patient-oriented and epidemiological research

Cholesterol esterification and atherogenic index of plasma correlate with lipoprotein size and findings on coronary angiography

Milada Dobiášová,* Jiri Frohlich,[1,†] Michaela Šedová,[§] Marian C. Cheung, and B. Greg Brown****

Institute of Physiology,* Academy of Sciences of the Czech Republic, Prague, Czech Republic; St. Paul's Hospital Healthy Heart Program/University of British Columbia,[†] Vancouver, BC, Canada; Center of Biomedical Informatics,[§] Institute of Computer Science, Academy of Sciences of the Czech Republic, Prague, Czech Republic; and Department of Medicine,** University of Washington, Seattle, WA

Abstract We examined the association between rate of cholesterol esterification in plasma depleted of apolipoprotein B-containing lipoproteins (FER_{HDL}), atherogenic index of plasma (AIP) [(log (TG/HDL-C)], concentrations, and size of lipoproteins and changes in coronary artery stenosis in participants in the HDL-Atherosclerosis Treatment Study. A total of 160 patients was treated with simvastatin (S), niacin (N), antioxidants (A) and placebo (P) in four regimens. FER_{HDL} was measured using a radioassay; the size and concentration of lipoprotein subclasses were determined by nuclear magnetic resonance spectroscopy. The S+N and S+N+A therapy decreased AIP and FER_{HDL}, reduced total VLDL (mostly the large and medium size particles), decreased total LDL particles (mostly the small size), and increased total HDL particles (mostly the large size). FER_{HDL} and AIP correlated negatively with particle sizes of HDL and LDL, positively with VLDL particle size, and closely with each other ($r = 0.729$). Changes in the proportions of small and large lipoprotein particles, which were reflected by FER_{HDL} and AIP, corresponded with findings on coronary angiography. Logistic regression analysis of the changes in the coronary stenosis showed that probability of progression was best explained by FER_{HDL} ($P = 0.005$). FER_{HDL} and AIP reflect the actual composition of the lipoprotein spectrum and thus predict both the cardiovascular risk and effectiveness of therapy. AIP is already available for use in clinical practice as it can be readily calculated from the routine lipid profile.—Dobiášová, M., J. Frohlich, M. Šedová, M. C. Cheung, and B. G. Brown. **Cholesterol esterification and atherogenic index of plasma correlate with lipoprotein size and findings on coronary angiography. *J. Lipid Res.* 2011.** 52: **566–571.**

Supplementary key words fractional esterification rate (FER_{HDL}) • log (TG/HDL-cholesterol) • AIP • biomarkers of cardiovascular risk • lipoprotein particle size • HDL-Atherosclerosis Treatment Study (HATS)

Many anthropometric, clinical, and biochemical factors can influence the composition and size of lipoprotein subpopulations. It has been demonstrated that the prevalence of small dense LDL particles increases cardiovascular (CV) risk (1–3) and that the distribution of differently sized particles in HDL influences its anti-atherogenic effects (4–8). In the HDL-Atherosclerosis Treatment Study (HATS), in which patients with coronary disease and low HDL-cholesterol (HDL-C) were treated with a combinations of simvastatin, niacin, and antioxidants, the therapy had a selective effect on composition of lipoprotein subpopulations and therefore on consequent changes in the coronary artery stenosis (9). Although the composition of lipoprotein subpopulations contributes substantially to plasma atherogenicity, it is impractical to measure its variations as the assays have not been standardized and are expensive and thus not suitable for routine use.

We have established that two markers of CV risk, namely cholesterol esterification rate in apolipoprotein

The study was supported by Grant NR/8328-3 from the Ministry of Health of the Czech Republic and grants from the National Institutes of Health (HL49546), the Clinical Nutrition Research Unit (DK35816), and the Diabetes Endocrinology Research Center (DK17047). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health or other granting agencies. A portion of this study was performed in the Clinical Research Center at the University of Washington under grant MO1 00037. Statistical analysis was performed in the Center of Biomedical Informatics ICS AS CR v.v.i. with the support of the grant no. 1M06014 of the Czech Ministry of Education, Youth and Sports.

Manuscript received 27 September 2010 and in revised form 6 January 2011.

Published, JLR Papers in Press, January 11, 2011
DOI 10.1194/jlr.P011668

Abbreviations: A, antioxidant; AIP, atherogenic index of plasma; apo, apolipoprotein; CE, cholesteryl ester; CV, cardiovascular; FER_{HDL}, rate of cholesterol esterification in plasma depleted of apolipoprotein B-containing lipoproteins; HATS, HDL-Atherosclerosis Treatment Study; HDL-C, HDL-cholesterol; log(TG/HDL-C), logarithmically transformed ratio of molar concentrations of triglyceride and HDL-cholesterol; N, niacin; P, placebo; S, simvastatin; TC, total cholesterol; TG, triglyceride.

