Atorvastatin Dose-Dependently Decreases Hepatic Lipase Activity in Type 2 Diabetes

Effect of sex and the *LIPC* promoter variant

INGRID I.L. BERK-PLANKEN, MD[1]
NICOLINE HOOGERBRUGGE, MD, PHD[1,2]
RONALD P. STOLK, MD, PHD[3]
AART H. BOOTSMA, MD, PHD[1]
HANS JANSEN, MRBC, PHD[1,4]
ON BEHALF OF THE DALI STUDY GROUP

OBJECTIVE — Hepatic lipase (HL) is involved in the metabolism of several lipoproteins and may contribute to the atherogenic lipid profile in type 2 diabetes. Little is known about the effect of cholesterol synthesis inhibitors on HL activity in relation to sex and the hepatic lipase gene, the *LIPC* promoter variant in type 2 diabetes. Therefore, we studied the effect of atorvastatin 10 mg (A10) and 80 mg (A80) on HL activity in 198 patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Patients (aged 45–75 years, without manifest coronary artery disease, total cholesterol 4.0–8.0 mmol/l, and fasting triglycerides [TG] 1.5–6.0 mmol/l) were included in a double-blind, randomized, placebo-controlled trial for 30 weeks (Diabetes Atorvastatin Lipid Intervention study).

RESULTS — HL activity at baseline was significantly higher in our population compared with an age-matched control group without type 2 diabetes (406 ± 150 vs. 357 ± 118 units/l). HL activity in men versus women (443 ± 158 vs. 358 ± 127 units/l), in carriers of the *LIPC* C/C allele versus carriers of the T/T allele (444 ± 142 vs. 227 ± 96 units/l), and in Caucasians versus blacks (415 ± 150 vs. 260 ± 127 units/l) all differed significantly ($P < 0.001$). Atorvastatin dose-dependently decreased HL (A10, −11%; A80, −22%; both $P < 0.001$). Neither sex nor the *LIPC* C→T variation influenced the effect of atorvastatin on HL activity.

CONCLUSIONS — Sex, *LIPC* promoter variant, and ethnicity significantly contribute to the baseline variance in HL activity. Atorvastatin treatment in diabetic dyslipidemia results in a significant dose-dependent decrease in HL activity, regardless of sex or the *LIPC* promoter variant.

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Hepatic lipase (HL) is involved in the metabolism of several lipoproteins (1) and is a key player in HDL metabolism (2,3). Hydrolysis of phospholipids and triglycerides by HL leads to the conversion of large, buoyant HDL2 to small, dense HDL3 and may induce cholesterol (ester) flux to the liver (4,5). In this way, HL is involved in reverse cholesterol transport and is a major determinant of plasma HDL concentration (6,7). HL also plays a role in the formation of small, dense LDL and contributes to the expression of the LDL subclass phenotype (8,9). Finally, HL is proposed to be involved in postprandial lipid clearing (10). Obviously, HL activity seems to be central in the metabolism of lipoproteins strongly associated with coronary artery disease (CAD) risk in type 2 diabetes. Zambon et al. (11) identified HL as a focal point for the development and treatment of CAD. Genetic variation, sex, and abdominal fat mass affect HL activity in humans (12–15).

In the human hepatic lipase gene (*LIPC*), variants are found that affect lipase activity (16–18). Besides rather rare variants leading to complete HL deficiency (19,20), common base substitutions in the proximal *LIPC* promoter affect HL activity up to twofold (15,21,22). Four base substitutions, −250 G→A, −514 C→T, −710 T→C, and −763 A→G, were found to be completely linked (23). Together, they constitute two alleles, indicated as the *LIPC* C and T allele, after the C→T variant at −514 bp, which is also indicated as C–480T. The C and T alleles are associated with high and low HL activity, respectively. The frequency of both alleles varies highly among different ethnic populations (21,24,25). The C allele is the most common allele in Caucasians, whereas the T allele is the major allele in black Americans. Asians have an intermediate frequency. Besides genetic variance, HL lipase activity is also hormonally affected. In particular, sex hormones influence *LIPC* expression and are believed to be responsible for HL activity being lower in women than in men (13,25,26). An increase in HL activity is associated with an increase in abdominal fat mass, BMI, and fasting insulin and fasting plasma triglyceride levels (27). These correlations are reflected in a high HL activity in type 2 diabetes.

