Progression of Retinopathy After Improved Metabolic Control in Type 2 Diabetic Patients

Relation to IGF-1 and hemostatic variables

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OBJECTIVE — To determine the impact of improved glycemic control on the development and progression of retinopathy after the institution of insulin therapy in patients with type 2 diabetes and to assess the relation to IGF-1 and hemostatic variables.

RESEARCH DESIGN AND METHODS — In a prospective observational study, 45 type 2 diabetic patients were examined at baseline and 1, 3, 6, 12, and 24 months after change to insulin therapy. Retinopathy was graded on fundus photographs using the Wisconsin scale; HbA_{1c}, IGF-1, and hemostatic variables were measured.

RESULTS — During the observation period of 2 years, 23 patients progressed in the retinopathy scale; 8 progressed ≥3 levels. After 2 years of insulin treatment, HbA_{1c} and IGF-1 were significantly lower than at baseline, whereas the hemostatic variables had not changed significantly. Progression of retinopathy ≥3 levels was related to the degree of HbA_{1c} reduction, the duration of diabetes, a higher prothrombin fragment 1+2 levels (F1+2), but not to other hemostatic variables or IGF-1. The relative risk for progression ≥3 levels was 2.6 when HbA_{1c} had been reduced ≥3 percent units (95% CI 1.1–6.1)

CONCLUSIONS — The magnitude of improvement of HbA_{1c} by the institution of insulin treatment over a 2-year period may be associated with progression of retinopathy in patients with type 2 diabetes.

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Improvement in glycemic control retards the development and progression of retinopathy in both type 1 and type 2 diabetes (1–3). There have, however, been reports on worsening of retinopathy after rapidly improved glycemic control (1,4–12). Such progression of retinopathy has in some reports been temporary (1,7), whereas in others progression has proceeded to retinopathy requiring treatment (5,8–12) and to blindness (9). A relationship between the degree of improvement in HbA_{1c} and early worsening of retinopathy was confirmed in a recent report from the Diabetes Control and Complications Trial (DCCT) (13).

The mechanism behind this so-called normoglycemic re-entry phenomenon has yet to be defined. Growth hormone (GH) is a counterregulating hormone, and its secretion is increased in poorly controlled diabetes (14). Elevated GH levels have also been associated with activation of the fibrinolytic and coagulation systems (15) and a development of retinopathy in type 2 diabetic patients (16), and in patients with mild carbohydrate intolerance (17). IGF-1, and not GH in itself, is responsible for GH's biological activity (14). Some studies suggest that IGF-1 is related to the development of retinopathy (18–20). A variety of hemostatic abnormalities have been demonstrated in diabetic patients, resulting in a state of hypercoagulability (21,22). Hyperglycemia probably determines the onset of these abnormalities, which may play a crucial role in the pathogenesis of diabetic vascular disease.

In patients with type 2 diabetes, the glycemic control gradually deteriorates, and many patients require insulin treatment after 10 years of diabetes (3,23). The aims of the study were to examine the progression of retinopathy in type 2 diabetic patients who changed treatment from oral agents to insulin and to assess whether progression was related to improvement in glycemic control and to changes in IGF-1 and hemostatic variables.

RESEARCH DESIGN AND METHODS

Patients

Forty-five consecutive orally treated type 2 diabetic patients aged <75 years were included in the study when they were referred to the Diabetic Day Care Unit at the Department of Endocrinology in Malmö, Sweden, to be changed to insulin therapy (Table 1). Patients suffering from severe concomitant diseases preventing a follow-up, patients with severe nonproliferative retinopathy or proliferative retinopathy, and patients with cataracts, making retinal photography impossible, were excluded from the study.

