

# Serum Lipids and the Progression of Nephropathy in Type 1 Diabetes

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**OBJECTIVE** — Dyslipidemia contributes to the progression of microvascular disease in diabetes. However, different lipid variables may be important at different stages of nephropathy. This study examines the pattern of dyslipidemia associated with the progression of nephropathy in patients with type 1 diabetes.

**RESEARCH DESIGN AND METHODS** — A total of 152 patients with type 1 diabetes were recruited in order to represent various phases of nephropathy. Patients were followed for 8–9 years, during which time they received standard care. Renal progression was defined a priori as a doubling in albumin excretion (in patients with normo- or microalbuminuria) or a decline in creatinine clearance (in those with macroalbuminuria). A panel of lipid variables was determined and correlated with indexes of progression.

**RESULTS** — In patients with normoalbuminuria ( $n = 66$ ), progression was associated with male sex ( $P < 0.05$ ), borderline albuminuria ( $P = 0.02$ ), and LDL-free cholesterol ( $P = 0.02$ ). In patients with microalbuminuria ( $n = 51$ ), progression was independently associated with triglyceride content of VLDL and intermediate-density lipoprotein (both  $P < 0.05$ ). In patients with macroalbuminuria ( $n = 36$ ), a significant decline in the renal function ( $>3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ ) was independently associated with poor glycemic control, hypertension, and LDL size ( $P < 0.05$ ). When all patients with progressive nephropathy were analyzed together, only LDL cholesterol was predictive on multivariate analysis ( $P < 0.05$ ), which masked the importance of triglyceride enrichment in microalbuminuria.

**CONCLUSIONS** — Lipid variables are associated with progression of diabetic kidney disease, but the relationship is not the same at all stages. This finding has implications for the design of renoprotective strategies and the interpretation of clinical trials in type 1 diabetes.

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The control of blood lipids is one of the cornerstones in the treatment of type 1 diabetes. Apart from effects on macrovascular outcomes (1), dyslipidemia potentially contributes to microvascular disease (2,3). Prospective studies have confirmed a link between serum lipids and nephropathy (4–10), although lipid fractions measured in these studies have been limited, and parameters asso-

ciated with kidney disease have not been consistently identified (8,9). It remains to be established which lipids or lipoproteins are most important in the pathogenesis of nephropathy and should therefore be targeted for intervention. Furthermore, it may be that different lipid variables are important at different stages of diabetic kidney disease. For example, triglycerides and cholesterol appeared to

have different effects on the progression of nephropathy, depending upon the duration of diabetes (10). Consequently, this study makes a detailed examination of the pattern of dyslipidemia associated with the progression of nephropathy, at each stage of renal disease, in patients with type 1 diabetes followed for 8–9 years.

## RESEARCH DESIGN AND METHODS

A total of 153 Caucasian patients with type 1 diabetes, including 84 men and 69 women, were recruited from Guy's Hospital and Kings College Hospital ( $n = 75$ ) and Helsinki University Central Hospital ( $n = 78$ ). Selection criteria for this study have been previously described (4,11–13). Briefly, 66 patients with normoalbuminuria, 51 with microalbuminuria, and 36 with macroalbuminuria were recruited in order to represent various phases of diabetic nephropathy. The three groups were matched for age, diabetes duration, and glycemic control (4). Other patient characteristics have been previously reported (4) and are also provided in the online appendix (available at <http://care.diabetesjournals.org>). Patients on lipid-lowering therapy, diuretics, or  $\beta$ -blockers at baseline were excluded. Participants were then followed for a mean of 8.7 years, during which time they received standard care, including the use of lipid-lowering agents where appropriate.

Full methods of baseline examination have been published elsewhere (4,11–13). Briefly, clinical data were obtained from patient records, including age, sex, duration of diabetes, medication history, anthropometric indexes, insulin dose, and the presence of microvascular complications. Blood pressure was determined from three separate measurements in the sitting position. Urinary albumin excretion rate (AER) was derived from overnight urinary albumin measurement and expressed as the geometric mean of three consecutive collections. Normoalbuminuria was defined by an AER  $<20 \mu\text{g}/\text{min}$ , microalbuminuria by an AER between 20 and  $200 \mu\text{g}/\text{min}$ , and macroalbuminuria by an AER  $>200 \mu\text{g}/\text{min}$  (4). Creatinine clearance was deter-

