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Lipids, Lipoproteins, and Cardiovascular Risk Factors

Fractional Esterification Rate of Cholesterol and Ratio of Triglycerides to HDL-Cholesterol Are Powerful Predictors of Positive Findings on Coronary Angiography

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Background: **We examined the predictive value of various clinical and biochemical markers for angiographically defined coronary artery disease (aCAD). Specifically, we assessed the value of the ratio of plasma triglyceride (TGs) to HDL-cholesterol (HDL-C) and the fractional esterification rate of cholesterol in plasma depleted of apolipoprotein B (apoB)-containing lipoproteins (FER_{HDL}), a functional marker of HDL and LDL particle size.**

Methods: **Patients (788 men and 320 women) undergoing coronary angiography were classified into groups with positive [aCAD(+)] and negative [aCAD(−)] findings. Patient age, body mass index, waist circumference, blood pressure (BP), medications, drinking, smoking, exercise habits, and plasma total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-unesterified cholesterol, HDL-C, TGs, FER_{HDL}, apoB, log(TG/HDL-C), and TC/HDL-C were assessed. Lipids and apoproteins were measured by standard laboratory procedures; FER_{HDL} was determined by a radioassay.**

Results: **Members of the aCAD(+) group were older and had a higher incidence of smoking and diabetes than those in the aCAD(−) group. The aCAD(+) group also had higher TG, apoB, FER_{HDL}, and log(TG/HDL-C) and lower HDL-C values. aCAD(+) women had greater waist circumference and higher plasma TC and TC/HDL-C. aCAD(+) men, but not women, had higher plasma LDL-C. In the multivariate logistic model, the significant predictors of the presence of aCAD(+) were FER_{HDL}, age, smoking, and diabetes. If only laboratory tests were included in the multivariate logistic model, FER_{HDL} appeared as the sole predictor of aCAD(+). Log(TG/HDL-C) was an independent predictor when FER_{HDL} was omitted from multivariate analysis.**

Conclusions: **FER_{HDL} was the best laboratory predictor of the presence of coronary atherosclerotic lesions.**

Several predictors of coronary atherosclerosis, such as age, male sex, smoking, presence of diabetes, hypertension, obesity, high serum LDL-cholesterol (LDL-C),[3] and low serum HDL-cholesterol (HDL-C) have been well established *(1–6)*. Many other predictors have been studied, including plasma concentrations of triglycerides (TGs) and TG-enriched lipoprotein particles *(7–10)*, lipoprotein particle size *(11, 12)*, apolipoprotein B (apoB) *(13)*, lipoprotein(a), homocysteine, C-reactive protein, and others *(14)*. Various algorithms for predicting coronary atherosclerosis have been established *(15)*, most of which are based on large epidemiologic, cohort, and cross-sectional studies *(16)*.

We assessed both clinical and laboratory predictors of atherosclerosis in 1108 consecutive patients undergoing coronary angiography with the following objectives: (*a*) to compare the associations between the various clinical and laboratory indices and the presence or absence of angiographically defined coronary artery disease (aCAD); and (*b*) to examine the potential usefulness of a new functional

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[3] Nonstandard abbreviations: LDL-C and HDL-C, LDL-and HDL-cholesterol, respectively; TG, triglyceride; apoB, apolipoprotein B; aCAD, angiographically defined coronary artery disease; FER_{HDL}, fractional esterification rate of cholesterol in plasma depleted of apoB-containing lipoproteins; MI, myocardial infarction; BP, blood pressure; TC, total cholesterol; HDL-UC, HDL-unesterified cholesterol; CI, confidence interval; and BMI, body mass index.

test for indirect assessment of HDL and LDL particle size, i.e., the fractional esterification rate in apoB-depleted plasma (FER_{HDL}) *(17–19)*. FER_{HDL} measures the rate of esterification of free cholesterol in HDL in plasma depleted of apoB-containing lipoproteins. The esterification is mediated by lecithin-cholesterol acyl transferase, an enzyme that transfers acyl of fatty acids from lecithin to unesterified cholesterol, which leads to the formation of cholesterol esters. The esterification rate is related to the particle size: it is fastest in the smallest HDL particles and slower in larger particles *(17–19)*.

