ASBMB
JOURNAL OF LIPID RESEARCH

Cholesterol esterification rate in plasma depleted of very low and low density lipoproteins is controlled by the proportion of HDL_2 and HDL_3 subclasses: study in hypertensive and normal middle-aged and septuagenarian men

Milada Dobiasova,[1,*] Jarmila Stribrna,[†] P. Haydn Pritchard,[**] and Jiri J. Frohlich[**]

Institute of Nuclear Biology and Radiochemistry,* Czechoslovak Academy of Sciences, Prague, Czechoslovakia; Institute for Clinical and Experimental Medicine,† Prague, Czechoslovakia; and University Hospital Lipoprotein Research Group, Department of Pathology,** University of British Columbia, Vancouver, British Columbia, Canada

Downloaded from www.jlr.org by guest, on October 19, 2017

Abstract The relationship between the fractional rate of cholesterol esterification (FER_{HDL}) in very low density lipoprotein (VLDL)- and low density lipoprotein (LDL) - depleted plasma and the particle size distribution of high density lipoproteins (HDL) were studied in: *a*) a control group of 9 apparently healthy men (42 ± 11 years); *b*) 15 septuagenarians (76 ± 6 years) who had no clinical signs of coronary artery disease; and *c*) 32 outpatients with essential hypertension of different stages of severity (51 ± 10 years). There were small differences between the groups with respect to their plasma total and HDL-cholesterol and plasma triglyceride levels. However, there was a highly significant increase in FER_{HDL} in patients with hypertension compared to control and older men. The HDL of hypertensive patients had a markedly increased relative content of HDL_{3b}, while their HDL_{2b} fraction was reduced by over 50% compared to the other groups. Overall, there was a strong positive correlation between FER_{HDL} and HDL_{3b} ($r = 0.89; P < 0.001$) and a negative correlation between FER_{HDL} and HDL_{2b} ($r = -0.61; P < 0.001$) and HDL_{3a} ($r = -0.77; P < 0.001$). These findings confirm our previous conclusions that FER_{HDL} reflects the relative HDL subclass distribution. In addition, we demonstrate that FER_{HDL} is increased in hypertensive male subjects regardless of the stage of hypertension, i.e., whether or not organic lesions have already become manifest (stage III and stages I plus II, respectively).—**Dobiasova, M., J. Stribrna, P. H. Pritchard, and J. J. Frohlich.** Cholesterol esterification rate in plasma depleted of very low and low density lipoproteins is controlled by the proportion of HDL_2 and HDL_3 subclasses: study in hypertensive and normal middle-aged and septuagenarian men. *J. Lipid Res.* 1992. **33:** 1411–1418.

Supplementary key words high density lipoprotein subclasses • lecithin:cholesterol acyltransferase • hyperlipidemia

Lecithin:cholesterol acyltransferase (LCAT) has been reported to regulate the transport of cholesterol between extravascular and intravascular pools (1-3). This important plasma enzyme, therefore, potentially plays a central role in the initial steps of a process known as reverse cholesterol transport. In this theoretical pathway, esterification of cholesterol in plasma serves to maintain a chemical concentration gradient for unesterified cholesterol between peripheral cells and plasma. In plasma, LCAT protein is bound to high density lipoproteins and its activity appears to be confined to this lipoprotein class. Since plasma levels of HDL cholesterol are inversely related to the risk of coronary artery disease (CAD), it is generally believed that an increased rate of cholesterol esterification may be beneficial because it contributes to HDL cholesterol content of plasma. However, the evidence supporting this hypothesis is scarce. In fact, LCAT activity in plasma appears to have no correlation with HDL concentration (3); moreover, the HDL_2 subpopulation believed to be highly protective, inhibits the activity of LCAT (4).

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; CAD, coronary artery disease; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TC, total cholesterol; TG, triglyceride; FER_{HDL}, fractional rate of esterification.

[1]To whom correspondence should be addressed at: Lipoprotein Research Group UBC, The Research Center, 950 West 28th Avenue, Vancouver, B.C., Canada V5Z 4H4.

ASBMB

JOURNAL OF LIPID RESEARCH

Similarly, previous studies carried out in our laboratory have suggested that (contrary to the expectation) there was a positive relationship between the esterification rate of cholesterol in the HDL plasma pool and the risk of CAD (5). We have developed a simple method that allows an assessment of the esterification rate of cholesterol in LDL- and VLDL-depleted plasma samples. This is expressed by the fractional rate of cholesterol esterification in the HDL fraction of plasma (FER_{HDL}) and its value is conceivably a parameter that adequately defines the capability of the HDL pool to esterify free cholesterol in individual plasma samples. We have shown that the FER_{HDL} value markedly differed among groups of patients with differing atherogenic risk. It was significantly greater in healthy men than in women, and it was significantly higher in hyperlipidemic patients suffering from manifest CAD. A significant inverse relationship between FER_{HDL} and the relative proportion of the HDL_{2b} subclass in the HDL pool indicates that FER_{HDL} reflects the relative capability of LCAT to utilize different sized HDL. Thus FER_{HDL} reflects the metabolic activity of the total HDL pool in individual subjects.

