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Atherogenic Lipoprotein Profile in Families with and without History of Early Myocardial Infarction

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Summary

In this study we compared several parameters characterizing differences in the lipoprotein profile between members of families with a positive or negative family history of coronary artery disease (CAD). In addition to regular parameters such as the body mass index (BMI), total plasma cholesterol (TC), low density (LDL-C) and high density (HDL-C) cholesterol and triglycerides (TG) we estimated the fractional esterification rate of cholesterol in apoB lipoprotein-depleted plasma (FER_{HDL}) which reflects HDL and LDL particle size distribution. A prevalence of smaller particles for the atherogenic profile of plasma lipoproteins is typical. Log (TG/HDL-C) as a newly established atherogenic index of plasma (AIP) was calculated and correlated with other parameters. The cohort in the study consisted of 29 young (< 54 years old) male survivors of myocardial infarction (MI), their spouses and at least one offspring (MI group; n=116). The control group consisted of 29 apparently healthy men with no family history of premature CAD in three generations, their spouses and at least one offspring (control group; n=124). MI families had significantly higher BMI than the controls, with the exception of spouses. Plasma TC did not significantly differ between MI and the controls. MI spouses had significantly higher TG. Higher LDL-C had MI survivors only, while lower HDL-C had both MI survivors and their spouses compared to the controls. FER_{HDL} was significantly higher in all the MI subgroups (probands 25.85±1.22, spouses 21.55±2.05, their daughters 16.93±1.18 and sons 19.05±1.33 %/h) compared to their respective controls (men 20.80±1.52, spouses 14.70±0.98, daughters 13.23±0.74, sons 15.7±0.76 %/h, $p<0.01$ to $p<0.05$). Log (TG/HDL-C) ranged from negative values in control subjects to positive values in MI probands. High correlation between FER_{HDL} and Log (TG/HDL-C) ($r = 0.80$, $p<0.0001$) confirmed close interactions among TG, HDL-C and cholesterol esterification rate. The finding of significantly higher values of FER_{HDL} and Log (TG/HDL-C) indicate higher incidence of atherogenic lipoprotein phenotype in members of MI families. The possibility that, in addition to genetic factors, a shared environment likely contributes to the familial aggregation of CAD risk factors is supported by a significant correlation of the FER_{HDL} values within spousal pairs (control pairs: $r = 0.51$ $p<0.01$, MI pairs: $r = 0.41$ $p<0.05$).

Key words

Cholesterol esterification rate (FER_{HDL}) • Atherosclerosis • Spouses • Offspring • Log (TG/HDL cholesterol)

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Introduction

Studies of families at risk for coronary artery disease (CAD) have contributed significantly to our understanding of genetic and environmental factors that cause atherosclerosis (Simons *et al.* 1988, Genest *et al.* 1991, Knutsen and Knutsen 1991, Knuiman *et al.* 1996a,b, Inoue *et al.* 1996). However, only a few studies examined plasma lipoprotein profiles in greater detail. Recently, the criteria for "atherogenic" plasma phenotype were defined (Campos *et al.* 1992, Coresh *et al.* 1993, Grundy 1999). The "atherogenic" profile of plasma lipoproteins is characterized by elevated concentration of plasma triglycerides (TG) and by a predominance of small dense LDL and low HDL cholesterol (HDL-C). Small HDL particles and a lack of larger HDL particles also contribute to the plasma atherogenic profile (Johansson *et al.* 1991, Drexel *et al.* 1992). The predominance of small sized HDL and LDL particles is reflected in a high rate of cholesterol esterification by endogenous lecithin cholesterol acyltransferase (LCAT) in apo B lipoprotein-depleted plasma (FER_{HDL}) (Dobiášová *et al.* 1991, 1992, Ohta *et al.* 1997a,b). On the other hand, the "nonatherogenic" lipoprotein profile is characterized by the presence of large HDL and LDL particles and low values of FER_{HDL}. Because of the high correlation between lipoprotein particle size and FER_{HDL} this method could be considered as an alternative of the more laborious gradient gel electrophoresis. As reported earlier, the FER_{HDL} correlates positively with plasma TG and negatively with plasma HDL-C (Dobiášová *et al.* 1991, 1992, Ohta *et al.* 1997a,b, Dobiášová and Frohlich 1996). This fact led us to hypothesize that it is the ratio of TG to HDL-C in the plasma that determines the esterification rate of cholesterol (Dobiášová and Frohlich 1999).

