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Relations Between Particle Size of HDL and LDL Lipoproteins and Cholesterol Esterification Rate

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Summary

Particle size of low density (LDL) and high density (HDL) lipoproteins and cholesterol esterification rate in HDL plasma (FER_{HDL}) are important independent predictors of coronary artery diseases (CAD). In this study we assessed the interrelations between these indicators and routinely examined plasma lipid parameters and plasma glucose concentrations. In 141 men, healthy volunteers, we examined plasma total cholesterol (TC), triglycerides (TG), HDL and LDL cholesterol (HDL-C, LDL-C) and HDL unesterified cholesterol (HDL-UC). Particle size distribution in HDL and LDL was assessed by gradient gel electrophoresis and FER_{HDL} was estimated by radioassay. An effect of particle size and FER_{HDL} on atherogenic indexes as the Log(TG/HDL-C) and TC/HDL-C was evaluated. Subjects in the study had plasma concentrations (mean ± S.D.) of TC 5.2±0.9 mmol/l, HDL-C 1.2±0.3 mmol/l, TG 2.1±1.7 mmol/l, glucose 5±0.8 mmol/l. Relative concentration of HDL_{2b} was 17.6±11.5 % and 14.6±11.8 % of $HDL_{3b,c}$. The mean diameter of LDL particles was 25.8±1.5 nm. The increase in FER_{HDL} significantly correlated with the decrease in HDL_{2b} and LDL particle size ($r = –0.537$ and $–0.583$, respectively, $P<0.01$) and the increase in $HDL_{3b,c}$ (0.473, $P<0.01$). Strong interrelations among TG and HDL-C or HDL-UC and FER_{HDL} and particle size were found, but TC or LDL-C did not have such an effect. Atherogenic indexes Log(TG/HDL-C) and TC/HDL-C correlated with FER_{HDL} (0.827 and 0.750, respectively, $P<0.0001$) and with HDL and LDL particle size.

Key words

Fractional esterification rate of cholesterol (FER_{HDL}) • Particle size of plasma lipoproteins • HDL • LDL • Atherogenic indexes

Introduction

Fractional esterification rate of cholesterol in LDL/VLDL depleted plasma has been shown to be the strongest predictor test of positive findings on coronary angiography (Frohlich and Dobiášová 2003) and one of best indicators of changes in the progression of coronary artery disease (CAD) after treatment with statins and antioxidants (Brown *et al.* 2001). Its predictive potential bears upon the interaction lecithin-cholesterol bilayers of differently sized HDL subclasses with lecithin cholesterol acyltransferase (LCAT). The size of lipoprotein particles is crucial for cholesteryl esters (CE) production and destination (Dobiášová and Frohlich 1999, Dobiášová

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2004). The destination of newly produced CE appears to be more essential for the origin of CAD than their total production. Differently sized lipoprotein particles play a protective (buoyant HDL and LDL particles) or an atherogenic role (small HDL and LDL particles) in CAD (Austin *et al.* 1990, Drexel *et al.* 1992). Thus FER_{HDL} as a marker of lipoprotein particle size (Dobiášová and Frohlich 1994) serves as a functional test of lipoprotein quality. Although it has been reported earlier that FER_{HDL} correlated with HDL particle size (Dobiášová *et al.* 1992) and also with LDL particle size (Ohta *et al.* 1997, Dobiášová and Frohlich 2001), these interrelations have not been assessed in more detail.

In this study, we have examined standard lipid parameters, estimated HDL and LDL particle size, determined FER_{HDL} and analyzed the relations among these parameters in 141 men. The effects of FER_{HDL} and lipoprotein particle size on atherogenic indexes such as Log(TG/HDL-C) (Dobiášová and Frohlich 2001) and TC/HDL-C were also evaluated.

Subjects and Methods

A total of 141 clinically healthy men aged 28 to 70 years participated in this study. These were volunteers, mostly employees of the Czech railways.

Blood samples were collected after an overnight fast. Plasma concentrations of TC, HDL-C, HDL-UC, TG were assessed by standard enzymatic methods (Wako Chemicals GmbH, Germany), LDL-C was calculated using the Freedewald formula.