In this study we also compared the predictive power of two lipid indexes: the commonly used ratio of plasma cholesterol to HDL-C and the logarithmically transformed ratio of plasma TGs to HDL-C *(20)*.

Materials and Methods

PATIENTS

The participants were consenting individuals undergoing coronary angiography at Vancouver General and St. Paul's Hospitals in 1993–1994. The indications for angiography included exercise-induced chest pain, previously diagnosed myocardial infarction (MI), atypical chest pain, aortic stenosis/regurgitation, and mitral regurgitation. Patients with unstable angina and MI within the preceding 2 months were excluded from the study. Coronary angiograms were obtained by the standard techniques with multiple views recorded. The angiograms were defined regarding the number of vessels involved (0, 1, 2, or 3) and the lesion severity, i.e., more than or less than 50% lumen obstruction. For the purpose of this study, angiograms with a >50% stenosis in one or more arteries were classified as aCAD(+). Those with a maximum stenosis of ≤10% in any artery were defined as aCAD(−). Each participant in the study filled out a questionnaire that included their demographic data and presence/absence of the major risk factors, including personal and family history of vascular disease. Height, weight, and waist circumference were measured at the same time. All participants signed a consent form that had been approved by University of British Columbia and St. Paul's Hospital ethics committees.

LABORATORY ASSAYS

Blood samples were collected after an overnight fast into EDTA-containing Vacutainer tubes on the day of the angiogram, kept at 4 °C, and centrifuged within 12 h. Aliquots of plasma were frozen at −70 °C until analyses. Plasma total cholesterol (TC), TGs, total HDL-C, and HDL-unesterified cholesterol (HDL-UC) were measured enzymatically. LDL-C was calculated using the Friedewald equation, and apoB was measured nephelometrically (Beckman Array System). The radioassay for FER_{HDL} had been described previously *(17–19)*. Briefly, apoB-containing lipoproteins are precipitated from EDTA plasma 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. After the incubation, lipids are extracted by ethanol and separated by thin-layer chromatography. FER_{HDL} (%/h) is calculated from the ratio of radioactive unesterified to radioactive esterified cholesterol.

STATISTICAL ANALYSIS

The data are presented as means (SD) for all individuals and separately for men and women with positive [aCAD(+)] and with negative angiography findings [aCAD(−)]. Differences between those with positive and negative findings were tested by unpaired Student and Wilcoxon rank-sum tests. For variables with high interindividual variation (3 SD > mean), statistical analyses were performed on log-transformed values (as has been done for TGs, FER_{HDL}, and TG/HDL). Correlation was tested by a bivariate Spearman nonparametric rank correlation test. The association of measured variables with the presence of aCAD(+) was evaluated in both univariate and multivariate logistic regression models. Stepwise logistic regression analysis was used to predict the presence or absence of aCAD. The model was based on values of the set of variables that had significant association on univariate analysis. Odds ratios and 95% confidence intervals (CIs) for aCAD(+) vs aCAD(−) were calculated (Table 5). Data were adjusted for the categorical variables, such as smoking and diabetes, and for continuous variables, such as age, body mass index (BMI), waist circumference, TC, LDL-C, apoB, TGs, HDL-C, HDL-UC, FER_{HDL}, log(TG/HDL-C), and TC/HDL-C. The statistical packages SPSS, Base 11.0, and SPSS Regression Models 11.0 were used to analyze the data.

Results

CHARACTERISTICS OF THE STUDY POPULATION

The study cohort consisted of 320 women and 788 men (age range, 18–88 years). Their characteristics are shown in Table 1. Of the initial diagnoses, the most common was CAD (52% of women, 64% of men). Twenty-one percent of the women and 33% of the men had received a previous diagnosis of MI, whereas peripheral vascular disease or cerebral vascular disease was previously diagnosed in 15% and 9% of the women and 10% and 6% of the men, respectively. Eighty-one percent of the men and 58% of the women had positive findings on coronary angiography. Adult-onset diabetes was diagnosed in 17% of women and 16% of men, hypertension in 42% of women and 36% of men, and the BMI was >30 in 27% of both genders. More than 80% of the women were postmenopausal. Fifty-seven percent of the women and 76% of the men were current or former smokers, ~11% of both men and women did not exercise regularly, and 20% the men and 40% of the women were nondrinkers. Only

Table 1. Characteristics of patient cohort.