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Abbreviations: APC, activated protein C; APCr, APC ratio; F1+2, prothrombin fragment 1+2; DCCT, Diabetes Control and Complications Trial; DR, diabetic retinopathy; ELISA, enzyme-linked immunosorbent assay; GH, growth hormone; Log MAR, logarithm of the minimal angle of resolution; NPDR, nonproliferative diabetic retinopathy; PAI-1, plasminogen activator inhibitor type 1; PDR, proliferative diabetic retinopathy; vWF, von Willebrand factor.

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

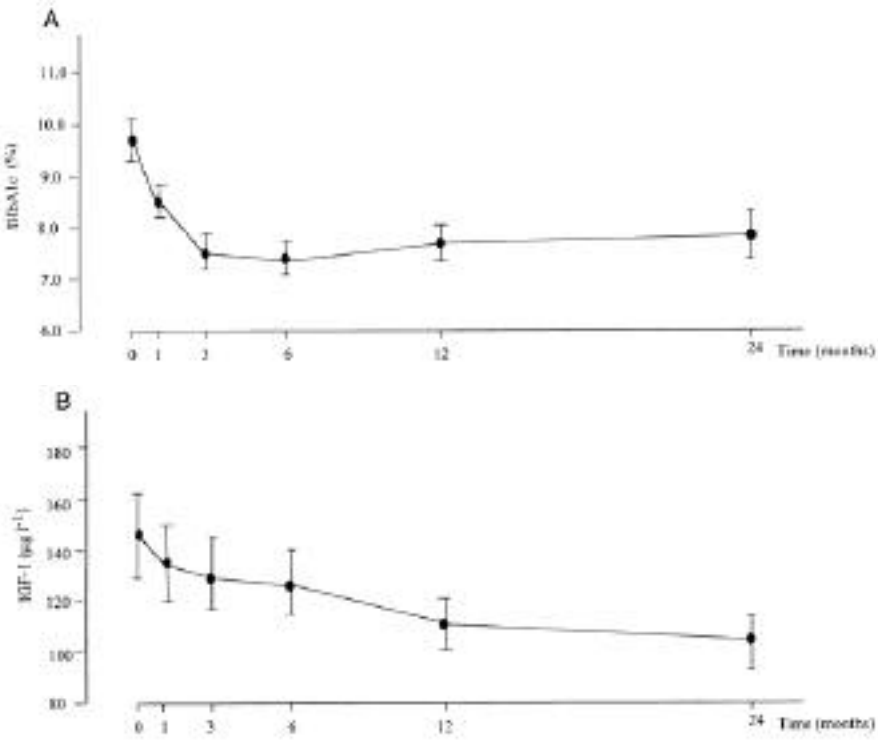

Figure 1—*HbA_{1c} (A) and IGF-1 (B) in the whole study group at baseline and after 1, 3, 6, 12, and 24 months (mean, 95% CI).*

The patients were examined at baseline, before the introduction of insulin therapy, and at follow-up after 1, 3, 6, 12, and 24 months. Fundus photography including visual acuity testing was performed, and blood samples for HbA_{1c}, IGF-1, and the hemostatic variables were collected at all examinations. At baseline, a simple neurological examination was performed; tendon reflexes and vibratory sensory thresholds by a biothesiometer at the ankle region (Bio-thesiometer, Bio.Medical Instrument, Newbury, OH) were assessed. Patients were asked about smoking and the current use of antihypertensive treatment. The blood pressure was measured in a supine position after a short rest. Hypertension was considered present if the patient used antihypertensive drugs or had a blood pressure >160/95 mmHg. Urinary albumin excretion was measured at baseline and then after 12 and 24 months. The study was approved by the Ethics Committee at the University of Lund, Sweden. Informed consent was obtained from all participating patients.

Analytical methods

BMI was calculated as kilograms per meter squared. Fasting plasma C-peptide was measured by radioimmunoassay (24) and HbA_{1c} by ion exchange chromatography (25); the reference interval was <5.3%.

