*Kidney International, Vol. 63 (2003), pp. 2171–2177*

# Very low-density lipoprotein-apoprotein CI is increased in diabetic nephropathy: Comparison with apoprotein CIII

**Tsutomu Hirano, Taro Sakaue, Asako Misaki, Satoru Murayama, Toshihiro Takahashi, Kenta Okada, Hiroko Takeuchi, Gen Yoshino, and Mitsuru Adachi**

*First Department of Internal Medicine, Showa University School of Medicine, Tokyo, Japan; and Department of Laboratory Medicine, Toho University School of Medicine, Tokyo, Japan*

**Very low-density lipoprotein-apoprotein CI is increased in diabetic nephropathy: Comparison with apoprotein CIII.**

*Background.* Recent studies have suggested that apoprotein (apo) CI in very low-density lipoprotein (VLDL) plays an important role in causing hypertriglyceridemia independent of apo CIII, which is associated with coronary heart disease (CHD). Because the incidence of CHD is increased in diabetic patients and is even higher when diabetic nephropathy is developed, we measured apo CI levels in VLDL from type 2 diabetic patients, with various degree of nephropathy, and compared the results with those for healthy controls or nondiabetic patients with chronic renal failure (CRF).

*Methods.* This study enrolled healthy control subjects, type 2 diabetic patients with normoalbuminuria, microalbuminuria, overt proteinuria, and CRF on hemodialysis and nondiabetic hemodialyis patients. VLDL (density <1.006) was separated by ultracentrifugation. Then the apo CI, CIII, and B concentrations in VLDL were measured by enzyme-linked immunosorbent assay (ELISA).

*Results.* The apo CI, CIII, and B concentrations in VLDL were respectively 3-, 2-, and 2-fold higher, respectively, in diabetic patients with overt proteinuria than in controls. Hemodialysis patients with diabetic nephropathy had levels of apo CI, CIII, and B in VLDL that were 2.6-, 2.7- and 2-fold higher, respectively, than those in controls. Nondiabetic hemodialysis patients also had a 2.7-fold higher level of VLDL apo CIII, whereas VLDL apo CI and VLDL apo B were not significantly increased. VLDL apo CI was significantly correlated with VLDL apo B independently of VLDL apo CIII level.

*Conclusion.* An increase of VLDL apo CIII is a prominent feature of dyslipidemia in CRF patients, regardless of whether they are diabetic or nondiabetic, whereas an increase of VLDL apo CI is more specific to diabetic nephropathy and is closely associated with an increase of VLDL particle numbers, a new risk factor for CHD.

**Key words:** apoprotein CI, apoprotein CIII, VLDL, diabetic nephropathy, chronic renal failure.

Received for publication August 24, 2002
and in revised form November 18, 2002
Accepted for publication January 28, 2003



It is well known that there is a high incidence of coronary heart disease (CHD) in patients with chronic renal failure (CRF) due to diabetic nephropathy, which results in a significantly higher morbidity and mortality rate than in their nondiabetic counterparts [1–3]. 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) occurs [4]. The pathogenesis of CHD in diabetic nephropathy is multifactorial, but dyslipidemia is thought to be a strong risk factor for coronary atherosclerosis [3–8]. Several studies, including ours, suggested that an atherogenic lipoprotein profile, such as an increase of very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) along with decrease of high-density lipoprotein (HDL)-cholesterol and LDL size, becomes more prominent in type 2 diabetic patients who have diabetic nephropathy [5–10]. Although lipid metabolism has been extensively investigated in diabetes, little information is available concerning the influence of nephropathy on diabetic dyslipidemia. It remains unclear how lipoprotein abnormalities worsen with increasing severity of diabetic nephropathy, and whether lipoprotein changes are attributable to diabetes-related metabolic abnormalities or renal dysfunction.

We recently reported that the number of VLDL particles [as estimated from the apoprotein (apo) B concentration in VLDL] was markedly increased in Japanese men with CHD, and that this was a superior discriminator for CHD/non-CHD when compared with established coronary risk factors such as LDL-cholesterol level or LDL size [11]. That preliminary report also showed that the apo CI level in VLDL was markedly increased in CHD and was associated with CHD events independently of apo CIII [11]. Apo CI was shown to inhibit VLDL particle removal by the liver as did apo CIII in a liver perfusion study performed using rats [12]. Recent studies have indicated that human apo C1 transgenic