The objective of the present study was to investigate the relationship between FER_{HDL} and subpopulations of the HDL_3 subclass, whose role in the esterification of cholesterol and in atherogenesis is unclear. We studied five groups of subjects who differed markedly in the magnitude of their atherogenic risk: healthy middle-aged men; fit, apparently healthy men in their seventies; and three subgroups of men with long-standing hypertension. The results obtained provide further evidence that the FER_{HDL} is higher in those individuals who are at increased risk of CAD, and that the FER_{HDL} is regulated by the ratio of HDL_{3b} to HDL_{2b} and HDL_{3a} subclasses.

METHODS

Subjects

The study was carried out on 9 apparently healthy middle-aged men with normal body weight and plasma lipid concentration, 15 slightly obese men over 70 years of age with mild hyperlipidemia but no clinical signs of atherosclerosis, and 32 middle-aged men suffering from long-standing arterial hypertension (**Table 1**). The last group was further subdivided according to WHO classification into three subgroups (the first and second stages, lacking any organic pathology, and a subgroup of the third stage characterized by organic lesions such as CAD, intermittent claudication, cerebrovascular attack, or myocardial infarction). All but three of the patients with hypertension had been previously subjected to prolonged treatment with antihypertensive drugs including β-adrenergic blocking agents, diuretics, or methyldopa, or various combinations (see Table 4).

Lipid analysis

Blood from subjects who had fasted overnight for 12 h was collected into EDTA-containing tubes, placed on ice, and centrifuged within 2 h at 1750 *g* for 10 min to separate plasma. Plasma was analyzed within 48 h if kept on ice, or within 3 months if stored at −20°C, or 12 months at −70°C. We have verified that these storage conditions did not affect the analysis of FER_{HDL} described below. Total and free cholesterol and triacylglycerols were estimated enzymatically (6, 7). VLDL/LDL-depleted plasma was prepared by precipitation of apoB-containing lipoproteins with phosphotungstate(PTA)–$MgCl_2$ (8). Briefly, to 100 μl plasma was added 10 μl of phosphotungstate solution (4% phosphotungstic acid in 1 M NaOH) and, after stirring, 2.5 μl of 2 M $MgCl_2 \cdot 6H_2O$. Precipitation of lipoproteins of lower densities was complete after 30 min standing in a refrigerator. The suspension was then centrifuged at temperature not exceeding 10°C for 30 min at 12,000 rpm.

Determination of esterification rates

The determination of FER_{HDL} is based on a method described previously (9) and was used in one of our earlier studies (5). As the method has now been adapted for use in clinical practice and improved so as to require a substantially reduced initial amount of material, we consider it useful to describe the procedure here in more detail. The essential step of the method consists in transferring a trace amount of [^{3}H]cholesterol from a paper disc to lipoproteins in a sample of whatever material may be analyzed: plasma, VLDL/LDL-depleted plasma, lymph, eye aqueous humor (10), etc. Spontaneous transfer of the label proceeds at low temperature and labeling homogeneity is attained after 18 h. The procedure makes it possible to minimize the undesirable interference caused by non-standard cholesterol carriers such as albumin or lysophosphatidylcholine (11) as well as inhibitors and activators of LCAT.

Preparation of paper discs. Discs of approximately 7–8 mm in diameter were cut off from Whatman 1 filter paper by means of a letter punch. Using a pair of tweezers and thin hypodermic needles fixed on a stand, the discs were pinned horizontally on the needles. [7(n)-^{3}H]cholesterol (sp act 5 Ci/mmol, Amersham, England) in an amount of 0.3 μCi dissolved in 3 μl ethanol was spread evenly onto each disc. After evaporation of the solvent, the discs were placed separately in stoppered 1- to 2-ml vials and kept in a refrigerator; under these conditions, samples remained unchanged for at least 3 months.

Labeling of lipoprotein samples at low temperature. Tris buffer

Downloaded from www.jlr.org by guest, on October 19, 2017

TABLE 1. Age, body mass index, and plasma lipids in the study groups

Group	n	Age	BMI	TC	TG	HDL-TC	HDL-FC
		yr			*mmol/l*		
Control men	9	42 ± 11	94 ± 7	5.1 ± 0.7	1.6 ± 0.5	1.13 ± 0.24	0.22 ± 0.04
Septuagenarians	15	76 ± 6[a]	103 ± 12[c]	6.1 ± 0.9[c]	1.3 ± 0.7	0.94 ± 0.15[c]	0.21 ± 0.04
HPT 1	7	41 ± 11[d]	113 ± 12[b]	6.1 ± 0.4[b]	1.4 ± 0.4	0.95 ± 0.30	0.21 ± 0.06
HPT 2	13	51 ± 10[d]	119 ± 14[b,e]	6.6 ± 1.2[b]	2.5 ± 1.6	1.06 ± 0.31	0.24 ± 0.05
HPT 3	12	56 ± 7[c,d]	116 ± 13[b,f]	7.0 ± 0.9[b,f]	4.1 ± 1.8[c,e]	0.87 ± 0.13[c]	0.17 ± 0.06