A change in HL activity may also be induced by hypolipidemic drugs. In

From the [1]Department of Internal Medicine, Erasmus MC, Erasmus University Rotterdam, Rotterdam, the Netherlands; the [2]Department of Human Genetics, University Medical Center Nijmegen, Nijmegen, the Netherlands; the [3]Julius Center for Patient Oriented Research, Utrecht, the Netherlands; and the [4]Departments of Biochemistry and Clinical Chemistry, Erasmus MC, Erasmus University Rotterdam, Rotterdam, the Netherlands.

Address correspondence and reprint requests to H. Jansen, Department of Internal Medicine, Rm. L175, Erasmus MC, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. E-mail: jansen@inw3.azr.nl.

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Abbreviations: CAD, coronary artery disease; DALI, Diabetes Atorvastatin Lipid Intervention; FFA, free fatty acid; HL, hepatic lipase; TG, triglycerides; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

males, but not in females, we found a decrease in HL activity during atorvastatin treatment of familial hypercholesterolemia (28). Zambon et al. (29) reported a drop in HL activity in participants of the Familial Atherosclerosis Treatment Study (FATS) during treatment with lovastatin-colestipol and niacin-colestipol. Interestingly, in both groups the effect of treatment on HL activity was strongly dependent on the presence of the *LIPC* T allele. HL activity was lowered less in carriers of the T allele compared with noncarriers (30). Thus, sex and genotype of the *LIPC* promoter may determine the efficacy of the statin treatment. Earlier studies (31–34) showed a beneficial effect of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors on the incidence of cardiovascular events in type 2 diabetes. Also, although atorvastatin, a powerful statin, effectively reduces total cholesterol and triglyceride concentrations, no data describing its effect on HL activity in type 2 diabetes are currently available. Therefore, we conducted a double-blind, placebo-controlled, randomized, 30-week study to evaluate the effect of atorvastatin 10 vs. 80 mg on HL activity in subjects with type 2 diabetes. Sex and the *LIPC* C→T variation as possible modifiers of the atorvastatin treatment effect were considered in this study.

RESEARCH DESIGN AND METHODS

Study population

This study is part of the Diabetes Atorvastatin Lipid Intervention (DALI) study. DALI is a randomized, double-blind, placebo-controlled, multicenter study conducted in the Netherlands. The subjects and methods have been described in detail (35).

In short, 217 patients (aged 45 to 75 years, with type 2 diabetes) participating in the DALI study were randomized to placebo, atorvastatin 10 mg (A10), or atorvastatin 80 mg (A80) during 30 weeks, to evaluate the effect on lipid metabolism, endothelial function, coagulation, and inflammatory parameters. The main inclusion criteria were plasma triglycerides between 1.5 and 6.0 mmol/l, total cholesterol between 4.0 and 8.0 mmol/l, and no history of coronary heart disease. Postheparin lipase activity blood samples of 198 DALI patients were available and evaluated in this study.

Analytical methods

Blood samples were drawn (after 12 h of fasting) at baseline and after 30 weeks, the end of the study. Standard lipid variables (total cholesterol, HDL cholesterol, and triglycerides [TG]), free fatty acids (FFAs), plasma glucose, HbA_{1c}, and HL activity were measured. Cholesterol and TG were determined by enzymatic colorimetric methods on a Hitachi 911 automatic analyzer (Boehringer Mannheim, Mannheim, Germany). Plasma HDL cholesterol was measured by a direct enzymatic HDL cholesterol method, after precipitation of VLDL and LDL by addition of manganese chloride. LDL cholesterol was estimated by the Friedewald formula (36). Fasting plasma glucose was determined on a Hitachi 917 analyzer using an ultraviolet-hexokinase method (cat. no. 18766899; Boehringer Mannheim). HbA_{1c} was determined by high-performance liquid chromatography, using the Bio-Rad Variant method (cat. no. 270-0003).