	Women (n = 320)		Men (n = 788)	
	n	%	n	%
BMI >30.0	88	27.5	211	26.8
CAD	166	51.9	502	63.7
PVD[a]	49	15.3	79	10.0
CVD	28	8.8	49	6.2
MI	66	20.6	258	32.7
aCAD(+)	186	58.1	638	81.0
DM	55	17.2	124	15.7
Hypertension	134	41.9	280	35.5
Smoke (ever)	177	56.9	591	76.3
Exercise (never)	35	11.3	82	10.6
Drinking (never)	123	39.5	165	21.3
Lipid medication	45	14.5	127	16.4
Menopause	258	83.0		

[a] PVD, peripheral vascular disease; CVD, cerebral vascular disease; DM, diabetes mellitus.

~15% of the men and women in this cohort were using lipid-lowering medications.

UNIVARIATE ANALYSES

The mean (SD) for each continuous variable in the negative [aCAD(−)] and positive angiography [aCAD(+)] groups are shown in Tables 2 and 3. Analyses of these variables were carried out separately for men (Table 2) and women (Table 3). The *P* values indicate the results of independent sample *t*-tests for differences between those with positive and negative angiographic findings. In the whole cohort, CAD(+) and aCAD(−) individuals differed significantly in age; TG, apoB, and HDL-C concentrations; FER_{HDL}; and log(TG/HDL-C). However, there were no significant differences in systolic BP and BMI. Men, but not women, with positive findings had higher LDL-C and lower HDL-UC. Women, but not men, with positive findings had significantly increased waist circumference, TC, unesterified cholesterol, and TC/HDL-C ratio.

Of the categorical variables, both smoking and diabetes were significantly higher in the cohort members with positive findings ($P < 0.001$). In addition, women with CAD(+) had a significantly higher frequency of hypertension ($P < 0.04$). Neither alcohol consumption nor physical inactivity were significantly associated with positive findings.

Table 2. Univariate analysis of CAD risk factors for men with negative and positive angiographic findings.

	Mean (SD)		
	aCAD(−) (n = 136)	aCAD(+) (n = 637)	*P*[a]
Age, years	54.6 (12.2)	60.9 (10.6)	**<0.0001**
BMI, kg/m^2	27.9 (5.1)	28.5 (4.7)	0.375
Waist circumference, cm	99.4 (12.8)	99.5 (11.8)	0.894
$BP_{Systolic}$, mmHg	134 (25)	133 (22)	0.900
$BP_{Diastolic}$, mmHg	80 (13)	78 (12)	0.237
TC, mmol/L	5.08 (1.03)	5.07 (1.00)	0.996
UC,[b] mmol/L	1.43 (0.30)	1.46 (0.38)	0.304
TGs, mmol/L	1.71 (0.98)	1.97 (1.40)	**0.032**
LDL-C, mmol/L	3.75 (0.95)	3.49 (0.91)	**0.003**
apoB, mmol/L	0.91 (0.22)	0.96 (0.22)	**0.020**
HDL-C, mmol/L	0.96 (0.27)	0.91 (0.22)	**0.013**
HDL-UC, mmol/L	0.21 (0.06)	0.20 (0.05)	**0.004**
FER_{HDL}, %/h	22.57 (7.72)	25.61 (8.62)	**0.0001**
Log(TG/HDL-C)	0.205 (0.27)	0.277 (0.29)	**0.008**
TC/HDL-C	5.57 (1.61)	5.92 (2.24)	0.074

[a] Statistically significant values are in bold font.
[b] UC, unesterified cholesterol.

Table 3. Univariate analysis of CAD risk factors for women with negative and positive angiographic findings.

