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Preβ1-HDL concentration is a predictor of carotid atherosclerosis in type 2 diabetic patients

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Running title: preβ1-HDL and carotid atherosclerosis in diabetics

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Preβ1-HDL is a minor high-density lipoprotein (HDL) subfraction that stimulates cholesterol efflux from cell membranes (1,2). However, the fasting preβ1-HDL concentration is elevated in patients with coronary artery disease (CAD), hyperlipidemia, obesity, and in hemodialysis patients (3-7). We examined whether the preβ1-HDL concentration is elevated in type 2 diabetic patients and whether elevated preβ1-HDL is a predictor of carotid atherosclerosis.

Research Design and Methods: We measured the preβ1-HDL concentration in 30 patients with type 2 diabetes (DM group: 58.5±12.3 years, 13 men and 17 women) and in 30 age- and sex-matched healthy controls. We excluded patients receiving hypolipidemic agents and those with renal dysfunction. The DM group had a long duration of disease (9.9±8.6 years), high HbA1c levels (10.4±2.0 %), and high body mass index (BMI; 26.1±6.5 kg/m^2). The preβ1-HDL in frozen plasma, pretreated with sucrose solution for stabilization, was measured using an immunoassay (8). In the DM group, we evaluated the severity of carotid atherosclerosis using ultrasonography with a 7.5-MHz probe. The greatest intima-media thickness (max IMT) and plaque score (PS) were determined as previously reported (9).

Results: The DM group had higher triglyceride (TG) concentrations than the controls (166.9±138.4 vs. 90.9±28.6 mg/dl, $p<0.01$), while the two groups did not differ significantly in the total cholesterol (215.1±49.9 vs. 201.0±20.9 mg/dl), low-density lipoprotein-cholesterol (LDL-C; 135.9±37.1 vs. 125.3±17.9 mg/dl), and HDL-C (55.5±18.7 vs. 58.3±16.6 mg/dl) concentrations. Although the DM group had lower apoAI (the only protein component of preβ1-HDL) levels than the controls (124.6±20.8 vs. 137.8±27.1 mg/dl, $p<0.05$), the former had higher absolute and relative preβ1-HDL concentrations than the latter (24.5±6.0 vs. 20.3±5.7 mg/L apoAI, $p<0.01$; 1.99±0.51 vs. 1.49±0.36 %apoAI, $p<0.001$). In the DM group, the absolute preβ1-HDL concentration was not correlated with HDL-C or ApoAI, while the relative preβ1-HDL concentration was negatively correlated with HDL-C and ApoAI (r=-0.49, $p<0.01$; r=-0.43, $p<0.05$). In comparisons using non-smokers only, the DM group (n=18) had greater absolute and relative preβ1-HDL concentrations than the controls (n=28) (24.3±6.7 vs. 20.0±5.7 mg/L apoAI, $p<0.05$; 1.86±0.58 vs.1.46±0.36 %ApoAI, $p<0.05$).

B-mode ultrasound imaging revealed that carotid atherosclerosis was more severe in the DM group than in the controls. The mean max IMT in the DM group was nearly twice that in the controls (1.20±0.70 vs. 0.64±0.12 mm, $p<0.001$). A higher IMT, defined as more than 1.0 mm, or at least one carotid plaque was detected in 80% of the DM patients.

In the DM group, the max IMT had significant correlations with the absolute and relative preβ1-HDL (Fig 1) and LDL-C concentrations (r=0.373, p=0.043). The PS had positive correlations with age (r=0.540, p=0.002), the duration of diabetes (r=0.472, p=0.008), and both the absolute and relative preβ1-HDL concentrations (Fig 1). Stepwise multiple regression analysis was performed using the max IMT or PS as the dependent variable, and preβ1-HDL (Model #1, absolute concentration; Model #2, relative concentration) together with other risk factors [age, sex, duration of DM, TG, LDL-C, HbA1c, systolic blood pressure, Brinkman Index (cigarettes per day multiplied by years smoked)] as the independent variables. The preβ1-HDL concentration was selected as an independent risk factor for the max IMT (Model #1, R=0.382, p=0.037, B=0.382, F=4.776; Model #2, R=0.563, p=0.006, B=0.454, F=7.024), and PS (Model #1, R=0.630, p=0.001, B=0.473, F=7.773; Model #2, R=0.681, p=0.0002, B=0.493, F=8.668; R, multiple correlation coefficient;

B, partial correlation coefficient). Of the other variables, LDL-C was also selected for the max IMT, and the duration of diabetes and age for PS.

Conclusions: Our results indicate that the preβ1-HDL concentration is elevated in the DM group and that a high preβ1-HDL concentration is a predictor of carotid atherosclerosis. Many prospective studies have reported positive correlations between the severity of carotid atherosclerosis and the cardiovascular risks in general populations and diabetics (9,10). Accelerated atherosclerosis in diabetics may be explained by insulin resistance, chronic inflammation, hyperglycemia, and dyslipidemia (11,12).

Interestingly, the absolute preβ1-HDL concentration was associated with carotid atherosclerosis, but not with glycemic control or other HDL markers. In the group combining hypertriglyceridemics and normolipidemics, the absolute preβ1-HDL concentration did not significantly correlate with either HDL-C or apoAI levels (5). Furthermore, patients with CAD or hemodialysis patients (high-risk patients for CAD) had an elevated preβ1-HDL despite a low HDL-C or apoAI (3,4,7). These data agree well with our study.

An increased preβ1-HDL in atherosclerotic disorders may result from either the impaired maturation of preβ1-HDL into α-HDL (3,6,7,13), or enhanced production of preβ1-HDL (5,14). Previously, we found that low lecithin-cholesterol acyltransferase (LCAT) activity was closely related to a high preβ1-HDL concentration (3,7). In diabetics, the relationship between the LCAT activity and atherosclerosis was inconsistent (15,16). In healthy Japanese, the most important determinant of the preβ1-HDL concentration was not the LCAT mass, but the rate of LCAT-dependent conversion of preβ1-HDL into α-HDL (17). Therefore, we need to determine the LCAT-dependent conversion rate of preβ1-HDL in diabetics in a future study.

Another possible explanation for the high preβ1-HDL concentration in diabetics is the enhanced production of preβ1-HDL from α-HDL. The rates of preβ1-HDL synthesis and recycling of α-HDL to preβ1-HDL were elevated in diabetics (14). The phospholipid transfer protein (PLTP) activity enhances preβ1-HDL synthesis, and is positively associated with carotid atherosclerosis in type 2 diabetes (18,19). As measuring PLTP activity is quite difficult, it might be more useful to measure the preβ1-HDL concentration when evaluating carotid atherosclerosis.

In conclusion, the preβ1-HDL concentration is elevated in type 2 diabetic patients, and an elevated preβ1-HDL concentration is a predictor of carotid atherosclerosis. A prospective study would confirm the clinical significance of preβ1-HDL in type 2 diabetic patients.

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Figure legend

Fig. 1. Positive correlation between the preβ1-HDL concentration and the indexes of carotid atherosclerosis defined as the max IMT and PS in type 2 diabetic patients.
The preβ1-HDL concentration is expressed as absolute (closed circles) or relative (open circles) concentrations. In the DM group (n=30), the max IMT (upper column) and PS (lower column) had significant positive correlations with both the absolute and relative preβ1-HDL concentrations.
max IMT, maximum intima-media thickness; PS, plaque score