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Insulin Resistance and Atherosclerosis in Diabetes Mellitus

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The aim of this study was to test our hypothesis that insulin resistance determines systemic atherosclerosis in type 2 diabetic patients. The design of the study was cross-sectional and included 28 type 2 patients with 48 type 1 patients as controls. The total daily insulin dose required to maintain glycosylated hemoglobin, HbA_{1c}, at 6.0% for 1 year was used as a measure of long-term insulin resistance. Systemic atherosclerosis was estimated by the toe systolic blood pressure index (TSPI). The results showed that total daily insulin dose was closely and independently associated with TSPI ($r = .4652$, partial $P = .0064$) in type 2 diabetic patients with secondary failure, even when adjusted for serum C-peptide ($r = .4443$, partial $P = .00123$). The association was absent in type 1 patients. Established risk factors were not associated with TSPI, but the products between individual risk factors and insulin dose were closely associated with TSPI. In conclusion, the daily insulin dose is associated with peripheral atherosclerosis in type 2 diabetic patients with high insulin resistance, but not in type 1 diabetes. The effect is additive to a lesser, underlying effect of established risk factors on atherosclerosis. Longstanding insulin resistance, as estimated by the daily insulin dose, is a determinant of atherogenesis.

DIMINISHED INSULIN action and pancreatic β-cell dysfunction are 2 genetically determined defects in glucose homeostasis[1] that predispose for type 2 diabetes and together cause the overt disease. Impaired insulin action is synonymous with increased insulin resistance. Insulin resistance increases with age and may eventually overwhelm the initial, compensatory increase in insulin secretion, thus generating overt diabetes.

Atherosclerosis is closely associated with established risk factors for cardiovascular disease (CVD) and with insulin resistance in the nondiabetic, Caucasian population.[2-5] Consequently, insulin resistance and cardiovascular risk factors are associated in nondiabetic persons, even when adjusting for age.[6-12]

In type 2 diabetes, risk factors for CVD are more prevalent than in the general population.[13] However, major risk factors can only explain 20% to 30% of the excess atherosclerosis found in patients with overt type 2 diabetes.[3, 14-16] The discrepancy suggests the existence of other atherogenic factors linked to the emergence of overt type 2 diabetes.[17]

Serum insulin is one candidate atherogenic factor in diabetic patients. Nevertheless, analyses of the associations between risk factors and CVD in type 2 patients have rarely been controlled by covariance adjustment for serum insulin.[17]

Insulin resistance per se is another hypothetical factor that might be related to both the emergence of overt type 2 diabetes and the development of excess atherosclerosis.[18]

Our hypothesis was that long-term insulin resistance is an independent determinant of atherogenesis in type 2 diabetic patients. Specifically, we hypothesized that long-term insulin resistance is associated with atherosclerosis independently of established cardiovascular risk factors and of total insulin exposure in these patients.

The insulin dose required for long-term tight glycemic control in type 2 patients with secondary failure of oral therapy is largely a measure of long-term insulin resistance and independent of other determinants of the insulin dose.[19,20] The relationship between insulin dose and insulin resistance will become apparent when insulin resistance overwhelms endogenous insulin secretion and secondary failure occurs. The relationship is then independent of the residual endogenous insulin secretion. Total insulin exposure can be expressed as the product of plasma C-peptide concentration and daily insulin dose.[6,15,21] In type 2 patients, total insulin exposure is less correlated with, and less a measure of, insulin resistance than is

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Table 1. Clinical Characteristics of 76 Diabetic Patients