HPT I–III stage of hypertension according to WHO classification; BMI, body mass index determined as {wt(kg)/height(cm) − 100} × 100; TC, total cholesterol; TG, triglycerides; HDL-TC, high density lipoprotein total cholesterol; HDL-FC, high density lipoprotein free cholesterol. Data are presented as means ± SD.
Significance of difference from control men: [a], $P < 0.001$; [b], $P < 0.01$; [c], $P < 0.05$.
Significance of difference from septuagenarians: [d], $P < 0.001$; [e], $P < 0.01$; [f], $P < 0.05$.

(Tris, 10 mmol/l; NaCl, 150 mmol/l; EDTA, 0.01%; NaN_3, 0.03%) was dispensed in 75-μl aliquots into 3–5 ml test tubes kept on ice. Thirty μl of precooled sample (VLDL/LDL-depleted plasma in our case) was added to the buffer. A radioactively labeled paper disc was immersed into each diluted sample solution and the tubes were stoppered and stored overnight on ice. Thereafter, the discs were removed and discarded.

Incubation and processing of the sample. Test tubes with labeled samples were placed in a shaking water bath and incubated for 30 min at 37°C. The tubes were then placed on ice, and the contents were mixed immediately with 98% ethanol (1.5 ml per tube). The mixture was stirred and left to stand for 2 h at room temperature. Samples were then centrifuged at 2000 rpm for 10 min and the supernatants were taken to dryness under a stream of nitrogen. The dry residue was dissolved in 100 μl of chloroform containing standards of unesterified and esterified cholesterol as carriers (15 mg of cholesterol and 10 mg of cholesteryl esters in 10 ml of chloroform) to make visualization of labeled components in the diluted sample possible. After separation by thin-layer chromatography (Merck, Germany), samples were visualized by I_2 vapor. The contours of the spots were outlined with a pencil and remaining traces of iodine were allowed to sublime. The spots were then cut out, put into vials with scintillation mixture, vigorously shaken, and left to stand for at least 3 h. Radioactivity was determined in a liquid scintillation counter. FER_{HDL} was calculated as the difference between the percentage of labeled esterified cholesterol before and after incubation. In VLDL/LDL-depleted human plasma, the percentage of cholesterol esterified before the incubation was always less than 0.3%.

Distribution of [³H]cholesterol in lipoproteins. Preliminary studies demonstrated that the [^{3}H]cholesterol equilibrated uniformly with the endogenous cholesterol of very low density, low density, and high density lipoproteins. Four samples of human plasma, differing in their lipoprotein pattern, were labeled as described above and the [^{3}H]cholesterol-labeled lipoproteins were prepared by ultracentrifugation at a density of 1.21 g/ml. After fractionation by gel filtration on Superose 6 (Pharmacia, Sweden), the fast protein liquid chromatography fractions were examined for radioactivity and unesterified cholesterol. Calculation of the specific radioactivity of unesterified cholesterol in each fraction demonstrated the complete equilibration of the [^{3}H]cholesterol into the lipoprotein pool.

Gradient gel electrophoresis of HDL

Plasma lipoproteins were removed by ultracentrifugation at a final plasma density of 1.21 g/ml in a 65 Ti rotor in a Beckmann 7-L ultracentrifuge (Palo Alto, CA) for 24 h at 15°C. The nondialyzed lipoprotein fraction was mixed with sampling buffer containing 40% sucrose, and 5 μl of sample was applied to each lane of a 4–30% polyacrylamide gradient gel (Pharmacia, Sweden). The samples were electrophoresed in Tris/borate/EDTA buffer, pH 8.3, as described by Nichols, Krauss, and Musliner (12). A mixture of globular proteins (HMW Calibration Kit, Pharmacia) was run concurrently as particle size markers. Gels were run for 21 h at 125 V and stained for proteins with Comassie Brilliant Blue. The migration distances of HDL subclasses were measured relative to migration distance of bovine serum albumin. Three of the HDL subclasses were distinctly resolved on the gel: HDL_{2b} (9.5–12.9 nm), HDL_{3a} (possibly with HDL_{2a}, 8.2–9.5 nm) and HDL_{3b} (with HDL_{3c}, 7.0–8.2 nm). These particle sizes were similar to those previously reported (13). The relative content of HDL subpopulations was estimated by determining the areas under the peaks of laser densitometer scans of the gels (LKB Ultroscan XL (LKB, Sweden).

Statistical analyses

Student's *t*-test was used to establish significant differences between the mean values of each group and correlation (*r*) among the parameters was calculated by linear regression analysis.