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**Abbreviations:** AER, albumin excretion rate; apo, apolipoprotein; IDL, intermediate-density lipoprotein. A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—The association of baseline clinical data and lipid variables with progression in patients with normoalbuminuria

	Nonprogressors	Progressors
n	47	14
Baseline AER (geometric mean) ( $\mu\text{g}/\text{min}$ )	5.1	9.5
End point AER (geometric mean) ( $\mu\text{g}/\text{min}$ )	7.1	63
Baseline CrCl (ml/min per 1.73 m <sup>2</sup> )	99 $\pm$ 2	105 $\pm$ 6
End point CrCl (ml/min per 1.73 m <sup>2</sup> )	100 $\pm$ 3	96 $\pm$ 4
Sex (% male)	78	40*†
HbA <sub>1c</sub> (%)	7.8 $\pm$ 0.2	8.1 $\pm$ 0.3
Blood pressure (mmHg)	121/78 $\pm$ 2/1	120/76 $\pm$ 3/2
LDL cholesterol (mmol/l)	2.6 $\pm$ 0.1	3.4 $\pm$ 0.3*†
Non-HDL cholesterol (mmol/l)	3.0 $\pm$ 0.1	3.8 $\pm$ 0.2*
Triglycerides (mmol/l)	0.97 $\pm$ 0.09	0.96 $\pm$ 0.07
HDL cholesterol (mmol/l)	1.6 $\pm$ 0.1	1.5 $\pm$ 0.1
LDL free cholesterol (mmol/l)	0.74 $\pm$ 0.02	0.92 $\pm$ 0.07*†
LDL mass (mg/dl)	296 $\pm$ 9	373 $\pm$ 23*†
ApoAI (mg/dl)	155 $\pm$ 3	138 $\pm$ 5*†
LDL size (nm)	25.7 $\pm$ 0.1	25.9 $\pm$ 0.2
LPL <sub>15</sub> ( $\mu\text{mol FFA} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ )	39 $\pm$ 1	37 $\pm$ 3
HL <sub>15</sub> ( $\mu\text{mol FFA} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ )	25 $\pm$ 1	30 $\pm$ 4

Data are means  $\pm$  SE unless otherwise indicated. \*Progressors vs. nonprogressors, univariate  $P < 0.05$ . †Progressors vs. nonprogressors, adjusted  $P < 0.05$ . CrCl, creatinine clearance; FFA, free fatty acid; HL<sub>15</sub>, hepatic lipase 15 min after an intravenous bolus of heparin; LPL<sub>15</sub>, lipoprotein lipase 15 min after an intravenous bolus of heparin.

mined using the Cockcroft Gault formula and adjusted for body mass.

### Lipid variables

Lipid parameters were measured in fasting serum samples as previously described (4,11–13). Total cholesterol, unesterified free cholesterol triglycerides, and phospholipid concentrations in whole serum and lipoprotein fractions were measured enzymatically using a Cobas autoanalyzer. Serum lipoprotein fractions, including chylomicrons, VLDL, intermediate-density lipoprotein (IDL), and LDL were separated by sequential preparative ultracentrifugation (11). The sum of all components in each lipoprotein fraction was used to estimate the mass concentration of each lipoprotein.

Serum apolipoprotein (apo)B concentrations were determined by immunoassay (Orion, Espoo, Finland) after separation from other apoproteins by isopropanol. ApoB-containing triglyceride-rich lipoprotein particles, VLDL<sub>1</sub> (Sf 60–400), VLDL<sub>2</sub> (Sf 20–60), and IDL (Sf 12–60) were isolated using density gradient ultracentrifugation. LDL size was resolved using nondenaturing gradient gel electrophoresis. Composition and density distribution of LDL was ascertained by density gradient ultracentrifugation, as previously described (11). Postheparin lipoprotein lipase and hepatic lipase activ-

ity were measured in samples taken 15 min after an intravenous bolus of heparin (100 IU/kg body wt) and expressed as micromoles of free fatty acid per milliliter per hour, using an established immunoassay (4).

