Fractional Esterification Rate of HDL Particles in Patients With Type 2 Diabetes

Relation to coronary heart disease risk factors

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OBJECTIVE — To study the fractional esterification rate of cholesterol on HDL particles (FER_{HDL}) in adults with type 2 diabetes and assess its correlation with serum lipids and other coronary heart disease (CHD) risk factors.

RESEARCH DESIGN AND METHODS — FER_{HDL} was measured in 90 adult (57 men, 33 women) patients by an isotopic assay method involving several steps, including preparation of VLDL- and LDL-depleted plasma, labeling of the sample with a trace amount of tritiated cholesterol, separation of free and esterified cholesterol fractions by chromatography post incubation, and subsequent counting of radioactivity in the individual fractions.

RESULTS — Male patients have higher FER_{HDL} values than their female counterparts. When HDL cholesterol was controlled for in a multivariate regression analysis, the sex factor was not significant. There was a significant positive correlation between FER_{HDL} and plasma total cholesterol ($r = 0.32$), triglycerides ($r = 0.82$), apolipoprotein B (apo B; $r = 0.48$), insulin ($r = 0.46$), BMI ($r = 0.31$), and waist-to-hip ratio (WHR; $r = 0.50$). There was a negative correlation between FER_{HDL} and HDL cholesterol ($r = -0.76$) and apolipoprotein AI ($r = -0.60$). When both HDL cholesterol and triglycerides were controlled for, the only significant correlation was between FER_{HDL} and BMI.

CONCLUSIONS — Non–insulin-requiring type 2 diabetic patients have FER_{HDL} , which correlated positively with triglycerides and negatively with HDL cholesterol. The positive correlation of FER_{HDL} with serum insulin, WHR, total cholesterol, and apo B, but not that with BMI, loses its significance when HDL cholesterol and triglycerides are controlled. The sex difference between men and women in FER_{HDL} also loses its significance when HDL cholesterol is controlled.

Quantitative and qualitative lipoprotein abnormalities occur in patients with type 2 diabetes, regardless of their mode of treatment (1). The concentration of HDL is on average reduced by 10–20%. In addition, there are significant changes in the composition of HDL subclasses (2). While a low plasma HDL cholesterol level has been clearly established as an independent risk factor for coronary heart disease (CHD), recent studies suggest that functional assessment of HDL subclasses may provide a better indication of its antiatherogenic potential (3–6). The fractional rate of cholesterol esterification in VLDL– and LDL–depleted plasma (FER_{HDL}) has been used to measure HDL heterogeneity based on the finding that the rate of esterification of cholesterol by lecithin:cholesterol acyltransferase (LCAT) reflects the relative size and composition of HDL particles. A significantly higher FER_{HDL} demonstrated in subjects with CHD (2), hypertension (4–6), hyperlipidemia (3), or hypoalphalipoproteinemia (5), correlates positively with the concentrations of plasma small $HDL_{3b,c}$ subfractions (4–7) and plasma triglyceride (3–7). Conversely, it showed an inverse correlation with the relative concentrations of anti-atherogenic particles such as HDL_{2b} (3–7) and apolipoprotein (apo) AI (5). FER_{HDL} was also found to be significantly higher in healthy men than in healthy women (3). However, FER_{HDL} measured in healthy septuagenarian men was no different from that in middle-aged men (4). Assessment of FER_{HDL} may, therefore, serve as an effective predictor of the risk for CHD. We measured the FER_{HDL} in patients with type 2 diabetes and evaluated its correlation with CHD risk factors.

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Abbreviations: apo, apolipoprotein; CHD, coronary heart disease; FER_{HDL}, fractional rate of cholesterol esterification; LCAT, lecithin:cholesterol acyltransferase; Lp(a), lipoprotein(a); WHR, waist-to-hip ratio.