**Table 1.** General and metabolic characteristics of subjects

| | Nondiabetic control | Type 2 diabetes | | | | Nondiabetic hemodialysis |
|---|---|---|---|---|---|---|
| | | Normo | Micro | Overt | Hemodialysis | |
| Number *M/F* | 42 (24/18) | 40 (21/19) | 15 (8/7) | 17 (10/7) | 17 (10/7) | 29 (18/11) |
| Age *years* | 59 ± 2 | 62 ± 2 | 64 ± 3 | 65 ± 2 | 60 ± 3 | 60 ± 3 |
| BMI $kg/m^2$ | 23.7 ± 0.6 | 23.6 ± 0.7 | 24.4 ± 1.0 | 24.9 ± 0.8 | 22.6 ± 1.0 | 20.2 ± 0.5[a,b,c,d] |
| Therapy mode for diabetes (DI:OHA:INS) | | 4:31:5 | 0:12:3 | 0:13:4 | 7:5:5 | |
| Albumin/creatinine index *mg/g* | 14 ± 3 | 17 ± 1 | 109 ± 19[a,b] | 2312 ± 478[a,b,c] | NA | NA |
| FPG *mg/100 mL* | 99 ± 22 | 144 ± 5[a,f] | 166 ± 13[a,f] | 184 ± 27[a,b,e,f] | 131 ± 7[a,f] | 95 ± 31 |
| $HbA_{1c}$ *%* | 5.4 ± 0.1 | 7.7 ± 0.2[a,e] | 8.3 ± 0.4[a,e] | 7.9 ± 0.5[a,e] | 5.9 ± 0.4 | NA |
| Albumin *g/100 mL* | 4.2 ± 0.1 | 4.1 ± 0.0 | 4.2 ± 0.0 | 3.7 ± 0.2[a] | 3.8 ± 0.1[a] | 3.9 ± 0.2 |
| Creatinine *mg/100 mL* | 0.9 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.0 | 1.4 ± 0.3[a,b,c] | 9.5 ± 0.5[a,b,c,d] | 10.8 ± 0.3[a,b,c,d] |

Data represent mean ± SEM. Abbreviations are: normo. normoalbuminuric diabetes; overt, overt proteinuric diabetes; micro, microalbuminuric diabetes; NA, not available; DI, diet; OHA, oral hypoglycemic agents; ISN, insulin; BMI, body mass index; $HbA_{1c}$, hemoglobin $A_{1c}$.

Significance ($P < 0.0033$) was determined by ANOVA. [a]vs. control; [b]vs. normo; [c]vs. micro; [d]vs. overt; [e]vs. diabetic hemodialysis; [f]vs. nondiabetic hemodialysis

mice are hypertriglyceridemic because of a defect in the catabolism of VLDL [13]. In addition, mice with overexpression of human apo CI and a lack of the LDL receptor develop severe hypertriglyceridemia [14], suggesting that apo CI impairs the catabolism of VLDL via LDL receptor-independent pathways. However, the role of apo CI in lipoprotein metabolism remains unknown in humans. Because some clinical studies have demonstrated that the apo B concentration of VLDL is substantially increased in diabetic nephropathy [5, 15], we speculated that VLDL apo CI levels would also be increased, leading to delayed clearance of VLDL and its accumulation in the plasma. This study was carried out to examine the apo CI and apo CIII levels in VLDL from diabetic patients with various stages of nephropathy and nondiabetic patients with CRF in order to determine whether apo CI was independent of apo CIII.

## METHODS

### Subjects

We studied 42 healthy control subjects, 72 type 2 diabetic patients with various degrees of albuminuria, and 46 CRF patients on hemodialysis due to diabetic nephropathy ($N = 17$) or nondiabetic kidney disease ($N = 29$). All of the diabetic patients fulfilled the World Health Organization (WHO) criteria (1986) for type 2 diabetes mellitus: their fasting plasma glucose levels were higher than 140 mg/dL and/or the plasma glucose level exceeded 200 mg/dL at 2 hours in the 75 g oral glucose tolerance test. Spot urine samples were collected on at least three different days and the average albumin/creatinine index in the urine was calculated. Urine specimens contaminated with bacteria, white blood cells, or red blood cells were excluded. The urinary albumin concentration was measured by the latex turbidimetric immunoassay method using a commercially available kit (LA-system; AIC Co., Tokyo, Japan) and the diabetic patients were classified into the following subgroups according to the level of albuminuria: normoalbuminuria, albumin/creatinine index in the urine less than 30 mg/g; microalbuminuria, albumin/ceatinine index in the urine of 30 to 300 mg/g; and overt diabetic nephropathy, albumin/creatinine index in the urine greater than 300 mg/g as described previously [16]. Diabetic patients with nephropathy had overt proteinuria but did not have clinical or laboratory evidence of kidney disease other than diabetic glomerulosclerosis. All of the diabetic patients with overt diabetic nephropathy had long duration of diabetes (more than 10 years) and all of these patients also had diabetic retinopathy (Table 1). All diabetic patients were fed an isocaloric diet (26 to 27 Kcal/kg ideal body weight) consisting of 17% protein, 23% fat, and 60% carbohydrate, regardless of the presence of nephropathy. Drugs affecting plasma lipid level were discontinued at least 1 month prior to blood examination. Most of the diabetic patients with normoalbuminuria, microalbuminuria, and overt proteinuria were being treated with sulfonylureas (glibenclamide 1.25-5.0 mg/day or gliclazide 20-80 mg/day) and/or alpha-glucosidase inhibitors (150 to 300 mg/day acarbose or 0.6 to 0.9 mg/day voglibose). Five normoalbuminuric, three microalbuminuric, four proteinuric, and five diabetic hemodialysis patients were on insulin therapy. Four diabetic patients with normoalbuminuria and seven diabetic hemodialysis patients were being treated with diet and exercise therapy only (Table 1). The nondiabetic CRF patients on hemodialysis consisted of 16 patients with chronic nephritis, three with immunoglobulin A (Ig A) nephritis, three with focal glomerulosclerosis, two with polycystic kidney, and five with disease of unknown etiology. Blood samples were obtained after an overnight fast. In the patients on hemodialysis, blood samples were obtained immediately before starting dialysis. Total cholesterol, triglyceride, HDL cholesterol, glucose, hemoglobin $A_{1c}$, albumin, and creatinine were measured by standard laboratory procedures. The LDL cholesterol level was measured by a direct assay using a commerially available kit (Choletest LDL, Daiichi Pure Chemical Co.,

