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Relationship between postheparin plasma lipases and high-density lipoprotein cholesterol in different types of diabetes

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Summary. We measured serum lipids, lipoproteins and postheparin plasma lipases, lipoprotein lipase and hepatic lipase, in 12 female patients with Type 1 (insulin-dependent) diabetes (postglucagon C-peptide undetectable), in 11 female insulin-treated patients with Type 2 (non-insulin-dependent) diabetes (postglucagon C-peptide > 0.60 nmol/l) and in 16 non-diabetic female control subjects. These three groups of subjects were similar with respect to age and obesity. Insulin dose was similar in patients with Type 1 and with Type 2 diabetes. HDL and HDL_2 cholesterol were lower in patients with Type 2 diabetes than in non-diabetic control subjects ($p < 0.05$) but did not differ between patients with Type 1 diabetes and non-diabetic control subjects. No difference in lipoprotein lipase activity was seen between the groups. The highest levels of lipoprotein lipase and hepatic lipase activities were observed in patients with Type 2 diabetes. Lipoprotein lipase activity correlated significantly with HDL cholesterol in patients with Type 1 diabetes ($p < 0.01$) and in patients with Type 2 diabetes ($p < 0.001$) but not in control subjects. Hepatic lipase activity did not correlate significantly with HDL cholesterol in any of the groups. In conclusion, postheparin plasma lipoprotein lipase and hepatic lipase activities do not seem to explain the difference in HDL cholesterol concentration between patients with Type 1 and Type 2 diabetes.

Key words: Type 1 (insulin-dependent) diabetes, Type 2 (non-insulin-dependent) diabetes, HDL cholesterol, lipoprotein lipase, hepatic lipase.

Major interest has been recently focused on serum high density lipoprotein fraction (HDL) and its main subfractions in diabetes because this lipoprotein class is known to have an inverse and independent association with atherosclerotic vascular disease [1-3]. Lipoprotein lipase (LPL) and hepatic lipase (HL) are important regulatory factors in HDL cholesterol metabolism. LPL catalyzes the degradation of triglyceride rich lipoproteins and thus high LPL activity tends to be associated with high HDL levels [4]. The other enzyme, HL probably exerts its physiological function as a phospolipase using HDL as substrate [5]. It has been postulated that the activity of LPL explains the difference in HDL cholesterol between the two main types of diabetes [6]. In insulin-treated patients with high or normal HDL cholesterol level [7-8] LPL activity has been reported to be high or normal and in Type 2 diabetic patients with low HDL cholesterol level [9-12] normal or decreased [13]. None of the studies have included both types of diabetes with insulin treatment and therefore the relationship between HDL cholesterol and LPL and HL activities has remained controversial. Therefore, we studied HDL cholesterol, LPL and HL activity in female non-diabetic subjects and in female patients with Type 1 and Type 2 diabetes, treated with insulin.

Subjects and methods

Subjects

The patients for this study were selected from a group of 170 insulin-treated diabetic patients (90 women, 80 men) and 124 non-diabetic control subjects (59 women, 65 men) who had previously participated in a study concerning lipid and lipoprotein abnormalities in diabetic patients as compared to non-diabetic control subjects [14]. The original study population comprised all insulin-treated diabetic patients aged 45 to 64 years living in the region of the Kuopio University Central Hospital (population 250,000) who were identified on the basis of a national register of drug-treated diabetic patients. Insulin-treated patients for this study were selected from 15 female patients with no C-peptide response to glucagon, representing patients with Type 1 diabetes, and from 38 female patients with postglucagon C-peptide exceeding 0.60 nmol/l representing patients with Type 2 diabetes. The cut-off level of 0.60 nmol/l was used in the classification of diabetic patients into the two main types as suggested by Madsbad et al. [15]. Non-diabetic control subjects aged 45 to 64 years were originally drawn randomly from the population register in the region of the Kuopio University Central Hospital. The study groups were formed so that they were similar with respect to body mass index and the diabetic groups also with respect to insulin dose. In non-diabetic subjects diabetes was excluded on the basis of a fasting plasma glucose measurement. None of the subjects studied was taking hypolipidaemic drugs because of diagnosed hyperlipidaemia and none of them had a significant impairment of renal function as assessed by serum creatinine level.

Approval for the study was given by the Ethical Committee of the University of Kuopio. Informed consent was given by all subjects.

Methods

Body mass index (BMI) was calculated according to the formula BMI = weight (kg)/height^2 (m). Exercise level was evaluated by a questionnaire. Those who had regular physical exercise during leisure time for at least 30 min at least twice per week were classified as physically active. The prevalence of angina pectoris and intermittent claudication was evaluated in an interview using Rose's cardiovascular questionnaire [16]. A subject was defined as having hypertension if his or her systolic blood pressure was ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg or if he or she was receiving drug treatment for hypertension.