RESEARCH DESIGN AND METHODS

Subjects

Of the adult patients with non–insulin-requiring type 2 diabetes, 90 (57 males, 33 females) participated in this study. The mean ± SD ages of the men and women were 53.5 ± 11.8 and 54.3 ± 13.4 years, respectively. Of the total group, 46 patients were managed with diet alone (Diabetes Meal Plan, Canadian Diabetes Association) and 36 were managed with diet and oral hypoglycemic agents (sulfonylureas and/or metformin). The average duration of diabetes for the male and female patients was 4.2 ± 5.5 and 4.0 ± 5.4 years, respectively. Informed consent was obtained from these patients who were randomly selected from type 2 diabetic patients who attended the education program of the Nova Scotia Diabetes Centre and participated in a survey of cardiovascular risk factor profile in patients with type 2 diabetes. All eligible patients, regardless of age, serum lipids, BMI, or glucose control were invited to participate. The protocol was approved by the institutional ethics committee.

Determination of cholesterol esterification rate

FER_{HDL} was determined by an isotopic assay method, which will be described briefly here as detailed methodology has been reported previously (3,8). VLDL- and LDL–depleted plasma was prepared by

Table 1—*CHD risk factor variables of type 2 diabetic patients*

CHD risk factor	Men	Women	*P* value
n	57	33	
FER_{HDL} (%/h)	35.3 ± 12.3	26.3 ± 10.8	<0.001
Cholesterol (mmol/l)	4.87 ± 0.97	4.85 ± 0.96	NS
Triglyceride (mmol/l)	2.90 ± 2.73	2.00 ± 1.65	<0.04
HDL (mmol/l)	0.71 ± 0.24	0.94 ± 0.24	<0.0001
Apo B (mg/dl)	130 ± 31	125 ± 31	NS
Apo A1 (mg/dl)	128 ± 19	147 ± 21	<0.0002
Lp(a) (mg/dl)	23.5 ± 24.3	34.5 ± 34.0	<0.05
Glucose (mmol/l)	8.65 ± 2.92	7.87 ± 2.11	NS
Insulin (mU/l)	18.0 ± 12.5	17.7 ± 10.5	NS
HbA_{1c} (%)	6.99 ± 1.47	6.81 ± 1.13	NS
BMI (kg/m^2)	30.5 ± 4.9	30.2 ± 4.7	NS
WHR	0.96 ± 0.06	0.86 ± 0.08	<0.0001

Data are *n* or means ± SD.

precipitation with phosphotungstate(PTA)-$MgCl_2$ of apolipoprotein (apo) B–containing lipoproteins. A trace amount of tritiated cholesterol from a paper disk is then added to plasma. Label transfer occurs spontaneously at low temperature (on ice) overnight for about 18 h. The labeled samples are then incubated at 37°C for 30 min to allow esterification by LCAT to proceed. The lipid extract is then subjected to thin layer chromatography, and the radioactivity of the respective free and esterified cholesterol fractions is determined by liquid scintillography. FER_{HDL} is calculated as the difference between the percentage of label found in esterified cholesterol before and after incubation. The interassay coefficient of variation of the FER_{HDL} assay varied from 4.5 to 7.3% (7).

Assessment of coronary risk factors

Historical and clinical data were obtained from the survey of cardiovascular risk factor profiles in patients with type 2 diabetes. Hypertension was defined as a systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg. The BMI was expressed as body weight (kilograms) divided by the square of height (meters squared). Undesirable BMI was defined as being ≥27 kg/m^2. The ratio of waist-to-hip circumference (WHR) was considered undesirable if ≥0.95.

The concentrations of total and HDL cholesterol and triglyceride were measured enzymatically with commercial kits (Boehringer Mannheim, Canada). The undesirable cut-offs for plasma levels of triglyceride and HDL cholesterol were set at ≥2.3 mmol/l and <0.9 mmol/l, respectively. The concentrations of lipoprotein(a) [Lp(a)], apolipoprotein A1 (apo AI), and apo B were determined by nephelometry (Behring Diagnostics, Hoechst-Roussel, Canada). The interassay coefficient of variation for the various lipids and apoproteins are as follows: total cholesterol 1.6%, triglyceride = 3.0%, HDL cholesterol = 1.9%, LDL cholesterol = 2.1%, apo AI = 3.1%, and apo B = 3.1%. Plasma insulin level was measured by radioimmunoassay method (Kabi Pharmacia Diagnostics, Uppsala, Sweden). HbA_{1c} was determined by high-performance liquid chromatography (HPLC) with a normal range of 4.3–5.6%.