Urinary albumin was measured by rate immunonephelometry (Beckman ARRAY instrument; Beckman, Fullerton, CA). Microalbuminuria was defined as an albumin excretion of 20–200 µg/min. IGF-1 was measured by an immunometric assay kit (IGF-1 IRMA) from Nichols Institute Diagnostics (San Juan Capistrano, CA) according to the instructions of the manufacturer. The intra-assay and interassay coefficients of variation were 5 and 15% respectively, at the level 60 g/l. The detection limit was 6 g/l.

For coagulation studies, 4.5 ml of blood was collected by venipuncture using evacuated glass tubes containing 0.5 ml of 0.13 mol/l sodium citrate. Plasma was recovered by centrifugation at 2,000*g* for 20 min and stored at −70°C. Resistance to activated protein C (APC) was tested with two different methods (22), without or, in a modified method, with predilution of sample plasma in factor V-deficient plasma. The modified APC resistance method, used only in the 2-year examination, is less sensitive to differences in plasma handling and clotting factor content except for factor V and has a near 100% specificity and sensitivity for the factor V:Q^{506} allele, which is the mutation responsible for APC resistance with low APC ratio (APCr). A cutoff level of 2.4 for the original method and of 1.9 for the modified method has been established as the level that best discriminates between normal individuals and those with the factor V gene mutation. Preparation of genomic DNA from EDTA blood and determination of the factor V:Q^{506} mutation was performed (26) as previously described. Genotyping of factor V was only performed in individuals with APCr below the cutoff level 1.9, as determined by the modified test. Factor VII:C and factor VIII:C were tested with chromogenic substrate methods (Coa-set Factor VII, Kabi Diagnostica, Mölndal, Sweden, and Coatest Factor VIII, Chromogenix AB, Mölndal, Sweden, respectively). Von Willebrand factor (vWF) antigen was measured by enzyme-linked immunosorbent assay (ELISA) (27), and fibrinogen with a turbidometric assay based on activation using *Bothrops atrox* venom (Fibrinogen Kinetic, Boehringer Mannheim, Mannheim, Germany). Prothrombin fragment 1+2 (F1+2) was measured by ELISA (Behring, Marburg, Germany) and plasminogen activator inhibitor type 1 (PAI-1) by the Spectrolyse/pL PAI kit (Biopool, Umeå, Sweden).

Fundus photography and visual acuity

Stereo color fundus photographs of the seven standard fields were taken during pharmacological mydriasis, at an angle of 30°, with a Topcon TRC-50 VT fundus camera (Tokyo Optical, Tokyo). The alternative classification of the Wisconsin Epidemiologic Study of Diabetic Retinopathy was used to define the retinopathy level (28,29). This classification provides an overall retinopathy scale. Level 10 represents no retinopathy; levels 21 through 51 represent nonproliferative diabetic retinopathy (NPDR) of increasing severity; and levels 60+ represent all forms of proliferative diabetic retinopathy (PDR), with and without laser treatment. The patient's retinopathy level was derived by giving the eye with the higher level a greater weight. This scheme provides an 11-step scale: 10, 21/10, 21/21, 31/ <31, 31/31, 41/ <41, 41/41, 51/ <51, 51/51, 60+/<60+, 60+/60+ (29). Thereafter, the retinopathy levels were divided into four groups; no diabetic retinopathy (DR) (level 10), mild retinopathy (levels 21/10–31/31), moderate NPDR (levels 41/ <41–51/ <51),

Table 1—*Clinical characteristics at entry of patients with progression of retinopathy ≤2 and ≥3 levels*