[1] To whom correspondence should be addressed.
e-mail: jifr@interchange.ubc.ca

Copyright © 2011 by the American Society for Biochemistry and Molecular Biology, Inc.

This article is available online at http://www.jlr.org

Downloaded from www.jlr.org by guest, on June 20, 2012

ASBMB JOURNAL OF LIPID RESEARCH

(apo)B-depleted plasma (FER_{HDL}) and atherogenic index of plasma [log (TG/HDL-C)] (AIP) reflect the size of LDL and HDL subpopulations and closely correlate with each other over a wide range of plasma lipid values (10–13). AIP is, of course, a transformation of triglyceride (TG)/HDL-C that better meets the assumption of normality of the errors in the statistical model being used to describe the treatment effects than does the untransformed variable.

The value of both FER_{HDL} and AIP can be seen in the context of intravascular cholesterol transport: FER_{HDL} measures esterification rate of cholesterol by lecithin: cholesterol acyltransferase within HDL differently sized subpopulations. In small HDLs the esterification rate is high but large particles reduce it (10, 14). The destination of newly produced cholesteryl esters (CEs) is also linked to subpopulations size and with added internal standards of unesterified cholesterol and cholesteryl oleate. Large HDLs reduce esterification rate and serve as the most effective vehicle for delivery of CE via scavenger receptor class B type 1 to catabolic sites in liver and steroidogenic tissues (15). The close association of FER_{HDL} with AIP can be explained by TG participation in the production of large VLDL and small dense LDLs and have also been proposed to be the major determinants of cholesterol esterification/transfer and HDL remodeling in particles that regulate the esterification rate.

The potential of FER_{HDL} and AIP to predict CV risk was shown in the study of 1,108 patients who underwent coronary angiography (16). The relationships between FER_{HDL} or AIP and CV risk have been well established (12, 16, 17). However, the changes of these risk biomarkers with different therapies and their relation to treatment outcomes have not been studied.

In this study, we related the changes on coronary angiography in HATS to the values of FER_{HDL} and AIP and investigated their relation to lipoprotein subpopulations in patients on different therapeutic regimens.

MATERIALS AND METHODS

Patients

The rationale, methods, and results of HATS have been described in detail (9). The study tested the hypothesis that a decrease in serum LDL-cholesterol (LDL-C) with a simultaneous increase in HDL-C induced by the statin-niacin combination therapy provides greater benefits than treatment with either placebo or antioxidants. One hundred and sixty patients were divided into four groups and each group was treated with one of four regimens: simvastatin plus niacin (S+N), antioxidants (A), simvastatin, niacin, and antioxidants (S+N+A), or placebos (P). Patients underwent coronary angiography before and after 3 years of treatment. Plasma samples obtained at baseline and at 1 year on therapy were examined in the present analysis.

Laboratory assays

Analyses of plasma lipids and apolipoproteins were previously described (9). The average particle sizes of HDL, LDL, and VLDL subpopulations were determined by NMR spectroscopy (18). Particle concentrations (nmol/L for VLDL and LDL; μmol/L for HDL) were calculated for each subclass based on existing knowledge about the lipoprotein structure and the link between particle diameter and total core lipid content. Lipoprotein size subpopulations were defined as follows: large VLDL/chylomicrons (>60 nm), medium VLDL (35–60 nm), small VLDL (27–35 nm), large LDL (21.2–23 nm), small LDL (18–21.2 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm). Measurement of FER_{HDL} was described in detail previously (4, 11, 19). Briefly, apoB-containing lipoproteins are precipitated from EDTA plasma (that can be stored at −20°C up to 4 months or at −70°C for up to 6 years without changes in absolute values of FER_{HDL}) by phosphotungstic acid and $MgCl_2$. To the supernatant, which contains plasma with HDL only, is added a filter paper disk containing a trace of 3-H cholesterol. After an overnight incubation at 4°C, the disk is removed and the plasma with labeled HDL is heated to 37°C and incubated for 30 min (the esterification reaction is always linear over this time period). After the incubation, lipids are extracted by ethanol, ethanol evaporated, and with added internal standards of cholesterol and cholesteryl oleate, separated by TLC. Spots of cholesterol and cholesteryl oleate are visualized by iodine, spots cut from TLC plates, and transferred to scintillation vials. The radioactivity is estimated by liquid scintillation counting. The fractional esterification rate is calculated from radioactivity in spots of free and esterified cholesterol as percentages of HDL-C esterified per h. AIP (12) was calculated as logarithmically transformed ratio of molar concentrations of TG and HDL-C [log (TG/HDL-C)] in plasma (20).

