Markers of Insulin Resistance Are Strong Risk Factors for Retinopathy Incidence in Type 1 Diabetes

The EURODIAB Prospective Complications Study

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OBJECTIVE — To determine the incidence of retinopathy and the relative importance of its risk factors in type 1 diabetes.

RESEARCH DESIGN AND METHODS — This is a 7.3-year follow-up of 764 of 1,215 (63%) people with type 1 diabetes across Europe, aged 15–60 years at baseline with no retinopathy (the EURODIAB Prospective Complications Study). Retinal photographs were taken at baseline and follow-up and risk factors were assessed to a standard protocol.

RESULTS — Retinopathy incidence was 56% (429/764, 95% CI 52–59%). Key risk factors included diabetes duration and glycemic control. We found no evidence of a threshold effect for HbA_{1c} on retinopathy incidence. Univariate associations were observed between incidence and albumin excretion rate, cholesterol, triglyceride, fibrinogen, von Willebrand factor, γ-glutamyltransferase, waist-to-hip ratio, and insulin dose. No associations were observed for blood pressure, cardiovascular disease, or smoking. Independent risk factors, as assessed by standardized regression effects, were HbA_{1c} (1.93, $P = 0.0001$), duration (1.32, $P = 0.008$), waist-to-hip ratio (1.32, $P = 0.01$), and fasting triglyceride (1.24, $P = 0.04$).

CONCLUSIONS — Retinopathy incidence in type 1 diabetes remains high. Key risk factors include diabetes duration and glycemic control, with no evidence of a threshold for the latter. Other independent risk factors, such as waist-to-hip ratio and triglyceride levels, both markers of insulin resistance, were strongly related to incidence.

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Retinopathy is the most common complication of type 1 diabetes, affecting 70–100% of all patients (1–3). The only proven preventive measure is strict glycemic control (4), which alone is not wholly satisfactory because retinopathy still develops in ~12% of intensively treated diabetic patients, and the institution of such control places great demands on both patients and health care systems, questioning its true practicality (5).

A review concluded that there was a threshold of glycated hemoglobin at which the risk of retinopathy could not be reduced further (6). The implications of such a conclusion, if true, would be far reaching in terms of health care guidelines and should be confirmed.

Thus, because tight control cannot abolish the risk of retinopathy, there is a continuing need to develop new interventions, and the potential existence of a threshold effect for glycemic control emphasizes the need to target the use of currently existing therapies. The development of new interventions is limited by a lack of knowledge about the relative importance of putative risk factors for retinopathy and how they relate to each other. Furthermore, the focus has shifted from the treatment of late-stage disease (i.e., proliferative retinopathy) to the prevention of incident retinopathy, and we cannot assume that risk factors for incidence are similar to those for progression. Therefore, we examined the risk and relative importance of risk factors for incident retinopathy in the EURODIAB Prospective Complications Study (PCS), a European-wide cohort study of individuals with type 1 diabetes (7).

RESEARCH DESIGN AND METHODS

— Baseline investigations were performed between 1989 and 1991 on 3,250 patients with type 1 diabetes, which was defined as a diagnosis made before the age of 36 years with a need for continuous insulin therapy within a year of diagnosis (7). Patients were recruited from 31 centers in 16 European countries and were aged between 15 and 60 years. These patients were invited back for reexamination on average between 6 and 8 years after baseline investigations. Women who were pregnant at the time of study were examined after delivery.

At both investigations, complication status was measured using the same standardized protocol as at baseline (7). Local

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Abbreviations: DCCT, Diabetes Control and Complications Trial; PCS, Prospective Complications Study; SRE, standardized regression estimate; vWF, von Willebrand factor.