Clinical Characteristics	Type 1 Diabetes		Type 2 Diabetes		*P* Value
	Mean	95% CI*	Mean	95% CI*	
Sex ratio, no. of males/no. of females	32/16		19/9		.8094
Age (yr)	50	45-54	58	52-63	.0338
Duration of diabetes (yr)	21	18-25	12	10-14	.0005
Total daily insulin dose (IU)	52	47-58	55	45-65	.6218
Serum C-peptide (nmol/L)	0.06	0.04-0.08	0.6	0.4-0.8	<.0001
Blood HbA_{1c} (%)	6.3	6.0-6.5	6.3	5.8-6.8	.8607
Serum LDL-cholesterol (mmol/L)	3.2	3.0-3.4	3.5	3.2-3.8	.1582
Serum HDL-cholesterol (mmol/L)	1.52	1.39-1.66	1.33	1.16-1.49	.0742
Serum total cholesterol/HDL-cholesterol ratio	3.7	3.4-3.9	4.6	4.0-5.1	.0019
Serum triglyceride (mmol/L)	1.14	0.96-1.32	1.68	1.33-2.02	.0027
Plasma tHcy (mmol/L)	10.5	9.6-11.4	11.2	10.0-12.4	.3266
Serum uric acid (μmol/L)	266	241-291	313	269-358	.0456
BMI (kg body weight/cm^2 height)	26	25-27	28	27-30	.0046
Urinary AER (μg/min)	8†	4-1,043‡	8†	4-293‡	.5396
GFR (mL/min/1.73 m^2)	100	92-109	100	89-111	.9983
Systolic blood pressure (mm Hg)	148	142-154	151	144-159	.5638
TSPI	0.80	0.74-0.85	0.80	0.74-0.87	.8658
Probability for CVD	0.19	0.13-0.25	0.31	0.22-0.40	.0253

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; tHcy, total homocysteine; BMI, body mass index; AER, albumin excretion rate; GFR, glomerular filtration rate.

*Confidence interval; †median; ‡minimum-maximum.

the exogenous insulin dose by itself.[19] This is so, because total insulin exposure incorporates the component of endogenous insulin, which depends on the status of the β-cell function in the individual patient. Our patients had type 2 diabetes with secondary failure, but with different residual β-cell functions.[22,23]

In diabetic patients, insulin resistance fluctuates over time in concert with glycemic control,[24] and measurement of momentary insulin resistance by an isoglycemic insulin clamp, as used in persons with intact glucose homeostasis, may be misleading in diabetic patients. We, therefore, estimated long-term insulin resistance indirectly as the daily insulin dose required to maintain tight glycemic control (ie, isoglycemia) at a glycosylated hemoglobin, HbA_{1c}, level around 6.0%. This surrogate estimate can be regarded as an integrated measure over time of fluctuating insulin resistance.

We measured atherosclerosis quantitatively as the toe systolic blood pressure index (TSPI), which closely reflects atherosclerosis in other major vascular beds of the body.[25-29] The association between insulin dose and atherosclerosis in type 2 patients was compared with that in type 1 patients of similar age, but with little insulin resistance.

SUBJECTS AND METHODS

Patients

Type 1 diabetes was defined as diabetes with fasting serum C-peptide less than 0.24 nmol/L and type 2 as diabetes with serum C-peptide greater than 0.23 nmol/L (lower limit of the normal serum C-peptide range). Inclusion criteria for the study were insulin requirement and informed consent to undergo intensive insulin treatment aimed at maintaining an HbA_{1c} level of 6.0%.

A total of 210 patients were referred for insulin treatment during the 2-year inclusion period, and 136 of these met the inclusion criteria. Ninety-nine patients completed the study (Table 1). Seventy-six patients had no missing values and were used for statistical analyses. None of the patients used estrogen replacement therapy or oral contraceptives. The number of patients with a clinical history of CVD events is presented in Table 2.

None of the patients had major intercurrent illnesses requiring hospital contact within 6 months before measurement of the TSPI or before having blood samples drawn for plasma total homocysteine (tHcy) determinations.

Most of the 43 patients who dropped out did so early in the study because they lacked motivation to continue intensified glycemic control, by that introducing a possible selection bias.

Protocol

The insulin treatment schedule for all patients was 2 daily injections of intermediate-acting insulin in the morning and late afternoon and 3 daily injections of fast-acting insulin at mealtime.

Identical HbA_{1c} targets were set for type 1 and type 2 patients, and the resulting long-term glycemic control was very similar in the 2 types of diabetic patients. All type 2 patients were in secondary failure on identical, maximal doses of glibenclamide and metformin, which were continued during insulin therapy. Under these conditions, in which β cells have reached the limit of their capacity to compensate for insulin resistance, the dose of exogenous insulin necessary for maintaining a target level of HbA_{1c} depends on insulin resistance.