Statistical analysis

Statistical analysis was performed using SPSS.15 .0 and R (21) software. The data are presented as means ± SD both before and during treatment for the four treatment groups. For descriptive purposes, the differences between measurements taken before and after treatment were tested by paired *t*-test within the four groups. The effect of treatment on FER_{HDL} and AIP was analyzed by one-way ANOVA. We tested the hypotheses that the mean values after 1 year of treatment are equal against the alternative that they differ at least for one treatment.

To investigate the correlations between FER_{HDL} and AIP on one hand and particle sizes and concentrations on the other hand, we calculated bivariate correlation coefficients for basal values of all subjects in the study and partial correlation coefficients for values obtained after treatment to eliminate the influence of the various treatments. To determine the after-treatment relationships between these measurements, we fitted two linear regression models with FER_{HDL} and AIP as the response variables and particle sizes and concentrations as explanatory variables.

We assessed association of changes in the coronary artery stenosis with FER-HDL, AIP, and other variables by logistic regression model. The progression of the coronary artery stenosis, defined as positive change versus no change or regression (i.e., dichotomous outcome) was considered as a response variable and the final model was found by the forward selection procedure (21). The initial set of explanatory variables was as follows: AIP, FER_{HDL}, total LDL and HDL cholesterol, triglycerides, apoAI, apoB, HDL, LDL, VLDL particle sizes, and HDL, LDL, and VLDL subpopulations' concentration. All models were adjusted for treatment.

RESULTS

Changes in the concentration and particle size of lipoproteins on treatment with S+N and S+N+A

Table 1 summarizes the data on lipoprotein subpopulations before and after 1 year of therapy with the four treatment regimens. The table also shows results of paired

Downloaded from www.jlr.org by guest, on June 20, 2012
ASBMB JOURNAL OF LIPID RESEARCH

t-tests between baseline and on treatment values performed for each treatment separately. Although the *p*-values are not adjusted for multiple comparisons, they indicate that there was a marked increase in the total HDL particles and decrease in the total LDL and VLDL particles induced by the S+N and S+N+A treatment. Total HDL increased mostly on account of large HDL (from 8.7% to 15.5% of total HDL in S+N, 6.% to 12.5% in S+N+A treatment). LDL decreased on account of small LDL particles whereas the number of large particles was not significantly changed. On the contrary, the treatment by S+N and S+N+A reduced practically all large, medium, and small VLDL particles. The mean particle size of HDL and LDL significantly increased by treatment with S+N and S+N+A. During these treatments, total cholesterol (TC), LDL-C, and TG markedly decreased while HDL-C increased. Placebo treatment had similar even lower significant effects on routine lipid profile with the exception of TG.

Effect of different treatment regimens on FER_{HDL} and AIP

Table 1 shows that after 1 year of treatment with S+N and S+N+A, FER_{HDL} decreased from 30.73 ± 7.05 and 32.0 ± 7.53%/h at baseline, respectively and to 19.53 ± 6.76 (−36%) and 21.96 ± 8.64%/h (−31%,), respectively. AIP decreased from 0.43 ± 0.22 and 0.49 ± 0.24 at baseline to 0.13 ± 0.25 (−71%) and 0.22 ± 0.31 (−51%), respectively. The placebo group also showed a small decrease in FER_{HDL} (−12.1%) whereas antioxidants had negligible effect. For the four treatment groups, the mean AIP and FER_{HDL} values after 1 year of treatment were compared by one-way ANOVA. In both cases, the hypothesis that mean values are the same in all groups was rejected ($P < 0.001$). Compared with placebo, antioxidant therapy had no effect, whereas S+N and S+N+A treatment decreased AIP and FER_{HDL} significantly.

Correlations between AIP and FER_{HDL} and concentrations and particle sizes of lipoproteins on treatment

We examined the relationship between FER_{HDL}, AIP, and the lipoprotein particles in plasma baseline and on the various treatment regimens (**Table 2**). At baseline, we used bivariate analysis, as the starting values of the patients were similar. The possible effects of the 1 year therapy were eliminated using appropriate adjustments. Table 2 shows that values of the correlation coefficients before and on treatment remained very close. FER_{HDL} and AIP values correlated with each other at baseline ($r = 0.721$); the partial correlation coefficient at 1 year of therapy (adjusted for treatment) was $r = 0.729$. The type of the treatment did not have a statistically significant effect on the linear relation between other variables. There was a significant correlation between FER_{HDL} and AIP and the number of total and small LDL and total, large, medium, and size of VLDL. Highly significant inverse correlations were observed in the atheroprotective variables such as large HDL. The inverse correlations were seen between LDL particle size and large LDL. Also significant association was found of FER_{HDL} and AIP with atherogenic apoB and atheroprotective apoAI.

