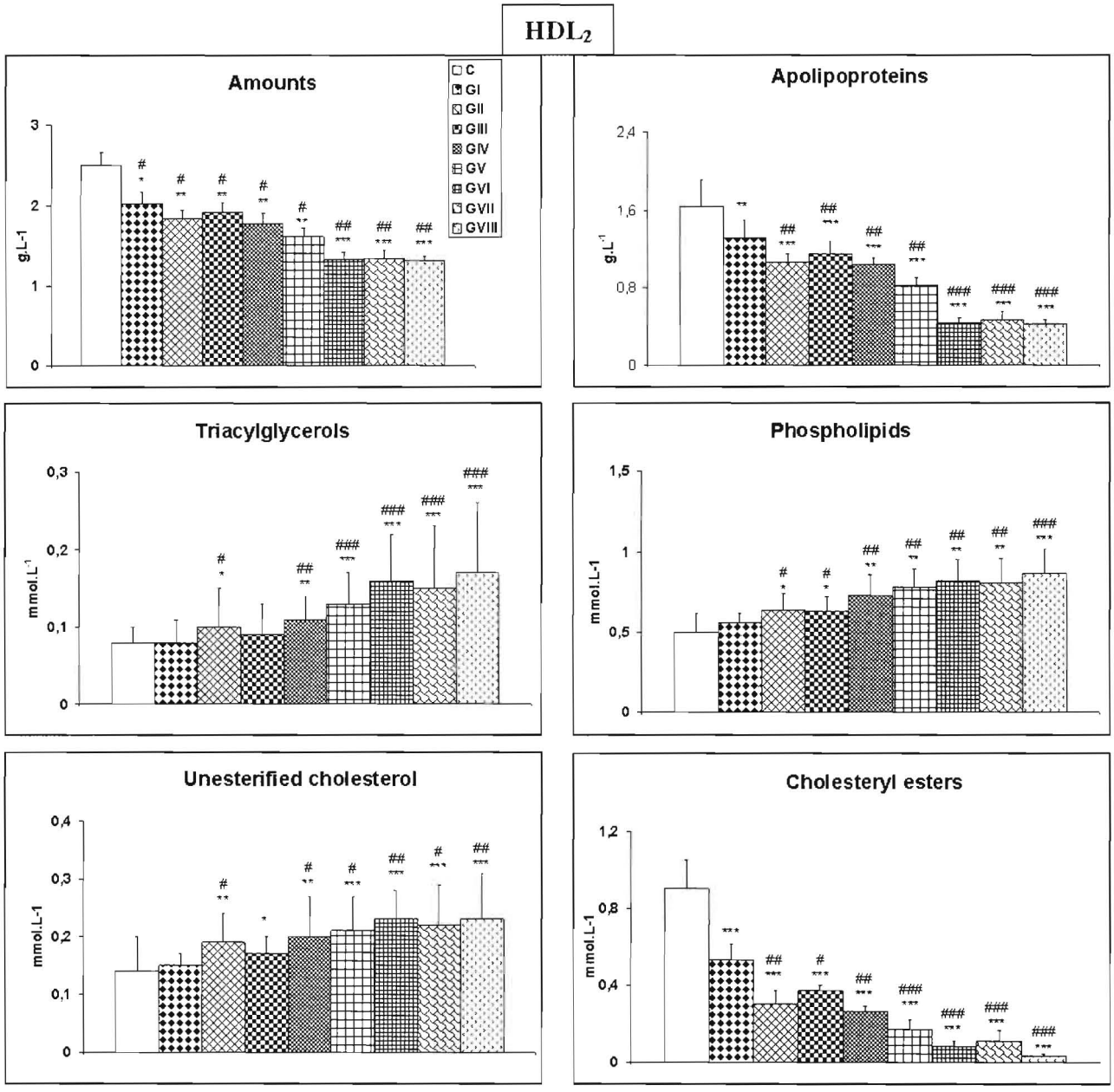

Fig.1. Serum HDL_2 Amounts and Composition. (Values are means ± SD; Controls, GI, hypertension alone; GII, hypertension + diabetes; GIII, hypertension + obesity; GIV, hypertension + diabetes + obesity; GV, hypertension + coronopathy; GVI, hypertension + diabetes + coronopathy; GVII, + obesity + coronopathy; GVIII, hypertension +diabetes + obesity + coronopathy. Comparisons of the means was performed by Kruskal Wallis 1- way Anova procedure.

*: High Risk Atherosclerosis Groups vs. Controls; #: GI vs. (GII, GIII, GIV, GV, GVI, GVII and GVIII).