**Table 2.** Plasma lipid and apoprotein profile of subjects

| | Nondiabetic control | Type 2 diabetes | | | | Nondiabetic hemodialysis |
|---|---|---|---|---|---|---|
| | | Normo | Micro | Overt | Hemodialysis | |
| Number | 42 | 40 | 15 | 17 | 17 | 29 |
| Triglyceride *mg/100 mL* | 93 ± 5 | 108 ± 9 | 128 ± 15 | 199 ± 26[a,b,c,f] | 146 ± 12[a] | 102 ± 9 |
| Total cholesterol *mg/100 mL* | 185 ± 5 | 207 ± 5 | 208 ± 9 | 233 ± 11[a] | 160 ± 7[b,c,d] | 167 ± 7[a,b,c,d] |
| LDL cholesterol *mg/100 mL* | 113 ± 4 | 132 ± 5[a] | 130 ± 5 | 139 ± 9[a] | 95 ± 7[b,c,d] | 100 ± 5[b,c,d] |
| HDL cholesterol *mg/100 mL* | 56 ± 3 | 46 ± 2[a] | 45 ± 3 | 47 ± 3 | 36 ± 2[a] | 47 ± 3 |
| Apoprotein A1 *mg/100 mL* | 130 ± 4 | 125 ± 3 | 121 ± 7 | 128 ± 6 | 102 ± 5[a,b,c,d,f] | 120 ± 6 |
| Apoprotein B *mg/100 mL* | 76 ± 2 | 95 ± 3[a] | 97 ± 5[a] | 108 ± 7[a,e,f] | 83 ± 7 | 81 ± 5 |
| Apoprotein CII *mg/100 mL* | 3.6 ± 0.3 | 4.5 ± 0.2 | 5.0 ± 0.5 | 7.5 ± 1.4[a,b,f] | 6.8 ± 0.7[a,b,f] | 4.0 ± 0.4 |
| Apoprotein CIII *mg/100 mL* | 9.9 ± 0.8 | 9.0 ± 0.4 | 10.2 ± 1.1 | 15.6 ± 2.0[a,b,c] | 14.5 ± 1.4[a,b] | 12.9 ± 0.9[a,b] |
| Apoprotein E *mg/100 mL* | 4.0 ± 0.1 | 4.6 ± 0.1[a] | 4.8 ± 0.4[a] | 5.8 ± 0.6[a,b] | 5.1 ± 0.5[a] | 4.6 ± 0.3 |

Data represent mean ± SEM. Abbreviations are: normo, normalbuminuric; micro, microalbuminuric diabetes; overt, overtproteinuric diabetes; and LDL, low-density lipoprotein.

Significance ($P < 0.0033$) was determined by ANOVA. [a]vs. control; [b]vs. normo; [c]vs. micro; [d]vs. overt; [e]vs. diabetic hemodialysis; [f]vs. nondiabetic hemodialysis