Measurement of FER_{HDL} in the HDL fraction of the plasma has previously been described in detail (Dobiášová and Schützová 1986, Dobiášová and Frohlich 1998, Dobiášová *et al.* 2000). Briefly, apoB-containing lipoproteins are precipitated from EDTA plasma (that can be stored at –20 ^{0}C up to 3 months or at –70 ^{0}C up to several years) by phosphotungstic acid and $MgCl_2$. A filter paper disk containing a trace of ^{3}H-cholesterol is added to the supernatant. After overnight incubation at 4 ^{0}C the disk is removed and the plasma with labeled HDL is incubated at 37 ^{0}C for 30 min (esterification reaction is always linear during this time period). After the incubation lipids are extracted by ethanol and separated by thin layer chromatography. The fractional esterification rate of cholesterol is calculated from the ratio of radioactivity of free and esterified cholesterol. Values are percentages of HDL cholesterol esterified per hour (%/h).

Subpopulations of HDL and LDL were analyzed by gradient gel electrophoresis (Alamo gels) as described in detail (Rainwater 1998). Briefly, EDTA plasma was centrifuged at density of 1.21 g/ml in a 70.1Ti rotor using a Beckmann L-90 ultracentrifuge at 42 000 rpm for 24 h at 15 ^{o}C. Non-dialysed lipoprotein fraction was mixed with sampling buffer containing 40 % sucrose, and 8 μl of sample was applied on each lane of polyacrylamide gradient gels (Alamo Gels, San Antonio, TX) in an electrophoresis system (C.B.S. Scientic Company, Inc. Del Mar, CA). For resolution of HDL subclasses 4-30 % and for LDL 2-16 % polyacrylamide gels were used. The samples were electrophoresed in Tris-borate/EDTA buffer. A mixture of globular proteins (HMW Calibration Kit, Pharmacia, Uppsala, Sweden) were run concurrently as particle size markers. Gels were run for 21 h at 125 V and stained for proteins by Commasie Brilliant Blue. The migration distances of HDL subclasses were measured relative to the migration distance of bovine serum albumin. Three of HDL subclasses were distinctly resolved on the gel: HDL_{2b} (9.5-11.9 nm), $HDL_{3a,2a}$ (8.3-9-5 nm) and $HDL_{3b,c}$ (7.0-8.2 nm). These particle sizes were similar to those reported by Williams *et al.* (1990). 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, Sweden). For the assessment of LDL size we measured diameters of predominant LDL species, yielding continuous variables. Thyreoglobulin (17 nm), ferritin (12 nm) and polystyrene microspheres (38 nm diameter, Duke Scientific Corporation, Palo Alto, CA) were used as calibrators for LDL.

The data were analyzed using statistical methods by SPSS Base and Regression Models 11.5. Continuous variables are presented as means ± SD. Differences between FER_{HDL} quartiles were tested by unpaired Student´s T-test and Leven statistic for the equality of group variances.

The relationship between FER_{HDL} and other examined parameters were calculated using Pearson and non-parametric Spearman bivariate correlation tests. Linearity of relationship between variables were tested using partial correlation by controlling for the effect of age, body parameters as BMI and waist and TC. A stepwise multiple regression was used for estimating coefficients which best predict the value of selected risk factors.

Results

Data of the subjects in the study

The study cohort consisted of 141 men, age range 28-70 years. The concentrations of plasma lipids, relative proportions of HDL subpopulations, particle size of LDL, esterification rate of cholesterol and calculated indexes of atherogenicity of subjects in the study are shown in Table 1.

Mean plasma concentration of TC (5.2 mmol/l) and HDL-C (1.2 mmol/l) were within the range recommended by NCEP Adult Treatment Panel III (2002), while TG (2.1 mmol/l) was moderately elevated. Relative composition of HDL subpopulations such as 18 % of HDL_{2b} and 15 % $HDL_{3b,c}$ corresponded to values reported for men (Dobiášová *et al.* 1992). Mean diameter of LDL particles 25.8 nm also ranked mostly within the normal LDL pattern A (Austin *et al.* 1990). Plasma level of glucose (5 mmol/l) was within the normal range.

Table 1. Descriptive data of subjects in the study (n = 141).

Age (years)	43.9±9.1
SBP (mm Hg)	126.8±12.8
Glucose (mmol/l)	4.97±0.80
TC (mmol/l)	5.19±0.90
TG (mmol/l)	2.13±1.74
LDL-C (mmol/l)	3.21±0.75
HDL-C (mmol/l)	1.21±0.30
HDL-UC (mmol/l)	0.20±0.05
FERHDL (%/h)	19.41±6.08
HDL2b (%)	17.60±11.49
HDL2a3a (%)	67.80±12.41
HDL3b3c (%)	14.60±11.82
LDL size (nm)	25.82±1.53
Log(TG/HDL-C)	0.12±0.31
TC/HDL-C	4.50±1.24

Data are means ± S.D.