Downloaded from www.jlr.org by guest, on October 19, 2017

JOURNAL OF LIPID RESEARCH ASBMB

TABLE 2. FER_{HDL} and HDL subclass distribution

Group	n	FER_{HDL}	HDL Subclasses		
			HDL_{2b}	HDL_{3a}	HDL_{3b}
		%/h		%	
Control men	9	17.1 ± 3.8	16.4 ± 6.9	65.8 ± 9.8	17.7 ± 7.9
Septuagenarians	15	17.7 ± 5.3	18.4 ± 8.5	63.2 ± 8.5	21.8 ± 11.3
HPT1	7	25.6 ± 7.9[c,f]	10.7 ± 5.5[c,f]	58.3 ± 7.2	30.5 ± 11.6[c]
HPT2	13	29.4 ± 8.7[a,d]	6.9 ± 3.5[b,d]	54.1 ± 8.2[b,e]	39.0 ± 9.6[a,d]
HPT3	12	33.1 ± 5.0[a,d]	8.4 ± 3.8[a,d]	49.7 ± 9.9[b,d]	42.2 ± 11.7[b,d]

Data are presented as means ± SD.
Significance of difference from control men: [a], $P < 0.001$; [b], $P < 0.01$; [c], $P < 0.05$.
Significance of difference from septuagenarians: [d], $P < 0.001$; [e], $P < 0.01$; [f], $P < 0.05$.

RESULTS

Age, body mass index, and plasma lipids in the study groups

Table 1 summarizes the data on subjects examined in this study. The septuagenarians and all groups of hypertensive men had significantly higher body mass index (BMI) and total cholesterol (TC) levels compared to the group of control men. A significantly increased concentration of plasma triglycerides (TG) was observed in patients with 3rd stage hypertension (HPT3). Total cholesterol in HDL (HDL-TC) was significantly lower in the HPT3 subgroup and in septuagenarians. Only minor nonsignificant differences were found between hypertensive patients and septuagenarians in the body mass index and plasma lipid level.

FER_{HDL} and distribution of HDL subclasses

FER_{HDL} was significantly elevated in all groups of hypertensive men compared to both control men and the septuagenarians (**Table 2**). The increase in FER_{HDL} correlated significantly with a change in particle size distribution. Data on the composition of HDL subclasses, also included in the table, demonstrate a distinct differentiation between healthy subjects regardless of age and all groups of hypertensive patients. It is apparent that the differences became wider as the hypertension advanced to more severe stages. When compared to the control groups, the proportions of HDL_{2b} and HDL_{3a} decreased, whereas the share of HDL_{3b} approximately doubled. It is worth noting that the differences in plasma lipid level were relatively small between septuagenarians and hypertensive patients (Table 1), whereas the changes in their composition of HDL and FER_{HDL} were highly significant (Table 2).

Correlation of FER_{HDL} with lipid, lipoprotein, and other parameters

The relationships between FER_{HDL} and other parameters measured during this study were determined by multiple linear regression analysis. When all subjects were considered together, there was a significant positive correlation between FER_{HDL} and body mass index, FER_{HDL} and plasma cholesterol, and FER_{HDL} and triglycerides (**Table 3**). In addition, there was a significant positive correlation between the FER_{HDL} and relative proportion of HDL_{3b} in the HDL pool (Table 3, **Fig. 1**). Conversely, there was a significant negative correlation with both the plasma level of HDL-TC and the proportion of HDL_{2b} and HDL_{3a} in the HDL pool (Table 3, **Fig. 2**).

TABLE 3. Correlation (r) of FER_{HDL} with lipoprotein subclasses and other parameters

	n	Age	BMI	TC	TG	HDL-TC	HDL_{2b}	HDL_{3a}	HDL_{3b}
Control men	9	0.44	0.60[c]	0.72[c]	0.25	0.07	−0.29	−0.82[b]	0.78[b]
Septuagenarians	15	0.38	0.28	0.33	0.49[c]	0.03	−0.49[c]	−0.56[c]	0.79[a]
HPT 1	7	0.17	0.39	0.08	0.43	−0.33	−0.61	−0.92[a]	0.87[b]
HPT 2	13	0.10	0.43	0.44	0.57[c]	−0.61[c]	−0.44	−0.89[a]	0.93[a]
HPT 3	12	0.15	0.15	0.13	0.34	−0.75[b]	−0.64[b]	−0.67[b]	0.78[a]
All subjects	56	0.10	0.53[a]	0.43[b]	0.63[a]	−0.34[b]	−0.61[a]	−0.77[a]	0.89[a]

Significance: [a], $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$.

