Apolipoprotein E Polymorphism and Renal Function in German Type 1 and Type 2 Diabetic Patients

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OBJECTIVE — To examine the association of renal function in diabetic patients with apolipoprotein (apo) E polymorphism.

RESEARCH DESIGN AND METHODS — Apo E genotypes, lipid and lipoprotein serum levels, creatinine clearance (CCr), and excretion of marker proteins were determined in German type 1 (IDDM; n = 162) and type 2 (NIDDM; n = 124) diabetic patients. Albumin and immunoglobulin (Ig) G are considered to reflect charge-size permselectivity of the glomerular capillary basement membrane, and increased α1-microglobulin (MG) excretion indicates compromised reabsorptive capacity of the renal tubules.

RESULTS — Patients with NIDDM had higher lipid levels and lower CCrs than patients with IDDM. In patients with IDDM, age- and sex-adjusted analysis of variance showed an association between apo E genotypes and CCr, and the Jonckheere-Terpstra test demonstrated a decreasing glomerular filtration rate in the following order of genotypes: ε4ε4/ε4ε3 > ε3ε3 > ε2ε2/ε2ε3. Multiple linear regression analyses revealed that in patients with IDDM, the ε2 allele was a negative predictor of CCr and a positive predictor of urinary excretion of albumin, IgG, and α1-MG independent from HDL and LDL cholesterol, TG concentration, age, and sex.

CONCLUSIONS — Apo E polymorphism influences serum lipoprotein levels in patients with IDDM and NIDDM. Apo E polymorphism may be a renal risk factor of clinical relevance in normolipidemic patients with IDDM.

Renal failure in diabetic patients is the most commonly recognized cause of irreversible uremia in the U.S. and Europe (1). Underlying mechanisms for the manifold higher atherosclerotic risk in patients with IDDM compared with age-matched control subjects are largely unclear (2). In contrast to patients with NIDDM, those with IDDM in good glycemic control have normal fasting lipid or lipoprotein levels (3–5). However, the lipoprotein remnant metabolism may be an underestimated risk factor for atherosclerosis (6). The loss of the portosystemic insulin gradient in patients with subcutaneous insulin injection may alter hepatic lipase activity (7) and impair function of hepatic lipoprotein receptors (6,8,9). Intraperitoneal insulin administration restores a positive portal-systemic blood insulin gradient (10) and normalizes lipid composition (7) and clearance of postprandial triglyceride (TG)-rich remnants (11) in patients with IDDM.

Common apolipoprotein (apo) E isoforms are coded by three codominant alleles (ε2, ε3, ε4). Apo E4 increases cholesterol (12) and uptake of dietary lipids, whereas apo E2 lowers cholesterol and delays clearance of atherogenic chylomicron and VLDL remnants. Accumulation of TG-rich lipoprotein remnants in serum and accelerated atherosclerosis characterizes type III hyperlipoproteinemia, which is caused by interaction of ε2 homozygosity with yet unknown genetic or environmental factors. Phenotype groups apo E2/3, apo E3/4, and apo E4/4 had similar increased risks (relative risks 1.5–1.7) for coronary artery disease compared with phenotype apo E3/3 (13), and apo E genotype E2/3 doubled the risk of carotid artery atherosclerotic disease independent from its effect on lipid levels and from other classical risk factors (14).

In the present study, our attention was attracted to the association of apo E polymorphism with main outcome measures of creatinine clearance (CCr) and urinary proteins as indicators of glomerular and tubular damage in patients with NIDDM and IDDM.

RESEARCH DESIGN AND METHODS

— A total of 286 diabetic patients without severe hepatic disorders or chronic infections were enrolled in the cross-sectional study. Fresh samples were used for immunonephelometric quantification of urinary proteins (Nephelometer Analyzer II; Behring Werke, Marburg, Germany). Stages of nephropathy were defined according to Mogensen et al. (15). The classification was based on at least three measurements in the first morning urine and after excluding other reasons for an increased albumin excretion. BMI was calculated (body weight [kg]/[height (m)]^2). HbA_1, determined by column chromatography (Boehringer Mannheim, Mannheim, Germany), had an upper reference limit of 8%.