TABLE 1. Effect of therapy on FER_{HDL}, AIP, and lipoprotein specific particles after 12 months of treatment

	PLACEBO		S+N		A		S+N+A	
n	Base-line 33	On treatment 33	Base-line 34	On treatment 34	Base-line 39	On treatment 39	Base-line 40	On treatment 40
Biomarkers								
FER_{HDL}	31. 5 ± 7.5	27.2 ± 7.2[a]	30.6 ± 7.4	18.5 ± 6.4[c]	30.0 ± 8.1	27.8 ± 9.5	32.0 ± 7.5	21.8 ± 8.6[c]
AIP	0.40 ± 0.23	0.35 ± 0.25	0.42 ± 0.21	0.11 ± 0.24[c]	0.40 ± 0.25	0.43 ± 0.29	0.48 ± 0.24	0.21 ± 0.31[c]
Lipoprotein particles								
HDL total (μmol/L)	28.4 ± 3.8	30.1 ± 3.8[b]	28.0 ± 3.6	30.4 ± 5.5[c]	29.0 ± 3.5	31.5 ± 4.5[c]	27.9 ± 4.8	31.3 ± 5.7[c]
HDL large (μmol/L)	1.8 ± 1.4	2.4 ± 1.6[a]	2.5 ± 1.6	4.7 ± 2.3[c]	2.2 ± 1.5	2.2 ± 1.9	1.8 ± 1.6	3.7 ± 2.2[c]
HDL small (μmol/L)	23.5 ± 4.2	24.3 ± 4.6	23.2 ± 4.7	22.8 ± 5.0	23.4 ± 4.3	26.3 ± 5.8[b]	22.7 ± 6.5	25.2 ± 7.1[a]
LDLtotal (nmol/L)	1763 ± 451	1694 ± 447	1670 ± 406	1027 ± 352[c]	1580 ± 426	1668 ± 451	1743 ± 500	1165 ± 347[c]
LDL large (nmol/L)	277 ± 253	309 ± 224	347 ± 308	327 ± 145	272 ± 212	270 ± 242	313 ± 298	271 ± 139
LDL small (nmol/L)	1431 ± 543	1333 ± 560	1275 ± 438	673 ± 393[c]	1260 ± 483	1342 ± 546	1371 ± 597	857 ± 379[c]
VLDL total (nmol/L)	106 ± 45	108 ± 46	94 ± 28	61 ± 28[c]	107 ± 41	108 ± 45	97 ± 40	60 ± 26[c]
VLDL large (nmol/L)	9.4 ± 8.0	8.3 ± 7.0	9.2 ± 8.1	4.1 ± 3.4[c]	7.3 ± 7.3	9.5 ± 10.0	11.4 ± 8.8	7.5 ± 6.8[b]
VLDL medium (nmol/L)	50.0 ± 28.3	49.1 ± 28.4	44.6 ± 23.6	24.7 ± 16.8[c]	48.6 ± 25.9	53.3 ± 32.8	48.2 ± 24.7	30.5 ± 25.7[b]
VLDL small (nmol/L)	46.6 ± 26.5	50.8 ± 20.2	40.3 ± 21.5	31.7 ± 15.7	51.2 ± 23.1	45.1 ± 22.3	37.3 ± 25.0	27.7 ± 20.5[a]
Lipoprotein size								
VLDL (nm)	55.2 ± 11.5	52.9 ± 9.6	54.5 ± 10.1	53.6 ± 8.9	52.7 ± 11.4	53.6 ± 12.7	58.4 ± 13.1	59.8 ± 14.4
LDL (nm)	20.2 ± 0.8	20.3 ± 0.8	20.3 ± 0.8	21.0 ± 0.7[c]	20.3 ± 0.7	20.2 ± 0.8	20.3 ± 0.9	20.7 ± 0.6[a]
HDL (nm)	8.4 ± 0.3	8.4 ± 0.3	8.4 ± 0.3	8.8 ± 0.5[c]	8.4 ± 0.3	8.4 ± 0.3	8.4 ± 0.4	8.6 ± 0.4[c]
Routine lipid profile[d]								
TC (mmol/L)	5.20 ± 0.79	4.90 ± 0.60[c]	5.13 ± 0.93	3.58 ± 0.63[c]	4.92 ± 0.61	4.95 ± 0.58	5.19 ± 0.90	3.81 ± 0.84[c]
LDL-C (mmol/L)	3.31 ± 0.67	3.02 ± 0.54[c]	3.31 ± 0.88	1.94 ± 0.54[c]	3.05 ± 0.67	2.95 ± 0.59	3.22 ± 0.78	2.06 ± 0.59[c]
HDL-C(mmol/L)	0.81 ± 0.11	0.87 ± 0.13[b]	0.81 ± 0.12	1.01 ± 0.24[c]	0.84 ± 0.12	0.86 ± 0.16	0.79 ± 0.11	0.93 ± 0.14[c]
TG (mmol/L)	2.35 ± 1.05	2.27 ± 1.05	2.34 ± 0.99	1.46 ± 0.76[c]	2.32 ± 1.29	2.69 ± 1.98	2.73 ± 1.3	1.87 ± 0.16[c]

Data are presented as mean ± SD.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] Current data estimated with NMR analyses.