Downloaded from www.jlr.org by guest, on October 19, 2017

ASBMB
JOURNAL OF LIPID RESEARCH

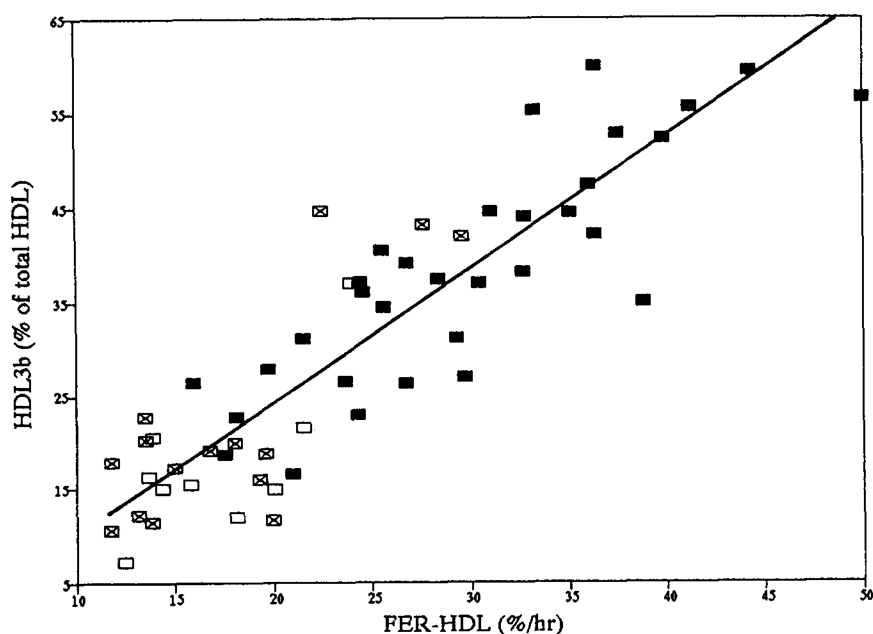

Fig. 1. Correlation between FER_{HDL} and the relative amount of HDL_{3b} in the HDL pool of all subjects in the study; (□) control men; (☒) septuagenarians; (■) hypertensive men.

The highly significant correlation observed between FER_{HDL} and HDL_{3b} even in individual groups (Table 3) indicates a causal relationship between the esterification rate of cholesterol in HDL and that specific component of the HDL_3 class. From the distribution of individual data from subjects belonging to the three groups (controls, septuagenarians, and hypertensive men), it is obvious that the degree of overlap is very low (Fig. 1). Conversely, the degree of overlap is considerable when data obtained in hypertensive patients of all three stages of severity are plotted individually (**Fig. 3**).

Effect of antihypertensive therapy on FER_{HDL} and distribution of HDL subclasses and other parameters

All the patients with hypertension except three had been previously treated. The patients were on five regimens

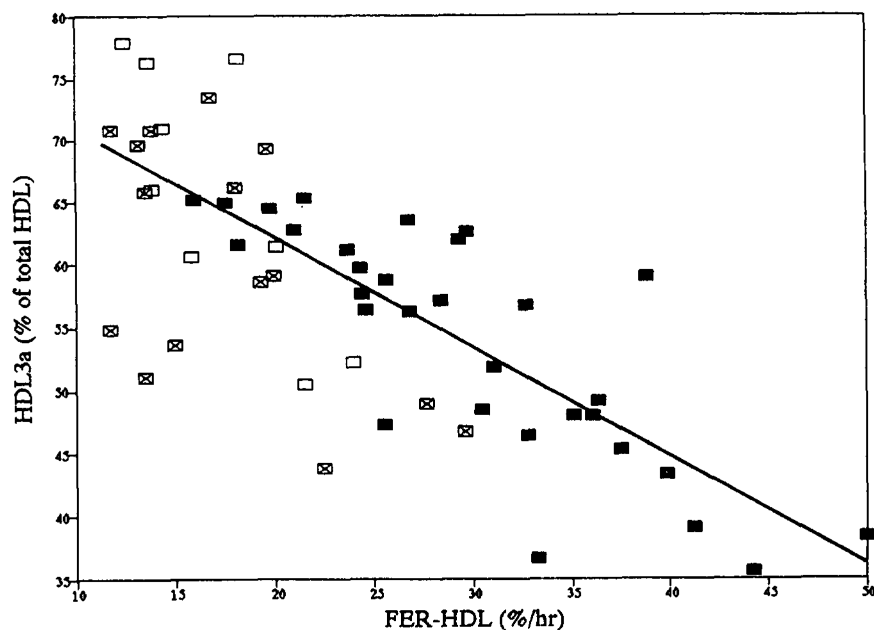

Fig. 2. Correlation between FER_{HDL} and the relative amount of HDL_{3a} in the HDL pool of all subjects in the study; (□) control men; (☒) septuagenarians; (■) hypertensive men.