Postheparin plasma HL activity

HL activity was measured using an immunochemical method as described previously (37) in plasma collected 20 min after contralateral intravenous administration of heparin (50 IU/kg) (Leo Pharmaceutical Products, Weesp, The Netherlands). HL control subjects consisted of 93 male and female volunteers, aged 45 to 75 years, without type 2 diabetes or hypertriglyceridemia.

DNA analysis

Genotyping of the *LIPC* gene for the C→T variance was carried out by determination of the G→A base substitution at –250 bp (38). Primers for the PCR amplification were 5′-GAT ACT TTG TTA GGG AAG ACT GCC-3′ and 5′-GGA TCA CCT CTC AAT GGG TC-3′. Amplification was carried out in a 25-μl reaction mixture with an initial denaturation at 95°C for 5 min, followed by 31 cycles of amplification at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s, with a final extension at 72°C, using Goldstar *Taq* polymerase (Eurogentec). Fifteen microliters of the PCR mix was digested with the restriction enzyme *Dra*I during 2 h at 37°C, followed by electrophoresis on a 2% agarose gel. The nondigested PCR product had a length of 560 bp. After digestion, the –250G variant yielded two products of 449 and 111 bp. Digestion of the −250A variant yielded a major product of 335 bp and minor products of 114 and 111 bp. It was shown previously that the –250G variant is 100% linked to the C variant at –514 and the –710T and –763A variants upstream in the *LIPC* promoter (18). These variants collectively form the *LIPC* C allele. The variant sequence containing −250 A, −514 T, −710 C, and −763 G are indicated as *LIPC* T allele.

Statistical analysis

Analyses were performed by SPSS for Windows (release 9.0). Mean differences between the study groups were analyzed using analysis of covariance (ANCOVA), adjusted for baseline levels of each treatment group and study location. Intervention effects were also further adjusted for additional potential confounders, using ANCOVA. The Student's *t* test was used to test significance between baseline and 30-week follow-up. Continuous variables are presented as mean values with the standard deviation (SD). *P* values <0.05 were considered statistically significant.

RESULTS

Patient characteristics

The baseline characteristics of the 198 patients are shown in Table 1.

There were no significant differences in characteristics between the three groups, except for the duration of diabetes, which was shorter in the placebo than the A80 group ($P < 0.05$). Fasting glucose and HbA_{1c} at baseline were relatively high, but equal in all groups. Fasting glucose did not change during the study. The A80 group had a slight increase in HbA_{1c} levels (8.4 ± 1.1% to 8.6 ± 1.3%; $P < 0.05$), whereas in patients using A10 and placebo, HbA_{1c} levels decreased slightly (both 8.3 ± 1.2% to 8.1 ± 1.2%; $P < 0.05$).

Atorvastatin effect on lipids and lipoproteins

At baseline, plasma lipids and lipoproteins were similar in all three groups (Table 1). As described before (35), administration of A10 and A80 resulted in significant reductions (25% and 35%, respectively) of plasma TG levels (both $P < 0.001$) and significant, dose-dependent reductions of total cholesterol (30% and 40%, respectively; both $P < 0.001$) and LDL (41% and 52%, respectively; both $P < 0.001$). HDL cholesterol levels signif-

Table 1—*Baseline characteristics*

	Placebo	Atorvastatin 10 mg	Atorvastatin 80 mg
n	65	67	66
Male sex (%)	48	62	54
Age (years)	58.5 ± 7.3	59.4 ± 7.6	60.5 ± 7.8
Diabetes duration (years)	9.5 ± 6.1	12.2 ± 7.7	13.2 ± 8.5*
Diabetes treatment (%)			
Diet	3.1	1.5	0
Tablets	40.6	50.0	43.9
Insulin	31.3	27.3	27.3
Combination	25.0	21.2	28.8
BMI (kg/m^2)	31.9 ± 6.1	30.1 ± 3.8	30.6 ± 4.5
WHR	0.99 ± 0.1	1.00 ± 0.1	1.00 ± 0.1
Hypertension (%)	48	49	62
Current smoking (%)	58	66	70
Fasting glucose (mmol/l)	10.5 ± 3.6	10.4 ± 3.0	10.6 ± 3.0
HbA_{1c} (%)	8.3 ± 1.2	8.3 ± 1.2	8.4 ± 1.1
Total cholesterol (mmol/l)	6.1 ± 0.9	5.9 ± 0.8	6.0 ± 1.0
LDL cholesterol (mmol/l)	3.7 ± 0.9	3.6 ± 0.8	3.7 ± 0.9
HDL cholesterol (mmol/l)	1.05 ± 0.2	1.06 ± 0.3	1.04 ± 0.2
Triglycerides (mmol/l)	2.64 ± 0.9	2.50 ± 0.9	2.82 ± 1.1