Polymerase chain reaction (PCR) was used for amplification of genomic DNA, and apo E genotyping was performed by restriction fragment length polymorphism (RFLP) (16,17). If requested by the physician or in case of elevated TG or total cholesterol levels (type 1 diabetes: 36/162, type 2 diabetes: 64/124), a micro-ultracentrifugation method using a special tube-slicing tech-

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Received for publication 10 September 1997 and accepted in revised form 12 February 1998.

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; apo, apolipoprotein; CCr, creatinine clearance; Ig, immunoglobulin; LDLp, pure LDL; Lp(a), lipoprotein(a); MG, microglobulin; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; TG, triglyceride.

Table 1—*Apo E genotypes and apo E alleles in 286 German diabetic patients*

	n	E2/2	E2/3	E2/4	E3/3	E3/4	E4/4	ε2	ε3	ε4
IDDM	162	0	33	4	92	30	3	37	247	40
NIDDM	124	1	21	7	72	23	0	30	188	30
IDDM and NIDDM	286	1 (0.3)	54 (18.9)	11 (3.8)	164 (57.3)	53 (18.5)	3 (1.0)	67 (11.7)	435 (76.0)	70 (12.2)
Hardy-Weinberg		4 (1.4)	51 (17.8)	8 (2.9)	165 (57.8)	53 (18.6)	4 (1.5)	—	—	—

Data are *n* or *n* (%). Data for Hardy-Weinberg are the expected apo E genotype distribution according to Hardy-Weinberg equilibrium.

nique was applied to determine VLDL, HDL, and LDL cholesterol (18). Otherwise, HDL cholesterol was measured in unfractionated serum (Boehringer, Mannheim), and LDL cholesterol was calculated according to Friedewald (19). A highly validated electroimmunodiffusion method (Immuno AG, Vienna, Austria) was used to measure lipoprotein(a) (Lp[a]) (20). Because the cholesterol content of Lp(a) contributes to the LDL cholesterol determined by the ultracentrifugation procedure, as well as that calculated according to Friedewald (19,21), we calculated the "pure" LDL (LDLp) cholesterol values by the following equation: LDLp cholesterol (mg/dl) = LDL cholesterol (mg/dl) − 0.3 × Lp(a) (mg/dl) (21).

Statistical analysis

Mean values and SEMs are given if not otherwise stated. Equality of means of normally distributed continuous variables was tested with analysis of covariance (ANCOVA) (SAS version 6.12, 1996; SAS Institute, Cary, NC). Variables with asymmetrical distribution (e.g., TG) were logarithmically transformed. The nonparametric Kruskal-Wallis and Mann-Whitney *U* (Wilcoxon rank-sum *W*) tests were used for analyzing variables with skewed distribution. Dichotomous variables were analyzed with the Pearson χ^2 test (SAS) or with Fisher's exact test when sample numbers became small. The Jonckheere-Terpstra test (SPSS Exact tests version 6.1 for Windows 1995; SPSS, Chicago, IL), which takes into account the natural a priori ordering of variables, was used to analyze associations between the apo E genotype and, for example, stages of diabetic nephropathy and CCr. Multiple regression techniques were applied to explain or predict the outcome variable, e.g., CCr (Proc REG; SAS). In stepwise multiple regression analysis, the effect of apo E was estimated by coding the gene dose: a value of 1 was assigned to homozygotes, 0.5 to heterozygotes, and 0 to those without this apo E allele under consideration (22). Associations between apo E polymorphism and CCr as well as urinary protein excretion as main outcome measures were specified a priori for confirmatory analysis in order to control type 1 statistical errors (α errors).

RESULTS — Lipid levels and urinary proteins were compared between both types of diabetes. Because age and sex were shown to influence lipid levels, analysis of variance (ANOVA) was performed after adjustment for these covariates. Compared with type 1 diabetic patients, type 2 diabetic patients had higher TG (193 ± 11.3 vs. 104 ± 5.6 mg/dl; $P < 0.001$), total cholesterol (223 ± 3.7 vs. 192 ± 2.7 mg/dl; $P < 0.05$), and VLDL cholesterol (41.2 ± 3.8 vs. 31.2 ± 4.8 mg/dl; $P < 0.06$) levels and lower HDL cholesterol (48.2 ± 1.6 vs. 59.7 ± 1.3; $P < 0.001$) levels. In contrast, LDLp cholesterol (134 ± 3.89 vs. 109 ± 2.71; NS) and LDL cholesterol (140 ± 3.97 vs. 113 ± 2.68 mg/dl; NS) levels were not significantly different according to adjusted ANOVA. Taken together, indicators of atherogenic risk were clearly elevated in type 2 diabetic patients (total cholesterol/HDL cholesterol: 5.28 ± 0.21 vs. 3.46 ± 0.10, $P < 0.001$; LDL cholesterol/HDL cholesterol: 3.32 ± 0.15 vs. 2.04 ± 0.07, $P < 0.002$). The Wilcoxon rank-sum *W* test, which was applied to compare urinary protein levels between type 1 and type 2 dia-