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

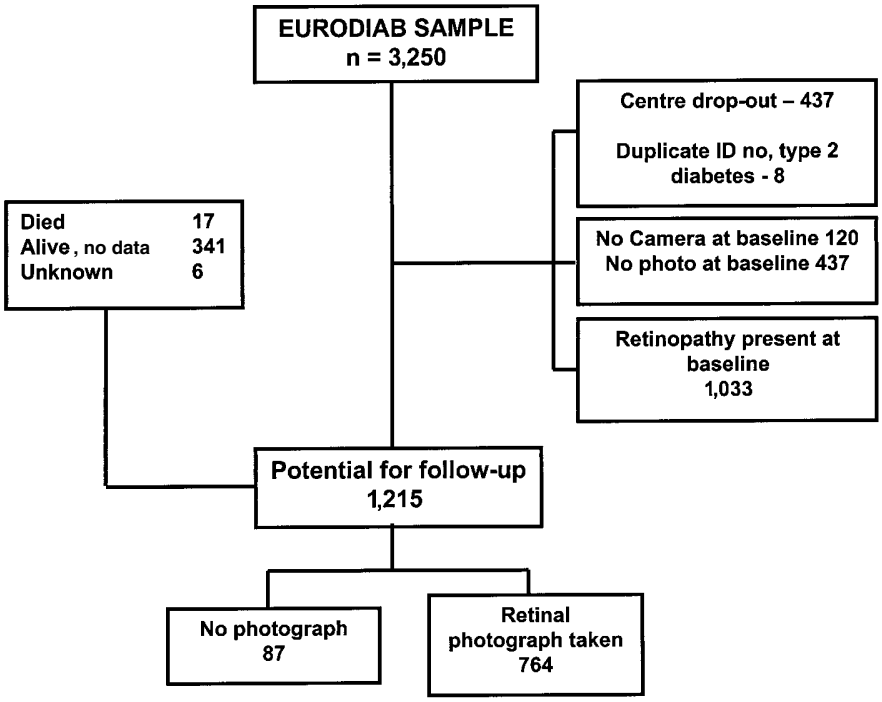

Figure 1—*Flow diagram for sample used in incidence of retinopathy analysis.*

HbA_{1c} measurements for the previous 2 years (a maximum of eight) were also recorded. Anthropometric measures were taken and resting blood pressure recorded (8). Retinal photographs were taken according to the EURODIAB protocol (9). This included a 45° or 50° macular and nasal field for each eye. Grading was performed by the retinopathy grading center at the Hammersmith Hospital of Imperial College (London) by observers masked to all information about the patient except an identification number (9). The same grading center was used for both baseline and follow-up investigations. The grading system has been described in detail previously (9), but in brief, retinal lesions are compared with standard photographs and patients assigned to one level out of a scale of six. We have demonstrated high validity when compared against the standard seven-field stereophotograph protocol (9).

Aliquots of baseline blood samples, fasting if possible, were sent to central laboratories. Measurements included total cholesterol, HDL cholesterol, and triglyceride (10–12). LDL cholesterol was calculated (13). The reference range for HbA_{1c} was 2.9–4.8% (14). Where possible, a sample was sent locally for measuring HbA_{1c}. Fibrinogen and von Willebrand factor (vWF) were also measured (15). γ-Glutamyltransferase levels were determined in plasma by a kinetic colorimetric method with L-γ-3-carboxyl-4-nitranilide and glycylglycine as substrates (Uni-Kit 2; Roche) using the Cobas-Bio centrifugal analyzer. Urinary albumin was measured on an aliquot from one 24-h collection (16).

Baseline cardiovascular disease was defined as a past history of a myocardial infarction, angina, or coronary artery bypass graft or stroke or major Q waves on an electrocardiogram (8).

Statistical analysis

Of the 3,250 patients recruited at baseline, 2,248 had usable photographs. Of these, 1,215 had no retinopathy at baseline, and 764 (63%) provided follow-up photographs. Linear regression was performed by the center to compare the result of the local HbA_{1c} measured at the same time as the central HbA_{1c}, both from baseline. This provided a conversion formula for each center's local HbA_{1c} assay to the centrally measured assay. An average of all local HbA_{1c} measures for each individual was then calculated and converted to the central measure, as described above, to allow comparison of local measures across centers.

Also, to allow comparisons with the Diabetes Control and Complications Trial (DCCT), a formula was derived from a linear regression plot of measures of HbA_{1c} by using values from the central London laboratory against those determined by using the DCCT method. The formula is as follows: DCCT HbA_{1c} = 1.0289 × London HbA_{1c} + 1.5263.

Baseline characteristics were calculated using regression methods for continuous variables and simple proportions for categorical variables. In both instances, adjustment was made for confounders, when appropriate. A break point or threshold effect for the relationship between HbA_{1c} and retinopathy was tested by using a two-phase segmented weighted regression analysis, which fits two straight lines through a series of defined points (17). These points were calculated by logistic regression adjusted for diabetes duration. This segmented regression was compared with the line of best fit using weighted linear regression. Logistic regression was also used to test for a threshold effect (18).