Continuous data are means ± SD. *$P < 0.05$ between atorvastatin 80 mg and placebo group.

icantly increased, independent of dose, during atorvastatin treatment by 5–6% ($P < 0.01$).

HL activity in type 2 diabetes

Baseline HL in the DALI population was significantly higher than in age-matched, nondiabetic control subjects without hypertriglyceridemia (406 ± 150 units/l, $n = 198$, vs. 357 ± 118 units/l, $n = 93$; $P < 0.001$). This was due to a significant increase in HL activity in the male patients compared with the male control subjects (443 ± 158 vs. 397± 125 units/l; $P < 0.001$). Female DALI patients had a comparable HL activity compared with female control subjects (358 ± 127 vs. 328 ± 105 units/l). Sex, *LIPC* genotype, ethnicity, and BMI were all significant and independent predictors of HL activity, demonstrated by stepwise linear regression. The *LIPC* genotype contributed 13%, sex 8%, ethnicity 4%, and BMI 3% to the variance in HL activity (adjusted R^2 of the model = 0.28, $P < 0.0001$).

Atorvastatin effect on postheparin lipase activity

Mean HL activity at baseline was comparable between the three study groups. In the placebo group, there was no significant reduction in HL activity at the end of the study. HL activity decreased in the A10 group by 11% ($P < 0.001$) and in the A80 group by 22% ($P < 0.001$) (Table 2). The additional reduction in HL activity by A80 compared to A10 was highly significant ($P < 0.005$).

Table 2—*Hepatic lipase activity (units/l) after 30 weeks of treatment*

Patients	*n*	Placebo	Atorvastatin 10 mg	Atorvastatin 80 mg
All	198			
Baseline		406 ± 156	402 ± 152	410 ± 138
30 weeks		382 ± 146	361 ± 149*	313 ± 112*
Males	107			
Baseline		449 ± 138	429 ± 154	451 ± 143
30 weeks		425 ± 128	390 ± 150*	343 ± 98*
Females	71			
Baseline		358 ± 131	360 ± 155	356 ± 109
30 weeks		343 ± 125	318 ± 138*	278 ± 120*

Continuous data are means ± SD. *$P < 0.001$, significantly different from baseline in each treatment group.

Atorvastatin effect on postheparin lipase activity in men and women

Because HL activity in the DALI participants was significantly higher in men than in women (443 ± 158 units/l, $n = 107$, vs. 358 ± 127 units/l, $n = 91$; $P < 0.001$), we analyzed men and women separately. The effect on HL activity by atorvastatin was not influenced by sex differences. Treatment with atorvastatin 10 or 80 mg reduced HL activity similarly, 11 and 22%, in both men and women (Table 2).

Atorvastatin effect on hepatic lipase activity in the *LIPC* promoter variant

The C→T variance in the *LIPC* promoter is a strong, significant predictor of HL activity; therefore we analyzed carriers and noncarriers of the T allele separately.

In the DALI study population, 55.0% of the patients were homozygous for the *LIPC* C allele, 37.4% were heterozygous for the T allele, and 7.6% were homozygous for the T allele, leading to a T allele frequency of 0.262. At baseline, the presence of the T variant significantly affected HL activity (Table 3). In heterozygous carriers of the T allele, HL activity was 19% lower, and in homozygous carriers of the T allele, 49% lower, than in C allele homozygotes ($P < 0.0001$).

The T allele lowered the baseline HL activity in men and women. Male heterozygous and homozygous T allele carriers, respectively, had 20% and 57% lower HL lipase activity than C homozygotes. Women had 16% and 37%, respectively, lower lipase activity (Table 3). In men and women, the frequency of the T allele was comparable, 0.252 vs. 0.278.