All patients visited the diabetes clinic at least 4 times yearly. The following laboratory determinations were performed at each visit to the clinic: fasting blood glucose, HbA_{1c} serum total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglyceride, uric acid, and creatinine, plasma fibrinogen, urinary albumin excretion rate (AER), supine blood pressure, and body weight. Blood samples were drawn in the morning before meals and before insulin administration and were analyzed at the central clinical laboratory of the hospital that participates in the Murex Quality Assessment Programmes (Murex Biotech Ltd, Dartford, UK). We calculated results as the mean of 4 different measurements within the last year of the

Table 2. Simple Associations Between Total Daily Insulin Dose and Positive History of Clinical CVD in Type 1 and Type 2 Diabetic Patients

Cardiovascular Diagnoses	Type 1 Diabetes				Type 2 Diabetes			
	N*	Percentage	Simple *r*	*P*	N*	Percentage	Simple *r*	*P*
Cardiovascular diseases†	14	29	.0460	.7589	8	29	.5408	.0030
Coronary heart disease	8	17	.1270	.3951	8	29	.4989	.0069
Left ventricular hypertrophy	6	13	.1786	.2298	4	14	.5036	.0063
Angina pectoris	5	10	.1275	.3930	8	29	.4741	.0108
Myocardial infarct	6	13	.0999	.5040	3	11	.3930	.0385
Stroke	2	5	.1113	.4562	0	0		
POAD of the lower extremities	8	17	.1429	.3378	2	7	.4245	.0243
Claudication	3	6	.1052	.4816	2	7	.4245	.0243

Abbreviation: POAD, peripheral arterial obstructive disease.

*No. of patients with positive clinical history of CVD.

†The variable "Cardiovascular diseases" is continuous for the number of CVD entities in each patient.

NOTE. All other variables are categorical for positive history of the disease.

observation period. All other tests were performed once or twice during the last year.

Analytical Methods

TSPI was calculated as the systolic blood pressure of the first toe, measured by mercury strain gauge technique and divided by the systolic blood pressure of the left arm. The procedure was performed according to the joint recommendations of the American Diabetes Association and the American Heart Association.[25,26] TSPI was measured on both feet, and the result was expressed as the mean of the 2 measurements. In diabetic patients, TSPI measures peripheral arterial obstructive disease (POAD) of the lower extremities more specifically than the ankle-brachial index (ABI), which is influenced by medial wall stiffness, common in diabetic patients.[25] Mean TSPI for normal asymptomatic persons is 0.90 with a normal range of 0.62 to 1.08.[30] All measurements of TSPI were performed in one vascular laboratory by the same nurse-technician.

Serum C-peptide was measured by radioimmunoassay as previously described.[31] Sensitivity of the assay is 0.02 nmol/L. The intra-assay coefficient of variation (CV) is 5.8% and interassay CV, 8.1%. The normal range is 0.24 to 0.64 nmol/L. Glucagon-stimulated serum C-peptide was measured 6 minutes after intravenous injection of 1 mg glucagon.

Plasma tHcy was measured as previously described, using an automated, high-performance liquid chromatography (HPLC) method.[32] The intra-assay CV is less than 3%. The normal range is 6.1 to 19.9 μmol/L ($\pm$ 2 SD of the mean). In statistical analyses, plasma tHcy concentrations were corrected for glomerular filtration rate (GFR).[32]

Serum lipoprotein (a) [Lp(a)] was measured by radioimmunoassay as also previously described.[31] Intra-assay CV is 5.4%. Sensitivity of the assay is 10 mg/L, and the normal range, 10 to 300 mg/L ($\pm$ 2 SD of the mean).

Plasma endothelin-1 was measured by radioimmunoassay (Nichols Institute Diagnostics B. V., Wijchen, The Netherlands). The intra-assay CV is 4.5%. Sensitivity of the assay is 2.0 pg/mL. The mean plasma ET-1 concentration for 21 normal subjects is 4.1 pg/mL.[33]

Urinary albumin concentration was analyzed by an automated nephelometric enzyme-linked immunosorbent assay (Behringwerke AG, Marburg, Germany). The AER was calculated from an overnight timed urine collection performed by the patient following written and oral instructions. The normal range is 4 to 15 μg/min ($\pm$ 2 SD of the mean).

GFR was determined as clearance of intravenous^{51} Cr-EDTA over a 4-hour period. The normal range for GFR in 20-year-old men and women is 85 to 135 mL/min/1.73m^2 and for 80-year-old men and women 45 to 95 mL/min/1.73m^2 ($\pm$ 2 SD).

The sagittal abdominal diameter was measured on the recumbent patient as the perpendicular distance between plane of support and highest point of the abdomen.[34] A specially constructed gallow was used for all measurements.