* # P<0.05; ** ## P<0.01; *** ### P<0.001.).

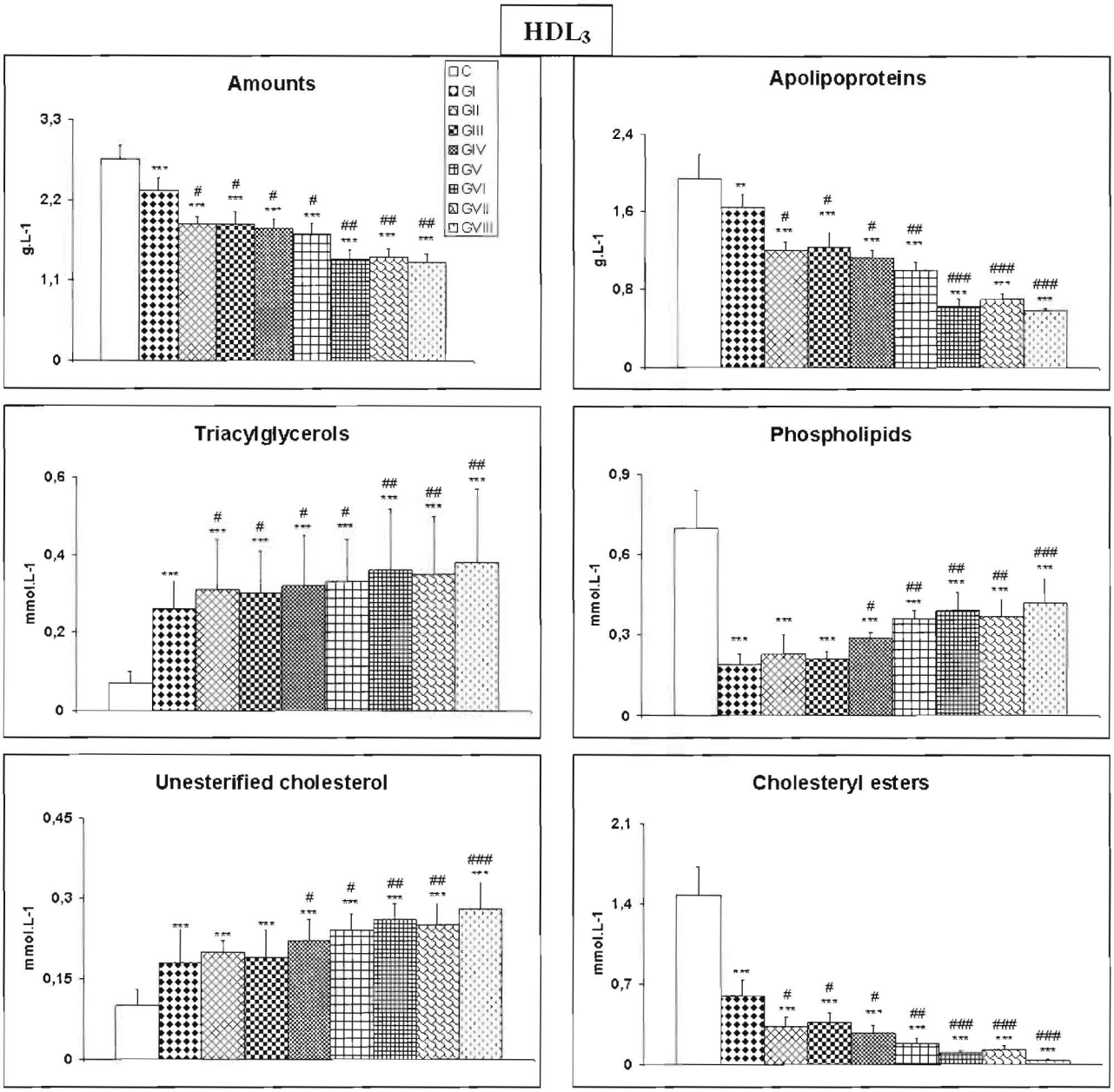

Fig.2. Serum HDL_3 Amounts and Composition. (Values are means ± SD; Controls, GI, hypertension alone; GII, hypertension + diabetes; GIII, hypertension + obesity; GIV, hypertension + diabetes + obesity; GV, hypertension + coronopathy; GVI, hypertension + diabetes + coronopathy; GVII, hypertension + obesity + coronopathy; GVIII, hypertension +diabetes + obesity + coronopathy. Comparisons of the means was performed by Kruskal Wallis 1- way Anova procedure.
*: High Risk Atherosclerosis Groups vs. Controls; #: GI vs. (GII, GIII, GIV, GV, GVI, GVII and GVIII).
* # P<0.05; ** ## P<0.01; *** ### P<0.001.).