Statistical analyses

As none of the variables were normally distributed, we used nonparametric tests to compare groups (scores from Wilcoxon's test) and determining correlation coefficients (Spearman's partial correlation coefficients). We also used multivariate least square regression analysis to determine significance of a variable while controlling for others.

RESULTS — Data obtained from the male and female type 2 diabetic patients are shown in Table 1. Men had significantly higher FER_{HDL} ($P < 0.001$) and triglycerides ($P < 0.04$) and lower HDL cholesterol ($P < 0.0001$) than women. FER_{HDL} was negatively correlated with HDL cholesterol and positively correlated with serum triglyceride (Table 2). We used multivariate regression analysis and controlled for triglyceride and/or HDL cholesterol to determine whether sex was a significant factor in determining FER_{HDL}. If triglyceride was controlled for, then sex was a significant factor. If HDL cholesterol was controlled for, then sex was no longer a significant factor. If triglyceride and HDL cholesterol were controlled for, then sex was not a significant factor. In view of this, we combined both CHD risk factors, controlling for triglyceride and/or HDL cholesterol (Table 2).

Table 2 summarizes the partial correlation coefficients between FER_{HDL} and the CHD risk factors measured in this study. In the initial multivariate regression analysis, FER_{HDL} showed significant positive correlation with plasma triglyceride ($r = 0.82$, $P < 0.0001$), total cholesterol ($r = 0.32$, $P < 0.002$), and apo B ($r = 0.48$, $P < 0.0001$). A positive correlation was also noted between FER_{HDL} and fasting plasma insulin levels ($r = 0.46$, $P < 0.0001$). No significant correlation was noted with HbA_{1c} levels. A positive correlation between FER_{HDL} vs. BMI ($r = 0.31$, $P < 0.01$) and WHR (r

Table 2—*Spearman's partial correlation coefficients between FER_{HDL} and various CHD risk factors*

CHD risk factor	No controls	Controlling for HDL	Controlling for triglyceride	Controlling for HDL and triglyceride
Cholesterol	0.32†	0.46‡	−0.15	0.08
HDL cholesterol	−0.76‡	—	−0.66‡	—
Triglyceride	0.82‡	0.75‡	—	—
Apo B	0.48‡	0.42‡	0.11	0.14
Apo AI	−0.60‡	0.11	−0.61‡	−0.15
Lp(a)	−0.14	−0.20	0.01	−0.08
HbA_{1c}	0.14	0.18	−0.12	−0.05
Insulin	0.46‡	0.30†	0.28†	0.19
BMI	0.31†	0.28†	0.19	0.21*
WHR	0.50‡	0.27†	0.23*	0.07

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.0001$.

Table 3—*FER_{HDL} in type 2 diabetic patients with and without specific CHD risk factor*

CHD risk factor	With risk factor	Without risk factor	*P* value
Obesity	33.5 ± 1.5 (68)	27.3 ± 2.7 (22)	<0.04
Android obesity	38.2 ± 2.0 (35)	28.0 ± 1.5 (55)	<0.0002
Blood pressure	34.3 ± 2.2 (30)	30.8 ± 1.7 (60)	NS
Cigarette smoking	36.9 ± 2.9 (23)	30.3 ± 1.4 (67)	<0.05
Triglycerides	42.9 ± 1.6 (34)	25.3 ± 1.2 (56)	<0.0001
HDL	38.6 ± 1.4 (55)	21.4 ± 1.2 (35)	<0.0001
Triglycerides and HDL	43.5 ± 1.6 (32)	25.6 ± 1.2 (58)	<0.0001

Data are means ± SD (*n*). The definitions of "With risk factor" for each CHD risk factor are as follows: obesity, BMI ≥27; android obesity, WHR ≥0.95; blood pressure, systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 mmHg; cigarette smoking, yes; triglyceride, ≥2.3 mmol/l; HDL, <0.9 mmol/l.