	Progression ≤2 levels	Progression ≥3 levels	*P* value
n (%)	35 (81)	8 (19)	—
Age at first eye examination (years)	61.4 ± 7.6	64 ± 6.3	0.37
Sex			
M	18 (51)	5 (63)	0.57
F	17 (49)	3 (38)	
Duration of diabetes at entry (years)	8.7 ± 5.1	13.5 ± 4.1	0.02
BMI (kg/m^2)	27.1 ± 4.1	26.0 ± 4.6	0.50
Serum creatinine (μmol/l)	92 ± 46	99 ± 23	0.69
C-peptide (nmol/l)	0.75 ± 0.43	0.76 ± 0.44	0.93
Anticoagulation treatment			
None	27 (77)	5 (63)*	0.38
Salicylate	6 (17)	3 (38)	
Waran	2 (6)		
Smokers	6 (17)	2 (25)	0.61
Hypertension	22 (63)	4 (50)	0.69
Degree of retinopathy at entry			
None (level 10/10)	18 (51)	3 (38)*	0.41
Mild NPDR (level 21/10–31/31)	14 (40)	3 (38)	
Moderate NPDR (level 41<41–51/<51)	3 (9)	2 (25)	
Macular edema or treatment	3 (9)	3 (38)	0.07
Visual acuity best eye (Log MAR)	0.02 (0.5 to −0.18)	−0.003 (0.44 to −0.14)	0.75
Albuminuria at entry (≥20 μg/min)	10 (27)	4 (50)	0.11
Peripheral pulses absent	4 (11)	2 (25)	0.32
Achilles reflexes absent	11 (31)	5 (63)	0.10
Patellar reflexes absent	2 (6)	3 (38)	0.01
Vibration sense, worse leg	25 (10 to 50)	38 (18 to 50)	0.03

Data are means ± SD, *n* (%), or medians (ranges). *Percentages do not add up to 100 because of rounding.

and severe NPDR or PDR (levels 51/51–60+/60+).

Macular edema was defined as the presence of hard exudates and/or retinal thickening within one disc diameter of the center of the macula in at least one eye. Macular treatment was defined as focal laser treatment in at least one eye.

Photographs were viewed against light boards using Donaldson's stereo viewer (5× magnification) (G J Davco, Holbrook, MA) and assessed by an independent grader with extensive experience with this classification. The grader had no knowledge of the patients' clinical data. No patients were excluded from the study because of media opacities.

For the testing of visual acuity, the Early Treatment Diabetic Retinopathy Study charts (logarithm of the minimal angle of resolution [Log MAR]) (30) were used. The visual acuity of the best eye was reported.

Main outcome measures

Change of retinopathy level from first to last examination was related to change in HbA_{1c}, hemostatic variables, and IGF-1. The group with progression ≥3 levels was compared with the group with progression ≤2 levels. A progression of ≥3 levels was considered a clinically significant deterioration.

Statistical analysis

The study was designed with a statistical power of 80% for detecting a relationship between lowered HbA_{1c} and a three-step progression of retinopathy given a significance level of 0.05. The statistical analyses were performed with the statistics program SPSS for Windows. Friedman's and Wilcoxon's signed-rank tests were used to analyze changes between repeated measurements. The area under the curve was estimated for each patient and variable measured serially (31). When analyzing differences between variables in two independent groups, the Student's *t* test, the Mann-Whitney *U* test, and the χ^2 test were used. Multiple regression analysis was used to assess the influence of HbA_{1c}, IGF-1, and retinopathy at baseline on progression ≥3 levels and was performed with all variables entered in one single step. Significance was considered for $P \leq 0.05$. Results were given with their 95% CI. Data were presented as means ± SD.

RESULTS — Two patients died during the 2-year follow-up; 43 completed the study and remained on insulin treatment after 2 years. When comparing baseline values with average values during follow-up (area under the curve divided by 24 months), HbA_{1c} and IGF-1 were significantly reduced (Fig. 1*A* and *B*) ($P < 0.001$), but the hemostatic parameters were unchanged. The decrease in HbA_{1c} levels occurred rapidly (within 3 months) after the institution of insulin therapy, whereas IGF-1 levels decreased gradually (maximal after 24 months).

