Impaired glucose tolerance, Type II diabetes mellitus and carotid atherosclerosis: prospective results from the Bruneck Study

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Abstracts

Aims/hypothesis. Cardiovascular disease is a well-known severe complication of impaired glucose tolerance and Type II (non-insulin-dependent) diabetes mellitus. The independent contribution of glucose intolerance to cardiovascular disease and the underlying pathogenic mechanisms are still, however, not clear.
Methods. In this prospective population-based study, 826 subjects aged 40–79 years underwent high resolution duplex ultrasound examinations of carotid arteries and extensive clinical and laboratory screenings for potential vascular risk factors at baseline and 5 years later. The ultrasound protocol involved measurements of maximum axial diameter of atherosclerotic plaques, if any, in common and internal carotid arteries on both sides and enable differentiation of two main stages in carotid artery disease, termed early non-stenotic and advanced stenotic atherosclerosis. Intima-media thickness was assessed at the follow-up examination.
Results. Type II diabetes and, to a lesser extent, impaired glucose tolerance were found to be statistically significant risk predictors of 5-year changes in carotid atherosclerosis. These associations were in part independent of other vascular risk factors typically clustering with glucose intolerance. Both impaired glucose tolerance and Type II diabetes mellitus were not independently related to early non-stenotic atherosclerosis. In contrast, Type II diabetes mellitus was the strongest single risk predictor of advanced stenotic atherosclerosis [odds ratio 5.0 (95% confidence intervals 2.3–11.1)] and impaired glucose tolerance was of relevance as well [odds ratio 2.8 (1.2–6.4)] ($p < 0.001$).
Conclusion/interpretation. Impaired glucose tolerance and, to a greater extent, Type II diabetes were strong independent predictors of advanced carotid atherosclerosis in our prospective population-based study. [Diabetologia (2000) 43: 156–164]

Keywords Impaired glucose tolerance, Type II diabetes mellitus, atherosclerosis, carotid arteries, cardiovascular risk factors.

Atherosclerotic vascular diseases such as myocardial infarction, stroke and lower extremity arteriopathy are well-known severe complications of impaired glucose tolerance (IGT) and Type II (non-insulin-dependent) diabetes mellitus [1–7] and account for up to 60–70% of deaths in diabetic subjects [8]. Impaired glucose tolerance and Type II diabetic patients often have, however, multiple metabolic, haemodynamic and haemocoagulative defects such as high triglycerides and low HDL cholesterol, high blood pressure and high fibrinogen [9–15], which are cardiovascular risk factors in themselves [16]. These defects can precede the development of glucose intolerance by several years [17, 18]. Thus, the independent contribution of glucose intolerance and its bio-

chemical hallmark, i.e. hyperglycaemia, to the clinical manifestations of atherosclerosis is still disputed [19–22].

The current prospective population-based study was designed to examine the role of IGT and Type II diabetes in carotid atherosclerosis. Differential effects of IGT and Type II diabetes on various stages of atherosclerosis and on the aetiologic relevance of hyperglycaemia and metabolic abnormalities typically associated with glucose intolerance was a special focus.

Materials and methods

Subjects. The Bruneck Study is a prospective population-based survey on atherosclerosis and its risk factors carried out in Bruneck, a small town of about 13,500 people in north-eastern Italy. As previously reported [23], the baseline evaluation was carried out between July and November 1990 on subjects aged 40 to 79 years. Of the 4793 subjects of the appropriate age range, 125 men and 125 women from each of the fifth to the eighth decade of age were selected randomly for inclusion. Of the 1000 subjects invited to participate, 936 volunteered after the purposes and the modalities of the study had been carefully presented. Complete data were available in 919 subjects (91.9% of the sample extracted from the general population).

From July to October 1995 the cohort was re-evaluated [24]. Of the original population sample, 62 subjects had died, 1 had moved away and could not be traced and 30 declined to participate in the follow-up study. Thus, the latter was 96.5% complete among survivors ($n = 826$ out of 856). The protocol was approved by the ethics committee of the Verona City Hospital.

Clinical data. In each subject information about cigarette smoking, alcohol consumption, physical activity and socio-economic status was collected by a standardized questionnaire, as described previously [23, 24].