Correlation Analysis

A negative correlation was noted between LCAT activity and HDL_3-PL in high risk patients ($r = -0.69$, $P<0.01$), positive correlation was noted between LCAT activity and HDL_2-CE ($r = 0.55$, $P<0.01$). Inverse correlation was noted between LCAT activity and triglyceridemia ($r = -0.76$, $P<0.01$), between HDL_2-CE and triglyceridemia ($r = -0.63$, $P<0.01$) and between serum total cholesterol and HDL_2-CE ($r = -0.87$, $P<0.01$). A positive correlation was noted between serum total cholesterol and serum triacylglycerols ($r = 0.99$, $P<0.01$).

DISCUSSION

The present study was undertaken to determine the effect of hypertension with or without associated risk factors (obesity, diabetes) and coronopathy on HDL_2, HDL_3 amounts and composition and LCAT activity. LCAT activity was strongly decreased in all patients, particularly in patients with coronopathy, compared with controls. According to risk factors atherosclerosis number, a progressive reduction by about 17% in GII, GIII and GIV patients with diabetes or obesity or the both, 43% in patients with coronopathy, about 56% in patients with diabetes and obesity and 61% in patients of GVIII (four risk factors), compared with GI (hypertensive patients). Theses results are in agreement with previous data (Raison, 1992) showing that hypertension accompanied with other risk factors as obesity and particularly diabetes accentuates LCAT activity decrease.

A progressive decrease was noted in serum apolipoprotein (apo) A-I according to risk factors number. Indeed, a decrease by about 23% in patients with diabetes or obesity or the both, 42% in patients with coronopathy, about 56% in patients with diabetes or obesity or the both and 59% in patients with four risk factors, compared with hypertensive patients. The fact that (apo) A-I concentrations were decreased in our patients confirmed that LCAT activity was due to its diminished activating cofactor. LCAT converts HDL_3-unesterified cholesterol to HDL_2-cholesteryl esters. LCAT and its cofactor apo A-I use cholesterol and a fatty acid from lecithin as substrate to form cholesteryl esters, producing HDL_2 (Tall, 1998). Cholesteryl esters are then transferred from HDL_2 to triglyceride rich-lipoproteins (TGRLs), such as VLDL. HDL_2 are regenerated through the action of hepatic lipase (Kwiterovich, 1998). Hypertriglyceridemia was due to decreased lipoprotein lipase and hepatic lipase activities in our patients (data non published). Loukidi-bouchenak recently reported that triglygeridemia was higher in hypertensive mothers compared with normal control women (Loukidi-Bouchenak, *et al.* 2008). Lipoprotein lipase hydrolyses TG carried by VLDL and hepatic lipase catalyzes the hydrolysis of HDL_2-TG and HDL_2-PL. Hepatic lipase activity reduction involves an increase in HDL_2-TG and HDL_2-PL (Von Eckardstein, *et al.* 2001a). Reduced LCAT activity and serum apolipoprotein A-I in patients is accompanied with decreased HDL_2-CE and HDL_3-CE. Reduced HDL_2-CE and HDL_3-CE, associated with enriched fractions in TG contributes to cardiovascular disease complications (Von Eckardstein, *et al,* 2001a). Indeed, all patients and particularly those with coronopathy and diabetes or obesity or the both presented the most elevated HDL_2-TG and HDL_3-TG levels. Després, *et al.* (Després, *et al.* 2000; Després, *et al.* 1990) have noted that hypertension associated with diabetes or abdominal obesity contributes to decrease HDL_2-cholesteryl esters and increase HDL_2 and HDL_3-unesterified cholesterol, with an enrichment of triacylglycerols (Rey, *et al.* 1999). LCAT activity reduction in patients could be associated to a low enzyme affinity for HDL_3 fraction which was enriched in TG and lowered in PL (Jiang, *et al.* 1999). Considering the role of LCAT in the esterification of plasma cholesterol, reduced LCAT activity is normally associated with increased UC and decreased CE concentrations. Indeed, in this study increased HDL_3-UC as well as decreased HDL_2-CE was observed.

HDL_3-PL is the substrate of LCAT (Von Eckardstein, *et al.* 2001a) and the fact that LCAT activity was negatively correlated with HDL_3-PL indicates that increased HDL_3-PL in all patients is a result of decreased LCAT activity. Reduced LCAT activity contributes to a retarded conversion

of HDL_3 to HDL_2 due to lowered cholesterol esterification. This metabolic disturbance might be related to altered HDL composition and contributed to the reduced efficacy of reverse cholesterol transport from peripheral tissues. Low HDL_2-CE levels are strongly associated with high risk of atherosclerosis (Castelli, *et al.* 1992).