### End points

The primary study outcome, progression of diabetic nephropathy, was defined a priori in patients with normo- or microalbuminuria at baseline as a doubling in AER that had to exceed 10  $\mu\text{g}/\text{min}$ . When dealing with small patient numbers, this definition avoids the lead-time bias inherent in categorical analysis and appropriately considers albuminuria as a continuous variable. In patients with established macroalbuminuria, change in creatinine clearance was used as the primary outcome measure because disease progression in these patients sees a decline in creatinine clearance rather than change in AER. The natural history of diabetic nephropathy is associated with a decline in creatinine clearance of 10–12  $\text{ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ , which is reduced to  $<3\text{--}4 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$  with conventional treatment. For the purpose of our study, progression was defined as a decline in renal function of  $>3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ .

### Statistical analysis

Continuous data are expressed as means  $\pm$  SE. Nonparametric variables were handled as log derivatives. Differences in continuous variables were compared using Student's  $t$  tests. Multivariate logistic regression was used to analyze associations between baseline variables and the primary outcome. The same panel of lipid measurements at baseline was used to analyze associations within baseline categories, except where lipid variables were significantly associated with one another (Pearson  $P < 0.05$ ) and where only one variable from each "factor" of associated variables was entered into the model at any one time.

**RESULTS**— The baseline characteristics of this study population have been previously described (4). Sixty-six patients had normoalbuminuria, 51 had microalbuminuria, and 36 had macroalbuminuria. These groups were matched for age ( $38 \pm 1$  year) and age of onset ( $13 \pm 1$  year). Within each group, there was no significant difference between the duration of diabetes ( $23 \pm 1$  year), mean insulin dose ( $0.7 \pm 0.1 \text{ unit} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ), or glycemic control at baseline (HbA<sub>1c</sub>  $8.3 \pm 0.1\%$ ) (4,11–13). At the study baseline, no patients were receiving lipid-lowering therapy, but, during the study, 20 patients subsequently received statin therapy, as indicated by then current guidelines. Sixty-one percent of patients were receiving antihypertensive agents at the study end point, including 55% receiving an ACE inhibitor. No patients in this study received angiotensin receptor blockers.

### Patients with normoalbuminuria at baseline

Follow-up data were available for 61 patients with normoalbuminuria at baseline ( $n = 61$  of 66 [92%]). Two patients died, and three were lost to follow-up. Over the 8 years of follow-up, 14 patients (23%) more than doubled their AER and exceeded 10  $\mu\text{g}/\text{min}$ , such that 12 patients had an AER within the range of microalbuminuria. The characteristics of these patients are shown in Table 1. Patients whose AER increased during the study had a higher AER at baseline than those who did not progress. There was no difference in treatment at baseline between patients who progressed and those who did not. However, progressing patients were more likely to be using an ACE inhibitor (42 vs. 20%,  $P = 0.02$ ) or a sta-

tin (35 vs. 9%,  $P = 0.01$ ) at end point, reflecting the development of an indication for intervention.

Progression in patients with normoalbuminuria at baseline was independently associated with an elevated baseline LDL cholesterol. Other lipid variables, including non-HDL cholesterol, LDL mass, LDL cholesterol ester, and free cholesterol content apoAI and -AII, were also associated with progression (all  $P < 0.05$  after adjusting for other risk factors). Of these factorially associated variables, LDL-free cholesterol had the strongest relationship to progression. On multivariate logistic analysis, adjusting for other variables, independent predictors for progression were male sex ( $P < 0.05$ ), albuminuria in the high-normal range (10–20  $\mu\text{g}/\text{min}$ ,  $P = 0.02$ ), and LDL-free cholesterol ( $P = 0.02$ ). Substitution of any other of the LDL-associated lipid variables did not significantly detract from the predictive value of the model, with dyslipidemia explaining ~9% of the variation in the rate of progression, after correcting for other variables.

### Patients with microalbuminuria at baseline

Follow-up data were available for 40 patients with microalbuminuria at baseline ( $n = 40$  of 51 [78%]). Four patients died, and seven were lost to follow-up. At follow-up, 15 patients (38%) more than doubled their AER, including 8 patients with an AER within the range of macroalbuminuria. The characteristics of the patients who progressed are shown in Table 2. Patients with microalbuminuria who progressed had a lower creatinine clearance at baseline, which declined during follow-up (~2–3  $\text{ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$  vs. baseline,  $P = 0.05$ ). Creatinine clearance did not change in patients whose AER did not increase. There was no difference in medical treatment at baseline between patients who subsequently progressed and those who did not progress. Patients who progressed were more likely to use an ACE inhibitor (93 vs. 64%,  $P = 0.02$ ) at end point, although the use of lipid-lowering therapy was similar (29 vs. 21%,  $P = \text{NS}$ ).