At baseline, 21 patients showed no DR, 17 had mild, and 5 patients had moderate DR (Tables 1 and 2). In 18 patients, the level of retinopathy remained unchanged; in 2, it was reduced by 1 level; in 15, it progressed by 1–2 levels; and in 8, it progressed by ≥3 levels. The progression affected all levels of retinopathy (i.e., from no retinopathy to

Table 2—*Distribution of retinopathy in patients at baseline and follow-up*

	At baseline		
At follow-up	No DR (*n* = 21)	Mild NPDR (*n* = 17)	Moderate NPDR (*n* = 5)
No DR (level 10)	15	2	0
Mild NPDR (levels 21/10–31/31)	5	9	0
Moderate NPDR (levels 41/<41–51/<51)	1	5	2
Severe NPDR or PDR (levels 51/51–60+/60+)	0	1	3

Data are *n*.

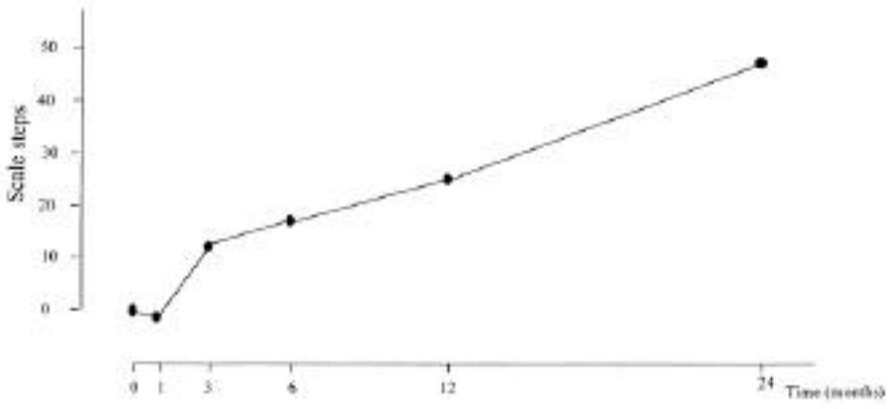

Figure 2—*Increase in the retinopathy scale (scale steps) in all patients during follow-up (i.e., all individual values have been summed up).*

moderate NPDR [Table 2]) and seemed to continuously increase during the 2-year study period: the increase in scale steps was 23, during both the 1st and the 2nd year in the whole patient group (Fig. 2).

The group with progression by ≥3 levels (n = 8) was compared with the group with progression by ≤2 levels (n = 35). Patients with progression by ≥3 levels had a longer duration of diabetes and a more pronounced degree of neuropathy than those with less progression (Table 1). There was no relationship between the degree of retinopathy at entry and progression by ≥3 levels. Visual acuity of the best eye at baseline and at follow-up was similar in both progression groups and deteriorated slightly during the study period. The number of patients with macular edema and/or treatment did not change during follow-up. There were no significant differences in urinary albumin excretion between the two progression groups, and the insulin doses per kilogram body weight were equal. One patient (nonprogressor) underwent cataract surgery during follow-up, and one patient with progression by ≥3 levels had vitrectomy performed because of recurrent vitreous hemorrhages.

Patients who progressed by ≥3 levels had a significantly lower APCr (P = 0.04) at entry and a higher average F1+2 (P = 0.01) during follow-up compared with those with progression by ≤2 levels (Table 3). The modified APC resistance test showed reduced ratios (≤1.9) in two (25%) of those who progressed by 3 levels, and in three (9%) of the group with less progression (P = 0.22). Genotyping showed that all were carriers of the FV:Q^{506} allele.

During the follow-up, the minimum HbA_{1c} value was determined, and the difference between the baseline and the minimum value was calculated. Patients who progressed by ≥3 levels had a significantly greater lowering of HbA_{1c} during the follow-up than those with less progression; 3.1 and 2.0% units, respectively (P = 0.05) (Fig. 3). The time to the minimum HbA_{1c} value was significantly longer in the progression group than in the group with less progression, 16.1 versus 8.1 months (P = 0.02).