Standardized regression effects were calculated by multiplying the β estimate from logistic regression models by the SD of that variable; here, all log-transformed variables were not back-transformed. This allows the direct comparison of the degree of importance of each variable in accounting for the risk of incidence of retinopathy. Multivariate models were restricted to the 460 of 764 individuals who had complete data on all included risk factors. The bulk of the missing data was due to the number of patients who did not have a fasting triglyceride value at baseline.

At first, all analyses were stratified by sex, because there were no appreciable differences in risk of retinopathy or risk factor relationships (combined data are presented).

RESULTS — Follow-up photographs were available from 63% (764/1,215) of the cohort who had no retinopathy at baseline (Fig. 1). Baseline distribution of risk factors did not differ significantly between those who did and did not have follow-up data apart from HbA_{1c}, which was significantly worse in those with no follow-up data (6.8 vs. 6.4%, $P = 0.001$).

The mean follow-up was 7.3 years. The cumulative incidence of retinopathy was 56% (95% CI 52–59%). Incidence peaked at between 10 and 20 years of baseline diabetes duration.

Risk factors for incidence (Table 1) included baseline duration and centrally measured HbA_{1c}. Local HbA_{1c} measured over the previous 2-year period and

Table 1—*Risk factors for incidence of retinopathy*

Risk factor	Incident case: Yes	Incident case: No	*P*
n	429	335	
Age (years)	29 ± 0.5	30 ± 0.4	0.3
Duration (years)	11 ± 0.3	9 ± 0.4	0.0002
Central HbA_{1c} (%)	6.9 ± 0.1	5.6 ± 0.1	0.0001
Local HbA_{1c} (%)*	6.7 ± 0.1	5.7 ± 0.1	0.0001
Systolic blood pressure (mmHg)	116 ± 0.7	115 ± 0.7	0.3
Diastolic blood pressure (mmHg)	74 ± 0.5	73 ± 0.6	0.6
AER (μg/min)	12 (6–19)	10 (6–14)	0.001
Cholesterol (mmol/l)	5.2 ± 0.05	5.0 ± 0.05	0.008
Fasting triglyceride (mmol/l)	0.94 (0.68–1.16)	0.80 (0.60–0.96)	0.0001
HDL cholesterol (mmol/l)	1.48 ± 0.02	1.54 ± 0.02	0.06
LDL cholesterol (mmol/l)	3.18 ± 0.06	3.07 ± 0.06	0.2
Fibrinogen (g/l)	3.19 ± 0.05	3.04 ± 0.06	0.05
vWF (U/ml)	1.23 ± 0.03	1.14 ± 0.03	0.04
γGT (U/l)	10.7 (7.5–14.0)	9.6 (7.0–12.5)	0.02
Height (cm)	170 ± 0.5	170 ± 0.5	0.4
Weight (kg)	67.7 ± 0.5	66.3 ± 0.6	0.1
Waist-to-hip ratio	0.87 ± 0.006	0.83 ± 0.007	0.0001
Current smokers (%)	32 ± 2	27 ± 2	0.2
Inject insulin >twice/day (%)	47 ± 3	50 ± 3	0.4
Insulin dose/weight (U/kg)	0.70 ± 0.01	0.65 ± 0.01	0.003
History of CVD (%)	5 ± 1	6 ± 1	0.4

Data are means ± SEM or means (25th–75th percentiles for log-transformed data). *Mean of previous 2 years worth of glycated hemoglobin, standardized to the central measurement. AER, albumin excretion rate; CVD, cardiovascular disease; γGT, γ-glutamyl transferase.

adjusted to the central London measurement was also predictive of subsequent incident retinopathy. Incidence of retinopathy was positively associated with HbA_{1c}, but we could detect no evidence of a significant break point or threshold effect in this relationship (Fig. 2).

Other significant risk factors included albumin excretion rate, total cholesterol, fasting triglyceride, fibrinogen, vWF, γ-glutamyltransferase, waist-to-hip ratio, and insulin dose per kilogram of body weight.