= 0.50, $P < 0.0001$) was noted. No significant correlation was observed between FER_{HDL} and Lp(a) levels. When HDL cholesterol was controlled for, FER_{HDL} showed significant positive correlation with plasma triglyceride ($r = 0.75$, $P < 0.0001$), total cholesterol ($r = 0.46$, $P < 0.0001$), and apo B ($r = 0.42$, $P < 0.0001$). A positive correlation was also noted between FER_{HDL} and fasting plasma insulin levels ($r = 0.30$, $P < 0.01$). No significant correlation was noted with HbA_{1c} levels. A positive correlation between FER_{HDL} vs. BMI ($r = 0.28$, $P < 0.01$) and WHR ($r = 0.27$, $P < 0.01$) was noted. No significant correlation was observed between FER_{HDL} and Lp(a) levels. When triglyceride was controlled for, an inverse correlation between FER_{HDL} and HDL cholesterol ($r = -0.66$, $P < 0.0001$) and apo A1 ($r = -0.61$, $P < 0.0001$) was noted. A positive correlation was also noted between FER_{HDL} and fasting plasma insulin levels ($r = 0.28$, $P < 0.01$). No significant correlation was noted with HbA_{1c} levels. A positive correlation between FER_{HDL} and WHR ($r = 0.23$, $P < 0.05$) but not BMI was noted. No significant correlation was observed between FER_{HDL} and Lp(a) levels. When both HDL cholesterol and triglyceride were controlled for, the only significant correlation was between FER_{HDL} with BMI ($r = 0.21$, $P < 0.05$).

Table 3 shows further analysis of FER_{HDL} in the subgroups categorized by the presence or absence of certain CHD risk factors. The mean FER_{HDL} was significantly higher in the subgroups with undesirable BMI ($P < 0.05$) or WHR ($P < 0.0002$). There were no significant differences in the mean FER_{HDL} between subgroups with and without hypertension. Those who smoked also had higher FER_{HDL}. The mean FER_{HDL} was significantly higher in subgroups whose concentrations of plasma triglyceride, HDL cholesterol, or both were in the undesirable range ($P < 0.0001$).

CONCLUSIONS — The results of our study in patients with type 2 diabetes corroborate the findings of previous studies evaluating FER_{HDL} in subjects with CHD, hypertension, dyslipidemia, or hypoalphalipoproteinemia (3–6). The FER_{HDL} in type 2 diabetic patients studied showed a significant positive correlation with plasma concentrations of triglyceride and apo B, and negatively with HDL cholesterol and apo A1 levels. The estimation of FER_{HDL} reflects the ratio between the small and large HDL particles in the plasma. Previous studies have shown that FER_{HDL} correlated with the various HDL subspecies: it was significantly lower in subjects with increased relative concentrations of the larger and anti-atherogenic HDL_{2b} species; and significantly higher in subjects with increased concentrations of the smaller, inert or probably atherogenic, $HDL_{3b,c}$ particles. This correlation has been explained by the nature of LCAT interaction with the various HDL subspecies: HDL_{2b} particles are known to inhibit the enzyme, while the small $HDL_{3b,c}$ subsets provide excellent substrates for LCAT activity (9,10).

Our observations in type 2 diabetic patients can be at least in part explained by the fact that these individuals often have relative lipoprotein lipase deficiency, leading to altered triglyceride metabolism, which is associated with reduced levels of HDL_2 and elevated levels of HDL_3 (2,11). Measurement of FER_{HDL} reflects the particle size distribution within the HDL pool, and serves as a quantitative indicator of CHD risk. This is of special relevance in diabetic patients as they often have subtle abnormalities in the composition of lipoproteins that may not be detected by the routine measurement of serum lipid levels (1,2). Another possible advantage of using the functional assay, as opposed to quantitative determination of HDL cholesterol subspecies, is that it may reflect the interaction of two opposing (as far as the apparent effect on atherogenesis is concerned) components, namely, HDL_{2b} and $HDL_{3b,c}$. The measurement of FER_{HDL} by the isotopic assay described has been demonstrated to be a highly reproducible technique with a coefficient of variation ranging between 5 and 8% (7).

Previous studies have reported that male control subjects/patients had higher FER_{HDL} than their female counterparts (3–6). We extend these previous observations by demonstrating that male type 2 diabetic patients had significantly higher FER_{HDL} than female patients. We also extend previous observations by showing this sex difference in FER_{HDL} loses its significance if HDL cholesterol, but not triglyceride, is controlled for in the multivariate regression analysis.