Duration of diabetes (per year) and a lower average HbA_{1c} (per percent) were significantly associated with retinopathy progression ≥3 levels in a logistic regression analysis (Table 4). The level of HbA_{1c} at baseline was of borderline significance with regard to progression. There was no statistically significant relationship between decrements in IGF-1 and progression of retinopathy.

CONCLUSIONS — The finding of a relationship between reductions of HbA_{1c} levels and the progression of retinopathy is in agreement with results from previous studies in both type 1 diabetes (4–9,13) and type 2 diabetes (10,11). The 7-year follow-up of the Oslo study reported that retinopathy progression was associated with HbA_{1c} at baseline, changes of HbA_{1c} from baseline to the average level during the 7 years, duration of diabetes, and retinopathy at the start (7). The DCCT confirmed these findings (13). Change in HbA_{1c} level between the screening and month 4–5 and duration of diabetes were shown to be the dominant risk factors for early worsening in the DCCT. There was no evidence in the DCCT to suggest that a gradual reduction of glycemia might be associated with a lesser risk of progression. This is in keeping with our findings. Indeed, in our study, the time period to the lowest HbA_{1c} value was longer in those who progressed by ≥3 levels than in those with less progression.

Table 3—*HbA_{1c}, IGF-1, and hemostatic parameters at entry and follow-up*

	Progression ≤2 levels	Progression ≥3 levels	*P* value
n (%)	35 (81)	8 (19)	—
HbA_{1c} at entry (%)	9.7 ± 1.2	10.1 ± 1.1	0.43
Average HbA_{1c}*	7.9 ± 0.8	7.4 ± 1.2	0.17
IGF-1 at entry (μg/l)	145 ± 52	157 ± 54	0.33
Average IGF-1*	117 ± 35	124 ± 47	0.62
Fibrinogen at entry (2.0–4.3 g/l)	3.9 ± 1.0	4.1 ± 0.6	0.72
Average fibrinogen*	4.0 ± 1.0	4.5 ± 0.8	0.18
PAI-1 at entry (0–16 U/ml)	18.8 ± 14.9	17.0 ± 11.5	0.71
Average PAI-1*	17.0 ± 11.7	14.7 ± 7.9	0.60
vWF at entry (0.5–2.0 U/ml)	1.7 ± 0.7	1.5 ± 0.4	0.58
Average vWF*	1.6 ± 0.6	1.9 ± 0.5	0.29
F VII:C at entry (60–160%)	105 ± 32	123 ± 36	0.12
Average F VII:C*	110 ± 25	114 ± 32	0.46
F VIII:C at entry (0.5–2.1 U/ml)	2.3 ± 0.7	2.5 ± 0.7	0.36
Average F VIII:C*	2.1 ± 0.6	2.5 ± 0.8	0.12
F1+2 at entry (0.44–1.1 nmol/l)	1.6 ± 0.7	1.7 ± 0.6	0.57
Average F1+2*	1.5 ± 0.7	2.3 ± 1.0	0.01†
APCr at entry (>2.4)	3.2 ± 0.5	2.7 ± 0.5	0.04†
Average APCr*	3.2 ± 0.6	2.9 ± 0.6	0.18

Data are means ± SD. Normal ranges for coagulation data are given in parentheses. *Area under the curve during the study period/24 months. †$P < 0.05$. C, clotting activity.