Progression in patients with microalbuminuria was independently associated with triglyceride content of VLDL and IDL (both  $P < 0.05$ , adjusted for sex, baseline creatinine clearance, and medication history) (Table 2). Baseline lipid variables associated with the triglyceride

**Table 2—The association of baseline clinical parameters and lipid variables with progression in patients with microalbuminuria**

	Nonprogressors	Progressors
<i>n</i>	25	15
Baseline AER (geometric mean) ( $\mu\text{g}/\text{min}$ )	52	50
End point AER (geometric mean) ( $\mu\text{g}/\text{min}$ )	35	407
Baseline CrCl (ml/min per 1.73 $\text{m}^2$ )	84 $\pm$ 3	74 $\pm$ 4*
End point CrCl (ml/min per 1.73 $\text{m}^2$ )	77 $\pm$ 4	57 $\pm$ 5*
Sex (% male)	66	40
HbA <sub>1c</sub> (%)	8.1 $\pm$ 0.3	8.5 $\pm$ 0.3
Blood pressure (mmHg)	129/80 $\pm$ 4/2	133/81 $\pm$ 4/1
LDL cholesterol (mmol/l)	3.2 $\pm$ 0.2	3.2 $\pm$ 0.3
Non-HDL cholesterol (mmol/l)	3.6 $\pm$ 0.2	3.8 $\pm$ 0.3
Triglycerides (mmol/l)	1.12 $\pm$ 0.08	1.13 $\pm$ 0.12
HDL cholesterol (mmol/l)	1.6 $\pm$ 0.1	1.4 $\pm$ 0.1
VLDL triglyceride content (%)	47 $\pm$ 1	54 $\pm$ 2*†
IDL triglyceride content (%)	25 $\pm$ 1	32 $\pm$ 2*†
VLDL mass (mg/dl)	89 $\pm$ 16	107 $\pm$ 12
IDL mass (mg/dl)	41 $\pm$ 5	47 $\pm$ 7
VLDL triglycerides (mmol/l)	0.52 $\pm$ 0.11	0.57 $\pm$ 0.07
LPL <sub>15</sub> ( $\mu\text{mol FFA} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ )	35 $\pm$ 2	39 $\pm$ 4
HL <sub>15</sub> ( $\mu\text{mol FFA} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ )	30 $\pm$ 3	31 $\pm$ 3

Data are means  $\pm$  SE unless otherwise indicated. \*Progressors vs. nonprogressors, univariate  $P < 0.05$ . †Progressors vs. nonprogressors, adjusted  $P < 0.05$ . CrCl, creatinine clearance; FFA, free fatty acid; HL<sub>15</sub>, hepatic lipase 15 min after an intravenous bolus of heparin; LPL<sub>15</sub>, lipoprotein lipase 15 min after an intravenous bolus of heparin.

content of VLDL and IDL were also linked to progression. For example, the cholesterol ester content of these particles was inversely associated with the particle triglyceride content (data not shown). However, there was no association between progression and VLDL or IDL mass (both  $P > 0.3$ ). Overall, patients with triglyceride-enriched particles had a greater risk of progression than those with lower triglyceride content, independent of the total or particulate triglyceride level. In this setting, dyslipidemia explained ~19% of the variation in the rate of progression. Notably, standard lipid variables failed to predict the risk for progression attributable to dyslipidemia. Nonetheless, the triglyceride content of the lipoprotein particles was significantly correlated with total serum cholesterol ( $P < 0.05$ ).

### Patients with macroalbuminuria at baseline

Follow-up data were available for 30 patients with macroalbuminuria at baseline ( $n = 30$  of 36 [86%]), with 1 patient lost to follow-up and 5 deaths. All patients received standard care that included an ACE inhibitor. Nevertheless, 12 patients (39%) experienced a decline in their renal function of  $>3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ , including 2 who required dialysis. The characteristics of the patients who pro-

gressed are shown in Table 3. Patients with macroalbuminuria who experienced a progressive decline in renal function had similar AER, creatinine clearance, and glycemic control at baseline compared with those who did not progress. However, pulse pressure was higher in patients with macroalbuminuria who progressed, as was the prevalence of smoking (both  $P < 0.05$ ). There was no significant difference in the use of lipid-lowering therapy between patients who subsequently progressed and those who did not.