Serum lipids and lipoproteins were determined from fresh serum samples drawn after a 12-h overnight fast. Lipoprotein fractions were isolated by the sequential ultracentrifugation according to Havel et al. [17]. Densities of the samples for the separation of each lipoprotein class were adjusted with NaCl-KBr salt solutions and EDTA (100 mg/l). VLDL was flotated at d 1.006 g/ml and 105,000 *g* for 18 h. Thereafter LDL was fractioned from HDL by spinning samples at d 1.063 and 105,000 *g* for 24 h. Remaining total HDL was separated into HDL_2 and HDL_3 fractions by raising the density of infranatant to 1.125 g/ml and spinning for 48 h at 105,000 *g*. After each ultracentrifugation step, lipoprotein fractions were isolated by the tube slicing technique.

Cholesterol and triglycerides from the whole serum and from the lipoprotein fractions were assayed by automated enzymatic methods (kits of Boehringer-Mannheim CmgH, FRG). The concentration of apoprotein B was determined with the radial immunodiffusion kit M-partigen-Apolipoprotein B (Behringwerke AG, Marburg, FRG), and apoprotein A-I and A-II with another immunodiffusion method [18].

LPL and HL activities were assayed from plasma samples taken 15 min after intravenous administration of 100 IU/kg of heparin by a selective immunochemical method [19]. The intra- and interassay variations for LPL and HL determinations were 5.0 and 4.0%, and 14.4 and 14.0% respectively.

The plasma C-peptide response to glucagon was determined according to Faber and Binder [20]. C-peptide concentration was measured by radioimmunoassay (antibody M1230, Novo, Copenhagen, Denmark). Glucosylated haemoglobin A_1 ($GHbA_1$) was determined by column chromatography (Quick-Sep Fast Hemoglobin Test System, Isolab, Akron, Ohio) after incubation in 0.9% saline solution for 12 h.

Statistical analysis

The results for continuous variables are expressed as mean ± SEM. The differences between the groups were assessed by the χ^2 test and Student's two-tailed t-test for independent samples. Adjustment for confounding factors in the comparison of lipid and lipoprotein values between different groups was done with the analysis of covariance (ANCOVA). Linear regression analysis was applied to assess the independent effects of LPL and HL activities on HDL cholesterol and VLDL triglycerides. Correlation analyses were performed using Spearman correlation coefficients.

Characteristics of the study population

Table 1 shows the characteristics of the study groups. No difference was observed between the groups in age, BMI, serum creatinine, physical activity and smoking. Patients with Type 2 diabetes had higher frequency of angina pectoris, claudication and use of diuretics than non-diabetic control subjects. The prevalence of retinopathy was higher among Type 1 diabetic patients than among control subjects. The duration of diabetes was longer in patients with Type 1 than in patients with Type 2 diabetes ($p < 0.001$). Insulin dose and the prevalence of micro- and macrovascular complications or the use of cardiovascular drugs did not differ between the two groups of diabetic patients.

Table 1. Characteristics of the study groups

	Non-diabetic subjects	Patients with Type 1 diabetes	Patients with Type 2 diabetes
No. of subjects	16	12	11
Age (years)	57 ± 1	57 ± 2	58 ± 2
Body mass index (kg/(m)^2)	25.4 ± 0.8	25.8 ± 0.9	27.2 ± 1.1
Fasting plasma glucose (mmol/l)	5.5 ± 0.2	10.6 ± 1.9	11.3 ± 1.6
Glycosylated haemoglobin (%)	3.9 ± 0.2	7.5 ± 0.6[c]	8.8 ± 0.8[c]
Serum creatinine (μmol/l)	78 ± 2	90 ± 8	82 ± 4
Physically active	13/16	7/12	6/11
Smokers	3/16	1/12	0/11
Prevalence of angina pectoris	3/16	5/12	7/11[a]
Prevalence of claudication	0/16	3/12	6/11[b]
Prevalence of hypertension	3/16	5/12	4/11
Prevalence of retinopathy	0/16	6/12[b]	2/11
Users of β-blockers	2/16	5/12	6/12
Users of diuretics	1/16	4/12	7/11[b]
Duration of diabetes (years)	-	21 ± 2	14 ± 1
Insulin dose (U/day)	-	39 ± 4	47 ± 6

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$, patients with Type 1 or with Type 2 diabetes vs non-diabetic control subjects

Results

Table 2 shows the lipid, lipoprotein and apoprotein concentrations in the different groups of subjects. Patients with Type 1 diabetes had higher levels of VLDL

Table 2. Serum lipids, lipoproteins and apoproteins in female patients with Type 1 and Type 2 diabetes and in control subjects

