OBSERVATIONS

Vitreous Levels of Placenta Growth Factor and Vascular Endothelial Growth Factor in Patients With Proliferative Diabetic Retinopathy

Placenta growth factor (PlGF) is a close homolog of vascular endothelial growth factor (VEGF), shares receptors with VEGF, and stimulates angiogenesis (1). Intravitreous PlGF levels are elevated in proliferative diabetic retinopathy (PDR) (2), but the relationship between PlGF levels and VEGF levels or clinical activity remains unclear. We attempted to ascertain whether intravitreous PlGF levels correlate with VEGF levels or clinical activity in PDR.

We assayed PlGF and VEGF levels in vitreous samples from 50 consecutive patients with PDR (31 patients) and macular hole (nondiabetic control subjects, 19 patients) who underwent pars plana vitrectomy. The PDR stage was classified as active (16 patients) if there were new preretinal capillaries and as quiescent (15 patients) if the vasoproliferation consisted of only large vessels (3, 4). Informed consent was obtained from each patient. The undiluted vitreous samples were collected during the vitrectomy before intraocular infusion. Vitreous PlGF and VEGF concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) for PlGF and VEGF (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol. The total protein concentration of the vitreous humor was measured using a BCA protein assay kit (Pierce Chemical, Rockford, IL). The Mann-Whitney *U* test was used to compare vitreous concentrations of PlGF and VEGF. Spearman's rank correlation test was used to examine correlations.

PlGF and VEGF levels (median range) in PDR (PlGF, 100.6 pg/ml, range 7.6–1,038.6; VEGF 653.9 pg/ml, 9.0–5,423.8) were significantly higher ($P > 0.0001$) than in the control (PlGF 7.0 pg/ml, 7.0–12.1; VEGF 9.0 pg/ml, 9.0–10.0). Moreover, the differences remained highly significant ($P < 0.0001$) when the ratio of PlGF and VEGF to protein was considered (PlGF 14.6, 1.5–250.7 vs. 2.6, 1.1–4.1; VEGF 95.5, 2.0–904.0 vs. 3.0, 1.4–5.3) (4,5).

The ratio of PlGF and VEGF to protein in active PDR patients was significantly higher than that in quiescent PDR patients (PlGF 33.5, 2.7–250.7 vs. 11.1, 1.5–35.8, $P = 0.0039$; VEGF 130.1, 7.8–904.0 vs. 73.9, 2.0–150.3, $P = 0.0328$).

Intravitreous PlGF levels significantly correlated with intravitreous VEGF levels in both PDR patients ($r = 0.824$, $P < 0.0001$) and total subjects ($r = 0.857$, $P < 0.0001$).

Neovascularization is the most important event in PDR. PlGF stimulates angiogenesis in vivo (1). PlGF is detected in the fibrovascular membranes of PDR (2), and PlGF mRNA expression significantly increases in retina during diabetic retinopathy (6). In this study, intravitreous PlGF levels were significantly higher in active PDR than in quiescent PDR, suggesting that PlGF is involved in the developing stages of PDR.

PlGF does not directly induce endothelial cell proliferation or vascular permeability but acts indirectly by potentiating the activity of VEGF (7,8). Genetic studies indicate a synergism between PlGF and VEGF in pathological angiogenesis (9). In the present study, intravitreous PlGF levels significantly correlated with VEGF levels. Taken together, these results suggest that PlGF might have a cooperative role with VEGF in the progression of PDR.

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Is Acanthosis Nigricans a Marker of Insulin Resistance in Obese Children?

Acanthosis nigricans (AN) was proposed as an insulin resistance marker and an independent risk factor for type 2 diabetes (1). A number of studies had associated AN with insulin resistance (IR) and much higher prevalence of type 2 diabetes in childhood. (2)

Although children with AN are often obese, only few studies have considered the roles of BMI and AN as independent markers of IR. The purpose of this research was to compare several indexes of IR (i.e., homeostasis model assessment of IR [HOMA-IR], insulin-like growth factor binding protein 1 [IGFBP1] levels, and base insulinemia) in obese children with and without AN and to determine the rate of association between AN and BMI, HDL, triglycerides, and other predictors of type 2 diabetes.