None of the standard lipid variables were independently correlated with renal progression in patients with macroalbuminuria at baseline (Table 3). However, LDL size was independently correlated with progression, after adjusting for other risk factors ( $P < 0.05$ ). This association was independent to LDL mass and other indexes of LDL quantity, including LDL cholesterol. In patients with macroalbuminuria at baseline, LDL size was correlated with postheparin hepatic lipase activity, HDL cholesterol, and serum triglycerides (all  $P < 0.01$ ) but not cholesteryl ester transfer protein or lipoprotein lipase activity. However, none of these factors individually explained the association between LDL size and progressive nephropathy. In addition, there

**Table 3—The association of baseline clinical data and lipid variables with declining renal function in patients with macroalbuminuria**

	Nonprogressors	Progressors
n	19	12
Baseline AER (geometric mean) ( $\mu\text{g}/\text{min}$ )	446	398
Baseline CrCl (ml/min per $1.73\text{ m}^2$ )	$72 \pm 6$	$66 \pm 4$
End point CrCl (ml/min per $1.73\text{ m}^2$ )	$67 \pm 9$	$26 \pm 6^*$
Sex (% male)	35	58*
HbA <sub>1c</sub> (%)	$8.1 \pm 0.3$	$8.5 \pm 0.3$
Systolic blood pressure (mmHg)	$132 \pm 4$	$145 \pm 6^*$
Diastolic blood pressure (mmHg)	$87 \pm 2$	$82 \pm 2$
Pulse pressure (mmHg)	$44 \pm 3$	$61 \pm 6^*\dagger$
Smoking at baseline (%)	12	50*†
Lipid-lowering therapy at end point (%)	17	33
LDL cholesterol (mmol/l)	$3.2 \pm 0.2$	$3.1 \pm 0.2$
Non-HDL cholesterol (mmol/l)	$3.7 \pm 0.3$	$3.7 \pm 0.2$
Total triglycerides (mmol/l)	$0.97 \pm 0.07$	$1.05 \pm 0.10$
HDL cholesterol (mmol/l)	$1.5 \pm 0.1$	$1.7 \pm 0.1$
LDL size (nm)	$26.2 \pm 0.1$	$25.3 \pm 0.2^*\dagger$
Percentage VLDL triglyceride (%)	$47 \pm 3$	$47 \pm 2$
LPL <sub>15</sub> ( $\mu\text{mol FFA} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ )	$37 \pm 3$	$39 \pm 4$
HL <sub>15</sub> ( $\mu\text{mol FFA} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ )	$30 \pm 2$	$29 \pm 4$

Data are means  $\pm$  SE unless otherwise indicated. \*Progressors vs. nonprogressors, univariate  $P < 0.05$ . †Progressors vs. nonprogressors, adjusted  $P < 0.05$ . CrCl, creatinine clearance; FFA, free fatty acid; HL<sub>15</sub>, hepatic lipase 15 min after an intravenous bolus of heparin; LPL<sub>15</sub>, lipoprotein lipase 15 min after an intravenous bolus of heparin.

was no association between progressive nephropathy in patients with macroalbuminuria and any of the triglyceride-linked indexes that were predictive in patients with microalbuminuria.

### Pooled analysis

Progression of nephropathy was considered in a pooled analysis of 131 patients for whom follow-up data were available. Using the same stage-specific criteria detailed above, kidney disease progressed in 41 individuals. On multivariate analysis, the predictors for progression were baseline AER, creatinine clearance, and LDL cholesterol (all  $P < 0.01$ ). Factorially associated lipid variables (total cholesterol, non-HDL cholesterol, LDL mass, apoB, LDL cholesterol ester, and free cholesterol) could be used interchangeably with LDL cholesterol as predictive variables on multivariate analysis ( $P < 0.05$ ). LDL cholesterol was a better predictor of progression outcomes than non-HDL cholesterol, total-to-HDL cholesterol ratio, or other lipid variables. Although triglyceride accumulation was predictive in patients with microalbuminuria, it was eliminated when pooling progression at all stages of diabetic kidney disease. The use of antihypertensive therapy, blockade of the renin-angiotensin system, or lipid-lowering therapy at baseline had no sig-

nificant effect on the risk of progression, after adjusting for other variables.

