Original Article

Significant Increase of Apolipoprotein B48 Levels by a Standard Test Meal in Type 2 Diabetic Patients with Nephropathy

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Aim: We investigated postprandial changes of apolipoprotein (apo) B48 in type 2 diabetics at different stages of diabetic nephropathy in order to explore non-traditional lipid abnormalities in diabetic nephropathy.

Methods: Twenty-two healthy controls and 56 type 2 diabetics with normoalbuminuria (NA), microalbuminuria (MA), and overt albuminuria (OA) were enrolled. Blood samples were taken at 0, 1, 2, 4, 6 h after the ingestion of Test meal A (460 Kcal, 18 g fat). The maximal increase of triglyceride (TG) was 40% above baseline in controls and 17% above baseline in diabetics. The incremental area under the curve (iAUC) of TG, however, was comparable among controls and diabetics with NA, MA, and OA. The maximal increase of apoB48 was 92% above baseline in controls and 56-88% above baseline in diabetics. Apo B48-iAUC was significantly higher in diabetics than in controls, and diabetics with OA exhibited the highest apoB48-iAUC among the diabetic subgroups. Small dense low-density lipoprotein-cholesterol (sd-LDL-C) was elevated in diabetic nephropathy, and apoB48-iAUC was positively associated with the level of sd-LDL-C.

Conclusions: ApoB48 is a sensitive marker for postprandial lipemia, a condition which is significantly increased in diabetic nephropathy and associated with an increase of potent atherogenic sd-LDL particles.


Key words: Apolipoprotein B48, Postprandial lipemia, Diabetic nephropathy, Small dense LDL

Introduction

Diabetics suffer from high rates of coronary heart disease (CHD), and the rates climb even higher in those with diabetic nephropathy. A recent prospective cohort study of patients with type 2 diabetes mellitus has demonstrated that diabetic nephropathy is significantly associated with subsequent mortality from CHD even before end-stage renal disease (ESRD). Among the multiple factors known to play roles in the pathogenesis of CHD in diabetic nephropathy, dyslipidemia is thought to be a powerful risk factor for coronary atherosclerosis. Although lipid metabolism has been extensively investigated in diabetes, little information is available on the influence of nephropathy on diabetic dyslipidemia. Several studies, including our own, have suggested that atherogenic lipoprotein profiles, namely, a reduced level of high density lipoprotein (HDL)-cholesterol in combination with elevated levels of very-low density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and small dense low-density lipoprotein (LDL), are more prominent in type 2 diabetics with diabetic nephropathy.

Chylomicrons are synthesized in the intestine for the transport of dietary triglyceride (TG) in the circulation. Chylomicrons are promptly hydrolyzed with lipoprotein lipase, and this chemical change leads to the production of chylomicron remnants. An accumulating body of evidence suggests that enhanced levels of chylomicron remnants are involved in the pathogenesis of atherosclerosis. The atherogenic nature of these particles is thought to be due to their ability...
to infiltrate the arterial wall, either directly or indirectly, by influencing the composition and metabolic fate of other lipoprotein classes via neutral lipid exchange. Apolipoprotein (apo)B48 is essential for the formation of chylomicrons. The plasma level of apoB48 can be used to precisely count the number of chylomicron particles, as each molecule of protein is contained in one chylomicron particle. The physiopathologic role of TG-rich lipoprotein (TGRL)-apoB48 lipoparticles in atherosclerosis has been documented. Our group previously reported that the postprandial increase of TGRL was markedly higher in nephropathic diabetics than in non-nephropathic diabetics or in patients with non-diabetic kidney disease. We also reported elevations in the fasting apoB48 level in the advanced stage of diabetic nephropathy. To proceed further in the direction mapped out in previous studies, we examined the postprandial increase of apoB48 levels in type 2 diabetic patients at various stages of diabetic nephropathy. “Test meal A”, a standard meal authorized by the Japanese Diabetes Association, has been established to be effective for the evaluation of postprandial hyperglycemia and hyperlipidemia. Our group used this standard meal in an experiment to determine how diabetic nephropathy affects postprandial increases of TG and apoB48 levels after test meal administration.