Skinfold thickness refers to the sum of the triceps, subscapular, and suprailiac skinfolds. The skinfold thickness was measured by one observer using a Holtain caliper (Holtain Ltd; Crymych, UK).

Left ventricular hypertrophy was estimated electrocardiographically as the sum of amplitudes of S in lead V1 and of R in lead V5.

Cardiovascular risk profiles were calculated according to prediction equations by Anderson et al.[35] The equations are based on data from the original Framingham and Framingham Offspring Cohorts, which include the following continuous cardiovascular risk factors: age, total and HDL cholesterol, systolic and diastolic blood pressure, and the following dichotomous risk factors: electrocardiogram (ECG) voltage criteria for left ventricular hypertrophy, cigarette smoking, diabetes, coronary heart disease incidence, and sex.

Data Analysis

Normality of distribution was tested by the Shapiro and Wilk's W statistic. For variables with normal distributions, results were presented as means with 95% confidence limits. Non-normally distributed variables were presented as medians with interquartile ranges and were log-transformed in analyses of regression and variance. Unpaired *t* tests were used for comparisons between male and female values.

Associations between variables were calculated as the Pearson coefficient of correlation or the partial coefficient of correlation.

Analysis of covariance with adjustments for age and sex was used to stratify mean TSPI by insulin dose and established risk factors and to estimate the type of interaction statistically (additive or synergistic) (see Fig 2).

An "all possible subsets regression" analysis was used for initial identification of the most predictive model among all possible combinations of variables in type 1 and type 2 patients separately. The following variables were tested for predictive value in the models: sex, age, duration of diabetes, fasting blood glucose, HbA_{1c}, basal- and glucagon-stimulated serum C-peptide, serum cholesterol, LDL-cholesterol HDL-cholesterol, Lp(a), triglyceride, uric acid, and creatinine, plasma fibrinogen, tHcy, and tCys, ET-1, body mass index (BMI), abdominal sagital diameter, skinfolds, reclining systolic and diastolic blood pressure, AER, GFR, TSPI, probability of CVD, total daily insulin dose adjusted for body weight, and number of cigarettes smoked daily. In the final multiple regression analyses, the 5 most predictive variables initially identified were included in the models along with the variables of interest for the hypothesis. A *P* value of greater than .05 was defined as nonsignificant (NS). All tests were 2-tailed. The BMDP

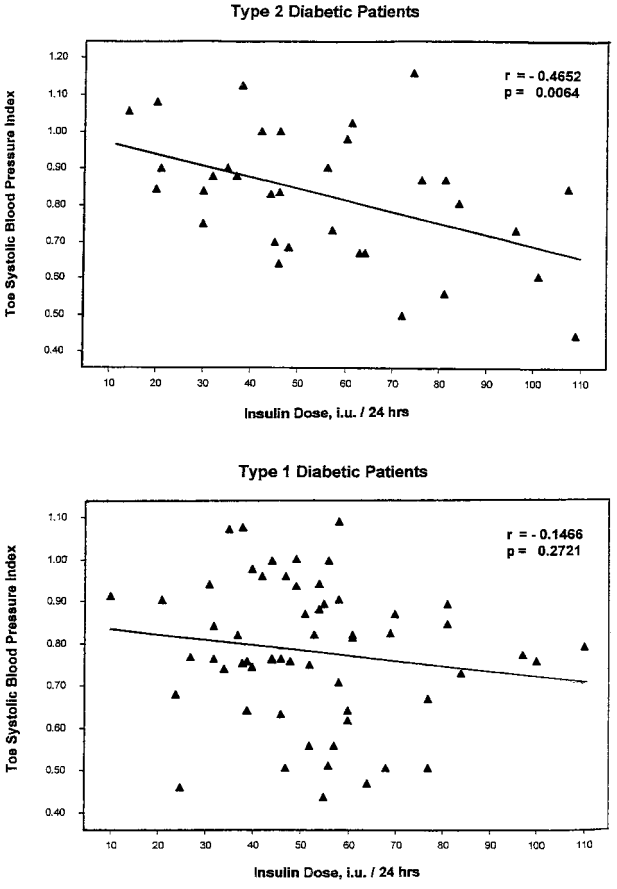

Fig 1. Type 1 and type 2 diabetic patients: simple regression analysis between daily insulin dose and TSPI.

Statistical Software (BMDP Classic, Release 7, SPSS Inc, Chicago, IL) was used for data management and calculations.