**CONCLUSIONS**— This study demonstrates the link between serum lipids and progression of diabetic nephropathy. This relationship was independent of glycemic and blood pressure control in a population of intensively treated patients. However, the nature of this interaction appeared to be different at various stages of renal disease. In patients with normoalbuminuria, progression of nephropathy was linked to LDL cholesterol, whereas in those with microalbuminuria, progression was linked to the triglyceride content of VLDL and IDL particles. In patients with macroalbuminuria at baseline, progressive renal impairment was associated with LDL size but not with triglyceride-linked indexes. Although this is a small and selected study, these observations are consistent with the findings by Coonrod et al. (10), who reported that triglycerides and cholesterol had differing effects on progression of nephropathy, depending upon the duration of diabetes.

The variable relationship between lipids and progression at different stages of nephropathy may also partly explain the differing associations observed in studies in type 1 diabetes (8,9). It is conceivable that the pooling of indexes of disease pro-

gression may have diminished the impact of pathogenetic lipid fractions. For example, when pooled progression was considered in our study, only LDL cholesterol and factorially associated variables were retained as predictive variables. However, this finding belies the association between triglyceride accumulation and progression in patients with microalbuminuria. In addition, while LDL cholesterol levels were higher in patients with normoalbuminuria who progressed (Table 1), they were lower in patients with established macroalbuminuria who progressed (Table 3). These observations suggest that the role of specific lipids may be different at different stages of diabetic kidney disease.

This study used doubling of AER as the criterion for progressive nephropathy in patients with normo- and microalbuminuria, as it may be considered a more appropriate marker for progression of nephropathy than traditional “stepwise” transition between arbitrary stages of diabetic renal disease. For example, a patient with an AER of  $18\ \mu\text{g}/\text{min}$ , who has an AER of  $20\ \mu\text{g}/\text{min}$  9 years later, may not be the same as one who changes from 1 to  $190\ \mu\text{g}/\text{min}$ , though both patients have developed microalbuminuria. It is possible that this kind of lead-time bias may have influenced the interpretation of previous lipid studies, particularly given the link between albuminuria and dyslipidemia. We acknowledge that this represents a paradigm shift for some. While it is clear that staging of nephropathy is an important means to stratify the risk of progressive kidney disease, albuminuria should be considered as a log-linear continuous variable for following the evolution of kidney disease in diabetes (14). For the same reasons, change in creatinine clearance was used as the primary outcome measure in patients with macroalbuminuria.

As part of this study design, a group of patients was selected with persistent normoalbuminuria and long duration of diabetes. Over the 8 years of follow-up, 23% more than doubled their AER. The risk factors for progression were male sex and elevated baseline AER within the normal range. In addition, lipid variables associated with LDL cholesterol were independently associated with the risk of progression. This finding is consistent with previous studies in normoalbuminuric patients with type 1 diabetes, which demonstrates that LDL cholesterol is an important risk factor for the development of microvascular complications (7,10), as it is for macrovascular disease. The asso-

ciations found in our study were not related to changes in the density distribution or composition of LDL, suggesting that this association was primarily due to the increased number of LDL particles in patients who subsequently increased their AER (4). It is possible that a decrease in the clearance or transit of LDL particles from the blood stream also may have contributed to this finding (15).

The specific lipid variables associated with progression in patients with normoalbuminuria clearly differed from those seen in patients with micro- or macroalbuminuria. This is consistent with findings from the Pittsburgh Epidemiology of Diabetes Complications study, in which LDL cholesterol was more important for subjects with shorter durations, whereas triglycerides were important for those with longer durations (10). In particular, progression in microalbuminuric individuals was associated with lipid indexes correlated with the triglyceride content of VLDL and IDL particles. This association has not been previously demonstrated in patients with type 1 diabetes, although some larger studies have documented an increased risk of progression in patients with microalbuminuria and elevated total cholesterol levels (9). It is possible that the correlation between total cholesterol and VLDL-triglyceride content, as demonstrated in our study, may have confounded such results. Whether this finding is a marker or mediator of progressive renal disease remains to be established. We have previously demonstrated the accumulation of triglyceride-rich lipid particles in diabetic patients with early renal disease (4). Similar changes have been reported to be associated with nondiabetic proteinuric renal disease (16). It is conceivable that the triglyceride enrichment of VLDL and IDL may be a marker of more advanced or aggressive renal disease in patients with microalbuminuria. This may explain why it was not associated with progression in normoalbuminuric individuals. However, direct vasculo- and nephropathic effects of triglyceride-rich lipid particles have also been suggested (3). It is conceivable that our findings reflect the pathogenic role of the metabolic syndrome and its dyslipidemic corollary in the progression of established renal disease in type 1 diabetes (17,18), while LDL cholesterol may be more important for its initiation (19).