Downloaded from www.jlr.org by guest, on October 19, 2017

ASBMB
JOURNAL OF LIPID RESEARCH

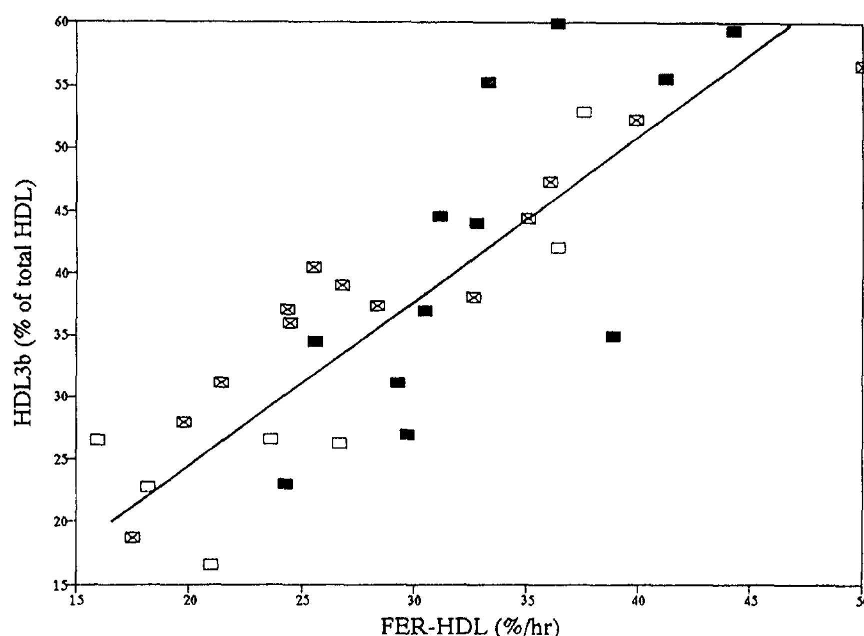

Fig. 3. Correlation between FER_{HDL} and the relative amount of HDL_{3b} in the HDL pool of hypertensive men; (□) patients in the 1st stage of hypertension; (⊠) patients in the 2nd stage; (■) patients in the 3rd stage.

(none, beta-blockers, diuretics, beta-blockers + diuretics, and beta-blockers + diuretics + methyldopa). **Table 4** summarizes the average values and their ranges, with each treatment regimen. As can be seen, the particle size distribution in HDL and the FER_{HDL} are similar in all treatment groups. In all five groups the proportions of HDL_{2b} and HDL_{3b} were, respectively, lower than 10% and higher than 30%, figures that are in fact multiples of the values found in control groups (Table 2). Despite the fact that patients differing in the severity of their hypertensive disease were not evenly represented in particulate groups, the data in Table 4 suggest that differences in the composition of HDL subpopulations and FER_{HDL} cannot be attributed to the effect of the treatment only.

DISCUSSION

The results of this study further confirm our working hypothesis, namely that the cholesterol esterification rate in VLDL/LDL-depleted plasma (FER_{HDL}) reflects the relative particle size distribution of the HDL pool. More specifically, we have previously provided evidence of a strong negative correlation between FER_{HDL} and HDL_{2b} (5). By studying subjects with much lower HDL_{2b} and higher HDL_{3b}, we have now also demonstrated a very strong positive correlation (r = 0.89) between FER_{HDL} and the relative contribution of HDL_{3b} to the total HDL pool. FER_{HDL} thus well corresponds with the mechanism of LCAT action described in vitro: whereas HDL_{2b} inhibits LCAT activity (4), small HDL molecules provide a preferable substrate for LCAT reaction (14–16). It follows that FER_{HDL} as an integral parameter of these antagonistic trends in HDL pool not only serves as a quantitative indicator of the particle size distribution, but may also acquire a prognostic value in assessing the risk of atherogenic coronary disease in individual subjects. This is particularly relevant in view of recent studies that demonstrated an inverse correlation between the relative content of HDL_{2b} in plasma and the severity of CAD (17, 18). Thus, Hamsten et al. (18) proved a significant negative correlation and the "severity score" of angiographically determined coronary atherosclerosis. Our results may shed some light on seemingly contradictory findings identifying either the HDL_2 or HDL_3 classes as the most important protective agents in the development of coronary disease. Most reports refer to the HDL_2 class as an unequivocally protective factor, while the HDL_3 class is largely considered as inert in this respect (19). However, since the HDL_3 class, even though it is regarded as uniform in the clinical sense when isolated by ultracentrifugation or precipitation methods, consists, in fact, of two components that differ in their ability to affect esterification of cholesterol (Table 3, Figs. 1 and 2), it is conceivable that it is their relative proportion that most affects the magnitude of the risk.

Another substantive result brought out by this study is the finding that, in comparison with healthy control men, hypertensive patients, regardless of the severity of their disease, exhibit significantly higher FER_{HDL} depending on a decrease in HDL_{2b} and an increase in HDL_{3b} subclasses (Table 2). Such a striking change in the relative