Table 2—*Demographic characteristics of diabetic patients according to apo E genotypes*

	Type 1 diabetes			Type 2 diabetes		
	E2/3	E3/3	E3/4 E4/4	E2/2 E2/3	E3/3	E3/4
n (M/W)	33 (16/17)	92 (46/46)	33 (20/13)	22 (13/9)	72 (38/34)	23 (13/10)
Age (years)	38.2 ± 2.5	39.1 ± 1.2	37.5 ± 2.4	64.5 ± 2.9	62.4 ± 1.1	64.4 ± 1.8
BMI (kg/m²)	23.6 ± 0.4	24.0 ± 0.3	24.3 ± 0.4	26.6 ± 0.6	27.1 ± 0.4	27.6 ± 1.0
HbA_1 (%)	7.5 ± 0.3	7.9 ± 0.2	7.8 ± 0.2	8.4 ± 0.4	8.2 ± 0.2	8.0 ± 0.3
Serum creatinine (mg/dl)	0.99 ± 0.11	0.90 ± 0.03	0.90 ± 0.04	0.98 ± 0.05	0.98 ± 0.03	1.03 ± 0.10
LDLp cholesterol (mg/dl)	94.8 ± 5.6†§	112 ± 3.6	117 ± 6.2	118 ± 8.5§	134 ± 4.6	154 ± 10.5*
LDL cholesterol (mg/dl)	102 ± 5.6§	116 ± 3.6	120 ± 6.0	125 ± 9.0§	139 ± 4.6	159 ± 11.0
HDL cholesterol (mg/dl)	58.2 ± 3.0	61.4 ± 1.8	56.7 ± 3.0	56.6 ± 4.8*	46.8 ± 1.9	46.0 ± 3.8
VLDL cholesterol (mg/dl)	43.0 ± 17.6	26.6 ± 5.9	28.6 ± 6.3	27.1 ± 5.2	42.3 ± 4.9	43.0 ± 9.3
Total cholesterol (mg/dl)	180 ± 6.4*‡	194 ± 3.5	196 ± 5.7	211 ± 9.7‡	220 ± 4.3†	241 ± 9.5*
TG (mg/dl)	99.1 ± 9.4	100 ± 7.8	108 ± 12.1	169 ± 16.8	188 ± 14.1	221 ± 36.6

Data are means ± SEM or *n* from 158 type 1 and 117 type 2 diabetic patients. Continuous variables were compared by ANOVA after adjustment for age and sex when lipid and lipoprotein concentrations were investigated. Serum creatinine was analyzed with nonparametric tests. LDL and LDLp cholesterol were measured by ultracentrifugation method or calculated by Friedewald formula. VLDL cholesterol was determined by ultracentrifugational analysis (type 1 diabetes: $n = 34$, type 2 diabetes: $n = 60$). TG values were log-transformed for ANCOVA. *$P < 0.05$ and †$P < 0.01$ for tested vs. ε3ε3. ‡$P < 0.05$; and §$P < 0.01$ for tested vs. ε3ε4/ε4ε4.