However, many risk factors may be confounded by diabetes duration and HbA_{1c}. Therefore, we adjusted all other risk factors for these, which attenuated or abolished many of the risk factor associations. The only risk factors that remained statistically significant were fasting triglyceride (0.90 vs. 0.83 mmol/l, $P = 0.04$), waist-to-hip ratio (0.86 vs. 0.83, $P = 0.001$), and locally measured HbA_{1c} (6.3 vs. 6.1%, $P = 0.03$). Interestingly, weight also became a significant risk factor (68.0 vs. 65.9 kg, $P = 0.02$). Diabetes duration, HbA_{1c}, waist-to-hip ratio, and fasting triglyceride remained significant predictors for retinopathy incidence when entered simultaneously into a logistic regression model (Table 2). The strongest influence on risk of retinopathy was glycemic control, with a standardized regression estimate (SRE) of 1.93. Diabetes duration, fasting triglyceride, and waist-to-hip ratio were then equally strong in predicting incidence of retinopathy. Sex-specific univariate analyses comfirmed that the impact of waist-to-hip ratio was similar in men (SRE 1.60, 95% CI 1.25–2.05) and women (SRE 1.21, 0.97–1.49). None of the other factors listed above, including locally measured HbA_{1c} (SRE 1.29, 0.93–1.81, $P = 0.1$), had any additional impact. There was no evidence of a significant interaction for any of these variables. The analyses for Table 2 were reperformed for all patients who had full data on diabetes duration, HbA_{1c}, and waist-to-hip ratio. The SREs were very similar, and the ranking of variables was identical to those presented here.

CONCLUSIONS — We demonstrate that the incidence of retinopathy in type 1 diabetes remains high, developing in 56% of patients over 7 years. This compares favorably with earlier work in which incidence was 59% over 4 years (19) and more recently 89% over 10 years (20). Incidence in the conventional treatment arm of the DCCT was ~30% over 7 years, which may reflect the selection of motivated patients with no other complications in the trial (4). While previous studies used seven-field stereo photographs as opposed to our two, we have previously shown that the EURODIAB system is highly valid and unlikely to miss lesions. Therefore, it is unlikely that we

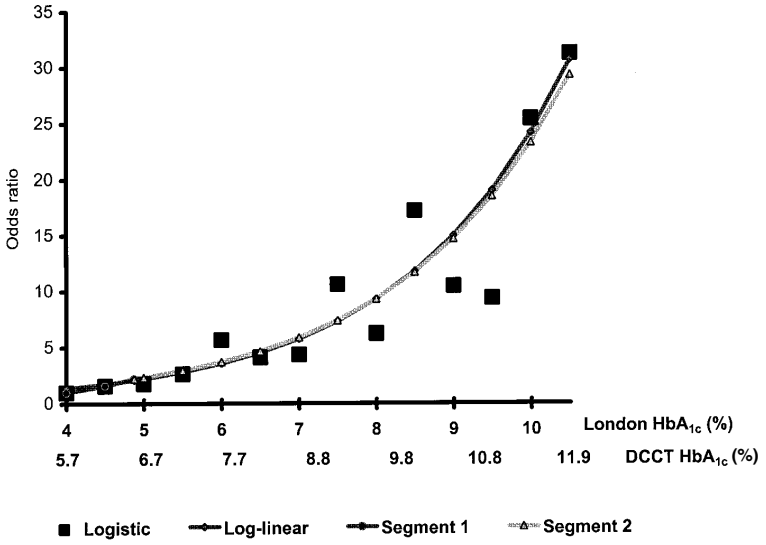

Figure 2—*Comparison of log-linear and break-point models for association between HbA_{1c} at baseline and incidence of retinopathy, adjusted for diabetes duration.*

Table 2—*SREs for relationship between key risk factors and incidence of retinopathy*

Risk factor	SRE (95% CI)	*P*
Duration	1.32 (1.07–1.61)	0.008
HbA_{1c}	1.93 (1.52–2.44)	0.0001
Fasting triglyceride*	1.24 (1.01–1.54)	0.04
Waist-to-hip ratio	1.32 (1.07–1.63)	0.01

*Analysis performed on log-transformed variables.

have seriously underestimated incidence for this reason alone.

The strongest risk factors for retinopathy include glycemic control and diabetes duration, as most other studies have shown (19–24). We also demonstrate that there is no glycemic threshold at which incidence of retinopathy escalates sharply, reflecting our cross-sectional findings (25) and other cohort studies (19,20,26). This is in contrast with a report of a glycemic threshold effect at 8%, based on a reevaluation of published data (6). This threshold was well within the range studied here, so discrepant findings cannot be ascribed to differences in the range of HbA_{1c}. However, the earlier analysis had not been subjected to formal statistical testing and did not distinguish between progression and incidence, although risk factor associations may vary at different stages of disease (6).

Strikingly, we observed strong associations with other risk factors that hitherto have either not been explored or have shown inconsistent relationships. Many relationships could be abolished once diabetes duration and glycemic control were taken into account. However, we observed a strong independent relationship between triglyceride and retinopathy. Others have been inconsistent and have variably adjusted for important confounders (21,22,27–29).