Subjects were regarded as diabetic if they were receiving insulin or oral hypoglycaemic agents or both or when their fasting plasma glucose exceeded 7.8 mmol/l or if their plasma glucose 2 h after oral glucose loading exceeded 11.1 mmol/l [25]. Impaired glucose tolerance was diagnosed when plasma glucose 2 h after glucose load exceeded 7.8 mmol/l [25]. Type II diabetes was diagnosed according to World Health Organisation (WHO) criteria [25].

Physical examination data. Weight (to the nearest 0.5 kg) and height (to the nearest 0.5 cm) were measured after an overnight fast and while the subjects were wearing only undergarments. Body mass index (BMI) was calculated as weight (kg) divided by height (m)^2. Waist and hip circumferences (to the nearest 0.5 cm) were measured by a plastic tape meter at the level of the umbilicus and of the greater trochanters, respectively and waist to hip ratio (WHR) was calculated.

Blood pressure was measured with a standard mercury sphygmomanometer on the left arm, after the subjects had been seated for at least 10 min. Mean values were determined from two independent measurements taken at intervals greater than 10 min. Hypertension was defined by a systolic blood pressure of 160 mmHg or higher or a diastolic blood pressure of 95 mmHg or higher or the use of anti-hypertensive treatment or more of them [26].

Laboratory data. In the morning, after an overnight fast, blood samples were collected for the measurement of glycated haemoglobin (HbA$_{1c}$), factor V Leiden mutation, plasma concentrations of glucose, serum concentrations of total, LDL and HDL cholesterol, triglycerides, apolipoproteins A1 and B, Lp(a), ferritin, fibrinogen and antithrombin III. Thereafter, a 75-g oral glucose load (OGTT) was given to all subjects, except for those with well established diabetes, and venous blood was collected after 2 h for the measurement of plasma glucose. Biochemical variables were assessed by standard and quality-controlled procedures [27–37].

Assessment of carotid atherosclerosis. At baseline and 5 years later carotid atherosclerosis (CA) was determined by high-resolution B-mode ultrasound (ATL UM8, Advanced Technology Laboratories, Bothel, Washington, D.C., USA), as described previously [23]. All measurements were carried out by a single specialist physician (J. Willeit), who had no information on the results of the first evaluation and was blind to all clinical and laboratory characteristics. Images were recorded on a videotape. Briefly, the scanning protocol included imaging of the common and internal carotid arteries in multiple longitudinal and transverse planes. The near and far walls at each of four well-defined imaging sites of both carotid arteries were explored: proximal common carotid artery: 15 to 30 mm proximal to the carotid bulb; distal common carotid artery: less than 15 mm to the carotid bulb; proximal internal carotid artery: carotid bulb and the initial 10 mm of the vessel; distal carotid artery: more than 10 mm above the flow divider [38]. Atherosclerotic lesions were defined by two ultrasound criteria: (1) wall surface (protrusion into the lumen or roughness of the arterial boundary) and (2) wall texture (echogenicity). In all eight regions of interest the maximum axial diameter of plaques, if any, was measured (in mm) and the measurements summed to obtain an atherosclerosis score [38]. Maximum intima-media thickness (IMT) was measured in 1995 at the far wall of the common carotid arteries. This surrogate of definite atherosclerosis was defined as the distance between the lumen-intima interface and the leading edge of the intima-adventitia interface. Values applied are averages of the IMT in the right and left common carotid arteries.

Based on the follow-up examination, we assessed 5-year changes in the vascular status. In particular, we evaluated the change in the atherosclerosis score during follow-up. In addition, the scanning protocol permitted a differentiation of two main stages of atherogenesis, termed (1) incident non-stenotic (early) atherosclerosis and (2) incident stenotic (advanced) atherosclerosis. The former was defined by the occurrence of new plaques in previously normal segments, the latter was defined by a relative increase in the maximum axial diameter of pre-existing plaques that exceeded the double measurement error of the method and a resulting narrowing of the lumen of more than 40% [39, 40].

Validity and reproducibility of the ultrasound method applied have been described in detail elsewhere [39, 40]. Maximum efforts were made to avoid drifts in ultrasound assessments between 1990 and 1995. These included special training courses, continuous analyses for time trends in ultrasound assessments and carrying out all scans with the same equipment and by the same experienced sonographer.