Table 3—*Demographic characteristics of diabetic patients according to nephropathy stage*

	Type 1 diabetes			Type 2 diabetes		
	Stage I/II	Stage III	Stage IV/V	Stage I/II	Stage III	Stage IV/V
n (M/W)	118 (57/61)	32 (19/13)	12 (7/5)	79 (42/37)	25 (17/8)	20 (8/12)
Diabetes (years)	13.2 ± 1.0‡¶	20.3 ± 1.6	22.1 ± 2.2	12.6 ± 0.9¶	13.7 ± 1.9	19.3 ± 1.8
BMI (kg/m²)	23.7 ± 0.2*	24.8 ± 0.6	25.1 ± 0.9	26.4 ± 0.3‡¶	28.4 ± 1.1	29.0 ± 1.0
LDLp cholesterol (mg/dl)	105 ± 3.1*‖	117 ± 6.1	129 ± 11.6	135 ± 4.7	135 ± 9.7	130 ± 9.9
LDL cholesterol (mg/dl)	109 ± 3.0*‖	121 ± 5.9	135 ± 11.8	140 ± 4.9	137 ± 9.7	141 ± 9.7
HDL cholesterol (mg/dl)	61.0 ± 1.6	57.0 ± 2.9	54.6 ± 5.0	51.3 ± 2.0*	40.4 ± 2.4	45.5 ± 4.6
VLDL cholesterol (mg/dl)	28.3 ± 6.0	26.3 ± 6.7	48.5 ± 16.3	33.6 ± 4.6‖	44.4 ± 7.4	55.7 ± 8.3
Total cholesterol (mg/dl)	188 ± 2.9¶	193 ± 6.3¶	223 ± 13.3	221 ± 4.7	219 ± 8.0	235 ± 8.7
TG (mg/dl)	98.7 ± 6.1‖	103 ± 12.1§	156 ± 30.0	175 ± 14.2§	213 ± 21.3	241 ± 30.7

Data are means ± SEM or *n*. In 162 type 1 and 117 type 2 diabetic patients, continuous variables were compared by ANOVA after adjustment for age and sex when lipid and lipoprotein concentrations were investigated. LDL and LDLp cholesterol were measured by ultracentrifugation method or calculated by Friedewald formula. VLDL cholesterol was determined by ultracentrifugational analysis (type 1 diabetes: $n = 36$; type 2 diabetes: $n = 64$). TG values were log-transformed for ANCOVA. *$P < 0.05$; †$P < 0.01$; and ‡$P < 0.005$ for tested vs. ε3ε3. §$P < 0.05$; ‖$P < 0.01$; and ¶$P < 0.005$ for tested vs. ε3ε4/ε4ε4.

betic patients (because these skewed distributed variables were neither gender-dependent nor correlated with age), was not significant (type 1 vs. type 2, albumin: 67.1 ± 208 vs. 334 ± 1,649 mg/l; immunoglobulin [Ig] G: 8.8 ± 14.5 vs. 22.7 ± 70.2 mg/l; and α1-microglobulin [MG]: 9.4 ± 7.5 vs. 11.8 ± 10.4 mg/l).

Table 1 summarizes apo E genotyping results, which were not related to stages of nephropathy. Men and women had similar allele frequencies (data not shown). Table 2 demonstrates associations of apo E genotypes with lipid levels grouped by diabetes type. Duration of diabetes was comparable among apo E genotypes.

Age, height, and HbA_1 were similar among different stages of nephropathy, whereas, of course, serum creatinine, CCr, urinary albumin, IgG, and α1-MG were highly significantly altered in stage IV/V of diabetic nephropathy (data not shown). Table 3 summarizes anthropometric data and lipid and lipoprotein levels grouped by stages of diabetic nephropathy and type of diabetes. In type 1 diabetic patients, BMI, LDL, LDLp, and total cholesterol, and TGs were significantly higher in stage IV/V of nephropathy in contrast to VLDL and HDL cholesterol. Type 2 diabetic patients in stage IV/V had higher BMI, TGs, and VLDL cholesterol levels, lower HDL cholesterol levels, but comparable LDLp, LDL, and total cholesterol levels than those in stage I/II of diabetic nephropathy (Table 3). Statistical associations between the stage of nephropathy and the apo E genotype or ε allele frequencies could not be found ($P > 0.7$; Fisher's exact test).