Atherosclerosis is strongly related to hypercholesterolemia and at least degree with hypertriglyceridemia (Fruchart and Duriez, 2001; Yarnell, *et al.* 2001; Turpin, 1989). Our data showed that all patients presented hypercholesterolemia (2.35 - 2.84 g/L). Theses values were higher than tolerance limit (2.4 g/L) (Turpin, 1989), therefore, atherosclerosis risk is very elevated and these results are in agreement with previous data (Benfante and Reed, 1990).

Our data show that all patients presented hypertriglyceridemia which seemed however to be moderate (1.57 - 1.96 mmol/L) compared with the developed countries values (1.8 - 3 mmol/L) (O'Hare, *et al.* 2002; Elisaf, *et al.* 1995; Attman, *et al.* 1993; Seres, *et al.* 1993). This could be due to the diet consumed by the population which was characterised by considerable intake of vegetable proteins (29 to 54% of total proteins intake) in all patients groups, complex carbohydrates (53 to 76% of total carbohydrates), fibres (19 to 31 g/day) and monounsaturated fatty acids (25 to 52% of total lipid intake) (data non published). This diet is known to have a beneficial effect on plasma triacylglycerols and cholesterol. In addition to diet, smoking among all men of this study, with its direct effect on arterial wall, cholesterol metabolism in particular HDL subfractions, platelet aggregation, lipoproteins oxidation (Desveaux, *et al.* 1997), insulin resistance (Ishizaka, *et al.* 2003) and fat tissues distribution (Després, *et al.* 1990), plays a major role by its interactions with nutritional factors such as antioxidant vitamins, directly or by the means of associated style feeding (Nuttens, *et al.* 1992). Exercise training absence among all subjects, independently of its effect on weight, could represent an independent risk factor as suggested several studies, whereas physical activity such as walking is associated with reduction of coronary heart diseases risk (Manson, *et al.* 1999). In all studied patients, a reduction of HDL_2 and HDL_3 amounts was noted, in particularl in coronary patients. Moreover, the majority of patients presented increased levels of HDL_3-PL and HDL_3-UC with decreased HDL2-CE in both fractions.

The inverse relationship between serum total cholesterol and HDL_2-CE in patients with coronopathy (GV, $r = -0.75$, $P<0.01$; GVI, $r = -0.79$, $P<0.01$, GVII , $r = -0.86$, $P<0.01$ and GVIII, $r = -0.97$, $P<0.01$) indicated that coronopathy was strongly associated to hypercholesterolemia and decreased HDL_2-amounts, these results are in agreement with previous data (Barrans, *et al.* 1996).

CONCLUSION

This study demonstrates that high risk atherosclerosis impairs HDL_2 and HDL_3 metabolism. Indeed, LCAT activity decrease was associated with strong alterations in HDL_2 and HDL_3 composition. LCAT activity reduction was more pronounced in patients with coronopathy (GV, GVI, GVII and GVIII). The reduced activity of this enzyme involved increase HDL_3-UC and HDL_3-TG and decreased HDL_2 and HDL_3 amounts. Theses changes in HDL metabolism suggest that cholesterol transport from the tissues to liver is impaired, which may have implications for accelerated occurrence of vascular diseases.

Our findings showed that risk factors such as hypertension, obesity and diabetes contributes to the apparition of coronary lesions and, therefore, predispose patients to high risk of atherosclerosis (angina pectoris or myocardial infarction). Moreover, hypercholesterolemia and hypertriglyceridemia are strongly associated and capable to modify the risk of atherosclerosis, because the main cause of cardiovascular accidents (myocardial infarction in particular) is atherosclerosis. Thus, this study demonstrates the effect of high risk atherosclerosis on serum LCAT activity, HDL_2 and HDL_3 amounts and composition and LCAT activity in patients with one, two, three, or four risk factors. According to risk factors atherosclerosis number, impaired HDL_2 and HDL_3 amounts and compositions associated with reduced serum apo A-I and LCAT activity were more pronounced in coronary

patients, diabetes and obesity, with no difference between the both in this study accentuates lipid metabolic disturbance in particularly in coronary patients. We conclude that according to the presence of one or several atherosclerosis risk factors, the alterations in HDL_2 and HDL_3 composition contribute to reduced efficacy of reverse cholesterol transport, which is another risk factor for cardiovascular disease.

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