Statistical analysis. All analyses were carried out by SPSS-X and BMDP software (Los Angeles, Calif., USA). Statistical evaluation of the association between risk factors and the category of glucose tolerance was based on chi-squared test, analysis of variance and analysis of covariance with sex, age, level of physical activity, smoking habits, daily alcohol intake and so-

Table 1. Main clinical and biochemical features in the Bruneck Study cohort at baseline ($n = 826$)

	NGT ($n = 700$)	IGT ($n = 68$)	Diabetes ($n = 58$)	*p* value
	Unadjusted means/proportions			
Sex (% men)	51	40	48	NS
Age (years)	57	63[c]	67[c]	< 0.001
Smokers (%)	25	24	16	NS
Alcohol (g/day)	21.5	18.2	18.0	NS
Physical activity (score)	4.5	3.8[c]	3.7[c]	< 0.001
Social status (% high)	17	12	24	NS
	Adjusted means[a]			
Body mass index (kg/m^2)	24.8	26.6[c]	27.3[c]	< 0.001
Waist: hip ratio	0.93	0.93	0.96[c]	0.014
Fasting glucose (mmol/l)	5.36	5.82[c]	7.84[c]	< 0.001
HbA$_{1c}$ (%)	5.47	5.58[c]	6.86[c]	< 0.001
Total cholesterol (mmol/l)	5.84	5.86	5.89	NS
LDL cholesterol (mmol/l)	3.67	3.50	3.71	NS
HDL cholesterol (mmol/l)	1.48	1.33[c]	1.26[c]	< 0.001
Triglycerides (mmol/l)	1.47	2.12[c]	2.16[c]	< 0.001
Apoprotein A1 (mg/dl)	164	154[b]	155[b]	0.003
Apoprotein B (mg/dl)	123	132[b]	135[c]	0.011
Lp(a) (mg/dl)	16.3	14.1	14.9	NS
Ferritin (µg/l)	140	168	228	< 0.001
Fibrinogen (g/l)	2.67	2.74[c]	2.94[c]	0.001
Antithrombin III (%)	97	94	99	NS
Factor V mutation (%)	2.8	8.8[b]	3.5	0.038
Systolic blood pressure (mm Hg)	146	155[c]	159[c]	< 0.001
Diastolic blood pressure (mm Hg)	89	94[c]	94[c]	< 0.001
Hypertensive (%)	32	47[c]	52[c]	< 0.001

Sex, age, smoking, alcohol, physical activity and social status are unadjusted means or proportions. *P* values were derived from analysis of variance or chi-squared test.
[a] Means were adjusted for sex, age, smoking, alcohol consumption, physical activity and social status. *P* values were derived from analysis of covariance.

Pairwise comparisons of NGT vs IGT and NGT vs Type II diabetes were made with the Scheffe test which takes multiple comparisons into account (all pairs possible). [b] $P < 0.05$; [c] $p < 0.01$

cio-economic status used as covariates. Skewed variables were $\log_e$-transformed to improve the approximation to a gaussian distribution. Pair-wise comparisons of IGT compared with normal glucose tolerance (NGT) and Type II diabetes compared with NGT were made by the Scheffe test [41], which takes into account multiple comparisons (all pairs possible) and thus is a conservative procedure.

The relation of IGT or Type II diabetes with 5-year changes in carotid atherosclerosis and with IMT was examined by standard linear regression analyses. Multivariate adjustment was done by forced entry of a fixed set of covariates, including potential confounders (age, sex, social status), typical vascular risk factors (hypertension, LDL cholesterol, smoking) and components of the metabolic syndrome associated with IGT or Type II diabetes (triglycerides, HDL cholesterol, BMI, fibrinogen).

Risk profiles of early and advanced carotid atherosclerosis were assessed by stepwise logistic regression analyses. The hypothesis test was based on likelihood-ratio statistics [42]. Separate equations were fitted for subjects without carotid atherosclerosis at the 1990 baseline (incidence of non-stenotic atherosclerosis during follow-up: yes vs no) and in those with pre-existing lesions (incident stenotic atherosclerosis during follow-up: yes vs no). The p values for entry and removal of variables, were set at 0.10 and 0.15 [42, 43]. The analysis selected variables for inclusion among all those given in Table 1. Categories of glucose tolerance were modelled with indicator variables (set of dummy variables). Subjects with normal glucose tolerance were defined as the reference category (odds ratio = 1.0).