Multiple linear regression analysis was used to determine predictors of the renal function in diabetic patients. LDLp and HDL cholesterol, TGs, apo E4, E3, and E2, age, and sex were included as possible regressors for CCr, urinary albumin, IgG, and α1-MG, which were log-transformed. In type 2 diabetic patients only, age and female sex were negative predictors of CCr (Table 4). In both the type 1 diabetic and the whole patient groups, CCr turned out to be predicted negatively by female sex, age, TGs, and apo ε2 allele, whereas LDLp cholesterol gained statistical significance only in the whole group. Some 32–42% of the variation of CCr was explained by these regressors. In type 1 diabetic patients, the strength of the ε2 allele effect was −23.9 ± 11.4 ml/min (Table 4). In the whole patient group, LDLp cholesterol and TGs were weak predictors of albumin, IgG, and α1-MG excretion, whereas in type 2 diabetes, marker protein excretion was not predicted by any of the regressors tested. In type 1 diabetes, LDLp cholesterol and age were regressors for IgG and albumin excretion ($P < 0.002$), TGs for α1-MG, and ε2 allele was a significant predictor of all three marker proteins ($0.02 < P < 0.04$).

In contrast to patients with NIDDM, age- and sex-adjusted ANOVA revealed significant differences of CCr between genotypes in patients with IDDM (Table 5). The median test ($P = 0.02$) showed significant differences between CCrs of ε2, ε3, and ε4 carriers, and the nonparametric Wilcoxon rank-sum *W* test showed that type 1 diabetic carriers of the ε2 allele had lower CCrs than ε4 carriers (two-tailed, $P < 0.05$). These results were confirmed by the Jonckheere-Terpstra test, demonstrating that CCr decreased in the following order of genotypes: ε4ε4/ε3ε4 > ε3ε3 > ε2ε3 ($P = 0.03$).

CONCLUSIONS — In the past decades, there have been innumerable reports documenting an atherogenic lipoprotein

Table 4—*Stepwise multiple regression analyses on CCr with age, sex, LDLp and HDL cholesterol, TG, and ε2, ε3, and ε4 alleles as possible predictors*

	Type 1 diabetes	Type 2 diabetes	Both types
R^2	0.32	0.37	0.42
Intercept	161 ± 10.1§	171 ± 15.8§	154 ± 6.7§
Age (years)	−1.04 ± 0.19§	−1.53 ± 0.23§	−0.98 ± 0.11§
Sex	22.1 ± 4.6§	15.6 ± 4.7‡	19.3 ± 3.3§
LDLp cholesterol (mg/dl)	−0.11 ± 0.07 (0.10)	—	0.10 ± 0.04*
HDL cholesterol (mg/dl)	—	—	—
TG (mg/dl)	−0.095 ± 0.034†	—	−0.032 ± 0.016‡
ε2 allele	−23.9 ± 11.4*	—	−18.5 ± 8.3*

Data are regression coefficients ± SEM (P). Intra-individual allele types are coded 0 if not present; 0.5 in heterozygous and 1 in homozygous patients. Women are coded 0, men 1. R^2 is defined as the proportion of variance of the response that is predictable from the regressor variables. *$P < 0.02$; †$P < 0.005$; ‡$P < 0.001$; §$P < 0.0001$.

Table 5—*Association of apo E polymorphism with renal function*

	Type 1 diabetes			Type 2 diabetes		
	E2/2 E2/3	E3/3	E3/4 E4/4	E2/2 E2/3	E3/3	E3/4 E4/4
CCr	100 ± 6.0†	108 ± 3.6	116.4 ± 5.8	85.6 ± 6.4	84.3 ± 3.5	83.0 ± 7.0
Albumin (mg/l)	75.8 ± 32.9	73.4 ± 25.4	23.7 ± 7.3	105 ± 47.8	482 ± 255	153 ± 65.7
IgG (mg/l)	12.0 ± 3.5	8.4 ± 1.5	6.1 ± 0.8	11.4 ± 4.2	27.6 ± 10.6	18.3 ± 7.6
α1-MG (mg/l)	12.2 ± 2.0*†	8.9 ± 0.7	8.0 ± 0.8	12.6 ± 2.3	11.7 ± 1.2	11.8 ± 2.8

Data are means ± SEM. CCr was tested with age- and sex-adjusted ANOVA. Albumin, IgG, and α1-MG were tested with the nonparametric Wilcoxon's rank-sum *W* test of urinary protein levels. *$P < 0.05$ compared with ε3ε3 genotype; †$P < 0.05$ compared with ε4 allele carriers.

pattern in NIDDM, regardless of the mode of treatment (4,5,23), that may be related to hyperinsulinemia and/or insulin resistance (24). However, patients with IDDM in good metabolic control have normal fasting lipid and lipoprotein levels. Exploratory statistical evaluation of our study population was in line with general findings of lipoprotein levels in patients with IDDM and NIDDM. We determined Lp(a) immunologically and additionally presented calculated LDLp cholesterol (21), since these two potential risk factors, LDL and Lp(a), are metabolized in a different manner (25,26). In recent years, lipoprotein receptors and compositional changes of lipoproteins in diabetes have attracted increasing attention because insulin or its deficiency considerably affects lipid metabolism (7,27).