RESULTS

TSPI

The mean TSPI in type 1 patients was 0.80 (95% confidence interval [CI], 0.74 to 0.85) and in type 2 patients 0.80 (95% CI, 0.74 to 0.87) (Table 1).

Females had lower mean TSPI than men at all ages in both types of diabetes. Females with type 2 had lower TSPI than those with type 1, whereas males with both types of diabetes had similar TSPI.

Metabolic Variables

The mean total daily insulin doses were similar in type 1 and type 2 patients (Table 1). Mean total daily insulin doses adjusted for body weight were also similar: 0.69 (95% CI, 0.63 to 0.74) IU/kg body weight in type 1 and 0.66 (95% CI, 0.56 to 0.76) IU/kg body weight in type 2 diabetic patients.

Type 1 and type 2 patients differed with respect to age and metabolic abnormalities listed in Table 1. In addition, they differed with respect to mean plasma fibrinogen, 3.2 and 4.2 g/L, respectively, $P = .0001$. Mean number of cigarettes smoked per day, 10 and 15, respectively, were not significantly different, $P = .1124$. The difference in BMI between type 1 and type 2 patients represented central obesity, as it was similar to the difference in abdominal sagittal diameter: 22 cm and 24 cm, respectively, $P = .0079$, whereas skinfold thickness was not significantly different in the 2 types of diabetes: 130 mm and 145 mm, respectively, $P = .1945$.

Bivariate Relationships

TSPI was inversely correlated with the total daily insulin dose in type 2 diabetic patients (Fig 1 and Table 3). In contrast, no relationship existed between TSPI and the dose of exogenous insulin in type 1 diabetic patients with the same degree of glycemic control (Fig 1 and Table 4). Associations between positive history of CVD and the total daily insulin dose were similar to those between TSPI and insulin dose (Table 2).

TSPI was closely correlated to the number of patients with CVD manifestations (myocardial infarction [MI], stable angina, stroke, or claudication) for both types of diabetes: r −.3723, $P = .0100$ in type 1 and r −.6274, $P = .0004$ in type 2. The presence or absence of intermittent claudication was more strongly correlated to TSPI than was any other specific symptom of CVD: r −.3697, $P = .0031$ in type 1 and r −.4943, $r = .0022$ in type 2.

TSPI and the number of past CVD entities were associated with age, but not with other established risk factors for CVD (serum total, LDL-, and HDL-cholesterol, serum triglyceride, systolic blood pressure, smoking status) in either type of diabetic patients (Tables 3 and 4)

Established risk factors were not related to the total daily insulin dose (Table 5). Risk factors were also unrelated to the product between insulin dose and serum C-peptide (total insulin exposure) in any of the 2 types of diabetic patients (data not shown). Serum C-peptide was not associated with GFR.

TSPI and the number of past CVD entities were, however, strongly associated with the products between total daily insulin dose and established risk factors in type 2 patients (Table 3), but not in type 1 patients (Table 4). This suggests that in type 2 patients, insulin resistance and established risk factors together have greater predictive strength with respect to TSPI and CVD, than either one alone. In patients with little insulin

Table 3. Associations of TSPI With Risk Factors for CVD and With the Product Terms Between Insulin Dose and Risk Factors in Type 2 Diabetes Mellitus

	Risk Factors TSPI		Product Terms TSPI	
Risk Factor for CVD	Simple *r*	*P* Value	Simple *r*	*P* Value
Total daily insulin dose	−.4652	.0064		
Age	−.4174	.0102	−.5992	.0002
BMI	−.4555	.0077	−.5120	.0023
Diastolic blood pressure	−.1720	.3886	−.4740	.0053
Serum total cholesterol	−.1865	.2986	−.5543	.0017
Serum HDL-cholesterol	−.0081	.9645	.4585	.0073
Serum total/HDL-cholesterol	−.2053	.2517	−.4505	.0085
Plasma tHcy	−.2257	.2392	−.5548	.0018
Serum triglyceride	−.1305	.4692	−.3145	.0746
Serum uric acid	−.3220	.0885	−.5064	.0051
Serum Lp(a)	−.3221	.0772	−.4138	.0230

Table 4. Associations of TSPI With Risk Factors for CVD and With the Product Terms between Insulin Dose and Risk Factors in Type 1 Diabetes Mellitus