Results

The prevalence of IGT in the cohort was 8.2% ($n = 68$ out of 826) and that of Type II diabetes 7.0% ($n = 58$) at baseline. Table 1 reports clinical and biochemical features of subjects with NGT, IGT and Type II diabetes at baseline. As expected, IGT and, to a greater extent, Type II diabetic subjects had greater BMI and WHR, higher triglycerides, apolipoprotein B, lower HDL cholesterol and apolipoprotein A, and an increased proportion of hypertension. In addition these subjects also showed higher ferritin and fibrinogen concentrations.

Subjects with IGT and, to a greater extent, subjects with Type II diabetes had higher sex-adjusted and age-adjusted means atherosclerosis score in both 1990 ($p < 0.001$) and 1995 evaluations ($p < 0.001$). Also the mean changes in the atherosclerosis score during the follow-up were higher ($p < 0.001$) in IGT and Type II diabetes than in subjects with normal glucose tolerance (Fig. 1). Linear regression analysis showed that 5-year change in the atherosclerosis score was related to IGT and Type II diabetes also after adjustment for smoking, LDL and HDL cholesterol, triglycerides, fibrinogen, hypertension and
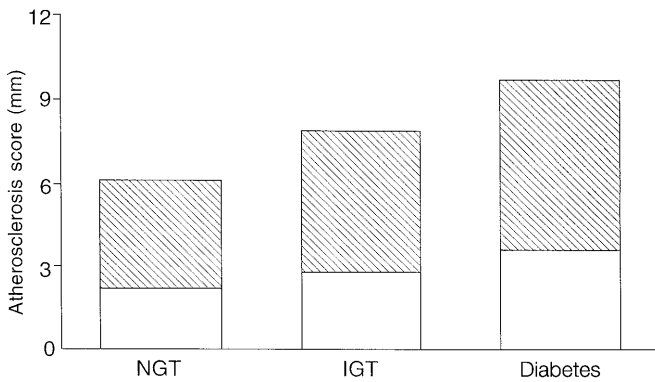
Fig. 1. Sex-adjusted and age-adjusted mean atherosclerosis scores in subjects with NGT, IGT and Type II diabetes mellitus from the Bruneck Study. The height of the bars represent mean scores at the end of follow-up. All differences (means in 1990, means in 1995 and mean changes in 1990–1995) were highly significant ($p < 0.001$), ☐ 1990, ▨ change in 1990–1995

Table 2. Linear regression analyses of 5-year change in the atherosclerosis score (1990 to 1995) on IGT, Type II diabetes and potential vascular risk factors ($n = 826$)

Variable	Regression coefficient (95 % CI)	p value
Unadjusted model		
IGT	1.090 (0.469 to 1.711)	< 0.001
Type II diabetes	1.698 (1.039 to 2.357)	< 0.001
Age/sex-adjusted model		
Age (per 10 years)	0.787 (0.641 to 0.933)	< 0.001
Male sex	0.799 (0.487 to 1.111)	< 0.001
IGT	0.717 (0.133 to 1.301)	0.016
Type II diabetes	0.908 (0.279 to 1.537)	0.004
Multivariate model		
Age (per 10 years)	0.628 (0.473 to 0.783)	< 0.001
Male sex	0.852 (0.533 to 1.171)	< 0.001
Cigarette smoking (yes vs no)	0.714 (0.347 to 1.081)	< 0.001
LDL cholesterol (per 1 mmol/l)	0.200 (0.050 to 0.350)	0.011
HDL cholesterol (per 1 mmol/l)	− 0.231 (− 0.459 to − 0.003)	0.048
Fibrinogen (per 1 g/l)	0.679 (0.379 to 0.979)	< 0.001
Hypertension	0.541 (0.176 to 0.906)	0.004
Triglycerides (per 1 mmol/l)	0.159 (0.017 to 0.301)	0.049
BMI (per 1 kg/m²)	0.007 (− 0.007 to 0.014)	0.304
IGT	0.631 (0.049 to 1.213)	0.034
Type II diabetes	0.726 (0.099 to 1.353)	0.024

The multivariate linear regression model was built by a forced entry of fixed set of covariates. Categorical variables were included as a set of dummy variables

BMI (Table 2). The use of means of covariates (1990–1995) in the analysis did not change the results [regression coefficients: IGT 0.645 (0.065 to 1.225), $p = 0.022$; Type II diabetes 0.734 (0.114 to 1.354), $p = 0.010$]. Compared with models including only sex and age, regression coefficients of IGT and Type II diabetes declined by 12 % and 25 %, respectively in the models including all classic risk factors.