In this study, we assessed the relation between the cholesterol esterification rate and the ratio of plasma TG to HDL-C logarithmically transformed. We determined FER_{HDL} and Log (TG/HDL-C) as well as the number of non-lipid and lipid risk factors in a cohort of young survivors of myocardial infarction and their first degree relatives. These findings were compared with those in an appropriately matched cohort of families with no history of early CAD in three successive generations.

Subjects and Methods

Subjects

1. Twenty-nine young male survivors of myocardial infarction (MI survivors) in whom the diagnosis was confirmed by typical clinical symptoms and enzyme elevations were recruited from out-patients of the Institute of Preventive and Clinical Medicine in Bratislava, Slovakia. All had myocardial infarction before the age of 54 years (range 31-54 years). Patients received standard therapy except for lipid lowering drugs. The beta-blockers used were cardioselective and thus did not affect lipid concentrations.

2. The spouses of MI survivors (MI spouses, n=29) shared the same family environment; only a few of them had risk factors. Table 1 summarizes the data on conventional risk factors in these subjects.

3. At least one offspring from each MI family was enrolled in the study (MI sons, n=30, MI daughters, n=28).

4. Control subjects were spouse pairs with negative personal and family history of premature atherosclerotic disorders (myocardial infarction, stroke and peripheral vascular disease before age 65) in three generations (control men, n=29, control spouses, n=29). Some of these subjects had risk factors for CAD (Tab. 1).

5. At least one offspring from each control family (control sons, n=24, control daughters, n=42).

Table 1. Additional risk factors in subjects in the study.

	MI probands	Control men	MI spouses	Control spouses
Hypertension	8 (28 %)	5 (17 %)	5 (17 %)	2 (7 %)
DM	2 (7 %)	2 (7 %)	3 (10 %)	0
Smoker	3 (10 %)	7 (24 %)	11 (38 %)	2 (7 %)

The offspring from MI and control cohorts, who shared the same family environment, were all apparently healthy. The only significant differences were in the frequency of smoking between women in the two groups (11 in MI vs. 2 in controls, $p<0.05$). Informed consent was obtained from all subjects and the study was approved by the Ethics Committee of the Institute of Preventive Medicine in Bratislava.

Clinical examination

Personal and family history as well as assessment of risk factors were noted down in a questionnaire and supervised by a physician. The physical examination included body weight and height, ECG and blood pressure measurement. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2).

Blood sampling and lipid analyses

Blood samples were collected after 12 or more hours of fasting into EDTA vacutainers. Plasma was isolated by centrifugation at 1750 x *g* for 10 min at 4 °C. Plasma samples were analyzed within 48 h if kept on ice, or within three months if stored at –20 °C. Plasma cholesterol (TC) and triglycerides (TG) were estimated enzymatically (Boehringer-Mannheim autoanalyzer Hitachi 911). High density lipoprotein total (HDL-C) and unesterified (HDL-UC) cholesterol were estimated using Wako kits (Wako Chemicals GmbH, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (the TG levels in all patients were less than 4.6 mmol/l).

Determination of cholesterol esterification rate

Fractional esterification rate of cholesterol in HDL plasma (FER_{HDL}) was determined as described in detail previously (Dobiášová and Frohlich 1996, 1998). The principle of the method is to measure the increase in radioactivity in cholesteryl esters (CE) in apoB lipoprotein-depleted plasma (HDL-plasma) prepared by precipitation of these lipoproteins with phosphotungstate (PTA)-$MgCl_2$ (Burnstein *et al.* 1970). HDL-plasma is labeled with a trace amount of ^{3}H-cholesterol (dispersed in filter paper discs) at 4 ^oC for 18 h. After incubation at 37 ^oC for 30 min is plasma extracted with 98 % ethanol, dried samples are solubilized in chloroform with UC/CE standards and separated by TLC. The spots visualized using iodine vapors are cut out from TLC and their counts were measured by a β-scintillation counter.

Statistical analyses

Differences between the lipid, lipoprotein, FER_{HDL}, BMI and age were determined by the two-tailed Student's t-test for normally distributed parameters and by the unpaired Wilcox test for non-normally distributed parameters. The relationship between these parameters was determined using partial correlation coefficients and ANOVA. Statistical analysis was carried out using the SAS statistical software (SAS Institute Inc.).