Tokyo, Japan), as described previously [17]. Plasma apoproteins were determined by the conventional immunoturbidometric assay. VLDL (density <1.006 g/mL) was separated from plasma by ultracentrifugation (Hitachi CP-65G; Hitachi Co., Ltd., Tokyo, Japan) with an RP 55T-708 rotor (Hitachi Co., Ltd.). Then, the apo B level in VLDL was measured by an enzyme-linked immunosorbent assay (ELISA) as described by Young et al [18] using monoclonal antibody to apo B (MABO 12, Chemicon International, Inc., Temecula, CA, USA). The ELISA methods for apo CI and CIII in VLDL were established in our laboratory. Briefly, antihuman apo C1 or CIII monoclonal antibodies (Biogenesis, Inc., Kingston, NH, USA) in carbonate buffer were coated on plates. Samples or standard apoproteins were added to each well and incubated. Goat antihuman apo CI or CIII antibodies (Ig G) (Chemicon International, Inc.) were added and incubated. After washing, alkaliphosphatase-labeled antigoat IgG (Chemicon International, Inc.) were added and incubated. After washing, enzyme substrate solution (p-nitrophenylphosphate in 1 mol/L diethanolamine, pH 9.8) was added. The resulting color was measured at 405 nm with an automated microplate reader (EL × 808; Bio-Tek, Winooski, VT, USA). The intra-assay coefficient of variation was 6% to 9% for measurement of these apoproteins by ELISA. Each ELISA was sensitive and suitable for detecting small amounts of apoproteins in isolated lipoprotein fractions, but plasma apo CI and CIII levels could not be measured probably because a component of plasma interfered with the assay. Therefore, the plasma apo CI concentration was not determined in this study and plasma apo CIII was measured by the conventional immunoturbidimetry method.

Differences between groups were assessed by analysis of variance (ANOVA) and then a post-hoc test was performed using Bonferroni/Dunn–type multiple comparison. Statistical significance was set at $P < 0.0033$. Correlation coefficients between two variables were calculated by Pearson's simple linear regression analysis. Statistical significance was accepted at $P < 0.05$.

Multiple linear regression analysis was performed to examine whether the associations between VLDL apo CI and kidney function, plasma lipids, or VLDL components were independent of VLDL apo CIII.

## RESULTS

As shown in Table 1, age and gender were comparable between the controls, the diabetic patients with normoalbuminuria, microalbuminuria, overt proteinuria, or CRF treated with hemodialysis and the nondiabetic hemodialysis patients. Body mass index (BMI) was comparable between these groups, except except that nondiabetic hemodialysis patients had lower BMI than the others. Serum creatinine levels were significantly higher and albumin was lower in the diabetic patients with proteinuria, while these levels in normoalbuminuric and microalbuminuric diabetic patients were comparable to controls. Both diabetic and nondiabetic hemodialysis patients had marked high serum creatinine and slightly low albumin concentrations, and these values were similar in diabetic and nondiabetic hemodialysis patients. Fasting plasma glucose and hemoglobin $A_{1c}$ levels were higher in the diabetic group than in nondiabetic subjects, but hemoglobin $A_{1c}$ levels were not elevated in diabetic hemodialysis patients. Hemoglobin $A_{1c}$ values were comparable among normoalbuminuric, microalbuminuric, and overt proteinuric diabetic groups.

Table 2 shows the levels of plasma lipids and apoproteins in each group. Plasma triglyceride levels increased with increasing severity of diabetic nephropathy, but the level was lower in diabetic hemodialysis patients than in diabetic patients with proteinuria. Total and LDL cholesterol levels were slightly higher in diabetic patients than in controls and were the highest in the diabetic patients with overt proteinuria, but these levels declined significantly in diabetic hemodialysis patients. Nondia-

**Table 3.** Very low-density lipoprotein (VLDL) concentration and composition

| | Nondiabetic control | Type 2 diabetes | | | | Nondiabetic hemodialysis |
|---|---|---|---|---|---|---|
| | | Normo | Micro | Overt | Hemodialysis | |
| Number | 42 | 39 | 15 | 17 | 17 | 29 |
| VLDL TG *mg/100 mL* | 43 ± 4 | 52 ± 7[a] | 63 ± 12[a,f] | 102 ± 6[a,b,c,f] | 80 ± 9[a,f] | 39 ± 5 |
| VLDL Chol *mg/100 mL* | 12 ± 1 | 16 ± 2[a] | 21 ± 3 | 32 ± 4[a,b,c,f] | 23 ± 3[a,f] | 13 ± 2 |
| VLDL Apo B *mg/100 mL* | 13 ± 1 | 12 ± 1 | 18 ± 3 | 26 ± 3[a,b,f] | 25 ± 3[a,f] | 15 ± 2 |
| VLDL Apo CI *mg/100 mL* | 1.5 ± 0.2 | 2.0 ± 0.4 | 2.6 ± 0.7 | 4.6 ± 0.9[a,b,e] | 3.9 ± 0.3[a,b] | 2.5 ± 0.2[a] |
| VLDL Apo CIII *mg/100 mL* | 2.9 ± 0.2 | 3.0 ± 0.4 | 3.0 ± 0.4 | 4.6 ± 0.6 | 7.7 ± 0.7[a,b,c,d] | 7.7 ± 0.8[a,b,c,d] |
| Apo CI/Apo B *w/w* | 0.12 ± 0.02 | 0.14 ± 0.02 | 0.12 ± 0.03 | 0.18 ± 0.03 | 0.18 ± 0.02 | 0.18 ± 0.03 |
| Apo CIII/Apo B *w/w* | 0.30 ± 0.04 | 0.31 ± 0.05 | 0.23 ± 0.05 | 0.21 ± 0.04 | 0.36 ± 0.05 | 0.51 ± 0.11[a,b,c,d] |

Data represent mean ± SEM. Abbreviations are: normo, normalbuminuric; micro, microalbuminuric diabetes; overt, overtproteinuric diabetes; TG, triglyceride; Chol, cholesterol; apo, apoprotein.