Subjects and Methods

Study subjects (n=78; male (M)/female (F), 44/34) included 56 (M/F: 30/26) type 2 diabetic patients with diabetic nephropathy at different stages and 22 (M/F: 14/8) non-diabetic healthy controls not receiving any medications. Subjects treated with lipid-lowering agents were excluded. The average albumin/creatinine index in urine (u-alb/cr) was calculated from spot urine samples collected from diabetic patients on three different days. Urine specimens contaminated with bacteria, white blood cells, or red blood cells were excluded. Diabetics were classified into the following subgroups based on urinary albumin concentrations measured by the latex turbidimetric immunoaassay method using a commercially available kit (LA-system; AIC Co., Tokyo, Japan): normo-albuminuria (NA), u-alb/cr less than 29 mg/g; micro-albuminuria (MA), u-alb/cr of 30-299 mg/g; overt albuminuria (OA). All diabetic patients enrolled in the study was placed on an isocaloric diet (26-27 Kcal/kg ideal body weight) consisting of 17% protein, 23% fat, and 60% carbohydrate. The dietary therapy was supervised by a diettian and exercise therapy was prescribed by a physician according to the recommendations of the Japanese Diabetes Association. The diet and exercise therapeutics were the sole treatments administered during the course of the study for 4 of the NA diabetics. Ten NA, 3 MA, and 6 OA diabetics were on insulin therapy. The other diabetic patients were treated with oral hypoglycemic agents concomitantly with the diet and exercise therapies (sulfonylureas, alpha-glucosidase inhibitors, metformin, pioglitazone, or a combination of the foregoing). Three of the NA diabetics received pioglitazone. There was no statistically significant difference in the frequency of metformin users among NA, MA, and OA diabetic subgroups. Informed consent was obtained from all subjects, and the study was approved by the local ethics committee.

One portion of Test meal A consisted of a bowl of cream of chicken soup, 5 crackers, and prynne. The total caloric value was 460 Kcal: 51.4% carbohydrate (55.6 g), 15.3% protein (17.2 g), and 33.3% fat (18 g). The subjects ate the test meal after an overnight fast, and gave blood samples at 1, 2, 4, and 6 h after ingestion. The plasma apoB48 level was measured by enzyme immunoassay (Lumipulse apoB48, Fujirebio, Tokyo, Japan) according to the method described by Sakai et al. ApoAI, B, and E were measured by immunoturbidimetry (Daiichi Pure Chemical Co.). Low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) were measured by direct assay using commercially available kits (Cholestest LDL and Cholestest N-HDL; Daiichi Pure Chemical Co.). Small dense (sd)-LDL-C was measured by the precipitation method (sd-LDL-C Seiken, Denka Seiken, Tokyo, Japan) according to the method described by Hirano et al. Large buoyant (lb)-LDL was determined by subtracting sd-LDL-C from total LDL-C. TG, glucose, hemoglobin (Hb) A1c, glycoalbumin, albumin, and creatinine were measured by standard laboratory procedures.

Postprandial response over a 6-h period was calculated as the incremental area under the curve (iAUC) using the trapezoid rule. The iAUC was adjusted for the baseline value by subtracting the fasting value from each postprandial value. Significant differences among the four groups were determined by one-way ANOVA with the Bonferroni/Dunn test. Correlation coefficients between two variables were calculated by Pearson’s simple linear regression analysis. Multivariate analysis was used to assess independent associations. Statistical significance was accepted at p<0.05.

Results

As we were unable to find age-matched healthy subjects who were willing to undergo the meal toler-
Table 1. General profile and serum lipid levels in healthy controls and type 2 diabetic patients with different degrees of albuminuria

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 22, M/F: 14/8)</th>
<th>Type 2 Diabetes (n = 56, M/F: 30/26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normo (n = 35)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>35 ± 3</td>
<td>59 ± 11*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23 ± 3</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>U-alb/cr (mg/g)</td>
<td>NA</td>
<td>11 ± 6</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>NA</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.0 ± 1.0</td>
<td>9.6 ± 2.2*</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>87 ± 34</td>
<td>160 ± 56*</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>118 ± 31</td>
<td>121 ± 33</td>
</tr>
<tr>
<td>Sd-LDL-C (mg/dL)</td>
<td>21 ± 11</td>
<td>25 ± 15</td>
</tr>
<tr>
<td>Lb-LDL-C (mg/dL)</td>
<td>97 ± 26</td>
<td>98 ± 29</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>63 ± 16</td>
<td>46 ± 10*</td>
</tr>
<tr>
<td>Apo AI (mg/dL)</td>
<td>149 ± 22</td>
<td>122 ± 19*</td>
</tr>
<tr>
<td>Apo B (mg/dL)</td>
<td>91 ± 23</td>
<td>107 ± 23*</td>
</tr>
<tr>
<td>Apo E (mg/dL)</td>
<td>3.9 ± 1.0</td>
<td>5.1 ± 1.3*</td>
</tr>
<tr>
<td>Apo B48 (mg/L)</td>
<td>4.5 ± 2.3</td>
<td>6.6 ± 4.2*</td>
</tr>
</tbody>
</table>

Normo-albuminuric, micro-albuminuric, and overt proteinuric patients were stratified by albuminuria of less than 30, 30-299, and higher than 300 mg/creatinine g, respectively. U-alb/cr, urinary albumin/creatinine ratio; Sd-LDL-C, small dense LDL-cholesterol; Lb-LDL-C, large buoyant LDL-cholesterol; NA, not available.