	Mean (SD)		
	aCAD(−) (n = 124)	aCAD(+) (n = 186)	*P*[a]
Age, years	61.2 (11.1)	64.2(10.3)	**0.017**
BMI, kg/m^2	26.8 (5.3)	27.7 (5.7)	0.164
Waist circumference, cm	89.6 (13.4)	93.8 (15.1)	**0.021**
$BP_{Systolic}$, mmHg	136 (22)	135 (27)	0.755
$BP_{Diastolic}$, mmHg	75 (11)	75 (11)	0.668
TC, mmol/L	5.17 (1.08)	5.51 (1.41)	**0.026**
UC,[b] mmol/L	1.43 (0.31)	1.59 (0.50)	**0.001**
TGs, mmol/L	1.38 (0.69)	1.94 (1.36)	**<0.0001**
LDL-C, mmol/L	3.74 (1.08)	3.84 (1.18)	0.444
apoB, mmol/L	0.93 (0.27)	1.03 (0.27)	**0.003**
HDL-C, mmol/L	1.16 (0.33)	1.08 (0.31)	**0.032**
HDL-UC, mmol/L	0.25 (0.08)	0.24 (0.08)	0.434
FER_{HDL}, %/h	17.86 (7.25)	21.04 (8.28)	**0.001**
Log(TG/HDL-C)	0.045 (0.24)	0.21 (0.22)	**<0.0001**
TC/HDL-C	4.78 (0.25)	5.53 (2.48)	**0.005**

[a] Statistically significant values are indicated by bold font.
[b] UC, unesterified cholesterol.

Table 4. Pearson correlations (*r*) between $logFER_{HDL}$ and anthropometric and biochemical variables.

	aCAD(−) (n = 260)		aCAD(+) (n = 823)	
	$LogFER_{HDL}$	*P* (two-tailed)	FER_{HDL}	*P* (two-tailed)
BMI	0.324	0.000	0.297	<0.0001
Waist circumference	0.401	0.000	0.323	<0.0001
$BP_{Systolic}$	−0.074	NS[a]	−0.0.047	NS
$BP_{Diastolic}$	0.117	NS	0.094	0.032
TC	0.138	0.036	0.215	<0.0001
UC	0.296	0.000	0.339	<0.0001
LogTG	0.592	0.000	0.631	<0.0001
LDL-C	NS	NS	0.009	NS
apoB	0.402	0.000	0.364	<0.0001
HDL-C	−0.648	0.000	−0.645	<0.0001
HDL-UC	−0.662	0.000	−0.605	<0.0001
Log(TG/HDL-C)	0.751	0.000	0.748	<0.0001
TC/HDL-C	0.580	0.000	0.555	<0.0001

[a] NS, not significant.

CORRELATION ANALYSIS

The relationships between plasma lipid/apoprotein measurements and FER_{HDL} are shown in Table 4. There was a strong correlation between FER_{HDL}, plasma TGs, HDL-C, and HDL-UC. Whereas the correlation between FER_{HDL} and either total or unesterified cholesterol was rather weak (albeit significant), the correlation between FER_{HDL} and apoB was much stronger. There was an even stronger correlation between FER_{HDL} and both calculated indexes [log(TG/HDL-C) and TC/HDL-C]. There was no significant difference in any the above correlations between the aCAD(+) and CAD(−) groups.

Conversion of the continuous variables (TGs, HDL-C, and apoB) into quartiles may better illustrate the relationships between these specific markers and FER_{HDL}: FER_{HDL} increased from 13.2%/h to 35.7%/h between the first and fourth quartiles of logTG and HDL-C. This increase was related independently to both of these variables (Fig. 1). The relationship between FER_{HDL} and apoB and HDL-C was similar (Fig. 2).