Significance ($P < 0.0033$) was determined by ANOVA. [a]vs. control; [b]vs. normo; [c]vs. micro; [d]vs. overt; [e]vs. diabetic hemodialysis; [f]vs. nondiabetic hemodialysis

betic hemodialysis patients had similar plasma triglyceride levels, but lower total and LDL cholesterol levels, when compared with diabetic groups. HDL cholesterol was lower in all diabetic groups and nondiabetic hemodialysis patients than in the controls, and the diabetic hemodialysis group had the lowest HDL cholesterol level. Similarly, apo AI levels were substantially lower in diabetic hemodialysis patients than in the other groups. Plasma apo B levels were significantly higher in diabetic patients than in controls, and diabetic patients with overtproteinuria had the highest level, while apo B was not elevated in diabetic or nondiabetic hemodialysis patients. Plasma apo CII, apo CIII, and apo E levels all increased significantly with increasing severity of diabetic nephropathy. The nondiabetic hemodialysis group had a higher plasma apo CIII level, but their apo CII and apo E levels were not elevated when compared with the controls.

Table 3 shows the VLDL level and its apoprotein components in each group. Like plasma triglyceride levels, VLDL triglyceride levels increased in diabetic patients with increasing severity of diabetic nephropathy, but the level in diabetic hemodialysis patients was lower than in diabetic patients with overt proteinuria. VLDL cholesterol concentrations showed a similar profile to VLDL triglyceride. VLDL apo B levels showed significant increase in diabetic patients with overt proteinuria and hemodialysis. In contrast, nondiabetic hemodialysis patients had comparable VLDL triglyceride, cholesterol, and apo B levels to those in controls. VLDL apo CI levels were significantly elevated in the diabetic patients with overt proteinuria and diabetic hemodialysis patients, being 3.1- and 2.6-fold higher than in controls, respectively. VLDL apo CI levels were also slightly elevated in nondiabetic hemodialysis patients, but this increase was not significant. VLDL apo CIII levels were not elevated in diabetic patients with normoalbuminuria, microalbuminuria, or overt proteinuria, but were significantly elevated in diabetic hemodialysis patients (being 2.7-fold higher than in controls). Unlike VLDL apo CI, the levels of VLDL apo CIII were markedly increased in nondiabetic hemodialysis patients and were similar to those in diabetic hemodialysis patients. The apo CI/apo B ratio, indicating the apo CI content of VLDL particles, was similar among all groups. The apo CIII/apo B ratio, indicating the apo CIII content of VLDL particles, was markedly increased in nondiabetic hemodialysis patients, but apo CIII enrichment was not clearly observed in patients with diabetic nephropathy.

Table 4 shows the correlations of VLDL apo CI or VLDL apo CIII with various plasma parameters in all subjects. VLDL apo CI was significantly related to urinary albumin excretion, but not to serum creatinine or albumin, whereas VLDL apo CIII was significantly related to serum creatinine or albumin, but not to urinary albumin excretion. Neither VLDL apo CI nor VLDL apo CIII was correlated with glycemic control. VLDL apo CI levels were closely associated with plasma triglyceride level and with apo B, CII, CIII, and E levels. VLDL apo CI levels showed also a significant inverse correlation with HDL cholesterol. VLDL apo CIII levels were closely associated with plasma triglyceride and apo B, CII, and CIII levels, but the correlation coefficients were lower than those for VLDL apo CI, except in the case of plasma apo CIII. VLDL apo CIII levels also showed significantly an inverse correlation with HDL cholesterol.

In an attempt to evaluate whether the correlation of VLDL apo CI with various parameters was independent of the influence of VLDL apo CIII, multiple linear regression analysis was performed using VLDL apo CI and VLDL apo CIII as the independent variables (Table 5). The VLDL apo CI level was significantly correlated with urinary albumin excretion and with the plasma levels of lipids and apoproteins. The VLDL apo CI level was also significantly ($P < 0.0001$) correlated with VLDL triglyceride, VLDL cholesterol, and VLDL apo B independently of VLDL apo CIII.