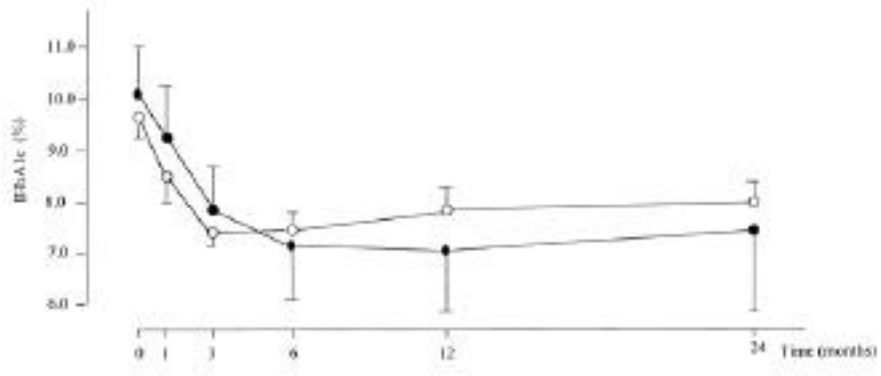

Figure 3—*HbA_{1c} in the two progression groups, ≥3 levels (●) and ≤2 levels (○) (mean, 95% CI).*

The risk for early worsening in our study, as in the DCCT, occurred in all subgroups of retinopathy.

No relationship was established between the levels of IGF-1 and the incidence and progression of retinopathy. Prior studies have shown conflicting data. Hyer et al. (32) reported a rise in IGF-1 in type 1 diabetes after subcutaneous insulin infusion—however, unrelated to the progression of retinopathy—and Wang et al. (33) found no relationship between the baseline level of IGF-1 and incidence or progression of retinopathy during 6 years. On the other hand, a transient rise in serum IGF-I has been found at the time of active PDR (34) and also preceding the progression of retinopathy in type 1 diabetes and Mauriac's syndrome (19,20). Our study does not support the concept that increased levels of circulating IGF are involved in the pathogenesis of the progression of DR in type 2 diabetes.

IGF-1 was significantly reduced during follow-up in our patients with no difference between those who progressed and those who did not. In a cross-sectional study, IGF-1 levels were lower in insulin-treated type 2 diabetic patients than in those on other therapies (35). This fits well with our observation of decrements in IGF-1 levels during insulin therapy.

In the present study, there were no overall changes in hemostatic variables during follow-up. A possible relationship between the progression of retinopathy and coagulation disorders has not, to our knowledge, been studied prospectively before. F1+2 was significantly increased in those who progressed by ≥3 levels in the retinopathy scale, indicating thrombin activation in those with retinopathy progression (36). A similar thrombin activation has been related to nephropathy in type 2 diabetic patients (37). A lowered APCr at entry among those who progressed by ≥3 levels could be explained by increased factor VIII activity causing an acquired APC resistance. APC inhibits the plasma coagulation by inactivation of factor VIII and V, and in our study there was a tendency toward increased factor VIII:C levels among those who progressed most in retinopathy. Five patients (12%) were found to be APC resistant and carriers of the factor V:Q^{506} allele, a frequency in the same range as previously reported in the diabetic and healthy population (22).

Table 4—*Logistic regression analysis of relating independent variables of progression ≥3 levels*

	Relative risk	95% CI	*P* value
Duration of diabetes (years)	1.37	1.0–1.9	0.05
HbA_{1c} at entry (per %)	4.1	1.0–17	0.06
Average HbA_{1c}* (%)	0.20	0.04–0.93	0.04
IGF-1 at entry (per 10 U)	1.20	0.8–1.8	0.36
Average IGF-1* (per 10 U)	0.69	0.4–1.2	0.21
Mild or moderate retinopathy at entry versus no DR	2.3	0.7–7	0.17

n = 8. *Area under the curve during the study period/24 months.

In conclusion, in previously orally treated type 2 diabetic patients, insulin treatment improved glycemic control for ≥2 years. A substantial HbA_{1c} reduction was, however, associated with an increased risk for progression of retinopathy. Patients with long duration of diabetes, neuropathy, and poor control appeared to be at greatest risk for progression, which was related to the magnitude, and not the rapidity, of the lowering of HbA_{1c}. The clinical implications of the present findings may be that insulin should be introduced at an earlier stage in the treatment of type 2 diabetes and that retinal status should be evaluated before glycemic control is intensified and then regularly monitored, especially in those individuals at greatest risk for progression.

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