	Non-diabetic control subjects	Patients with Type 1 diabetes	Patients with Type 2 diabetes
Total-C (mmol/l)	6.03 ± 0.35	6.26 ± 0.43	6.77 ± 0.43
HDL-C (mmol/l)	1.76 ± 0.08	1.67 ± 0.10	1.43 ± 0.13[a]
HDL_2-C (mmol/l)	1.22 ± 0.06	1.18 ± 0.07	0.99 ± 0.09[a]
HDL_3-C (mmol/l)	0.53 ± 0.02	0.49 ± 0.03	0.44 ± 0.04
LDL-C (mmol/l)	3.99 ± 0.29	4.15 ± 0.37	3.95 ± 0.36
VLDL-C (mmol/l)	0.28 ± 0.03	0.44 ± 0.06[a]	1.38 ± 0.52
Total-TG (mmol/l)	1.22 ± 0.10	1.66 ± 0.12[b]	3.27 ± 1.01
HDL-TG (mmol/l)	0.09 ± 0.10	0.09 ± 0.01	0.16 ± 0.03[a]
LDL-TG (mmol/l)	0.29 ± 0.02	0.50 ± 0.06[b]	0.58 ± 0.10[a]
VLDL-TG (mmol/l)	0.84 ± 0.10	1.06 ± 0.08	2.52 ± 0.90
Apo A-I (g/l)	1.74 ± 0.06	1.64 ± 0.07	1.57 ± 0.08
Apo A-II (g/l)	0.50 ± 0.02	0.44 ± 0.01[a]	0.52 ± 0.05
Apo B (g/l)	1.13 ± 0.07	1.27 ± 0.12	1.32 ± 0.08

C = cholesterol, TG = triglycerides. [a] $p < 0.05$, [b] $p < 0.01$; patients with Type 1 or with Type 2 diabetes vs non-diabetic control subjects

Table 3. Postheparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities ($\mu mol \cdot ml^{-1} \cdot h^{-1}$) in female patients with Type 1 and Type 2 diabetes and in control subjects

	Non-diabetic control subjects	Patients with Type 1 diabetes	Patients with Type 2 diabetes
LPL	22.5±2.0	26.6±2.1	29.3±3.4
HL	17.5±1.8	13.7±1.9	19.6±2.2

Table 4. Spearman correlation coefficients between HDL cholesterol, HDL_2 cholesterol, VLDL triglycerides, apoproteins and LPL and HL activities in female patients with Type 1 and Type 2 diabetes and in control subjects

	Non-diabetic control subjects		Patients with Type 1 diabetes		Patients with Type 2 diabetes	
	LPL	HL	LPL	HL	LPL	HL
HDL-C	+0.252	+0.231	+0.713[a]	−0.334	+0.840[b]	−0.348
HDL_2-C	+0.263	+0.181	+0.702[a]	−0.393	+0.841[b]	−0.348
VLDL-TG	−0.369	+0.279	−0.197	+0.053	−0.769[a]	+0.153
Apo A-I	−0.005	+0.436[b]	+0.794[a]	−0.380	+0.774[a]	−0.509
Apo A-II	−0.063	+0.521[b]	+0.012	+0.330	−0.205	−0.212
Apo B	−0.276	−0.042	+0.668[a]	+0.001	−0.288	+0.181

C = cholesterol, TG = triglycerides, LPL = lipoprotein lipase, HL = hepatic lipase. [a] $p < 0.01$, [b] $p < 0.001$

Table 5. Linear regression analysis: LPL and HL activities as independent variables and HDL cholesterol and VLDL triglycerides as dependent variables, each one separately in female patients with Type 1 and Type 2 diabetes and in control subjects

	HDL cholesterol as dependent variable		
	Non-diabetic control subjects	Patients with Type 1 diabetes	Patients with Type 2 diabetes
	Regression coefficient	Regression coefficient	Regression coefficient
LPL	+0.0152	+0.0334[a]	+0.0329[b]
HL	+0.0165	−0.0007	+0.0003
R^2	0.176	0.523	0.707
	VLDL triglycerides as dependent variable		
	Non-diabetic control subjects	Patients with Type 1 diabetes	Patients with Type 2 diabetes
	Regression coefficient	Regression coefficient	Regression coefficient
LPL	−0.0275	−0.0006	−0.0968[b]
HL	+0.0008	+0.0001	−0.0225
R^2	0.243	0.029	0.711

LPL = lipoprotein lipase, HL = hepatic lipase. [a] $p < 0.05$, [b] $p < 0.01$

cholesterol, total triglycerides and LDL triglycerides and lower level of apoprotein A-II than control subjects. Patients with Type 2 diabetes had lower levels of HDL and HDL_2 cholesterol and higher levels of HDL triglycerides and LDL triglycerides than control subjects. The differences in HDL cholesterol between control subjects and Type 2 diabetic patients persisted even after adjustment for LPL and HL activities ($p = 0.001$, ANCOVA). As shown in Table 3 no difference was observed in LPL and HL activities between control subjects and diabetic patients. Patients with Type 2 diabetes tended to have higher levels of HL activity than patients with Type 1 diabetes but this difference did not reach statistical significance ($n = 0.06$).