Downloaded from www.jlr.org by guest, on June 20, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

TABLE 2. The correlations (r) between FER_{HDL}, AIP, and lipoprotein subpopulations before and after 12 months of treatment

	Before treatment		On treatment	
	Bivariate correlations		Partial correlations	
	FER_{HDL}	AIP	FER_{HDL}	AIP
FER_{HDL}		0.721[a]		0.729[a]
AIP	0.721[a]		0.729[a]	
Particles				
HDL total	−0.186[c]	−0.145	−0.077	−0.078
HDL large	−0.536[a]	−0.597[a]	−0.630[a]	−0.598[a]
HDL small	−0.227[b]	−0.272[b]	−0.070	−0.004
LDL total	0.236[b]	0.230[b]	0.573[a]	0.468[a]
LDL large	−0.591[a]	−0.670[a]	−0.505[a]	−0.562[a]
LDL small	0.458[a]	0.477[a]	0.497[a]	0.451[a]
VLDL total	0.380[a]	0.410[a]	0.592[a]	0.685[a]
VLDL large	0.629[a]	0.816[a]	0.560[a]	0.733[a]
VLDL medium	0.453[a]	0.556[a]	0.436[a]	0.593[a]
VLDL small	−0.072	−0.184[c]	0.148	0.135
Sizes				
HDL	−0.309[a]	−0.344[a]	−0.491[a]	−0.466[a]
LDL	−0.573[a]	−0.620[a]	−0.640[a]	−0.628[a]
VLDL	0.481[a]	0.668[a]	0.296[a]	0.425[a]

HDL particles are in μmol/L, LDL and VLDL particles in nmol/L. Sizes of HDL, LDL and VLDL in nm.
[a] *p*-value<0.0001.
[b] *p*-value<0.002.
[c] *p*-value<0.01.

Associations of FER_{HDL} and AIP with changes in coronary artery stenosis

The HATS study participants were divided into two groups based on changes (negative vs. positive) in coronary artery stenosis after 3 years of treatment to show association between plasma lipoproteins and their subpopulations to the angiographic changes (**Table 3**). Both FER_{HDL} and AIP had higher values in the group with increased stenosis ($P < 0.001$ and 0.008), together with increased total particles of LDL ($P < 0.007$) and VLDL ($P < 0.033$), small LDL ($P < 0.005$), and large and medium VLDL ($P < 0.044$ and 0.036). Although the total number of HDL particles was not significant in relationship to the changes in stenosis, the decreased stenosis was characterized by an increase of large HDL particles ($P < 0.001$) and reduction of large VLDL and small LDLs. From traditional lipids, namely TC, LDL-C, HDL-C, and TG, only HDL-C has shown significant reduction in the progression group.

The forward stepwise logistic regression analysis (adjusted for treatment) of changes in the coronary artery stenosis showed that albeit the probability of progression was significant in the first step with FER, AIP, HDL-C, ApoA1, ApoB, large HDL, total and small LDL particles, total and medium VLDL particles, and sizes of LDL and HDL, this probability was best explained by FER_{HDL} only (**Table 4**) (odds ratio = 1.07, $P = 0.005$). No other variable was significant in this model. When FER_{HDL} was not included in the initial set of predictors in the model 2 selection procedure, the final model adjusted for treatment contained again only one significant predictor of probability of progression/regression, which was the concentration of the large HDL subpopulation (odds ratio = 0.80, $P = 0.016$). If AIP was tested in the model (adjusted for treatment), its *p*-value was borderline significant ($P = 0.055$).

DISCUSSION

The objective of this study was to assess the relation between the novel biomarkers FER_{HDL} and AIP and the distribution of lipoprotein subpopulations before and during lipid-lowering treatment in patients with coronary disease, low HDL-C, and normal LDL-C in HATS (9). We also studied the association between these markers and the changes in coronary artery stenosis.