Table 2. Correlations between FER_{HDL}, atherogenic indexes and lipoprotein particle size and estimated variables.

	FER_{HDL}	**Log(TG/HDL-C)**	**TC/HDL-C**	**HDL_{2b}**	**HDL_{2a3a}**	**HDL_{3b3c}**	**LDL size**
Age	0.072	0.143*	0.104	–0.152	–0.010	0.158	–0.162*
BPS	0.169*	0.213**	0.175*	0.016	–0.158	0.147	–0.200*
Glucose	0.177*	0.171*	0.253**	–0.237**	0.071	0.155	–0.095
TC	0.087	0.236**	0.395**	–0.087	0.141	–0.063	–0.040
TG	0.653**	0.881**	0.655**	–0.338**	–0.147	0.483**	–0.270**
LDL-C	0.025	0.038	0.485**	–0.109	0.161	–0.073	–0.060
HDL-C	–0.708**	–0.647**	–0.728**	0.348**	0.102	–0.446**	0.438**
HDL-UC	–0.638**	–0.568**	–0.663**	0.300**	0.045	–0.339**	0.440**
FER_{HDL}	1	0.827**	0.750**	–0.537**	0.046	0.473**	–0.582**
Log(TG/HDL-C)	0.827**	1	0.807**	–0.390**	–0.131	0.516**	–0.447**
TC/HDL-C	0.750**	0.807**	1	–0.394**	–0.084	0.471**	–0.404**
HDL_{2b}	–0.537**	–0.390**	–0.394**	1	–0.513**	–0.434**	0.210*
HDL_{2a3a}	0.046	–0.131	–0.084	–0.513**	1	–0.551**	0.124
HDL_{3b3c}	0.473**	0.516**	0.471**	–0.434**	–0.551**	1	–0.327**
LDL size	–0.582**	–0.447**	–0.404**	0.210*	0.124	–0.327**	1

*P<0.05, ** P<0.01

Correlation analysis

Interrelations among FER_{HDL}, HDL and LDL particle size and atherogenic indexes on the one hand and lipid parameters on the other are shown in Table 2. There were many similarities between large particles of HDL and LDL. They were inversely related to small HDL particles, to TG, to FER_{HDL} and both atherogenic indexes. Large particles positively correlated with HDL-C and HDL-UC, while relation to TC or LDL was not significant. It is of interest that LDL size inversely correlated with age and blood pressure. A similar relation between HDL_{2b} and age was found only if controlling for

TG and TC in partial correlation. The significance of the inverse correlation between HDL_{2b} and plasma glucose was maintained even if controlled for TC, TG and HDL-C. Small $HDL_{3b,c}$ particles correlated inversely with LDL size, HDL_{2b} particles, HDL-C and HDL-UC. On the other hand, they significantly correlated with atherogenic markers such as FER_{HDL}, TG and both atherogenic indexes. Again, there was no correlation between small HDLs and TC or LDL-C. On the contrary, the relative proportion of small $HDL_{3b,c}$ particles increased with increasing TG and reduced HDL-C. The intermediate $HDL_{3a,2a}$, constituting the bulk of HDL particles, were related to small and large HDLs but not to the other parameters. FER_{HDL} was strongly related to particle size of both HDL and LDL. The combination of positive correlation between FER_{HDL} and TG and inverse correlation between FER_{HDL} and HDL-C or HDL-UC resulted in a relation of FER_{HDL} with logarithmically transformed TG/HDL-C ratio ($r = 0.827$, $p<0.00001$). FER_{HDL}, similarly like lipoprotein particle size did not correlate with TC and LDL-C.

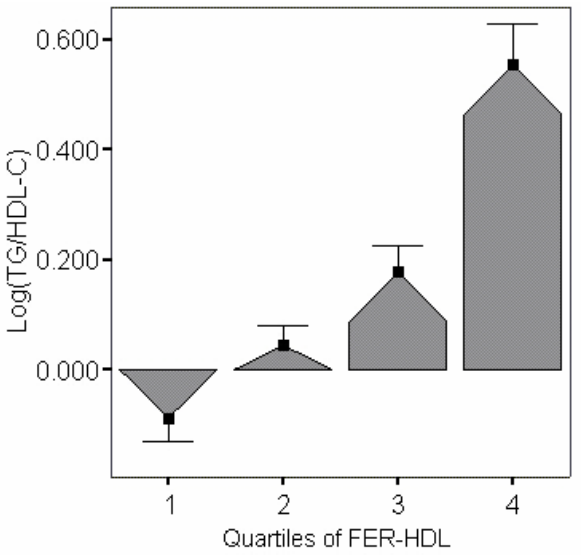

Fig. 1. Atherogenic index Log(TG/HDL-C) in FER_{HDL} quartiles. Values of FER_{HDL} quartiles: 1) 12.15±2.23 (%/h), 2) 16.75±1.18 (%/h), 3) 20.62±1.20 (%/h), 4) 26.95±3.77 (%/h). Bars show means. Error bars show 95.0 % of mean.