Table 4 shows the correlation between HDL cholesterol, HDL_2 cholesterol, VLDL triglycerides, and apoprotein concentrations and LPL and HL activities. In non-diabetic control subjects neither lipoproteins nor apoproteins correlated with LPL activity but apoA-I and apoA-II were positively correlated with HL activity. In patients with Type 1 diabetes HDL cholesterol, HDL_2 cholesterol, apoA-I and apoB correlated significantly ($p < 0.01$) with LPL. In patients with Type 2 diabetes the correlation between HDL and HDL_2 cholesterol and LPL was even stronger and a significant positive correlation of LPL with apoA-II and a negative correlation with VLDL triglycerides were also observed. HL did not correlate significantly with lipoproteins or apoproteins in patients with Type 1 or Type 2 diabetes.

Table 5 shows the results of linear regression analyses with HDL cholesterol and VLDL triglycerides as dependent variables, each one separately, and LPL and HL activities as independent variables. LPL and HL activities were not significantly associated with HDL cholesterol or VLDL triglycerides in control subjects. In patients with Type 1 diabetes LPL activity was positively associated with HDL cholesterol but HL activity did not show any association with HDL cholesterol or with VLDL triglycerides. In patients with Type 2 diabetes LPL activity was positively associated with HDL cholesterol and negatively with VLDL triglycerides. HL activity was not associated with HDL cholesterol or VLDL triglycerides in patients with Type 2 diabetes. LPL and HL activities explained 17.6% of the variation in HDL cholesterol in controls, 52.3% in patients with Type 1 diabetes and 70.7% in patients with Type 2 diabetes. The corresponding values for VLDL triglycerides were: 24.3% in control subjects, 2.9% in patients with Type 1 diabetes and 71.1% in patients with Type 2 diabetes.

Discussion

We included only women in the study because HDL cholesterol and LPL and HL activities are sex dependent. Female subjects have higher levels of HDL cholesterol [21], higher LPL activity [7] and lower HL activity than men [13]. The study groups were formed similarly with respect to obesity (BMI) and the diabetic groups to insulin dose because obesity can have an effect on HDL cholesterol and LPL activity has been shown to be positively correlated with obesity [22]. Insulin is an activator of both LPL and HL activities and therefore our patients with Type 2 diabetes included only those who were treated with insulin.

Our study shows that HDL and HDL_2 cholesterol are lower in patients with Type 2 diabetes than in control subjects but no difference in HDL cholesterol is evident between patients with Type 1 diabetes and control subjects. Our results are in accordance with several reports which have shown that HDL cholesterol is low or normal in Type 2 diabetes but high or normal in Type 1 diabetes as compared to non-diabetic control subjects [7-12]. However, we found no statistically significant differences either in LPL or HL activities between control subjects and diabetic patients which could explain the difference in HDL cholesterol.

When the two diabetic groups were compared, the LPL activity was highest in patients with Type 2 diabetes who showed the lowest HDL cholesterol levels. Patients with Type 2 diabetes had also slightly higher HL activity than patients with Type 1 diabetes (significance of this difference, $p = 0.06$). Thus LPL activity cannot explain the difference in HDL cholesterol levels between patients with Type 1 and Type 2 diabetes but it is not excluded that the slightly higher HL activity in patients with Type 2 diabetes could at least in part explain this difference.

LPL activity correlated significantly with HDL and HDL_2 cholesterol and with apoprotein A-I in both types of diabetes but not in control subjects. Even the regression analyses including both LPL and HL activities as independent variables showed that LPL was independently and positively associated with HDL in both types of diabetes but not in control subjects. We also found a negative correlation between VLDL triglycerides and LPL activity but this correlation was significant only in patients with Type 2 diabetes. Our results are in accordance with the results previously reported by Nikkilä and Hormila [7]. In patients with Type 2 diabetes LPL activity was inversely associated with VLDL triglycerides. In the regression analyses HL activity was not significantly associated with HDL cholesterol or with VLDL triglycerides in any of the groups studied.

In conclusion, our results show that the difference in HDL cholesterol between patients with Type 1 and Type 2 diabetes cannot be explained on the basis of LPL activity but that HL activity may contribute to the lower level of HDL cholesterol in Type 2 diabetes as compared with Type 1 diabetes. Other mechanisms, like differences in the rate of synthesis of HDL particles in the liver and intestine may be the explanation for the difference in HDL cholesterol between the two main types of diabetes.

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