The lipid-lowering treatment changed the sizes and concentrations of the lipoprotein subpopulations as well

TABLE 3. The association of FER_{HDL}, AIP, and lipoprotein subpopulations with change in coronary artery stenosis (mean ±SD)

Variable	Stenosis ≤ 0 n = 50	Stenosis > 0 n = 95	*p*
FER_{HDL} (%/h)	20.2 ± 8.4	25.8 ± 8.6	<0.001
AIP	0.189 ± 0.297	0.328 ± 0.296	0.008
Lipoprotein particles			
HDL total (μmol/L)	31.80 ± 4.79	30.75 ± 4.80	0.212
HDL large (μmol/L)	4.1 ± 2.2	2.8 ± 2.2	<0.001
HDL small (μmol/L)	24.9 ± 5.761	24.8 ± 5.9	0.93
LDL total (nmol/L)	1228 ± 515	1457 ± 456	0.007
LDL large (nmol/L)	323 ± 179	277. ±197	0.176
LDL small (nmol/L)	866 ± 559	1134 ± 518	0.005
VLDL total (nmol/L)	73.90 ± 46.12	91.46 ± 45.73	0.033
VLDL large (nmol/L)	5.7 ± 6.4	8.4 ± 7.9	0.044
VLDL medium (nmol/L)	32.5 ± 28.2	43.3 ± 29.1	0.036
VLDL small (nmol/L)	34.8 ± 21.3	39.8 ± 22.2	0.198
Lipoprotein particle sizes			
HDL (nm)	8.7 ± 0.4	8.5 ± 0.4	<0.001
LDL (nm)	20.8 ± 0.8	20.4 ± 0.8	0.002
VLDL (nm)	56.9 ± 13.276	54.5 ± 11.43	0.255
Routine lipid profile			
TC (mmol/L)	4.11 ± 0.95	4.41 ± 0.86	0.061
LDL-C (mmol/L)	2.36 ± 0.78	2.55 ± 0.73	0.143
HDL-C (mmol/L)	0.98 ± 0.17	0.90 ± 0.18	0.009
TG (mmol/L)	1.79 ± 1.23	2.20 ± 1.47	0.088

ASBMB
JOURNAL OF LIPID RESEARCH
Downloaded from www.jlr.org by guest, on June 20, 2012

TABLE 4. Logistic regression models for progression of the coronary artery stenosis

Model	Predictor	Coef	exp(coef)	St.E	p
1	FER_{HDL}	0.072	1.075	0.025	0.005
2	HDL (large)	−0.22	0.803	0.092	0.016

Model 1: Model found by stepwise selection procedure, initial set of predictor variables: FER_{HDL}, AIP, TC, LDL-C, HDL-C, TG, apoAI, apoB, HDL (total, large, medium, small, size), LDL (total, large, small, size), VLDL (large, medium, small, size) and age. Model 2: Model found by stepwise selection procedure, the initial set of predictor variables without FER_{HDL}, other variables the same as in Model 1. Models are adjusted for treatment.

as the values of FER_{HDL} and AIP. Statin+niacin-containing regimens lowered the atherogenic VLDL and LDL and increased the protective HDL. There was a decrease in the proportion of large and medium sized VLDL and small dense LDL particles. On the other hand, there was an increase in the atheroprotective large HDL.

Treatment with antioxidants alone did not affect either FER_{HDL} or AIP, or VLDL or LDL subpopulations. However, use of antioxidants either alone or in combination with S+N tended to increase small HDL particles (Table 1). Placebo treatment had a minor positive effect on the distribution of the various subpopulations (Table 1), significant only for the increase of large HDL. More pronounced was the decrease in FER_{HDL} and improvement of routine lipid profile. This effect was probably related to the participants' compliance with lifestyle recommendations and to the protocol use of simvastatin among the roughly 8% of placebo patients with baseline LDL-C > 3.5mmol/L. The correlation between FER_{HDL} and AIP was highly positive ($r = 0.717$) both at baseline and at 1 year on treatment ($r = 0.729$).

FER_{HDL} and AIP strongly correlated with the size and concentration of individual lipoprotein subpopulations (Table 2). Increased concentration of medium and large VLDL and small LDL particles resulted in higher FER_{HDL} and AIP whereas the values of these parameters decreased with increasing concentration of large LDL and large HDL subpopulations. To further investigate the relation between FER_{HDL}, AIP, and particle sizes, we used two linear regression models to assess the potential of the explanatory variables (not shown). The variability of AIP was best explained by all VLDL concentrations and VLDL size (positive effect) and concentrations of large HDL and large LDL (negative effect). The coefficient of determination was 0.75, which means that the model explained 75% variability of AIP. The variability of FER_{HDL} was best explained by concentration of large HDL and HDL and VLDL particle sizes with coefficient of determination 0.62.