Homozygous carriers of the C allele and heterozygous carriers of the T allele showed similar percentage reductions in HL activity after atorvastatin treatment (Table 4). In both groups, A10 resulted in a 10–12% decrease and A80 in a 21–22% decrease in HL activity ($P < 0.001$ vs. placebo; dose-dependent, $P < 0.005$). Four of the 15 T/T homozygotes were assigned to the A10 treatment group, 3 to the A80 group, and 8 placebo. After atorvastatin treatment, HL activities were available in only five patients. A10 treat-

Table 3—*Effect of the LIPC C → T variance on HL activity (units/l) at baseline*

	Total	Males	Females
CC genotype	444 ± 142	489 ± 131	385 ± 137
n	109	61	48
CT genotype	358 ± 135†	390 ± 158†	324 ± 95†‡
n	74	38	36
TT genotype	227 ± 96†	212 ± 56†	244 ± 131†
n	15	8	7

Data are means ± SD. *$P < 0.001$ vs. males; †$P < 0.001$ vs noncarriers; ‡$P = 0.03$ vs. males.

ment lowered HL activity in two cases (249 to 216 units/l, −15%; 349 to 259 units/l, −25%). In one case, A10 did not reduce HL activity (319 to 349 units/l, 9% increase). In both subjects assigned to A80, HL activity was lowered (248 to 218 units/l, −12%; 266 to 200 units/l, −25%). A10 lowered HL activity to a similar degree in male and female C/C and C/T carriers (10–13%). A80 resulted in a further reduction of 16% in the female C/C homozygotes and 31% in the female C/T heterozygotes. In men, the additional decrease was 27 and 19%, respectively, not significantly different from the values in women.

Ethnic differences, *LIPC* polymorphism, and HL activity

It has been reported that the prevalence of *LIPC* C and T alleles strongly varies among subjects with different ethnic heritage, thereby affecting HL activity. Thus, we studied the influence of ethnic heterogeneity in our study population. Mean HL activity at baseline was significantly lower among Asian ($n = 10$), Mediterranean ($n = 9$), and black ($n = 11$) patients compared with Caucasians ($n = 168$), respectively (350 ± 95, 269 ± 124, and 260 ± 127 units/l vs. 415 ± 150 units/l; $P < 0.01$). The T allele frequency was very high in black (0.590) and Asian (0.400) patients. Caucasians and Mediterranean patients had T allele frequencies of 0.235 and 0.222, respectively. At baseline, HL activity was strongly influenced by the *LIPC* gene variance in all ethnic groups. HL activity was significantly higher in C/C homozygotes than in C/T heterozygotes or T/T homozygotes. To study a possible effect of ethnic background, Caucasians were separately analyzed for the effect of atorvastatin in relation with the *LIPC* genotype. Atorvastatin had an effect in Caucasians similar to that of the whole study population (data not shown). The other groups were not analyzed separately, because of the small numbers.

CONCLUSIONS — This prospective, randomized study demonstrated a dose-dependent decrease in HL activity by atorvastatin in type 2 diabetes patients. HL activity decreased 11 and 22% after treatment with atorvastatin 10 and 80 mg, respectively ($P < 0.001$). In type 2 diabetes, HL is considered an important factor in the development of the atherogenic lipid profile. Our study results are the first published effects in type 2 diabetes of low- versus high-dose statin influencing HL activity. Sex and the *LIPC* C>T polymorphism both influence HL activity. Men have a higher HL activity than women because of a number of endogenous factors, such as central adiposity and sex steroid hormones (14,15,26,39,40). Recently, Carr et al. (41) also demonstrated that intra-abdominal fat is a major component of the sex difference in HL activity. In our diabetic study population as well, men exhibited higher HL activity than women. Whereas the male patients tended to have a higher HL activity compared with control subjects without diabetes, in the female patients HL activity was similar to control values. Baseline HL activity correlated significantly with waist-to-hip ratio (WHR) ($R = 0.20$, $P = 0.004$). Men had a higher WHR compared with women ($P < 0.001$), and the increased HL activity in the men may be partially explained by this increased WHR. Atorvastatin treatment abolished the correlation between HL activity and WHR. In preliminary results, we found that fatty acids may stimulate *LIPC* expression in HepG2 cells and that this effect is abolished by atorvastatin (27). Our present results are in line with these observations and suggest that increased HL activity in our male patients may be due to stimulation of HL expression by increased supply of fatty acids derived from a higher abdominal fat mass as reflected in the high WHR. By interfering with the stimulation of HL by FFAs, atorvastatin may then lower HL activity with the consequent loss of the association between HL activity and WHR. Besides sex, genetic variation of the *LIPC* promoter strongly affected HL activity at baseline and contributed 13% to the variance in HL activity. In the whole study population, *LIPC* T/T homozygotes had a 50% lower HL activity than the C/C homozygotes. Atorvastatin lowered HL activity in all subjects independent of sex and genotype. Zambon et al. (30) found an attenuating effect of the T allele on HL lowering by hypolipidemic drug treatment in hyperlipidemic patients. In the present study, the already low dose of statin decreased HL activity in all genotypes similarly. It is possible that atorvastatin has a higher intrinsic capacity to lower HL than other hypolipidemic drugs. Alternatively, HL activity in type 2 diabetes may be affected in a different way than in patients without