Initima-media thickness was measured at the end of follow-up. Sex-adjusted and age-adjusted mean values of IMT in NGT, IGT and Type II diabetes were 0.975, 1.032 and 1.062 mm, respectively ($p < 0.05$). When this surrogate measure of atherosclerosis replaced the 5-year change in the atherosclerosis score as outcome variable in the multiple linear regression analysis (Table 3) the associations of IMT with IGT and Type II diabetes only approached statistical significance ($p = 0.07$ and 0.10). The use of means of covariate (1990–1995) did not change the results [regression coefficients: IGT 0.048 (–0.004 to 1.000), $p = 0.106$; Type II diabetes 0.069 (–0.004 to 0.142), $p = 0.095$]. About one fifth of the relation between IGT or Type II diabetes and IMT appeared to be mediated by classic vascular risk factors.

All these results were consistent in men and women. For example, regression coefficients of 5-year progression of atherosclerosis were 0.89 and 0.99 for IGT and Type II diabetes in men, respectively ($p < 0.05$ each; analysis adjusted for age and sex). The corresponding figures in women were 0.58 and 0.75 ($p < 0.05$ each). In non-smokers regression coefficients were 0.82 and 1.10 ($p < 0.01$ each).

In diabetic subjects no statistically significant association was found between the degree of glucose control (fasting glucose or HbA_{1c} at baseline or at follow-up or average of 1990 and 1995 determinations) and atherosclerosis progression or IMT. Likewise, diabetic subjects receiving oral hypoglycaemic agents

faced a similar risk of atherosclerosis progression as those on diet or newly diagnosed: regression coefficients of 5-year change in atherosclerosis score 1.01 and 0.73, respectively ($p < 0.05$ each).

There were 500 subjects free of atherosclerosis in 1990. Of these subjects, 451 had NGT (mean age 52 years, women 54 %), 31 had IGT (mean age 56 years, women 58 %) and 18 had Type II diabetes (mean age 64 years, women 61 %). Incident cases of non-stenotic atherosclerosis in these subjects were as follows: NGT 103 (22.8 %), IGT 15 (48 %), Type II diabetes 8 (44 %). Unadjusted odds ratios (OR) in the three categories were 1.0, 3.2 (95 % confidence intervals 1.5–6.6), 2.7 (1.1–6.9) ($p < 0.001$ for differences). In a multivariate analysis the category of glucose tolerance lost its predictive significance. In this stepwise logistic regression model age, hypertension, ferritin, smoking (smoking-years), Lp(a), apolipoprotein B, and heavy alcohol intake were identified as independent risk attributes of incident non-stenotic (early) atherosclerosis (Table 4). When means of covariates (1990–1995) were used in the multivariate model, the results were similar [OR: IGT 2.43

Table 3. Linear regression analyses of intima-media thickness in 1995 on IGT, Type II diabetes and potential vascular risk factors ($n = 826$)

Variable	Regression coefficient (95 % CI)	p value
Unadjusted model		
IGT	0.097 (0.032 to 0.162)	0.004
Type II diabetes	0.192 (0.096 to 0.288)	< 0.001
Age/sex-adjusted model		
Age (per 10 years)	0.133 (0.120 to 0.146)	< 0.001
Male sex	0.092 (0.064 to 1.120)	< 0.001
IGT	0.057 (0.004 to 1.110)	0.046
Type II diabetes	0.087 (0.009 to 0.135)	0.024
Multivariate model		
Age (per 10 years)	0.118 (0.104 to 0.132)	< 0.001
Male sex	0.099 (0.070 to 0.128)	< 0.001
Cigarette smoking (yes vs no)	0.064 (0.031 to 0.097)	< 0.001
LDL cholesterol (per 1 mmol/l)	0.030 (0.015 to 0.046)	< 0.001
HDL cholesterol (per 1 mmol/l)	− 0.027 (− 0.065 to 0.011)	0.190
Fibrinogen (per 1 g/l)	0.037 (0.012 to 0.055)	0.007
Hypertension	0.072 (0.039 to 0.148)	< 0.001
Triglycerides (per 1 mmol/l)	0.009 (− 0.009 to 0.018)	0.322
BMI (per 1 kg/m^2)	0.001 (− 0.003 to 0.005)	0.500
IGT	0.046 (− 0.003 to 0.105)	0.103
Type II diabetes	0.072 (− 0.002 to 0.146)	0.072