**Table 4.** Correlation coefficients of very low-density lipoprotein (VLDL) apolipoprotein (apo) CI and VLDL apo CIII with various plasma parameters in total subjects

| | VLDL apo CI *r* | Significance *P* | VLDL apo CIII *r* | Significance *P* |
|---|---|---|---|---|
| Creatinine | 0.117 | NS | 0.172 | <0.05 |
| Albumin | −0.091 | NS | −0.172 | <0.05 |
| Albumin/creatinine | 0.337 | <0.0005 | 0.149 | NS |
| Hemoglobin $A_{1c}$ | 0.192 | NS | 0.137 | NS |
| Plasma glucose | 0.156 | NS | 0.152 | NS |
| Plasma triglyceride | 0.731 | <0.0001 | 0.432 | <0.0001 |
| Plasma total cholesterol | 0.225 | <0.01 | 0.049 | NS |
| LDL cholesterol | 0.100 | NS | 0.089 | NS |
| HDL cholesterol | −0.309 | <0.0001 | −0.227 | <0.005 |
| Plasma apo AI | −0.218 | <0.01 | −0.160 | NS |
| Plasma apo B | 0.370 | <0.0001 | 0.192 | <0.02 |
| Plasma apo CII | 0.595 | <0.0001 | 0.389 | <0.0001 |
| Plasma apo CIII | 0.516 | <0.0001 | 0.644 | <0.0001 |
| Plasma apo E | 0.411 | <0.0001 | 0.245 | <0.005 |
| VLDL triglyceride | 0.750 | <0.0001 | 0.391 | <0.0001 |
| VLDL cholesterol | 0.734 | <0.0001 | 0.365 | <0.0001 |
| VLDL apo B | 0.599 | <0.0001 | 0.436 | <0.0001 |
| VLDL apo CI | — | | 0.296 | <0.0002 |
| VLDL apo CIII | 0.296 | <0.0002 | | |

**Table 5.** Multiple regression analysis of very low-density (VLD) apolipoprotein (apo) CI or very low-density lipoprotein (VLDL) apo CIII as an independent variable with various dependent variables in total subjects

| | Independent variables | | | | | | |
|---|---|---|---|---|---|---|---|
| | VLDL apo CI | | | VLDL apo CIII | | | |
| Dependent variables | Standardized coefficient | *t* value | *P* value | Standardized coefficient | *t* value | *P* value | $R^2$ |
| Creatinine | −0.057 | −0.807 | NS | 0.588 | 8.369 | <0.0001 | 0.329 |
| Albumin | −0.044 | −0.486 | NS | −0.154 | −1.721 | NS | 0.030 |
| Albumin/creatinine | 0.332 | 2.704 | 0.008 | 0.012 | 0.099 | NS | 0.113 |
| Plasma triglyceride | 0.665 | 11.976 | <0.0001 | 0.223 | 4.013 | <0.0001 | 0.580 |
| Plasma total cholesterol | 0.263 | 3.171 | 0.002 | −0.128 | −1.510 | NS | 0.066 |
| High density lipoprotein cholesterol | −0.273 | −3.3376 | 0.001 | −0.122 | −1.510 | NS | 0.109 |
| Plasma apo AI | −0.191 | −2.171 | 0.032 | −0.084 | −0.950 | NS | 0.055 |
| Plasma apo B | 0.348 | 4.153 | <0.0001 | 0.067 | 0.796 | NS | 0.141 |
| Plasma apo CII | 0.525 | 6.253 | <0.0001 | 0.214 | 2.540 | 0.013 | 0.395 |
| Plasma apo CIII | 0.337 | 4.559 | <0.0001 | 0.542 | 7.330 | <0.0001 | 0.528 |
| Plasma apo E | 0.375 | 4.574 | <0.0001 | 0.114 | 1.395 | NS | 0.181 |
| VLDL triglyceride | 0.699 | 12.73 | <0.0001 | 0.173 | 3.153 | 0.002 | 0.590 |
| VLDL cholesterol | 0.691 | 12.118 | <0.0001 | 0.201 | 2.550 | 0.012 | 0.558 |
| VLDL apo B | 0.519 | 7.906 | <0.0001 | 0.271 | 4.130 | <0.0001 | 0.426 |

NS = $P > 0.05$.

## DISCUSSION

The physiologic role and clinical significance of apo CI are largely unknown. However, Bjorkegren et al [19] have recently reported that the apo CI content of VLDL particles shows a significant increase after fat loading in patients with CHD. We have also found that high levels of VLDL apo CI are significantly associated with CHD [11]. The available evidence therefore suggests that apo CI plays an important role in increasing the atherogenic potential of VLDL. Steiner et al [20] and Tkac et al [21] have demonstrated that VLDL apo B levels are associated with the angiographic severity of CHD in both diabetic and nondiabetic individuals. Sacks et al [22] recently reported that the prospective CARE study showed that the VLDL apo B level is an independent and powerful predictor of recurrent coronary events, being superior to plasma triglyceride or LDL cholesterol levels. We recently reported that VLDL triglyceride, cholesterol, and apo B levels were all increased in CHD patients, with the increase of VLDL apo B being more prominent than that of VLDL lipids [ 23]. These results suggest that the number of circulating VLDL particles may be strongly associated with the progression of CHD. In this study, we found that VLDL apo B was significantly correlated with VLDL apo CI. Experimental studies have suggested that apo CI plays an important role

in the clearance of VLDL through inhibiting uptake by the liver [12–14]. Therefore, it is reasonable to assume that an increase of apo CI delays particle clearance, which results in an increase of VLDL particles in the circulation.