Downloaded from www.jlr.org by guest, on October 19, 2017

ASBMB

JOURNAL OF LIPID RESEARCH

TABLE 4. Data of hypertensive patients sorted according to therapy

	Therapy				
	None	Beta-Blocker	Diuretics	Beta-Blocker + Diuretics	Beta-Blocker + Diuretics + Methyldopa
n	3	7	9	6	7
n (I, II, III stage)	(2, 1, 0)	(3, 3, 1)	(1, 5, 3)	(1, 3, 2)	(0, 1, 6)
AGE (yr)	49	43	53	55	52
	(43-59)	(22-59)	(34-67)	(45-65)	(38-61)
BMI	108	116	120	120	115
	(95-115)	(104-132)	(106-143)	(105-137)	(91-144)
TC (mmol/l)	6.9	6.6	6.5	6.9	6.5
	(5.8-8.7)	(5.5-8.2)	(5.1-8.2)	(6.0-8.4)	(4.7-8.1)
TG (mmol/l)	2.0	2.9	2.9	2.3	3.6
	(1.3-3.4)	(0.6-8.7)	(0.8-6.5)	(1.6-3.3)	(1.3-5.4)
HDL-TC (mmol/l)	0.90	0.97	1.10	0.93	0.84
	(0.87-0.97)	(0.73-1.22)	(0.58-1.66)	(0.55-1.42)	(0.57-1.09)
FER_{HDL} (%/h)	26.7	27.4	29.6	33.6	31.3
	(21.0-37.5)	(15.9-38.9)	(18.2-39.9)	(24.4-50.0)	(24.2-41.2)
HDL_{2b} (%)	8.5	9.3	6.7	8.7	9.1
	(1.7-20.6)	(6.2-16.0)	(4.6-15.7)	(4.8-14.2)	(5.3-17.3)
HDL_{3a} (%)	57.8	57.7	52.9	48.5	51.9
	(45.3-65.3)	(48.0-65.1)	(34.8-64.5)	(38.4-63.5)	(36.7-62.5)
HDL_{b3} (%)	33.6	32.8	40.7	42.8	39.1
	(16.6-52.9)	(18.7-44.5)	(22.8-59.9)	(26.3-59.4)	(27.0-55.6)

Data are presented as means and (range).

proportions of HDL subpopulations has not yet been reported for hypertensive patients. In order not to overestimate the significance of the described results we attempted to analyze the data in relation to the type of treatment, degree of overweight, and hypercholesterolemia. The data in Table 4 suggest that there were no such differences that could be ascribed to particular medication. Overweight and plasma concentration of cholesterol were other criteria used for re-sorting the data obtained from the group of hypertensive patients (**Table 5**). Again, no differences were found among such rearranged groupings either in FER_{HDL} or in distribution of HDL particle size. Deviations from normal control levels found in hypertensive patients appear to be particularly striking when they are compared to analogous data recorded in the group of septuagenarians, who have increased body weight, increased plasma cholesterol level, and reduced HDL cholesterol, yet who have no signs of CAD in spite of their age.

The exact mechanism of the process that affects the distribution of subclasses in plasma HDL and consequently the rate of cholesterol esterification in HDL pool remains to be established. It is conceivable that the basic pattern is determined genetically and may become modified later in the course of life. Two interpretations may be offered to explain the increased esterification rate in HDL popu-

TABLE 5. Data of septuagenarians and patients with hypertension sorted according to body mass index (BMI) and total cholesterol (TC)

	Septuagenarians	Hypertension Sorted According to BMI		Hypertension Sorted According to TC	
		BMI < 115	BMI ≥ 115	TC < 6.3	TC ≥ 6.3
n	n = 15	n = 17	n = 15	n = 16	n = 16
n (I, II, III stage)	(0)	(4,8,5)	(3,5,7)	(5,7,4)	(2,6,8)
Age (yr)	76	51	51	48	53
BMI	103	106	129	117	116
TC (mmol/l)	6.1	6.5	6.7	5.45	7.46
TG (mmol/l)	1.3	3	2.7	2	3.8
HDL-TC (mmol/l)	0.94	0.99	0.93	0.99	0.94
FER_{HDL} (%/h)	17.7	29.1	30.9	27.91	32
HDL_{2b} (%)	18.4	8	8.7	9.8	6.8
HDL_{3a} (%)	65.8	54.9	51.6	55	51.7
HDL_{3b} (%)	21.8	37.1	40	35.1	41.6

Data are presented as means of selected groups.

Downloaded from www.jlr.org by guest, on October 19, 2017

lations in patients at risk. Either it is a process that has a protective function as it induces an increase in the capacity of HDL to esterify cholesterol and facilitates its efflux out of cells or, alternatively, an increased rate of cholesterol esterification may accelerate the atherogenic process. Accordingly, if the free cholesterol in plasma HDL were esterified at a higher rate, the newly produced cholesteryl esters would accumulate in potentially atherogenic particles such as VLDL remnants or LDL, as we have recently discussed (20). The significant differences in FER_{HDL} associated with the specific changes in HDL_{2b} and especially in HDL_{3b} are not invalidated by the fact that it is not possible to determine whether the HDL subfractions are evenly labeled and therefore whether FER_{HDL} corresponds to real mass esterification within HDL subpopulations. However, we found a good correlation between FER_{HDL} and mass increment of cholesteryl esters in plasma VLDL/LDL with time (data not shown). This suggests that possible small changes in the specific activity of different HDL subclasses do not affect the validity of the present findings. Furthermore, a recent report (21) on association of HDL_{3b} with risk factors for coronary artery disease is entirely consistent with our data.

Thus, regardless of the mechanism underlying the changes in HDL subclass distribution, these findings support our proposal that measurement of FER_{HDL} is a useful, simple, and reproducible method for quantitation of the changes in HDL subspecies distribution; FER_{HDL} may thus provide valuable information on risk of coronary artery disease.