Results

Comparison of the cohort age, BMI and plasma lipids

Table 2 summarizes the data on age, BMI and plasma lipids in probands, spouses and offspring (sons and daughters) in control and MI cohorts. MI probands,

Table 2. Data of the men, spouses, sons and daughters of MI and control families.

	n	AGE	BMI	TC	TG	LDL-C	HDL-C	HDL-UC
Control								
Men	29	46.6±1.1	26.0±0.6	5.71±0.17	1.96±0.26	3.57±0.16	1.25±0.05	0.187±0.008
Spouses	29	45.3±1.0	24.5±0.9	5.19±0.14	1.14±0.1	3.17±0.13	1.50±0.07	0.231±0.013
Sons	24	19.8±1.1	20.6±0.5	4.20±0.17	0.97±0.11	2.44±0.41	1.32±0.06	0.211±0.008
Daughters	42	16.8±0.6	19.5±0.6	4.53±0.12	1.08±0.15	2.62±0.12	1.43±0.05	0.240±0.009
MI								
Men	29	48.1±1.0	28.3±0.5**	5.96±0.14	2.38±0.25	4.01±0.10*	1.05±0.05**	0.177±0.007
Spouses	29	45.3±1.0	26.8±1.0	5.69±0.25	2.11±0.41*	3.31±0.24	1.25±0.06**	0.204±0.010
Sons	30	19.3±1.2	22.8±0.8*	4.16±0.15	1.16±0.16	2.37±0.15	1.20±0.07	0.216±0.017
Daughters	28	18.7±1.0	21.3±0.6*	4.74±0.19	1.36±0.22	2.84±0.14	1.27±0.06	0.232±0.012

*Data are means ± S.E.M., t-test * $p<0.05$, ** $p<0.01$, BMI – body weight/height (kg/m^2), TC – plasma total cholesterol (mmol/l), TG – triglycerides (mmol/l), LDL-C, HDL-C – LDL and HDL cholesterol (mmol/l), HDL-UC – HDL unesterified cholesterol (mmol/l).*

sons and daughters (with a trend only in the spouses) had significantly higher BMI than the controls (probands $p<0.01$, offspring $p<0.05$). The MI probands had also significantly lower HDL-C ($p<0.01$) and higher LDL-C ($p<0.05$) values than control men. MI spouses had significantly higher TG ($p<0.05$) and lower HDL-C ($p<0.01$) than control spouses. There were no significant differences in plasma lipids between MI and control offspring.

Offspring from both MI and control families had significantly lower BMI and serum concentration of TC and LDL-TC than their parents. However, there were no differences in the concentration of HDL-C between mothers and daughters in MI and control families or between fathers and sons in the controls while the MI probands had a significantly lower HDL-C than their sons ($p<0.05$).

FER_{HDL}

As was to be expected, the men in both MI and control families had higher FER_{HDL} than mothers and children. Furthermore, the values were significantly higher in MI men ($p<0.01$), MI spouses ($p<0.001$), and sons and daughters ($p<0.05$) compared to their respective controls (Fig. 1). The differences in FER_{HDL} remained significant even after adjustment for TC, TG, HDL-FC. However, after correction for BMI only the differences between MI and control spouses remained significant.

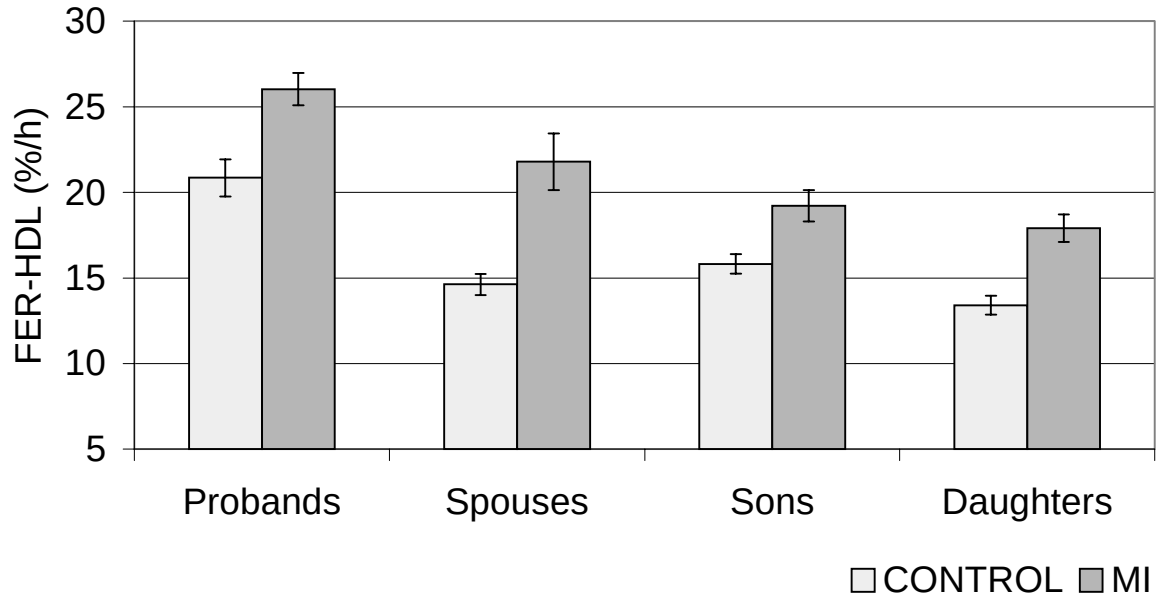

Fig. 1. *Fractional esterification rate of cholesterol in plasma depleted of Apo B lipoproteins FER_{HDL} in MI and control cohorts. Empty bars: control families, black bars: MI families. Means ± S.E.M., * $p<0.05$,** $p<0.01$*