*p < 0.05-0.001 vs healthy controls. †p < 0.05-0.01 vs diabetics with normo-albuminuria

In the oral glucose tolerance test, we had to rely on a control population that was significantly younger than the diabetic patients in this study. Table 1 lists the general profiles and serum lipid levels in healthy controls and type 2 diabetic patients at different stages of nephropathy. The body mass index (BMI) was significantly lower in the control group than in the diabetics with OA. Serum creatinine levels were significantly higher in the OA subgroup than in NA and MA subgroups. The fasting plasma glucose and HbA1c levels were very high, as expected in view of the poor diabetic control of the patients enrolled. HbA1c was comparable between NA and MA subgroups, but the OA subgroup had higher HbA1c than the MA subgroup. LDL-C, sd-LDL-C, and apoB increased with the progression of diabetic nephropathy, whereas lb-LDL-C remained unchanged. HDL-C and apo AI levels were lower in diabetics than controls, irrespective of the stage of diabetic nephropathy, whereas apo E levels were higher in the diabetics. Fasting TG and Apo B48 were higher in diabetics than in controls, and tended to increase with the progression of nephropathy, albeit not by a significant degree. These increases may have attained statistical significance if there had been more patients in the MA and OA subgroups.

Fig. 1 shows changes of serum glucose levels before and 1, 2, 4, and 6 h after ingestion of the test meal. Glucose levels at the baseline were markedly higher in diabetics than in controls, but there were no significant differences in baseline glucose levels among the different stages of diabetic nephropathy. Glucose levels peaked 1 h after ingestion in all diabetic subgroups, and there were no statistical differences of glucose levels among the subgroups at any time point thereafter. Glucose-iAUCs were substantially elevated in all of the diabetic subgroups compared with controls.

Fig. 2 shows changes of serum TG levels over the 6-h period after ingestion of the test meal. The TG level in control subjects rose mildly, peaking at 40% above the baseline at 2 h. TG levels in diabetic patients rose even more mildly, peaking at only 17% above the baseline. TG levels at baseline and 1, 2, 4, and 6 h after the meal were markedly higher in diabetics than in controls, but there were no significant differences in TG levels among the diabetic subgroups at any sampling point. TG-iAUCs were comparable between controls and diabetics, irrespective of the stage of diabetic nephropathy.

Fig. 3 shows changes of serum apoB48 levels over the 6-h period after ingestion of the test meal. Apo B48 levels at the baseline were markedly higher in diabetics than in controls, but there were no significant differences in baseline apoB48 levels among the diabetic subgroups. The maximum increase of apoB48 was 92% above the baseline in controls and 56-88% in diabetics, or more than twofold the maximum increases of TG. Apo B48-iAUC was significantly in-
creased in all diabetic subgroups compared with controls, and OA diabetics exhibited the highest apoB48-iAUC among subgroups.

**Table 2** lists correlation coefficients between apoB48-iAUC and various parameters. In the total study population, apoB48-iAUC was significantly correlated with glucose-iAUC, HbA1c, TG-iAUC, sd-LDL-C, apoB, and apo E levels. In control subjects, apoB48-iAUC was significantly correlated with TG-iAUC, sd-LDL-C, lb-LDL-C, and apoB levels. In diabetic patients, apoB48-iAUC was significantly correlated with logarithmic transformed albuminuria, HbA1c, TG-iAUC, sd-LDL-C, and apoB levels. Multivariate analysis revealed that apoB48-iAUC was significantly associated with TG-iAUC ($\beta=0.405$, $p<0.002$) and sd-LDL-C ($\beta=0.264$, $p<0.05$), independently of the HbA1c levels.

**Discussion**

Our group recently reported that fasting apoB48 levels increase continuously with the progression of diabetic nephropathy and reach peak levels in diabetic ESRD\(^{18}\). In the present study we were unable to examine the postprandial level of apoB48 in diabetic ESRD, as we had difficultly finding diabetic ESRD.
patients who would consent to the meal tolerance test. We found, nevertheless, that nephropathic diabetics had higher postprandial levels of apoB48 compared with controls and diabetics without nephropathy. At the outset of our study, we expected that the small number of patients with diabetic nephropathy might prevent us from finding any significant elevation of fasting apoB48 levels; however, the present study is the first to demonstrate a marked elevation of postprandial apoB48 level in type 2 diabetic patients with nephropathy.