	Risk Factors *v* TSPI		Product Terms *v* TSPI	
Risk Factor for CVD	Simple *r*	*P* Value	Simple *r*	*P* Value
Total daily insulin dose	−.1446	.2721		
Age	−.2520	.0482	−.3318	.0109
BMI	−.1947	.1431	−.1808	.1744
Diastolic blood pressure	−.0760	.5706	−.1647	.2166
Serum total cholesterol	−.1336	.3216	−.2003	.1389
Serum HDL-cholesterol	−.0757	.5757	−.0916	.5018
Serum total/HDL-cholesterol	−.0852	.5284	−.1562	.2504
Plasma tHcy	−.1113	.4464	−.2150	.1379
Serum triglyceride	−.2167	.1055	−.2290	.0896
Serum uric acid	−.3925	.0058	−.3139	.0298
Serum Lp(a)	−.4684	.0005	−.4405	.0012

resistance, ie, type 1 patients, the product terms, in contrast, were not associated with TSPI or CVD.

Serum uric acid was closely associated with TSPI and the number of past CVD entities in both type 1 and type 2 patients, and the associations were independent of insulin dose (Table 6).

All variables shown to be related or unrelated with TSPI had similar associations with the number of CVD entities (Table 2).

Multivariate Relationships

Analysis of variance was performed with TSPI as dependent variable, with insulin dose and risk factors as grouping variables, and with age and sex as covariates (TSPI was associated with both age and sex). In type 2 diabetic patients, the effect on TSPI was significantly dose-response related to insulin dose, but was independent of risk factor levels, when adjusted for age and sex as covariates (Fig 2). The dose-response relationship had a steeper slope at the higher level of risk factors. However, no significant interaction existed between insulin dose and any of the risk factors (Fig 2). The association of TSPI with the products between insulin dose and risk factors disappeared when risk factors were added separately to the model. This shows that the association between TSPI and the product terms represented additive effects on TSPI, rather than modification by one predictor of the other (Fig 2). Similar results were obtained with the number of past CVD entities as dependent variable (not shown). The effects of risk factors on TSPI and CVD were too weak to attain statistical independence of insulin dose in type 2 diabetic patients. In type 1 diabetic patients, neither insulin dose nor risk factors were related to TSPI or past CVD entities.

Results of an 'All Possible Subsets Regression' analysis with TSPI as dependent variable and with all initially selected variables and variables of interest to the hypothesis in the pool are shown in Table 6. The total daily insulin dose was strongly and independently associated with TSPI and with the number of past CVD entities in type 2 patients, but not in type 1 patients. In a model with total daily insulin dose as dependent variable, TSPI (partial $P = .0226$), HbA_{1c} (partial $P = .0003$), serum uric acid (partial $P = .0002$), BMI (partial $P = .0068$), and serum C-peptide (partial $P = .0115$) explained the total daily insulin dose with $R^2 = .77$ in type 2 patients. For type 1 patients, the same model showed no statistically significant effect on total daily insulin dose ($R^2 = .42$). Best predicting variables for the daily insulin dose in type 1 patients were HbA_{1c} (partial $P = .0009$) and BMI (partial $P = .0018$).

Similar multivariate associations as with TSPI existed with the number of CVD entities (not shown). Thus, the relationships between TSPI or CVD and total daily insulin dose in type 2 patients were independent of other variables tested in this study, including risk factors for CVD. Risk factors only became related to TSPI or CVD when their effects were added to the effect of insulin resistance.

DISCUSSION

We have shown that the mean daily insulin dose is one important determinant of the TSPI in type 2 diabetic patients with high insulin resistance. The association is absent in type 1 patients with little insulin resistance and is independent of total insulin exposure. Established risk factors have underlying, but additive effects on TSPI in insulin-treated type 2 diabetic patients.

Insulin sensitivity varies with glycemic control.[24] In diabetic patients, therefore, time-extended estimates of insulin resistance are more likely to be related to atherosclerosis, than insulin resistance determined at one point in time. We estimated insulin resistance over a period of 1 year by the mean daily insulin dose, continuously adjusted to maintain a near-normal level of HbA_{1c}. In secondary β-cell failure in type 2 diabetic subjects, Ryysy et al[19] showed that the daily dose of exogenous insulin needed for long-term maintenance of a target HbA_{1c} is determined by insulin resistance independently of other determinants of insulin dose. This insulin dose can be considered a long-term equivalent of the point-estimate of insulin resistance by an insulin clamp. We found a close, independent association between this estimate of insulin resistance and POAD in type 2 patients (Fig 1). Furthermore, the same association existed with the number of CVD entities in type 2 patients. Investigating another vascular bed in type 2