The multivariate linear regression model was built by a forced entry of fixed set of covariates. Categorical variables were included as a set of dummy variables

Table 4. Risk profile of incident non-stenotic atherosclerosis in subjects free of carotid atherosclerosis at the 1990 baseline evaluation (early atherogenesis; $n = 500$)

Variable	OR (95 % CI)	P value
Age (per 10 years)	1.80 (1.39–2.32)	< 0.001
Hypertension (yes vs no)	2.41 (1.43–4.06)	< 0.001
Ferritin (per 10 µg/l)	1.28 (1.08–1.52)	0.006
Smoking-years (per 10 years)	1.06 (1.02–1.10)	0.032
Lp(a) (per 10 mg/dl)	1.12 (1.01–1.23)	0.040
Apolipoprotein B (per 10 mg/dl)	1.08 (1.01–1.16)	0.041
Alcohol consumption		0.045
Abstainers	1.00	
1–50 g/day	0.70 (0.51–0.98)	0.040
51–99 g/day	1.92 (0.65–5.62)	0.236
≥ 100 g/day	2.16 (1.07–4.37)	0.036
Glucose tolerance		0.114
NGT ($n = 451$)	1.00	
IGT ($n = 31$)	1.90 (0.81–4.52)	0.070
Diabetes ($n = 18$)	0.62 (0.22–2.14)	0.408

OR were derived from forward stepwise logistic regression analyses which allowed for all variables given in Table 1. Alcohol consumption and glucose tolerance were modelled as indicator variables with the first category defined as the reference group (abstainers and NGT)

tions more than 32 mg/dl, fibrinogen, age, factor V mutation, antithrombin III (low levels) and mild alcohol intake (inverse association). Notably, hypertension and apolipoprotein B (or other lipid variables) did not rank among these risk attributes. When IGT or Type II diabetes was associated with anyone of the prothrombotic factors (increased fibrinogen, low antithrombin III, factor V mutation, Lp(a) more than 32 mg/dl, smoking) the occurrence of stenosis was remarkably increased (21 cases out of 31 subjects, i.e. 68 % in 5 years).

Discussion

The concept that diabetes is associated with a pronounced increased risk of atherosclerotic cardiovascular disease is widely accepted [44]. The idea that increased cardiovascular disease risk is an attribute of diabetic hyperglycaemia has less consensus [22]. The few intervention trials aimed at reducing the risk of cardiovascular disease by control of hyperglycaemia did not achieve or only marginally achieved this goal [45–47]. On the other hand, it has been estimated that in diabetic subjects the typical risk factors (smoking, cholesterol, blood pressure) in concert account for no more than 25–30 % of excess cardiovascular risk, thereby strongly suggesting that other undefined factors featuring the diabetic milieu could have a key role [48]. Furthermore, most epidemiological studies in this field targeted on clinical endpoints such as myocardial infarction, stroke, lower extremity amputation or cardiovascular death [8] and thus do not enable conclusions on atherogenic mechanisms in dia-

(1.00–5.27), $p = 0.07$; Type II diabetes 0.93 (0.31–2.75), $p = 0.89$].