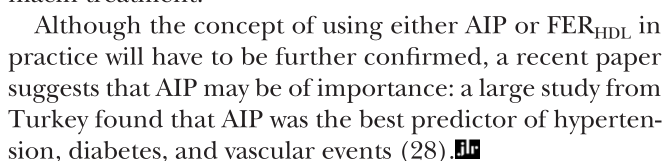
In the HATS, patients with normal LDL-C and low HDL-C level benefited significantly from the combination treatment with simvastatin and niacin that resulted in regression of coronary atherosclerosis (9). As previously reported, niacin increases the large particle size of HDL (21–23), and decreases the small HDL subpopulations (22, 23). Statins also increase the large α-1 HDL subpopulation (24). That was probably why the combination of niacin and simvastatin in the HATS not only decreased the concentration of plasma LDL-C and increased HDL-C (9) but also changed favorably the distribution of HDL subpopulations by increasing the proportion of large HDL. These changes resulted in markedly decreased values of both AIP and FER_{HDL}. Fibrates also decrease TGs and increase HDL but alter HDL distribution, in contrast to niacin, by increasing the proportion of small HDL and decreasing the large HDL (25).

In the logistic regression model adjusted (also nonadjusted) for treatment regimens, the probability of progression of the coronary artery stenosis (Table 4) was best explained by changes in FER_{HDL} with no other variable being significant in this model. When FER_{HDL} was not included in the set of initial predictors, the final model adjusted for treatment again contained only one predictor, namely the (−) concentration of large HDL subpopulation. If FER_{HDL} was replaced by AIP in the model, the p-value for AIP was borderline significant ($P = 0.055$). The effect of large HDL on the change of coronary stenosis, assessed by different methods, was previously reported (9, 22).

We hypothesize that the increased number of the large HDL particles, while suppressing the esterification rate of cholesterol, enhances the catabolism of the newly produced CE via scavenger receptor class B type 1. Our idea that differently sized HDL particles may affect the targeting of CE produced in plasma to either atherogenic or atheroprotective targets (26) is supported by the recent finding that rosuvastatin therapy may induce the regression of coronary atherosclerosis by raising plasma HDL-C, specifically by increasing HDL particle size (27). Thus, we believe that FER_{HDL} is a good measure of the atherogenic (or atheroprotective) pathways. It is not surprising that AIP, which is also associated with the lipoprotein size and correlates highly with FER_{HDL}, has a similar predicting potential as FER_{HDL}.

Our results confirm the importance of not only quantitative but also qualitative changes in HDL that occur with niacin treatment.

Although the concept of using either AIP or FER_{HDL} in practice will have to be further confirmed, a recent paper suggests that AIP may be of importance: a large study from Turkey found that AIP was the best predictor of hypertension, diabetes, and vascular events (28).



The authors thank Dr. J. Otvos for the NMR analyses.

REFERENCES

1. Austin, M. A., J. L. Breslow, C. H. Hennekens, J. E. Buring, W. C. Willett, and R. M. Krauss. 1988. Low-density lipoprotein subclass patterns and the risk of myocardial infarction. *JAMA*. **260:** 1917–1921.
2. Campos, H., J. J. Jr. Genest, E. Blijlevens, J. R. McNamara, J. L. Jenner, J. M. Ordovas, P. W. Wilson, and E. J. Schaefer. 1992. Low density lipoprotein particle size and coronary artery disease. *Arterioscler. Thromb.* **12:** 187–195.
3. Stampfer, M. J., R. M. Krauss, J. Ma, P. J. Blanche, L. G. Holl, F. M. Sacks, and C. H. Hennekkens. 1996. A prospective study of triglyceride level, low-density particle diameter, and risk of myocardial infarction. *JAMA*. **276:** 882–888.