Log (TG/HDL-C)

Men of MI and control families and spouses of MI survivors had positive values of the logarithmically transformed ratio of plasma triglycerides to HDL cholesterol – Log (TG/HDL-C). Spouses of the control group and all offspring of MI and control families had negative values of Log (TG/HDL-C). The values in the MI cohorts were higher ($p<0.001$) compared to their respective controls (Fig. 2). This was true for men, spouses and daughters.

Correlation analyses

The previously reported (Dobiášová *et al.* 1991, Ohta *et al.* 1997a) positive correlations between FER_{HDL} and BMI or triglycerides and an inverse correlation between FER_{HDL} and HDL-C or HDL-UC were

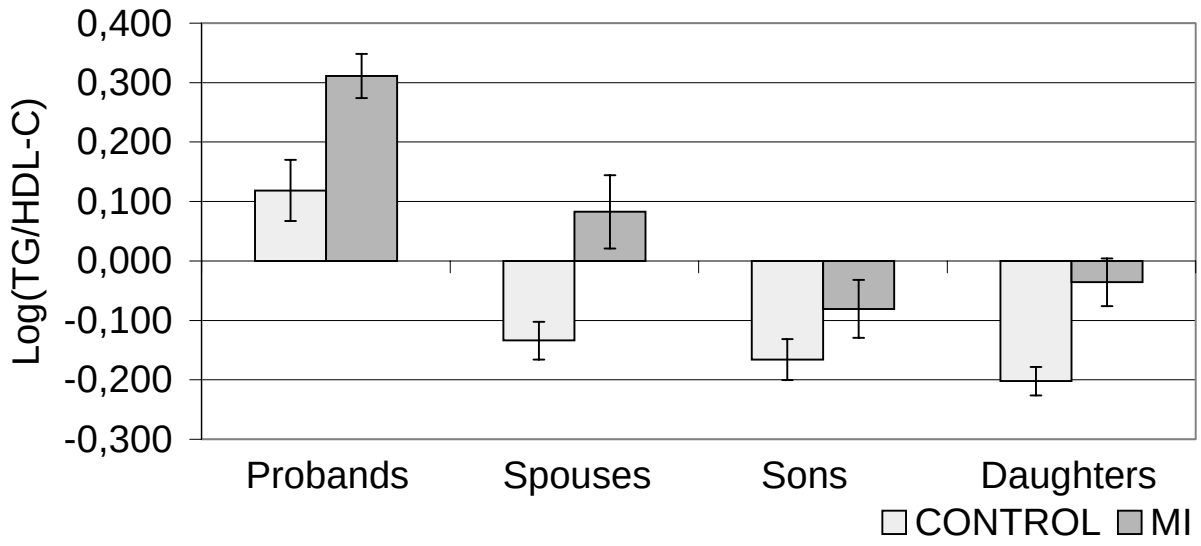

Fig. 2. *Atherogenic index of plasma calculated as Log (TG/HDL-C) in control and MI cohorts. Empty bars: control families, black bars: MI families. Means ± S.E.M., * p<0.05, ** p<0.01.*

confirmed in all studied groups (Tab. 3). A highly significant correlation was found (r ranging from 0.51 to 0.82, p<0.001) between FER_{HDL} and Log (TG/HDL-C) in all groups (Tab. 3).

Interestingly, significant correlations of the FER_{HDL} values were found within spousal pairs (control: r = 0.51, p<0.01; MI: r = 0.41, p<0.05). A less significant correlation of HDL-C values was also found within spousal pairs (control: r = 0.39; MI: r = 0.37, p<0.05 in both groups). However, when adjusted for BMI, both TG and HDL-C partial correlation for FER_{HDL} decreased below the level of significance.

Table 3. Correlation coefficients (r) between FER-HDL and other parameters.