A total of 1,250 Hispanic subjects (mean age 12.4 ± 1.4 years) who consulted the pediatric department between April and November 2001 were evaluated; 288 of these children were obese (BMI ≥95th percentile). Of these children, we took a randomized sample of 74 obese children (40 girls). Data for birth weight (BW), positive family history for obesity and/or type 2 diabetes, BMI, presence of AN, blood pressure, and Tanner stage were obtained. An oral glucose tolerance test (OGTT) and measurements of lipid profile, insulinemia, and IGFBP1 were performed. All of the children were of Tanner stage ≥2 (all were in puberty) and had a positive family history. There was a high rate of AN ($n = 41$; 55.4%). There was no statistical difference regarding age and sex between the group with AN ($n = 41$) and the group without AN ($n = 33$). In the group with AN, four were glucose intolerant; in the group without, only two were glucose intolerant. None presented type 2 diabetes. A Student's t test was used to compare both groups. The group with AN showed a statistical difference with the other group in BMI (30.6 vs. 27.3 kg/m^2, $P = 0.00039$), basal glucose (5.3 vs. 5.0 mmol/l, $P = 0.01$), HDL (39.2 vs. 45.1 mg/dl, $P = 0.02$), and BW (3.23 vs. 3.61 kg, $P = 0.0021$). AN showed an univariate association with BMI ($r^2 = 0.45$, $P = 0.00038$), BW ($r^2 = -0.37$, $P = 0.0021$), basal glucose ($r^2 = 0.30$, $P = 0.009$), and HDL ($r^2 = -0.25$, $P = 0.03$). There was no difference for all the IR indexes (HOMA-IR [6.6 vs. 4.9, $P = 0.19$], base insulinemia [27.3 vs. 21.5, $P = 0.27$], IGFBP1 [8.2 vs. 8.3, $P = 0.98$]) between the two groups. Likewise, there was no univariate association between AN and the markers of IR (base insulinemia [$r^2 = 016$, $P = 0.16$], HOMA-IR [$r^2 = 0.2$, $P = 0.06$], and IGFBP1 [$r^2 = 0.07$; $P = 0.69$]). Even though there were greater fasting insulin levels and HOMA-IR in the group with AN, the difference between both groups was not significant. Consistent with these results, a previous study showed that even though fasting insulin levels and HOMA-IR in obese children with AN were twice as high as those without AN; after adjusting for fat mass, there was no difference between both groups (3). The presence of AN showed a positive correlation with BMI (odds ratio [OR]1.30, 95% CI 1.08–1.57; $P = 0.018$) and a negative correlation with BW (OR 0.23, 95% CI 0.07–0.71; $P = 0.03$) in the multivariate analysis. BMI of subjects in the group with AN was significantly greater than in the group without AN, suggesting that AN may reflect only increased obesity. We conclude that AN predicts obesity and is not an independent marker of IR in our population.

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A Subtype of Markedly Abrupt Onset With Absolute Insulin Deficiency in Idiopathic Type 1 Diabetes in Japanese Children

Idiopathic type 1 diabetes, by definition, has no known etiology. Although only a minority of type 1 diabetic patients fall into this category, it is reported that the majority are of African or Asian descent. Individuals with this form of diabetes are considered to present heterogeneous clinical manifestations (1). Imagawa et al. (2) reported a new subtype of type 1 diabetes characterized by fulminant onset, absolute insulin deficiency, and absence of β-cell autoimmunity in Japanese adults. We reported similar cases of idiopathic type 1 diabetes in Japanese children.

Among 85 Japanese children with type 1 diabetes, 14 (16.5%) were classified as having idiopathic type 1 diabetes with no evidence of anti-islet autoantibodies. Of these 14 patients, 6 had a subtype of markedly abrupt onset. They consisted of five girls and one boy, aged 1.9–15.1 years at onset. Five of the six children had suffered viral infections before the onset of disease: two with Coxsackie virus B, one with influenza virus, one with mumps virus, and one with virus of unknown origin. The duration of hyperglycemic symptoms before the onset of overt diabetes was <18 days (range 5–18) in all six patients. Three patients had a particularly shorter symptomatic period of <7 days. Although all the patients showed high plasma glucose levels (565–772 mg/dl), all but one had HbA_{1c} values <8.0% (6.6–7.6) at diagnosis. This suggests that the short symptomatic period before the onset of overt diabetes might be reflected by a low HbA_{1c} value. All had ketoacidosis with bicarbonate levels <18 mmol/l (13–18) at onset. Four patients experienced impaired consciousness. They exhibited low or undetectable

values (0.3–0.4 ng/ml) of serum C-peptide. This implies that β-cells were completely destroyed from the time of onset. One patient had first-degree relatives with type 2 diabetes, while none had family members with type 1 diabetes. All the patients had high-risk HLA typing (either HLA-DR4 or -DR9) for type 1 diabetes.