Downloaded from www.jlr.org by guest, on June 20, 2012

4. Dobiášová, M., J. St íbrná, D. L. Sparks, P. H. Pritchard, and 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.
5. Drexel, H., F. W. Aman, K. Rentsch, C. Neunschwander, A. Leuthy, and S. I. Khan. 1992. Relation of high-density lipoprotein subfraction to the presence and extent of coronary artery disease. *Am. J. Cardiol.* **70:** 436–440.
6. Freedman, D. S., J. D. Otvos, E. J. Jeyarajah, J. J. Barboriak, A. T. Anderson, and J. A. Walker. 1998. Relation of lipoprotein subclasses as measured by proton nuclear magnetic resonance spectroscopy to coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1046–1053.
7. Asztalos, B. F., D. Collins, L. A. Cupples, S. Demissie, K. V. Horvath, H. E. Bloomfield, S. J. Sander Robins, and E. J. Schaefer. 2005. Value of high-density lipoprotein (HDL) subpopulations in predicting recurrent cardiovascular events in the Veterans Affairs HDL Intervention Trial. *Arterioscler. Thromb. Vasc. Biol.* **25:** 2185–2191.
8. Cheung, M. C., B. G. Brown, A. C. Wolf, and J. J. Albert. 1991. Altered particle size distribution of apolipoprotein A-I-containing lipoproteins in subjects with coronary artery disease. *J. Lipid Res.* **32:** 383–394.
9. Brown, B. G., Z. Xue-Qiao, A. Chait, L. D. Fisher, M. C. Cheung, J. S. Morse, A. A. Dowdy, E. K. Marino, E. L. Bolson, P. Alaupovic, et al. 2001. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N. Engl. J. Med.* **345:** 1583–1592.
10. Dobiasova, M., J. Stribrna, P. Pritchard, and J. Frohlich. 1992. Cholesterol esterification rate in plasma depleted of very low and low density lipoprotein 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.* **33:** 1411–1418.
11. Dobiášová, M., and J. Frohlich. 1996. Measurement of fractional esterification rate of cholesterol in apoB containing lipoproteins depleted plasma: methods and normal values. *Physiol. Res.* **45:** 65–73.
12. Dobiášová, M., and J. Frohlich. 2001. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER_{HDL}). *Clin. Biochem.* **34:** 583–588.
13. Dobiášová, M., Z. Urbanová, and M. Šamánek. 2005. Relations between particle size of HDL and LDL lipoproteins and cholesterol esterification rate. *Physiol. Res.* **54:** 159–165.
14. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
15. Rigotti, A., B. Trigatti, J. Babitt, M. Fenman, S. Xu, and M. Krieger. 1997. Scavenger receptor BI–a cell surface receptor for high density lipoprotein. *Curr. Opin. Lipidol.* **8:** 181–188.
16. Frohlich, J., and M. Dobiášová. 2003. Fractional esterification rate of cholesterol and ratio of triglycerides to HDL-cholesterol are powerful predictors of positive findings on coronary angiography. *Clin. Chem.* **49:** 1873–1880.
17. Tan, M. H., K. C. Loh, M. Dobiasova, and J. Frohlich. 1998. Fractional esterification rate of HDL particles in patients with type 2 diabetes: relation to coronary heart disease risk factors. *Diabetes Care.* **21:** 139–142.
18. Jeyarajah, E. J., W. C. Cromwell, and E. J. Otvos. 2006. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin. Lab. Med.* **26:** 847–870.
19. Dobiášová, M., and J. Frohlich. 1998. Assays of lecithin cholesterol acyltransferase (LCAT). *In*: Methods in Molecular Biology. Lipoprotein Protocols. J. M. Ordovas, editor. Humana Press, Totowa, NJ. 217–30.
20. Dobiášová, M. Calculator of atherogenic risk. http://www.biomed.cas.cz/fgu/aip
21. R Development Core Team. 2008. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.
22. Cheung, M. C., Z. Xue-Qiao, A. Chait, J. J. Albers, and B. G. Brown. 2001. Antioxidant supplements block the response of HDL to simvastatin-niacin therapy in patients with coronary artery disease and low HDL. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1320–1326.
23. Shepherd, J., C. J. Packard, J. R. Patsch, A. M. Jr. Gotto, and O. D. Taunton. 1979. Effects of nicotinic acid therapy on plasma high density lipoprotein subfraction distribution and composition and on apolipoprotein a metabolism. *J. Clin. Invest.* **63:** 858–867.
24. Johansson, J., and L. A. Carlson. 1990. The effect of nicotinic acid treatment on high density lipoprotein particle size subclass levels in hyperlipidaemic subjects. *Atherosclerosis.* **83:** 207–216.
25. Asztalos, B. F., K. V. Horvath, J. R. McNamara, P. S. Roheim, J. J. Rubinstein, and E. J. Schaefer. 2002. Comparing the effects of five different statins on the HDL subpopulation profiles of coronary heart disease patients. *Atherosclerosis.* **164:** 361–369.
26. Dobiášová, M., and J. Frohlich. 1998. Understanding the mechanism of LCAT reaction may help to explain the high predictive value of LDL/HDL cholesterol ratio. *Physiol. Res.* **47:** 387–397.
27. Asztalos, B. F., F. Le Maulf, G. E. Dallal, E. Stein, P. H. Jones, K. V. Horvath, F. McTaggart, and E. J. Schaefer. 2007. Comparison of the effects of high doses of rosuvastatin versus atorvastatin on the subpopulations of high-density lipoproteins. *Am. J. Cardiol.* **99:** 681–685.
28. Onat, A., G. Can, H. Kaya, and G. Hergenc. 2010. "Atherogenic index of plasma" (log10 triglyceride/high-density lipoprotein-cholesterol) predicts high blood pressure, diabetes and vascular events. *J. Clin. Lipidol.* **4:** 89–98.

ASBMB JOURNAL OF LIPID RESEARCH JLR

Downloaded from www.jlr.org by guest, on June 20, 2012