There were 326 subjects with pre-existing atherosclerosis in 1990. Of these subjects, 249 had NGT (mean age 65 years, women 40 %), 37 had IGT (mean age 69 years, women 62 %) and 40 had Type II diabetes (mean age 69 years, women 46 %). Incident stenosis above 40 % (atherothrombosis) occurred in 54 subjects with NGT (21.7 %), in 17 with IGT (45.9 %) and in 21 with Type II diabetes (52.5 %). Unadjusted OR in the three categories were 1.0, 3.1 (1.5–6.3), 4.0 (2.0–7.9) ($p < 0.001$ for differences). In a multivariate logistic regression analysis Type II diabetes turned out to be the strongest independent predictor of advanced stenotic atherosclerosis (Table 5). Impaired glucose tolerance was significantly associated to carotid stenosis as well. When means of covariates (1990–1995) were used in the multivariate model, the results were almost identical [OR: IGT 2.72 (1.22–6.11), $p = 0.002$; Type II diabetes 5.29 (2.41–11.58), $p < 0.0001$]. The risk profile of advanced stenotic atherosclerosis was further composed of smoking (cigarettes/day), Lp(a) concentra-

Table 5. Risk profile of incident carotid stenosis (atherothrombosis) in subjects with pre-existing atherosclerosis at the 1990 baseline evaluation ($n = 326$)

Variable	OR (95 % CI)	*P* value
Glucose tolerance		< 0.001
NGT ($n = 249$)	1.00	–
IGT ($n = 37$)	2.80 (1.20–6.35)	0.009
Diabetes ($n = 40$)	5.01 (2.28–11.08)	< 0.001
Smoking (per 10 cigarette/day)	1.98 (1.37–2.88)	< 0.001
Lp(a) > 32 mg/dl	4.29 (2.04–9.03)	< 0.001
Fibrinogen (per 1 g/l)	2.31 (1.39–3.86)	0.001
Age (per 10 years)	1.79 (1.22–2.64)	0.003
Alcohol consumption		0.005
Abstainers	1.00	–
1–50 g/day	0.39 (0.18–0.88)	0.023
51–99 g/day	1.05 (0.42–2.76)	0.737
≥ 100 g/day	1.92 (0.65–5.36)	0.236
Factor V mutation	4.08 (1.08–15.48)	0.041
Antithrombin III (per 10 %)	0.80 (0.66–0.98)	0.042

OR were derived from forward stepwise logistic regression analyses which allowed for all variables given in Table 1. Alcohol consumption and glucose tolerance were modelled as indicator variables with the first category defined as the reference group (abstainers and NGT)

betes to be drawn. In all, the precise pathogenic mechanisms linking diabetes and atherosclerosis are still a mystery.

In our study IGT or Type II diabetes showed an independent association with the progression of atherosclerosis (Table 2) but a modest association to early atherosclerosis which disappeared, however, after adjusting for typical vascular risk factors (Table 4). In other words, IGT or Type II diabetes was only of moderate relevance in early atherogenesis and its injurious effects were mediated, at least in part, by the risk complex associated with glucose intolerance. On the contrary, Type II diabetes was strongly and independently related to advanced stenotic atherosclerosis for which it constituted one of the leading risk conditions (Table 5). This association, which extended to IGT, was consistent in men and women, was independent of other vascular risk factors clustering with diabetes and persisted after adjusting for several potential confounders.

In recent years a number of studies attempted to make an objective evaluation of atherosclerosis by the use of imaging techniques. They yielded highly controversial results, with some studies indicating an independent association between atherosclerosis and diabetes [23, 49–56] and others obtaining no association at all [57–62]. The reason for the considerable heterogeneity in the results obtained is not yet clear but might include differences in the study cohorts and in the methods used to evaluate atherosclerosis, accuracy in defining the glucose tolerance status and in the assessment of confounding risk factors, as well

as the type of statistical analysis. Results of previous studies are, however, not directly comparable to those of the current study given the differences in the study design. Except for three [49–51], the previous studies were not population-based. In addition, none of the previous studies yielded prospective data. Furthermore, atherosclerosis (plaques) was not accurately quantified but IMT was used as a precursor/surrogate measure in most of these studies. Wall thickening can, however, occur before atherosclerosis manifests (precursor lesion) but it also accompanies the atherogenic process, thereby reflecting atherosclerosis severity (surrogate lesion). Thus, results of studies using IMT as the outcome variable do not exclusively refer to the early stage of atherosclerosis but also to atherosclerosis in general. This might explain why IMT in our study and in other studies was a weaker correlate of diabetes. For instance in a recent paper from the investigators of the Insulin Resistance Atherosclerosis Study, only subjects with established Type II diabetes but not those with IGT or newly diagnosed Type II diabetes showed a marginally increased IMT after adjustment for confounding factors [63].