The atherogenic indexes – TC/HDL-C and Log(TG/HDL-C)-correlated mutually, and with high significance, with FER_{HDL}, particle size and other lipids. It is worth noting that correlation between TC/HDL-C and TG was much higher than between TC/HDL-C and TC itself or LDL-C. Both indexes also correlated with SBP and glycemia.

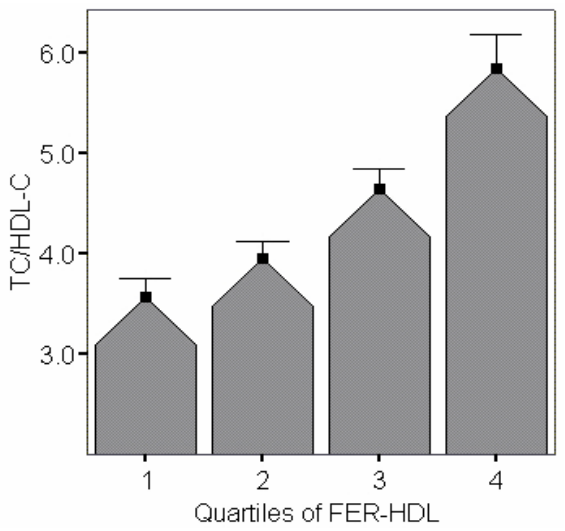

Fig. 2. Atherogenic index TC/HDL-C in FER_{HDL} quartiles. Values of FER_{HDL} quartiles the same as in Fig. 1.

Table 3. Predictors of selected variables in stepwise regression analysis

Dependent variable	Independent variables	Significance
FER_{HDL}	Log(TG/HDL-C)	0.0001
	HDL-C	0.0001
	HDL_{2b}	0.0001
	LDL size	0.0001
HDL_{2b}	FER_{HDL}	0.0001
	$HDL_{3b,c}$	0.0001
	Log(TG/HDL-C)	0.0001
$HDL_{3b,c}$	Log(TG/HDL-C)	0.0001
	HDL_{2b}	0.001
LDLSIZE	FER_{HDL}	0.0001
Log(TG/HDL-C)	TG	0.0001
	HDL-C	0.0001
	FER_{HDL}	0.006
	TC/HDL-C	0.045

Variables in analysis: Age, TG, HDL-C, LDL-C, HDL-UC, FER_{HDL}, GLY, Log(TG/HDL-C), TC/HDL-C, HDL_{2b}, $HDL_{3b,c}$ and LDL SIZE. Method: Stepwise (Criteria: Probability-of-F-to-enter ≤ 0.050, Probability-of-F-to-remove ≥ 0.100).

Regression analysis

The regression analysis was performed to identify the determinants of FER_{HDL} and lipoprotein particle size among the estimated variables (Table 3), significant with FER_{HDL} in bivariate correlation. The dominant predictor of FER_{HDL} was Log(TG/HDL-C). The next predictors were HDL-C, HDL_{2b} and LDL size.

FER_{HDL}, $HDL_{3b,c}$, and Log(TG/HDL-C) have contributed significantly to variations in HDL2b. Variations in $HDL_{3b,c}$ were dependent on Log(TG/HDL-C) and HDL_{2b}. FER_{HDL} alone was the predictor of LDL particle size. The predictors of Log(TG/HDL-C) were, besides the components of this ratio, also FER_{HDL} and ratio of TC/HDL-C.

Quartiles of FER_{HDL}

Considering FER_{HDL} as an independent metabolic marker of the lipoprotein phenotype, we compared differences of routinely used atherogenic indexes Log(TG/HDL-C) and TC/HDL-C between individual FER_{HDL} quartiles (Figs 1 and 2). In independent samples the t-test showed that the differences between successive FER_{HDL} quartiles were significant for both indexes.

Discussion

The main finding of this report was that the particle size of HDL and LDL lipoproteins is well interrelated and that the size of both lipoproteins is reflected in the rate of cholesterol esterification in the plasma depleted of VLDL and LDL.