Gene targeting mouse studies [13] have demonstrated that apo CI inhibits VLDL particle uptake but does not interfere with glycosaminoglycan binding or triglyceride hydrolysis of VLDL, while apo CIII predominantly interferes with glycosaminoglycan binding or lipolysis of VLDL. It has also been reported that apo CIII inhibits VLDL removal by suppressing its binding to the LDL receptor, while apo CI predominantly inhibits LDL receptor-related protein (LRP) or VLDL receptors [24]. Cross breeding of apo CI transgenic mice with LDL receptor knockout mice leads to severe hypertriglyceridemia [14], suggesting that apo CI impairs the catabolism of VLDL via LDL receptor-independent pathways. If VLDL is promptly taken up by LRP or the VLDL receptor in certain circumstances, apo CI may be a crucial factor for VLDL removal. There is a possibility that metabolic derangement in diabetic nephropathy might lead to changes of apo CI.

Attman et al [5, 25] extensively examined the apoprotein concentrations in lipoprotein fractions from diabetic and nondiabetic patients with CRF not on hemodialysis, and reported that VLDL apo CIII was significantly increased in both diabetic and nondiabetic patients. Similarly, we found that VLDL apo III levels were elevated in both diabetic and nondiabetic hemodialysis patients to about the same extent. These studies suggest that an increase of VLDL apo CIII is a common lipoprotein abnormality in CRF patients, regardless of the presence of diabetes or hemodialysis. VLDL apo CI levels increased with the increasing severity of diabetic nephropathy, but nondiabetic hemodialysis patients did not have significantly high levels of VLDL apo CI, suggesting that the VLDL apo CI level is not dependent upon renal function. An increase of VLDL apo CI was only observed in diabetic patients with overt nephropathy, but not in normo- or microalbuminuric patients. We assume that hepatic apo CI production is increased by the proteinuric-hypoproteinemic state. To investigate this possibility, it would be important to examine the VLDL apo CI concentration in nondiabetic patients with nephrotic syndrome. Nondiabetic hemodialysis patients had lower BMI than diabetic patients on hemodialysis and those with overt proteinuria, which might be associated their lower plasma lipids and VLDL concentrations than diabetic nephropathy. However, VLDL apo CIII concentration in the nondiabetic hemodialysis patients was as high as that in diabetic hemodialysis patients, which may also support that an increase of VLDL apo CIII is specific for CRF. Hemodialysis patients usually have moderate hypertriglyceridemia with elevated VLDL levels, but our nondiabetic hemodialysis patients were normotriglyceridemic. Accordingly, these patients may not have all of the typical lipid abnormalities of CRF patients. Further studies will be required to elucidate the mechanism of lipoprotein abnormalities in nondiabetic hemodialysis patients with hypertriglyceridemia. Sacks et al [22] found that the apo CIII concentration of the VLDL + LDL fraction was an independent coronary risk factor in the CARE study. Thus, we should also pay attention to an increase of VLDL apo CIII as a strong risk factor for CHD in patients with CRF regardless of the presence of diabetes. In this study, we only focused on VLDL abnormalities associated with apo CI and CIII, but IDL abnormalities represent another important risk factor for renal disease that we have not assessed, and apo CI or apo CIII may also play a role in such abnormalities. Therefore, further studies will be required to elucidate the role of these proteins in dyslipidemia associated with diabetic nephropathy or CRF.

## CONCLUSION

Our results suggest that an increase of VLDL apo CIII is a prominent feature of dyslipidemia in CRF patients, regardless of whether they are diabetic or nondiabetic, whereas an increase of VLDL CI is more specific to diabetic nephropathy and is closely associated with an increase of VLDL particle numbers, a new risk factor for CHD.

## ACKNOWLEDGMENT

This study was partly supported by a grant-in-aid (no 11671136) from the Japanese Ministry of Education, Science, and Culture.