In patients with macroalbuminuria, reduced LDL size was correlated with a

progressive decline in renal function ( $P < 0.05$ ). A number of previous studies have shown that the accumulation of small dense LDL in patients with established proteinuria is associated with adverse outcomes (20,21), although a direct effect remains to be established. Certainly, reduced LDL size is associated with increased oxidative stress (22), the accumulation of AGEs (23), and postprandial lipemia (18). Small dense LDL particles can be produced by the action of hepatic lipase on larger triacylglycerol-enriched LDL species. In our patients with macroalbuminuria at baseline, LDL size was correlated with postheparin lipase activity, HDL cholesterol, and serum triglycerides (all  $P < 0.01$ ). However, none of these factors fully explained the association between LDL size and nephropathy in microalbuminuria.

The variable relationship between lipids and progression at different stages of renal disease may also have contributed to the apparent failure of trials of lipid lowering to retard nephropathy in type 1 diabetes (24–26), with only one small study showing a marginal effect on albumin excretion (25). While these studies aimed to reduce cholesterol in established nephropathy, our findings would suggest lowering LDL cholesterol might be more important in patients with normoalbuminuria. This targeted use of statins has proved successful as a renoprotective strategy in experimental diabetes (3). In addition, simvastatin slowed the rate of decline of renal function in the Heart Protection Study in patients with type 2 diabetes without proteinuria (27). Thus, a differential therapeutic approach would seem to be required in the different phases of the diabetic kidney disease. Nonetheless, in our study, we were unable to demonstrate any significant effect of lipid lowering at any stage of nephropathy, possibly reflecting the intensive management applied to all patients in this cohort.

In summary, lipid variables are associated with progression of diabetic kidney disease, but the relationship is not the same at all stages. This finding has implications for the design of renoprotective strategies and the interpretation of clinical trials in patients with type 1 diabetes.

## References

1. Armitage J, Bowman L: Cardiovascular outcomes among participants with diabetes in the recent large statin trials. *Curr Opin Lipidol* 15:439–446, 2004
2. Wilens SL, Elster SK, Baker JP: Glomerular lipidosis in intercapillary glomerulosclerosis. *Ann Intern Med* 34:592–607, 1951
3. Bonnet F, Cooper ME: Potential influence of lipids in diabetic nephropathy: insights from experimental data and clinical studies. *Diabetes Metab* 26:254–264, 2000
4. Groop PH, Elliott T, Ekstrand A, Franssila-Kallunki A, Friedman R, Viberti GC, Taskinen MR: Multiple lipoprotein abnormalities in type 1 diabetic patients with renal disease. *Diabetes* 45:974–979, 1996
5. Hovind P, Rossing P, Tarnow L, Smidt UM, Parving HH: Progression of diabetic nephropathy. *Kidney Int* 59:702–709, 2001
6. Mulec H, Johnsen SA, Wiklund O, Bjorck S: Cholesterol: a renal risk factor in diabetic nephropathy? *Am J Kidney Dis* 22: 196–201, 1993
7. Watts GF, Powrie JK, O'Brien SF, Shaw KM: Apolipoprotein B independently predicts progression of very-low-level albuminuria in insulin-dependent diabetes mellitus. *Metabolism* 45:1101–1107, 1996
8. Colhoun HM, Lee ET, Bennett PH, Lu M, Keen H, Wang SL, Stevens LK, Fuller JH: Risk factors for renal failure: the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia* 44 (Suppl. 2): S46–S53, 2001
9. Krolewski AS, Warram JH, Christlieb AR: Hypercholesterolemia: a determinant of renal function loss and deaths in IDDM patients with nephropathy. *Kidney Int Suppl* 45:S125–S131, 1994
10. Coonrod BA, Ellis D, Becker DJ, Bunker CH, Kelsey SF, Lloyd CE, Drash AL, Kuller LH, Orchard TJ: Predictors of microalbuminuria in individuals with IDDM: Pittsburgh Epidemiology of Diabetes Complications study. *Diabetes Care* 16:1376–1383, 1993
11. Lahdenpera S, Groop PH, Tilly-Kiesi M, Kuusi T, Elliott TG, Viberti GC, Taskinen MR: LDL subclasses in IDDM patients: relation to diabetic nephropathy. *Diabetologia* 37:681–688, 1994
12. Kahri J, Groop PH, Elliott T, Viberti G, Taskinen MR: Plasma cholesteryl ester transfer protein and its relationship to plasma lipoproteins and apolipoprotein A-I-containing lipoproteins in IDDM patients with microalbuminuria and clinical nephropathy. *Diabetes Care* 17:412–419, 1994
13. Groop PH, Viberti GC, Elliott TG, Friedman R, Mackie A, Ehnholm C, Jauhainen M, Taskinen MR: Lipoprotein(a) in type 1 diabetic patients with renal disease. *Diabet Med* 11:961–967, 1994
14. Jerums G, Allen TJ, Campbell DJ, Cooper ME, Gilbert RE, Hammond JJ, Raffaele J, Tsalamandris C: Long-term comparison between perindopril and nifedipine in normotensive patients with type 1 diabetes and microalbuminuria. *Am J Kidney*