We would like to thank Mrs. Marie Schutzova and Mrs. Lida Adler for their invaluable assistance with this project. In addition, we are grateful to Dr. Karmin O for her help in the FPLC analysis. The study was, in part, supported by Czechoslovak Health Care Foundation, BC Heart Foundation, and the BC Health Research Foundation.

Manuscript received 30 July 1991, in revised form 4 March 1992, and in re-revised form 6 May 1992.

REFERENCES

1. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
2. Fielding, C. J. 1990. Lecithin:cholesterol acyltransferase and the genesis of HDL. *In* Disorders of HDL. L. Carlson, editor. Smith Gordon, London. 19–24.
3. Dobiasova, M. 1983. Lecithin:cholesterol acyltransferase and the regulation of endogenous cholesterol transport. *Adv. Lipid Res.* **20:** 107–194.
4. Barter, P. J., G. J. Hopkins, L. Gorjatschko, and M. E. Jones. 1984. Competitive inhibition of plasma cholesterol esterification by human high density lipoprotein-subfraction 2. *Biochim. Biophys. Acta.* **793:** 260–268.
5. Dobiasova, M., J. Stribrna, D. L. Sparks, P. H. Pritchard, and J. J. Frohlich. 1991. 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.* **11:** 64–70.
6. Allain, C. C., L. S. Poon, C. S. Chan, and W. Richmond. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **29:** 470–475.
7. Bucolo, G., and H. David. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.* **19:** 476–482.
8. Burstein, M., H. R. Scholnick, and R. Morfin. 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11:** 583–595.
9. Dobiasova, M., and M. Schutzova. 1986. Cold labeled substrate and estimation of cholesterol esterification rate in lecithin:cholesterol acyltransferase radioassay. *Physiol. Bohemoslov.* **35:** 319–327.
10. Dobiasova, M., Z. Zouchova, and J. Obenberger. 1989. Lecithin:cholesterol acyltransferase and possible origin of lysolecithin in rabbit aqueous after a damage of blood-aqueous barrier. *Curr. Eye Res.* **8:** 441–448.
11. Yen, F. T., and T. Nishida. 1990. Rapid labeling of lipoproteins in plasma with radioactive cholesterol. Application for measurement of plasma cholesterol esterification. *J. Lipid Res.* **31:** 349–353.
12. Nichols, A. V., R. M. Krauss, and T. A. Musliner. 1986. Nondenaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol.* **128:** 417–431.
13. Williams, P. T., R. M. Krauss, A. V. Nichols, K. M. Vranizan, and P. D. S. Wood. 1990. Identifying the predominant peak diameter of high-density and low-density lipoproteins by electrophoresis. *J. Lipid Res.* **31:** 1131–1139.
14. Sparks, D. L., and P. H. Pritchard. 1989. The neutral lipid composition of recombinant high density lipoproteins regulates lecithin:cholesterol acyltransferase activity. *Biochem. Cell Biol.* **67:** 358–364.
15. Barter, P. J., G. J. Hopkins, and L. Gorjatschko. 1985. Lipoprotein substrates for plasma cholesterol esterification. Influence of particle size and composition of the high density lipoprotein subfraction 3. *Atherosclerosis.* **58:** 97–107.
16. Hopkins, G. J., and P. J. Barter. 1982. An effect of very low density lipoproteins on the rate of cholesterol esterification in human plasma. *Biochim. Biophys. Acta.* **712:** 152–160.
17. Johansson, J., L. A. Carlson, C. Landou, and A. Hamsten. 1991. High density lipoproteins and coronary atherosclerosis: a strong inverse relation with the largest particles is confined to normotriglyceridemic patients. *Arterioscler. Thromb.* **11:** 174–182.
18. Hamsten, A., J. Johansson, P. Nilsson-Ehle, and L. A. Carlson. 1990. Plasma high density lipoprotein subclasses and coronary atherosclerosis. *In* Disorders of HDL. L. Carlson, editor. Smith Gordon, London. 155–162.
19. Stampfer, M. J., F. M. Sacks, S. Salvini, W. C. Willett, and C. H. Hennekens. 1991. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N. Engl. J. Med.* **325:** 373–381.
20. Sparks, D. L., J. J. Frohlich, and P. H. Pritchard. 1991. Lipid transfer proteins, hypertriglyceridemia and reduced high density lipoprotein cholesterol. *Am. Heart J.* **122:** 601–607.
21. Williams, P. T., R. M. Krauss, K. M. Vranizan, M. L. Stefanick, P. D. S. Wood, and F. T. Lindgren. 1992. Associations of lipoproteins and apolipoproteins with gradient gel electrophoresis estimates of high density lipoprotein subfraction in men and women. *Arterioscler. Thromb.* **12:** 332–340.

JOURNAL OF LIPID RESEARCH ASBMB

Downloaded from www.jlr.org by guest, on October 19, 2017