Table 4—*LIPC C → T variance and HL activity (units/l) after 30 weeks of treatment*

			Atorvastatin	
Genotype	*n*	Placebo	10 mg	80 mg
CC genotype	109			
Baseline		437 ± 124	448 ± 140	447 ± 129
30 weeks		441 ± 139	402 ± 156*	348 ± 98*
CT genotype	74			
Baseline		352 ± 144	357 ± 121	366 ± 157
30 weeks		349 ± 137	319 ± 128*	280 ± 128*
TT genotype	15			
Baseline		224 ± 76	306 ± 104	257 ± 76
30 weeks		236 ± 137	273 ± 34	209 ± 45

Data are means ± SD. *$P < 0.001$, significantly different from baseline in each treatment group.

diabetes. HL activity also varies among different ethnic groups. Our results showed that the allele frequency for the *LIPC* T allele in black patients was much higher than in Caucasian patients, consistent with earlier studies (21). This higher prevalence of the T allele in black patients completely accounted for the lowered HL activity at baseline compared with Caucasian patients (260 vs. 415 units/l). However, Nie et al. (24) stated that 97% of African Americans have at least one HL allele that is associated with low HL activity. The effect of ethnic background on the frequency of the T allele underscores the importance of this factor, when *LIPC* allele frequency between groups, with or without diabetes, is compared. The T allele frequency in our Caucasian subjects was 0.235. Jansen et al. (16) reported a frequency of 0.189 in a healthy, nondiabetic, Caucasian population. Without correction for ethnicity, T allele frequency in the patients with type 2 diabetes appeared to be higher than in Caucasians without diabetes (0.262 vs. 0.189).

The clinical significance of our findings needs further investigation. HL seems to be involved in the metabolism of almost all lipoprotein classes. Among other things, it may modulate LDL and HDL metabolism and postprandial lipid clearing. How and to what extent HL lowering by atorvastatin in type 2 diabetes affects these processes, and thereby CAD risk, is presently unknown.

In conclusion, sex, *LIPC* promoter variant, and ethnicity greatly contribute to the baseline variance in HL activity in type 2 diabetes. This should be taken into account in studies evaluating the effect of lipid-lowering therapy on HL expression. Atorvastatin treatment results in a dose-dependent decrease in HL activity, regardless of sex or the *LIPC* promoter variant.

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APPENDIX

The DALI study group (in alphabetical order): Erasmus Medical Center Rotterdam, Department of Internal Medicine (I.I.L. Berk-Planken, N. Hoogerbrugge, H. Jansen), Department of Biochemistry and Clinical Chemistry (H. Jansen); Gaubius Laboratory TNO-PG Leiden (H.M.G. Princen); Leiden University Medical Center (M.V. Huisman, M.A. van de Ree); University Medical Center Utrecht, Julius Center for General Practice and Patient Oriented Research (R.P. Stolk, F.V. van Venrooij); University Center Utrecht, Division of Internal Medicine (J.D. Banga, G. Dallinga-Thie, F.V. van Venrooij).

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