	AGE	BMI	TC	TG	LDL-C	HDL-C	HDL-UC	AIP
Control								
Men	0.113	0.582***	0.175	0.725***	–0.139	-0.678***	-0.664***	0.804***
Spouses	0.462**	0.584***	0.521**	0.760***	0.648***	-0.654***	-0.557**	0.820***
Sons	0.389	0.561**	0.325	0.295	0.544**	-0.581**	-0.466*	0.559**
Daughters	–0.009	0.423**	–0.111	0.116	0.042	-0.517***	-0.652***	0.733***
MI								
Men	–0.126	0.299	0.495**	0.496**	0.171	-0.313	-0.423*	0.506**
Spouses	0.159	0.679***	0.433*	0.707***	–0.132	-0.577***	-0.661***	0.838***
Sons	0.508**	0.567***	0.509**	0.793***	0.412*	-0.592***	-0.642***	0.795***
Daughters	–0.078	0.507**	0.131	0.688***	–0.025	-0.688***	-0.636***	0.736***

*AIP – atherogenic index of plasma calculated as Log (TG/HDL-C). Significance of r coefficients: * p<0.05, ** p<0.01, *** p<0.001.*

Discussion

The results on FER_{HDL} are in agreement with our previous findings in patients with coronary artery disease (Dobiášová *et al.* 1991), hypertension (Dobiášová *et al.* 1992, 1995), hypoalpha-lipoproteinemia (Dobiášová and Frohlich 1994), and type 2 diabetes (Tan *et al.* 1998). These studies have shown that FER_{HDL} correlated with markers of the atherogenic lipoprotein profile. There was a positive correlation of FER_{HDL} with BMI, plasma triglycerides, apoB and a negative correlation with HDL-C and apoA. The rate of cholesterol esterification in the plasma from which LDL and VLDL particles are removed, is regulated by the ratio of large "protective" HDL particles (HDL_{2b}) to the smallest, possibly atherogenic HDLs ($HDL_{3b,c}$). The findings of Ohta *et al.* (1997a,b) and also the data from our laboratory (unpublished results) indicate a highly significant correlation between FER_{HDL} and plasma LDL particle size. The esterification rate in the plasma with a prevalence of small dense atherogenic LDL particles is increased, while it is slowed down in the presence of large particles. However, since the LDL and VLDL particles can not directly affect the reaction itself (as they are absent from the plasma in which FER_{HDL} is measured), it follows that HDL particle size distribution in the plasma is closely related to the LDL particle size. In addition, previous data suggested that the particle size distribution in lipoproteins is closely related to the concentration of triglycerides and HDL cholesterol. We have thus suggested that FER_{HDL} and the ratio of TG and HDL-C likely determines the particle size distribution in lipoproteins, i.e. the atherogenic or nonatherogenic phenotype of the plasma.

We have found in this study that FER_{HDL} highly correlated with logarithmically transformed TG/HDL-C ratio. Table 3 shows that the highest correlation coefficients were found between FER_{HDL} and Log (TG/HDL-C), while correlation coefficients between FER_{HDL} and individual lipids were lower. The role of TG in the formation of an atherogenic phenotype with a predominance of small dense LDL has recently been reported (Superko 1996). New data (Gaziano *et al.* 1997, Gotto 1998, Jeppesen *et al.* 1998) reveal more significant relationship between triglycerides and atherosclerosis than was previously assumed. A new finding in this study is that of all the studied parameters only FER_{HDL} discriminates between all subgroups of the MI and control cohorts examined (Fig. 1). The highest FER_{HDL} values included survivors of myocardial infarction while the lowest values were found in women of the control groups. Log (TG/HDL-C) index also showed the significant differences between the MI and control cohorts (with exception of the sons). Based on the finding that LDL particle size of 25.5 nm (suggested as a transition point between LDL atherogenic and non-atherogenic phenotype, see Austin *et al.* 1990) corresponds to the Log (TG/HDL-C) index equal to 0.06 (Dobiášová and Frohlich 1999), we may select the estimated values to "risk" or "non-risk" groups. In this study we have shown that about 90 % of MI probands and about 40 % of control men belong to the "risk" group while more than 80 % of spouses, sons, and daughters from control families had "non-risk" values. We suggest that this taken together with significant correlation of FER_{HDL} within spousal pairs points to the possibility that the risk of CAD is, at least in part, determined by shared family environment and lifestyle (including diet and exercise habits). However, the extent to which such differences are determined by genes and by shared cultural values remains to be answered.

In conclusion, our study has shown that both FER_{HDL} and the index Log (TG/HDL-C) in patients with proven CAD and in their family members are elevated compared to the controls suggesting a more frequent occurrence of the atherogenic phenotype in these individuals. Both these parameters indicate the individual's atherogenic risk, particularly when plasma lipids are within the normal range.

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Reprint requests

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