The strong impact of waist-to-hip ratio on retinopathy incidence has also not been properly examined before. This association was similar in men and women and could not be accounted for by glycemic control or by the association with adverse lipid profiles or obesity itself. It is also striking that when the standardized regression effects were compared, waist-to-hip ratio was second only to glycemic control in the importance of its impact on retinopathy incidence and was equivalent to diabetes duration. Both waist-to-hip ratio and triglyceride are key markers of the insulin resistance syndrome, which in turn is implicated in the development of albuminuria in type 1 diabetes (30). However, cross-sectional analyses only demonstrated a weak relationship between waist-to-hip ratio and proliferative retinopathy, which disappeared on adjustment for confounders (31). A study of insulin resistance and degree of retinopathy (again cross-sectional) showed no association (32) in contrast to patients with type 2 diabetes (33).

We could not demonstrate an association between blood pressure at baseline and retinopathy risk. This is consistent with some (22,28,34) but not all earlier studies, which show a weak relationship between blood pressure and retinopathy cross-sectionally (35) or with incidence (23), which can be accounted for at least in part by confounding with either diabetes duration or glycemic control (23). A number of features of the EURODIAB study could account for our findings. First, stronger relationships appear to be present with progression rather than incidence (3) and illustrate the need to distinguish between these two. Second, mean blood pressures were relatively low at baseline in the EURODIAB study; a relationship may only be observable at higher levels or with a greater range. Finally, blood pressure was one of the few key risk factors measured locally, and the degree of variability due to the use of several observers may reduce the likelihood of observing a relationship. The other key risk factor measured locally is waist-to-hip ratio, which is usually measured with greater accuracy than blood pressure.

We demonstrated a modest but statistically significant association between fibrinogen, vWF, and retinopathy incidence, but this could be accounted for by duration and glycemic control. Others have previously demonstrated no association between these factors and retinopathy (36) or have shown an association but not taken into account confounding (37–40).

The EURODIAB PCS is the largest cohort study of type 1 diabetes, with standardized measures of both risk factors and outcomes and may overcome limitations of previous studies, which have often produced conflicting findings. While there was inevitably loss to follow-up, apart from HbA_{1c}, there were no differences in risk factors between those individuals who were and were not followed up. Further, it would be hard to hypothesize a situation in which a risk factor, such as triglyceride, was positively related to retinopathy risk in those attending for follow-up and negatively related in those who did not attend. Of course our findings may only be relevant to European populations and extrapolations should be performed with caution.

Our findings have important implications for further research and interventions. The striking associations between triglyceride and waist-to-hip ratio, independent of glycemic control and not reflected by other features of dyslipidemia and obesity, indicate that there is some special unifying feature of these factors to account for their relationship with retinopathy. The most likely candidate is insulin resistance, and this association requires further evaluation in observational studies. If true, interventions that improve insulin resistance may also reduce the risk of retinopathy. Previous studies of lipid lowering have been disappointing but may be due to the use of less efficient lipid-lowering medication in the past than is available now (41,42). Many of these studies were in type 2 diabetes, in which the association between lipids and retinopathy may not be the same as in type 1 diabetes. Further, the sample sizes of these early studies were relatively modest and may have been underpowered. More recently, a small ($n = 6$) uncontrolled study in type 1 diabetic patients showed regression of retinal lesions in response to lipid-lowering therapy, indicating that studying the effect of newer therapies may be valuable (43).

In conclusion, we demonstrate that the incidence of retinopathy remains high, and glycemic control was the strongest risk factor. We emphasize that there appears to be no glycemic threshold for retinopathy incidence, supporting guidelines for tight glycemic control. Despite the poor association with blood pressure, clinical trials indicate that the use of antihypertensive therapy may be a promising therapeutic area. Indeed, studies indicate that antihypertensive therapy may have a more marked effect on retinopathy incidence than improvements in glycemic control and certainly much greater than would be anticipated from observational data, suggesting that the

beneficial effects of antihypertensive therapy on retinopathy go beyond blood pressure lowering (44,45). Our intriguing finding of an association with waist-to-hip ratio deserves further exploration. This is not simply an effect of obesity, because no clear association with weight was observed. Given the size of the standardized regression effect, which implies that the role of waist-to-hip ratio is second only to that of glycemic control, therapeutic interventions designed to reduce central obesity may be particularly successful.

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APPENDIX

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