Only about one fifth of the association between Type II diabetes and atherosclerosis was mediated by classic vascular risk factors (metabolic complex). Furthermore, potential effects of diabetes on atherosclerosis was not related to the degree of glucose control or diabetes treatment. In other words, IGT or Type II diabetes seems to be associated with the progression of atherosclerosis independently of other vascular risk factors clustering with diabetes and also of the severity of hyperglycaemia.

Coagulation abnormalities have been proposed as a further potentially important pathophysiological link between glucose intolerance and atherosclerosis and arterial diseases, [8, 16, 48, 64]. Several clues indicate that IGT or Type II diabetes represent a prothrombotic state due to a dysbalance between procoagulant and antithrombotic factors and between activators and inhibitors of plasminogen activation. Insulin resistance typical of IGT and Type II diabetes might contribute to this dysbalance [65, 66]. In our study, stenosis higher than 40 % was found to predominantly originate from atherothrombosis [39, 40] and to rest on a risk profile including almost exclusively factors directly or indirectly related to coagulation and fibrinolysis: fibrinogen, antithrombin III (higher concentrations being protective), Lp(a), factor V Leiden mutation, smoking, alcohol intake (protective if mild) (Table 5). Type II diabetes was the strongest single risk predictors in this analysis and IGT was of relevance as well. The associations remained highly significant even after adjusting for the above mentioned prothrombotic variables. It might be that the adverse effect of glucose intolerance on atherothrombosis is predominantly mediated by fac-

tors of coagulation or fibrinolysis or both that we were not able to assess (e. g. plasminogen activator inhibitor-1). Notably, typical risk factors without procoagulant properties were not related to this advanced stenotic stage of atherosclerosis.

The Bruneck Study seems to be particularly suitable for investigating atherogenesis in diabetes on account of its high representativeness of the general population (near complete participation and follow-up), prospective design, assessment of several risk factors and potential confounders associated with diabetes, accurate identification of IGT and diabetes, and the extensive ultrasound assessments which enable standard analyses of diabetes effects on 5-year changes in carotid atherosclerosis to be carried out as well as special analyses addressing aetiologically distinct stages in the complex atherosclerosis process (atherothrombosis). In contrast to most previous studies, we have done an OGTT to identify IGT and undiagnosed Type II diabetes and to accurately distinguish these disorders from the condition of normal glucose tolerance. This is not a trivial point as many studies documented a substantial increase in cardiovascular disease in IGT and undiagnosed Type II diabetes [7, 8, 22, 67]. Accordingly, in our study undiagnosed diabetic subjects carried a risk of carotid stenosis as high as that in diagnosed Type II diabetes (data not shown) and an increased risk was found in the IGT condition as well. Inclusion of these subjects in the reference group, as is unavoidable in studies limited to self-reported information on diabetes status or measurement of fasting blood glucose with no OGTT or both, could weaken or even eliminate true associations between Type II diabetes and atherosclerosis.

Our findings, with some caution, can be extrapolated to atherosclerosis in other vascular territories. Indeed, high coincidence exists between carotid atherosclerosis and both coronary and peripheral artery disease [68, 69]. In addition, ultrasonographically determined carotid atherosclerosis and increased IMT are excellent risk predictors of cardiovascular events in longitudinal studies [70, 71].

In conclusion, IGT and, to a greater extent, Type II diabetes are prominent risk predictors of 5-year progression of carotid atherosclerosis in this large prospective population survey. The associations obtained were at least in part independent of hyperglycaemia and other vascular risk factors featuring the diabetic milieu. Because Type II diabetes and IGT seem to be particularly strong risk attributes of advanced atherothrombotic stages in atherosclerosis, coagulation abnormalities could be a major culprit of cardiovascular disease in IGT or Type II diabetes. It is striking that the risk of advanced stenotic atherosclerosis in diabetes is amplified by the presence of other inherited or acquired coagulation disorders. About 70 % of subjects with IGT or Type II diabetes and additional procoagulant risk factors developed carotid stenosis during the follow-up period of 5 years and, consequently, were at high risk of subsequent cardiovascular events. We suggest that strategies to prevent cardiovascular disease in IGT and Type II diabetes, in addition to a tight control of hyperglycaemia and other vascular risk factors, should focus on anti-thrombotic or anti-coagulant measures or both more vigorously than at present.

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