LOGISTIC STEPWISE REGRESSION MODEL

The results of logistic regression analysis of the differences between the aCAD(+) and aCAD(−) groups are shown in Table 5. Although the univariate models demonstrated significant differences in several variables between the aCAD(+) and aCAD(−) groups (Tables 2 and 3), many did not appear in the model adjusted for age, BMI, waist circumference, diabetes, smoking, logTG, LDL-C, apoB, HDL-C, HDL-UC, $logFER_{HDL}$, and the indices log(TG/HDL-C) and TC/HDL-C. In the logistic regression model that included the whole cohort, the most powerful predictors were FER_{HDL}, age, smoking, and diabetes (Table 5). When log FER_{HDL} was omitted from the model, the logarithmically transformed ratio of TGs to HDL-C became the best independent predictor of aCAD(+). The model examining the predictive power of laboratory tests [TC, logTG, LDL-C, apoB, HDL-C, HDL-UC, $logFER_{HDL}$, log(TG/HDL-C), and TC/HDL-C] only indicated that FER_{HDL} was the sole significant predictor (odds ratio = 28.33; $P < 0.0001$).

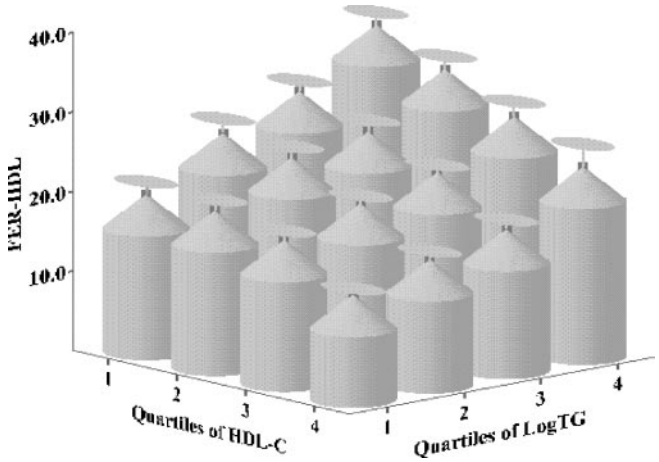

Fig. 1. Three-dimensional model relating FER_{HDL} values to the quartiles of logTG and HDL-C.

The *columns* indicate the means. *Error bars*, 95% CIs of the means.

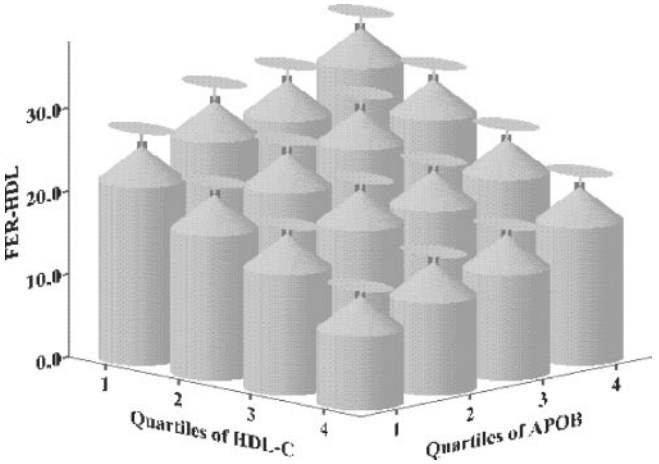

Fig. 2. Three-dimensional model relating FER_{HDL} values to the quartiles of apoB and HDL-C.

The *columns* indicate the means. *Error bars*, 95% CIs of the means.

Discussion

We examined the predictive value of different demographic, clinical, and biochemical indices for findings on selective coronary angiography in a cohort of 1108 patients.

We have shown that individuals with positive angiographic findings tend to be older, smokers, have a history of diabetes, and also have higher TG and apoB concentrations, lower HDL-C, and only mildly increased TC and LDL-C. These results are in agreement with previous reports of association of these "classic" markers with angiographically confirmed CAD *(21)*. We have shown that FER_{HDL}, an indirect measure of lipoprotein particle size *(22–24)*, is a better predictor of positive angiographic findings than the classic markers. Recent reports confirmed that the smallest LDL *(25)* and small HDL *(26)* particles are most strongly related to coronary disease progression. In our cohort, FER_{HDL} was the most effective predictor of positive angiographic findings among all clinical and biochemical markers assessed (Table 5). In women, in agreement with previous findings *(8)*, there was a strong relationship between plasma TGs and positive coronary findings. In the logistic regression model in which FER_{HDL} was omitted, the ratio of TGs to HDL-C was the most effective predictor. This index, log(TG/HDL), strongly correlated with FER_{HDL} and several other risk factors for CAD *(20)*. In the current study, log(TG/HDL-C) appeared to be a better predictor of positive angiographic findings than the commonly used ratio TC/HDL-C *(27, 28)*.