Another novel finding in our study is the positive correlation observed between the FER_{HDL} and the fasting plasma insulin levels. This may be of pathogenic significance to the increased CHD risk observed in patients with insulin resistance and hyperinsulinemia, described as metabolic syndrome X by Reaven (12). This correlation is present even when HDL cholesterol and triglyceride are controlled individually. However, when both are controlled simultaneously, the correlation loses its significance ($P < 0.07$). The significance of hyperinsulinemia in relation to the heterogeneity of HDL subspecies and its attendant propensity for atherogenesis needs further study.

There may also be a potential association between FER_{HDL} and the compositional abnormality in LDL particles (13). Diabetic patients have increased CHD risk attributed to the predominance of small, dense LDL particles, called LDL subclass phenotype B (14). Our study patients who are predisposed to LDL subclass phenotype B, namely, those with elevated plasma triglycerides and reduced HDL cholesterol concentrations, demonstrated significantly higher FER_{HDL} as compared with those with normal lipids profile. It is therefore likely that similar determinants or a "common soil" phenomenon exist for diabetic individuals manifesting a higher FER_{HDL} as well as LDL subclass phenotype B (15). These individuals also have in common a plasma lipids profile characterized by a

raised triglyceride and apo B levels, and a reduced HDL cholesterol and apo AI levels.

An interesting observation in our study on diabetic patients is the lack of correlation between FER_{HDL} and glycemic control, as reflected by HbA_{1c}. It is likely that alterations in relative HDL subspecies distribution in patients with type 2 diabetes occur independently of glycemic control. This abnormality may also persist in diabetic patients despite apparent normalization of dyslipidemia, as the diabetic cohorts have significantly increased risk of CHD compared with nondiabetic populations with similar levels of plasma lipids (16).

Another interesting finding is the positive correlation between FER_{HDL} and BMI. This correlation is also present even when HDL cholesterol and triglyceride are controlled in the multivariate regression analysis. A study in 909 type 2 diabetic patients revealed that the association between high plasma insulin level and CHD was significant only in subjects with a BMI >27 kg/m^2 (17). BMI does not accurately reflect visceral obesity; but WHR does. Our study shows a positive correlation between FER_{HDL} and WHR, a relationship that persists when HDL cholesterol and triglyceride are controlled individually. When they are controlled simultaneously, the correlation just loses its significance ($P < 0.07$). A study on 146 healthy elderly subjects showed that plasma HDL_2 levels were inversely correlated with upper-body obesity, plasma insulin levels, and the presence of glucose intolerance (18). These findings suggest that a higher FER_{HDL} noted in patients with upper body obesity could either be a marker of or responsible for the increased risk of CHD.

A previous study on FER_{HDL} estimation in hypertensive men showed that this was elevated regardless of the stage of hypertension (4). We did not, however, observed any significant differences in FER_{HDL} between the hypertensive and normotensive patients with type 2 diabetes. This observation certainly needs reevaluation with larger patient numbers to avoid possible beta-error in our analysis. The high FER_{HDL} levels of both normotensive and hypertensive diabetic patients might eliminate the effect of hypertension on FER_{HDL}. Smokers also showed higher FER_{HDL} levels and this may be due to their lower HDL cholesterol.

In summary, we found that in non-insulin-requiring patients with type 2 diabetes, their FER_{HDL} correlated positively with total cholesterol, triglyceride, apo-B, insulin, BMI, and WHR and correlated negatively with HDL cholesterol and apo AI. When HDL cholesterol was controlled for in the regression analysis, these correlations, except that for apo AI, remained significant. When triglyceride was controlled for in the regression analysis, these correlations, except that for apo B and BMI, remained significant. When both HDL cholesterol and triglycerides were controlled in the regression analysis these correlations, except that for BMI, lost their statistical significance. Male non–insulin-requiring type 2 diabetic patients had a significantly higher FER_{HDL} than their female counterparts. This sex difference disappeared when HDL cholesterol, but not triglyceride, is controlled for in the multivariate analysis. As in healthy control subjects, FER_{HDL} was higher in those with known CHD risk factors. Despite the lack of understanding of the mechanism involved in the functional heterogeneity of HDL pool, the determination of FER_{HDL} may provide another useful risk indicator for individuals predisposed to CHD.

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