Apo E polymorphism influences lipid levels and clearance of chylomicron and VLDL remnants and, thereby, may contribute in a clinically relevant way to atherogenesis (28,29). The main concern of the present study is whether apo E polymorphism is a risk factor for diabetic nephropathy. Genotypes were determined with PCR-RFLP (17), since erroneous phenotyping may result from posttranslational modifications (30), especially in diabetic patients in poor metabolic control. Apo E allele genotypes were in Hardy-Weinberg equilibrium, and allele frequencies in diabetic patients were within the range previously reported for Caucasians (31).

Discrepant results have been obtained concerning apo E polymorphism and atherosclerotic risk in diabetic patients (32,33) as well as LDL cholesterol values in patients with IDDM (34,35). We used an ultracentrifugation method when necessary instead of calculating all LDL cholesterol values by the Friedewald formula. Our results clearly show the expected association of apo E genotypes with LDL and LDLp cholesterol concentrations in both types of diabetes.

Our data in type 1 diabetic patients confirm recent data (35) on elevated TG and total cholesterol levels in macroalbuminuric patients with IDDM and additionally present LDL cholesterol concentrations. In type 2 diabetes, however, only TG and VLDL cholesterol levels reached statistical significance in age- and sex-adjusted ANOVA among stages of nephropathy. These relationships between lipid levels and stages of nephropathy may indicate that an atherogenic lipid profile accelerates development of diabetic nephropathy in IDDM, as suggested by a longitudinal study (36). However, a hepatic overproduction of apo B–containing lipoproteins in proteinuric patients may also contribute to this observation (37).

A few investigations have been performed on the regulation of lipoprotein receptors in human liver or nonhepatic tissues by insulin or its deficiency (8,9,38, 39). The portosystemic insulin gradient, which may contribute to the hepatic LDL receptor expression despite high ligand levels, might be impaired by subcutaneous insulin application in patients with IDDM. These pathophysiological considerations led to the hypothesis of a proatherogenic postprandial lipoprotein metabolism affecting mesangial cells (6,11).

Renal function was analyzed by measuring CCr and urinary proteins. Albumin and IgG are considered to reflect charge and size permselectivity of the glomerular capillary basement membrane, and increased α1-MG excretion indicates compromised reabsorptive capacity of the renal tubules. Including age, sex, TGs, total, LDL, and HDL cholesterol, and ε2, ε3, and ε4 alleles as possible regressors, multiple linear regression analysis showed that ε2 gene-dose was an independent predictor of urinary excretion of all three urinary proteins measured. Moreover, CCr was negatively influenced by the apo E2 isoform to a clinically relevant degree in patients with IDDM. This apo E2–CCr relationship was independent from apo E2 effects on fasting lipid levels. In contrast to patients with NIDDM, confirmatory statistical analysis (age- and sex-adjusted ANOVA; Jonckheere-Terpstra test) demonstrated in the target variable, i.e., the CCr, a significant difference between ε2, ε3, and ε4 carriers with IDDM. To the best of our knowledge, this association between glomerular filtration rate and apo E polymorphism has not been investigated thus far. The pathophysiological basis may be a lipoprotein-stimulated mesangial expansion that ultimately restricts glomerular capillary luminal volume and diminishes filtration surface.

Taken together, the present data are consistent with the view that apo E polymorphism may be one of the multiple risk factors involved in the progression of atherosclerosis in IDDM. However, prospective longitudinal studies are required to support this hypothesis and to clarify whether therapeutic intervention should be intensified in normolipidemic type 1 diabetic patients at high risk for nephropathy.

Acknowledgments— The authors thank Marion Künstler for skillful technical assistance.

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