The value of FER_{HDL} is strongly related to changes in HDL-C, TGs, and apoB (Figs. 1 and 2). The concentrations of all these analytes are known to influence lipoprotein

Table 5. Stepwise logistic regression model for all study participants.

Independent predictor of aCAD(+)[a]	*B*[b]	SE	exp(*B*)	95% CI for exp(*B*)	*P*
Regression model with FER_{HDL}					
$LogFER_{HDL}$	3.795	0.557	44.495	(14.927–132.633)	<0.0001
Age	0.048	0.008	1.050	(1.035–1.068)	<0.0001
Smoking	0.841	0.179	2.319	(1.624–3.267)	<0.0001
DM	0.641	0.297	1.899	(1.060–3.402)	0.031
Regression model without FER_{HDL}					
Log(TG/HDL-C)	1.620	0.330	4.055	(2.646–9.660)	<0.0001
Age	0.042	0.008	1.043	(1.027–1.059)	<0.0001
Smoking	0.852	0.178	2.343	(1.652–3.323)	<0.0001
Waist circumference	0.015	0.007	1.015	(1.001–1.029)	0.031
DM	0.715	0.296	2.044	(1.144–3.652)	0.016

[a] Set of predictor variables: age, BMI, waist circumference, smoking, diabetes mellitus, logTG, LDL-C, apoB, HDL-C, HDL-UC, $logFER_{HDL}$, log(TG/HDL-C), TC/HDL-C.
[b] *B*, coefficient; SE, standard error of *B*, exp(*B*), estimated odds ratio; DM, diabetes mellitus.

particle size and number. For example, higher TG concentrations are associated with the presence of small VLDL and LDL particles *(29)*, and the apoB concentration is directly linked to the number of LDL particles *(13)*.

It has been well documented that small, dense LDL particles are more susceptible to oxidation and are a better substrate for formation of foam cells than large cholesterol-rich LDL particles *(6)*. On the other hand, increased HDL-C is usually associated with an increase in protective HDL_{2b} particles *(30)*, which inhibit esterification of cholesterol, i.e., reduce FER_{HDL} *(23)*, whereas low HDL-C is seen in the presence of small particles *(11)*, which increase FER_{HDL} *(23)*. The value of FER_{HDL} as an independent indicator of angiographic endpoints was reported recently in the HDL-atherosclerosis study *(31)*.

The size of our cohort and the number of markers measured compare favorably with other studies in this field *(32–84)*. Most of these studies were limited in the number of patients examined, relative to one gender or age or race group, and many of them examined only some of the markers assessed in our study.

There are several limitations to our findings. The angiographic assessment of vascular disease, although currently an accepted "gold standard", is less sensitive and specific than newer methods, such as intravascular ultrasound. Furthermore, the assessment of the angiographic findings was only semiquantitative. The effects of beta-blockers and lipid-lowering drugs when taken into account in both the CAD(+) and CAD(−) groups had no effect on statistical analyses; however, they may have influenced several of the biochemical markers measured. Finally, it may be disputed that our control group (patients with negative findings on angiography) were not truly healthy individuals because they had risk factors that led to their angiography in the first place. We believe that this actually makes our data stronger because the differences observed might have been much greater if a control group of patients without any risk factors have been used. There were only 25 patients with 10–50% obstruction.

In conclusion, after correcting for the presence of classic risk factors and several other variables, FER_{HDL}, age, smoking, and diabetes were the best independent predictors of the presence of angiographically confirmed CAD in both genders. In women, serum TGs were also an independent predictor. Thus, the particle size of lipoproteins assessed by a functional test (FER_{HDL}) appeared to be the superior biochemical predictor of positive findings on coronary angiography.

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