Table 5. Associations Between Total Daily Insulin Dose and Established Risk Factors for CVD in Type 1 and Type 2 Diabetic Patients

	Type 1 Diabetes		Type 2 Diabetes	
Risk Factor for CVD	Simple *r*	*P* Value	Simple *r*	*P* Value
Age	−.2829	.0314	.1803	.3153
BMI	.4321	.0007	.5832	.0004
Diastolic blood pressure	.0063	.9624	.1866	.2984
Serum total cholesterol	−.0829	.5438	−.0664	.7136
Serum HDL-cholesterol	−.0854	.5314	−.2902	.1013
Serum total/HDL-cholesterol	−.1810	.1818	−.3203	.0692
Plasma tHcy	−.0404	.7944	.1197	.5688
Serum triglyceride	.2266	.0930	.2750	.1213
Serum uric acid	.3171	.0281	.5557	.0018
Serum Lp(a)	−.0321	.8231	−.0663	.7278

Table 6. Multivariate Associations With TSPI in Diabetic Patients

	Type 1 Diabetes		Type 2 Diabetes	
	R^2 = .5720*		R^2 = .6147*	
Independent Variables	Partial r†	*P* Value	Partial r†	*P* Value
Sex		NS	−.5138	.0073
Age		NS	−.4954	.0101
Total daily insulin dose		NS	−.5847	.0017
Serum uric acid	−.4395	.0028		NS
Serum Lp(a)	−.5959	.00004		NS
Plasma endothelin-1	−.4566	.0027		NS

Abbreviation: NS, not significant ($P > .05$).

*Coefficient of determination, squared.

†Coefficient of correlation with the dependent variable after including all other tested, independent variables in the model.

diabetes, Bonora et al[11] found an association between insulin resistance and carotid intima media thickness. In contrast, the insulin dose was not associated with POAD or other CVD in our type 1 diabetic patients, a group characterized by low insulin resistance, particularly when in strict glycemic control.

In nondiabetic persons with intact glucose homeostasis, a similar, close association has been shown between insulin resistance, measured by the isoglycemic clamp technique, and carotid intima media thickness, estimated by ultrasound[10,36] or by other techniques.[9,12,16,22]

Since serum insulin cannot be used as a measure of long-term insulin exposure in diabetic patients being treated with exogenous insulin, we used the product between total daily insulin dose and serum C-peptide to represent total insulin exposure. The product term did not determine POAD in either type 1 or type 2 diabetes. In nondiabetic persons, fasting serum insulin is associated with atherosclerosis both in longitudinal and cross-sectional studies.[2,14,37,38] However, the relationship is not linear[12,17,39] and not consistent,[40] which suggests confounding by some unknown factor(s) associated with both serum insulin and atherosclerosis, like insulin resistance.[41] Nevertheless, few investigators have adjusted associations between se-