- Dis 37:890–899, 2001
15. Jensen JS, Feldt-Rasmussen B, Jensen KS, Clausen P, Scharling H, Nordestgaard BG: Transendothelial lipoprotein exchange and microalbuminuria. *Cardiovasc Res* 63: 149–154, 2004
  16. Antikainen M, Holmberg C, Taskinen MR: Growth, serum lipoproteins and apo-proteins in infants with congenital nephrosis. *Clin Nephrol* 38:254–263, 1992
  17. Thorn LM, Forsblom C, Fagerudd J, Thomas MC, Pettersson-Fernholm K, Saraheimo M, Waden J, Ronnback M, Rosengard-Barlund M, Bjorkesten CG, Taskinen MR, Groop PH: Metabolic syndrome in type 1 diabetes: association with diabetic nephropathy and glycemic control (the FinnDiane study). *Diabetes Care* 28:2019–2024, 2005
  18. Hirano T, Oi K, Sakai S, Kashiwazaki K, Adachi M, Yoshino G: 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
  19. Kamanna VS: Low density lipoproteins and mitogenic signal transduction processes: role in the pathogenesis of renal disease. *Histol Histopathol* 17:497–505, 2002
  20. Sibley SD, Hokanson JE, Steffes MW, Purnell JQ, Marcovina SM, Cleary PA, Brunzell JD: Increased small dense LDL and intermediate-density lipoprotein with albuminuria in type 1 diabetes. *Diabetes Care* 22:1165–1170, 1999
  21. Skyrme-Jones RA, O'Brien RC, Luo M, Meredith IT: Endothelial vasodilator function is related to low-density lipoprotein particle size and low-density lipoprotein vitamin E content in type 1 diabetes. *J Am Coll Cardiol* 35:292–299, 2000
  22. Kondo A, Muranaka Y, Ohta I, Notsu K, Manabe M, Kotani K, Saito K, Maekawa M, Kanno T: Relationship between triglyceride concentrations and LDL size evaluated by malondialdehyde-modified LDL. *Clin Chem* 47:893–900, 2001
  23. Bucala R: Site-specific modification of apolipoprotein B by advanced glycosylation end-products: implications for lipoprotein clearance and atherogenesis. *Nephrol Dial Transplant* 11 (Suppl. 5):17–19, 1996
  24. Zhang A, Vertommen J, Van Gaal L, De Leeuw I: Effects of pravastatin on lipid levels, in vitro oxidizability of non-HDL lipoproteins and microalbuminuria in IDDM patients. *Diabetes Res Clin Pract* 29: 189–194, 1995
  25. Hommel E, Andersen P, Gall MA, Nielsen F, Jensen B, Rossing P, Dyerberg J, Parving HH: Plasma lipoproteins and renal function during simvastatin treatment in diabetic nephropathy. *Diabetologia* 35: 447–451, 1992
  26. Fried LF, Forrest KY, Ellis D, Chang Y, Silvers N, Orchard TJ: Lipid modulation in insulin-dependent diabetes mellitus: effect on microvascular outcomes. *J Diabetes Complications* 15:113–119, 2001
  27. Collins R, Armitage J, Parish S, Sleight P, Peto R: MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet* 361: 2005–2016, 2003