Lipoprotein particle size and CAD

Although it is well known that prevalence of small LDL particles (Campos *et al.* 1992) as well as small HDL subpopulations (Drexel *et al.* 1992) represent a high risk of increased CAD, the question why these particles are preferentially deposited in peripheral cells and how they originate, still remain far from clear. It is speculated that TGs play a decisive role in the remodeling lipoprotein subpopulations (Murakami *et al.* 1995). The possible pathway is that hypertriglyceridemic VLDL stimulates exchange of cholesteryl esters from both HDL and LDL for VLDL-TG by means of cholesteryl ester transferring protein (CETP). TG-enriched LDL then undergo lipolysis and become smaller and more dense, whereas from TG-enriched HDL can dissociate apoAI. The clearance of apoAI may induce a change in the HDL size pattern. Thus TG may have a direct effect on the formation of both HDL and LDL particles.

LCAT and reverse cholesterol transport (RCT)

LCAT-mediated esterification of plasma UC promotes RCT and thus creates a gradient for transfer of cholesterol from cell membranes (Glomset 1968). However, two findings counteract the attractive and simple conception that the higher the esterification rate the more of the cell's stores of UC would be depleted. The first finding is the fact that LCAT is able to esterify any UC which is located on small particles of HDL regardless of whether they are transferred from peripheral cells or from LDL (Dobiášová and Fohlich 2000). It has been reported that these LDLs become the major source of UC for LCAT reaction (Francone and Fielding 1990, Fielding *et al.* 1991, Huang *et al.* 1993). It has also been shown that excess of LDL inhibit esterification of cellular UC (Nakamura *et al.* 1993). The second finding is the prevalence of HDL small particles reroute newly formed CE by means of CETP to the VLDL with subsequent transformation to LDL thus increasing their atherogenic capacity. In the case when large HDL_{2b} particles are present, they may deliver their CE to specific (SR-BI) liver and steroidogenic tissue receptors where they are depleted of lipids (contrary to LDL) and released into the extracelular fluid (Rigotti *et al.* 1997). These are the reasons why knowledge of the total production of CE cannot provide complete information about the power of RCT and why conclusions about relations between LCAT and CAD have so far been inconsistent.

FER_{HDL} and lipoprotein particle size

FER_{HDL} is neither a measure of total mass of CE produced by LCAT nor a measure of esterification rate in total plasma. Depletion of apoB containing lipoproteins makes it possible to omit a secondary source of UC from LDL and VLDL and to preserve the natural plasma protein components for binding the other product of the LCAT reaction such as lysophosphatidyl choline which is able to inhibit the LCAT reaction *in vitro.* As FER_{HDL} is related to the plasma lipoprotein pattern, it is thus considered as a biological scanner of differently sized HDL particles which compete between each other as a better substrate for LCAT or for binding or releasing newly formed CE.

The relation between FER_{HDL} and HDL particle size is clear because both components are actually present in the reaction medium. However, the highly significant correlation between FER_{HDL} and size of LDL particles that had been removed previously, can be explained only by the closest relations between populations of apoA and apoB containing lipoproteins. In this study we have confirmed this hypothesis as there was a good accordance of these populations in the particle size pattern. We have also shown that FER_{HDL}

was the first important predictor of HDL_{2b} and LDL size. The close association of FER_{HDL} with TG and particle size of LDL, which were not actually present in the reaction medium, further demonstrates the interrelationship between HDL and LDL.

Our results seem to support the above hypothesis of triglycerides as a promoter of the remodeling of all plasma lipoproteins. On the other hand, the fluctuation of TG level by postpradial lipemia has a negligible effect on lipoprotein particle size and FER_{HDL} (results not shown). The rather invariable lipoprotein phenotype may be consistent with such a genetic influence.

FER_{HDL}, atherogenic indexes and CAD

It was shown previously that logarithmically transformed ratio TG/HDL-C highly correlated with FER_{HDL} and LDL particle size. The high value of FER_{HDL} and Log(TG/HDL-C) were consistent with the extent of atherogenic risk. Lowest values were found in children, women in premenopause and octagenarian men, while middle aged men and subjects of all known risk factors for CAD such as obesity, type 2 diabetes or hypertension had increased values. The logarithmically transformed ratio TG/HDL-C as shown in this study was the best determinant of the functional risk marker FER_{HDL}. Thus plasma TG and HDL-C, in fact their ratio is associated with particle size of both HDL and LDL.

Conclusions

Our results have shown the significant interrelations between HDL and LDL particle size and FER_{HDL} as the most powerful marker of lipoprotein phenotype.

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