In contrast to our findings with apoB48, we did not observe a significant postprandial increase of TG in patients with diabetic nephropathy. Indeed, TG-iAUC was comparable among control and diabetic groups, irrespective of the stage of nephropathy. We previously studied the postprandial increase of TG in diabetes with and without nephropathy in a fat tolerance test using 50 g/m² fat. This high dose of fat substantially elevated TG two fold above the baseline, and the fat tolerance test revealed that incremental TG levels over a 9-h period were two fold higher in nephropathic diabetics than in non-nephropathic diabetics. Tentolouris et al. recently reported that diabetics with MA had higher postprandial TG levels than those with NA after mixed meal of 783 Kcal (52.5% fat). In the current study we used “Test meal A”, a standard meal authorized by the Japan Diabetes Association for the assessment of postprandial increases of glucose, insulin, and TG. Test meal A is formulated to mimic a common Japanese breakfast (total calorific value: 460 Kcal), but with a slightly elevated fat content (from the standard 27% to 35%). A lower fat content of 18 g is insufficient to fully elevate the serum TG level, and thus cannot bring about apparent postprandial lipemia in patients with diabetic nephropathy taking the fat tolerance test. ApoB48 is a structural protein of chylomicrons, which is produced only in the intestine, and only in a postprandial state. ApoB48 disappears in the fasting state, whereas VLDL particles are produced constantly in the liver during both fasting and nonfasting states. ApoB48 therefore
serves as a more sensitive marker for postprandial lipemia than TG. Test meal A dose not contain enough fat to fully elevate serum TG levels; however, this could become a sensitive fat-tolerance test if postprandial apoB48 levels are monitored.

Given that B48 is secreted postprandially, the serum B48 level in the post-absorptive state is virtually regulated by the efficiency of remnant removal. How is the catabolism of chylomicron remnants impaired in diabetic nephropathy? Our group previously reported marked elevations of apo C1 and apoCIII levels in patients with diabetic nephropathy, and the results of our present study corroborate this finding. Apo C1 inhibits LDL-receptor-related protein (LRP)-mediated remnant uptake, and apoCIII inhibits the catabolism of TGRLs by suppressing lipolysis with lipoprotein lipase and particle uptake by the liver. Thus, increases of apoC1 and CIII may be associated with catabolic defects of chylomicron remnants in diabetic nephropathy. Lipoprotein lipase, a limiting enzyme for TG hydrolysis, was not measured in this study. Previously, however, we found that heparin-releasable lipoprotein lipase mass was significantly reduced in type 2 diabetics with MA. It thus seems likely that reduced lipoprotein lipase activity is involved in the mechanisms behind the postprandial increase of apoB48 in diabetic nephropathy. Hepatic triglyceride lipase also plays an important role in the removal of chylomicrons as a bridging protein to the liver. Noting that hepatic triglyceride lipase levels decline in ESRD, we speculate that reduced hepatic triglyceride lipase activity might also be involved in diminished remnant removal in diabetic nephropathy.

Sd-LDL-C was associated with apoB48-iAUC in both diabetic and nondiabetic subjects. Sd-LDL is characterized by the presence of cholesterol-poor particles generated by TG-cholesteryl ester exchange between TGRLs and LDL. Our group previously demonstrated marked TGRL-TG elevation (density <1.006 g/mL) in nephropathic diabetics with normal fasting TG levels, and the postprandial increase of TG in those subjects was negatively correlated with LDL size. Our previous and current studies both support the notion that postprandial lipemia is deeply involved in the generation of s-d LDL particles.

The present study had several limitations. First, we were unable to enroll nondiabetic subjects of comparable age to diabetic patients. Second, the small numbers of diabetics in our MA and OM subgroups diminished the statistical power of comparisons among diabetic subgroups. Third, no diabetic patients with ESRD were enrolled. To overcome these limitations, future studies will need to be conducted to elucidate postprandial lipemia in diabetic nephropathy using sufficient numbers of patients, and to compare with age-adjusted nondiabetic populations. Data from clinical studies have yet to clarify whether apoB48 is actually a risk factor for CHD. Valero et al. found no connection between apoB48 and the presence or severity of CHD. Karpe et al., on the other hand, found that the fasting apoB48 concentration in VLDL <1.006 g/mL was higher in patients with CHD than in controls. A large cohort study will be required to elucidate whether elevated serum apoB48 serves as an independent risk factor for CHD.

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