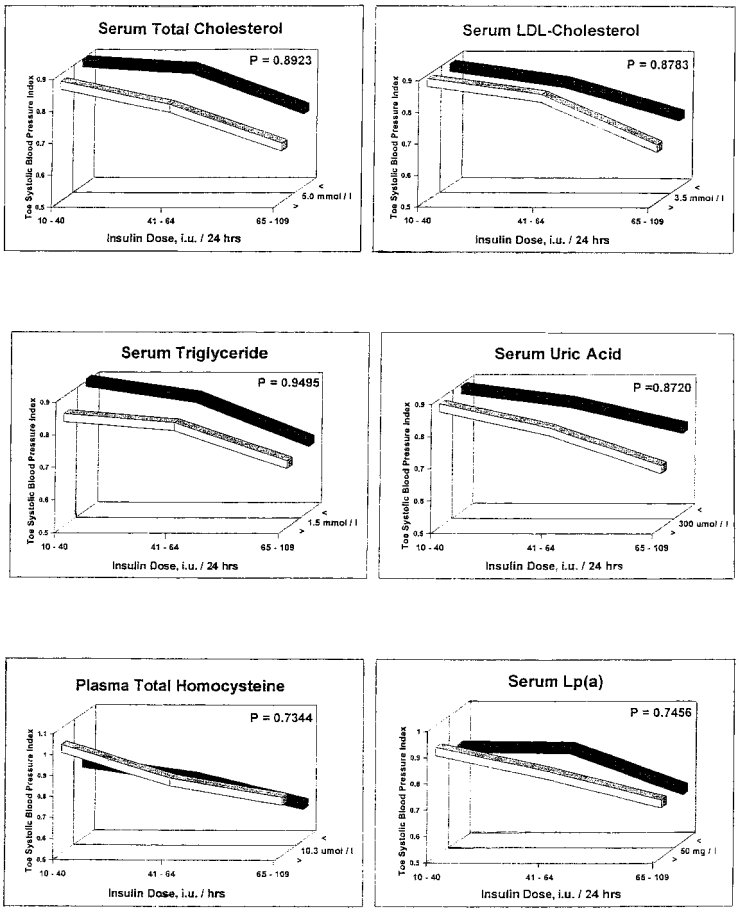

Fig 2. Type 2 diabetic patients: additive effects of established risk factors and insulin dose on TSPI. Means of TSPI adjusted for sex and age and stratified over insulin dose and over means of the upper and lower tertiles of established risk factors. *P* is the significance level of interaction.

rum insulin and atherosclerosis for insulin resistance. In a study of hypertensive, nondiabetic patients, however, Suzuki et al[18] showed that the association between fasting serum insulin and atherosclerosis depends on insulin resistance. Our results do not prove that the daily insulin dose is equivalent to long-term insulin resistance in type 2 patients, as shown by Ryysy et al.[29] However, the presence of a strong association between dose and POAD in itself supports their findings. Furthermore, the fact that a similar insulin dose determines POAD and other CVD in type 2 patients with high insulin resistance, but not in type 1 patients with little insulin resistance, suggests that it is insulin resistance, rather than insulin exposure, that influences peripheral atherosclerosis.[18] Our study and the study by Suzuki et al[18] show that the association between insulin resistance and atherosclerosis is independent of total insulin exposure.

Established risk factors for CVD were neither associated with POAD nor related to the total daily insulin dose in insulin-treated type 2 diabetic patients. However, the products between risk factors and insulin dose were strongly associated with POAD. As risk factors and insulin dose did not interact statistically, their individual effects on POAD consequently are additive (Fig 2). In contrast to our results in insulin-treated type 2 patients, Kannel et al [42] and Ford and DeStefano[4] concluded that the relationship between the increased levels of established risk factors and the increased prevalence of atherosclerosis in diabetic patients was not different from that seen in the general population. However, our type 2 patients were selected for secondary failure, and all had normal or high insulin secretion, thus representing a highly insulin-resistant population, which may explain why insulin resistance, and not established risk factors, emerged as an independent determinant of POAD.

In other studies of type 2 diabetic patients, adjustment for insulin resistance also eliminated associations between risk factors and atherosclerosis,[11,43,44] whereas in nondiabetic persons, risk factors and atherosclerosis are associated independently of insulin resistance.[9,10,12,18,41,45] Risk factors may predict an additive, underlying level of atherosclerosis in type 2 diabetes. As the insulin requirement in type 2 diabetic patients is determined by insulin resistance,[19] we interpret our results to show that the higher the insulin resistance the greater the atherogenesis, but the weaker its association with traditional risk factors. Just as insulin resistance has been shown to determine atherosclerosis in nondiabetic patients,[2,14,37,38] we have shown that it does so in type 2 patients also, and thus the particularly high insulin resistance in type 2 patients may explain their excess atherosclerosis.

Prevention of atherogenesis in type 2 diabetes is presently based on reduction of traditional risk factors by implication from prospective studies in nondiabetic populations. However, recently Minamikawa et al[46] showed rapid decline of atherosclerosis during reduction of insulin resistance by troglitazone. Furthermore, the outcome of the metformin arm in the United Kingdom Prospective Diabetes Study (UKPDS) suggests that insulin resistance is an important determinant of CVD morbidity and mortality in type 2 diabetes.[47] Consequently, considering that atherosclerosis in type 2 diabetes may be determined by both insulin resistance and established risk factors, it is prudent to treat both variables simultaneously to reduce atherosclerosis.

We conclude that insulin resistance independently predicts atherosclerosis in type 2 diabetic patients, whereas insulin exposure does not. Established risk factors may predict a basal, underlying level of atherosclerosis, but the excess atherogenesis of these patients may be predicted by their particularly high insulin resistance.

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