*Reprint requests to Tsutomu Hirano, M.D., First Department of Internal Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan.*
*E-mail: hirano@med.showa-u.ac.jp*

## REFERENCES

1. United States Renal Data System: USRDS 1998 Annual Data Report, Excerpts from V and VI. *Am J Kidney Dis* 32(Suppl 1): S69–S88, 1998
2. Hahn R, Oette K, Mondorf H, *et al*: Analysis of cardiovascular risk factors in chronic hemodialysis patients with special attention to the hyperlipoproteinemias. *Atherosclerosis* 48:279–288, 1983
3. Cheung AK, Sarnak MJ, Yan G, *et al*: Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. *Kidney Int* 58:353–362, 2000
4. Valmadrid CT, Klein R, Moss SE, *et al*: The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. *Arch Intern Med* 160:1093–1100, 2000
5. Attman P-O, Knight-Gibson C, Tavella M, *et al*: The compositional abnormalities of lipoproteins in diabetic renal failure. *Nephrol Dial Transplant* 13:2833–2841, 1998
6. Hirano T: Lipoprotein abnormalities in diabetic nephropathy. *Kidney Int* 71:S22–24, 1999
7. Jensen T, Borch-Johnsen K, Kofoed-Enevoldsen A, *et al*: Coronary heart disease in young type 1 (insulin-dependent) diabetic

patients with and without diabetic nephropathy: Incidence and risk factors. *Diabetologia* 30:144–148, 1987
8. De Cosmo S, Bacci S, Piras GP, *et al*: High prevalence of risk factors for cardiovascular disease in parents of IDDM patients with albuminuria. *Diabetologia* 40:1191–1196, 1997
9. Kashiwazaki K, Hirano T, Yoshino G, *et al*: Decreased release of lipoprotein lipase is associated with vascular endothelial damage in NIDDM patients with microalbuminuria. *Diabetes Care* 21:2016–2020, 1998
10. Hirano T, Oi K, Sakai S, *et al*: High prevalence of small dense LDL in diabetic nephropathy is not directly associated with kidney damage: A possible role of postprandial lipemia. *Atherosclerosis* 141:77–85, 1998
11. Hirano T, Sakaue T, Koba S: Increased VLDL particle number is a leading cause for coronary heart disease independent of IDL or dense LDL in Japanese men. *Circulation* 102(Suppl II):506, 2000
12. Windler E, Havel RJ: Inhibitory effects of C apoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and remnants by the perfused rat liver. *J Lipid Res* 26:556–565, 1985
13. Shachter NS, Ebara T, Ramakrishnan R, Steiner G, *et al*: Combined hyperlipidemia in transgenic mice overexpressing human apolipoprotein Cl. *J Clin Invest* 98:846–855, 1996
14. Jong MC, Dahlmans VE, van Gorp PJ, *et al*: In the absence of the low density lipoprotein receptor, human apolipoprotein C1 overexpression in transgenic mice inhibits the hepatic uptake of very low density lipoproteins via a receptor-associated protein-sensitive pathway. *J Clin Invest* 98:2259–2267, 1996
15. Sakaue T, Hirano T, Yoshino G, *et al*: The influence of diabetic nephropathy on remnant lipoproteins. *J Japan Diab Soc* 42:439–446, 1999
16. Hirano T, Oi K, Sakai S, *et al*: High prevalence of small dense LDL in diabetic nephropathy is not directly associated with kidney damage: A possible role of postprandial lipemia. *Atherosclerosis* 141:77–85, 1998
17. Sakaue T, Hirano T, Yoshino G, *et al*: Reactions of direct LDL-cholesterol assays with pure LDL fraction and IDL: Comparison of three homogeneous methods. *Clin Chim Acta* 295:97–106, 2000
18. Young SG, Smith RS, Hogle DM, *et al*: Two new monoclonal antibody-based enzyme-linked assays of apolipoprotein B. *Clin Chem* 32:1484–1490, 1986
19. Bjorkegren J, Boquist S, Samnegard A, *et al*: Accumulation of apolipoprotein C–I-rich and cholesterol-rich VLDL remnants during exaggerated postprandial triglyceridemia in normolipidemic patients with coronary artery disease. *Circulation* 101:227–230, 2000
20. Steiner G, Schwartz L, Shumak S, *et al*: The association of increased levels of intermediate-density lipoproteins with smoking and with coronary artery disease. *Circulation* 75:124–130, 1987
21. Tkac I, Kimball BP, Lewis G, *et al*: The severity of coronary atherosclerosis in type 2 diabetes mellitus is related to the number of circulating triglyceride-rich lipoprotein particles. *Arterioscler Thromb Vasc Biol* 17:3633–3638, 1997
22. Sacks FM, Alaupovic P, Moye LA, *et al*: VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation* 102:1886–1892, 2000
23. Hirano T, Sakaue T, Koba S: Increased VLDL partice number is a leading cause for coronary heart disease independent of IDL or dense LDL in Japanese men. *Circulation* 102(Suppl II):506, 2000
24. Shachter NS: Apolipoproteins C–I and C–III as important modulators of lipoprotein metabolism. *Curr Opin Lipidol* 12:297–304, 2001
25. Attman P-O, Alaupovic P, Tavella A, *et al*: Abnormal lipid and apolipoprotein composition of major lipoprotein density classes in patients with chronic renal failure. *Nephrol Dial Transplant* 11: 63–69, 1996