From these findings, the rapid onset form of idiopathic type 1 diabetes may not be rare in the Japanese population. This form is characterized by markedly abrupt onset with a severe metabolic disorder and absolute deficiency of insulin secretion at onset. Viral infection may be associated with the rapid destruction of β-cells. Ethnic and environmental factors may play a role in the etiology and clinical heterogeneity of idiopathic type 1 diabetes.

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Blood Pressure Profiles in Adolescents With Recently Diagnosed Type 2 Diabetes

Type 2 diabetes in children is a recent phenomenon, and current evidence about diabetes and hypertension is based on adult studies. Adults with diabetes have changes in 24-h ambulatory blood pressure (BP) profiles, including a decrease in sleep dip (the decline in BP that normally occurs during sleep) and increased systolic blood pressure (SBP), that are associated with early cardiovascular disease (1).

This cross-sectional study compared 24-h BP and heart rate (HR) profiles of Hispanic and African-American adolescents recently diagnosed with type 2 diabetes with the profiles of two groups of 10 healthy control subjects matched for age, sex, height, and race/ethnicity (2). BP and HR were monitored every 30 min for 24 h using an ambulatory monitor (model 90207; SpaceLabs, Redmond, Washington). Simultaneous recording of activity was monitored by the Motionlogger actigraph (Ambulatory Monitoring, Ardsley, New York). A mixed-model approach was used to examine differences in individual BP and HR measurements in the study and comparison groups.

The study group consisted of 10 adolescents with type 2 diabetes (6 males and 4 females). Mean age at diagnosis was 13.58 years, mean length of diagnosis was 10 months, and mean blood glucose on the day of monitoring was 149.30 mg/dl (SD 81.99). Mean weight was 82.53 kg (range 62.7–129.3) in the study group and averaged 60.89 kg (43–85) in the comparison groups. Compared with adolescents without diabetes, adolescents with type 2 diabetes had significantly smaller mean sleeping SBP dip even after controlling for weight ($P = 0.008$).

The model for SBP indicated a significant difference by group, [F (2, 799) = 4.84, $P = 0.0082$], position [F (2, 799) = 28.28, $P < 0.0001$], and group by position interaction [F (4, 799) = 4.92, $P = 0.0006$]. Reclining and sitting positions were associated with lower SBP compared with a standing position in the comparison groups, whereas only reclining was associated with lower SBP in the group with diabetes. The least-squares mean SBP decreased an average 12.5 points while reclining in the comparison groups, but it was only 3.8 points lower in the subjects. The model for HR indicated a significant difference in group-by-activity interaction, [F (2, 801) = 3.55, $P = 0.0291$], group effect [F (2, 801) = 24.13, $P < 0.0001$], position [F (2, 801) = 77.62, $P < 0.0001$], and activity [F (1, 801) = 4.12, $P = 0.0428$]. HR was significantly lower in the reclining and sitting positions for all subjects, but the group with diabetes had higher HR on average than either comparison group. Activity was unrelated to HR in the group with type 2 diabetes, while it was positively related in the two comparison groups.

This study provided evidence that changes in BP and HR are evident early in the course of type 2 diabetes in adolescents and may predict increased cardiovascular morbidity during young adulthood.

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White Blood Cell Count Is Positively Correlated With Albumin Excretion Rate in Subjects With Type 2 Diabetes

White blood cell (WBC) count is one of the main inflammatory markers that predict cardiovascular events (1) and is a component (in nondiabetic subjects) of the insulin resistance syndrome (2), a clustering of cardiovascular risk factors showing insulin

resistance as a common denominator (3). It is not known whether WBC count correlates with albumin excretion rate (AER), a component of the insulin resistance syndrome conferring a particularly strong risk of cardiovascular morbidity and mortality (3).

To evaluate whether WBC count correlates with AER in type 2 diabetes, we evaluated 659 Italian type 2 diabetic patients (354 men and 305 women) followed-up at the University Diabetes Unit of the San Luigi Gonzaga Hospital in Orbassano, Turin, Italy, who were not affected by neoplastic, inflammatory, infective, or liver diseases. AER was determined by nephelometric method (Beckman, Milan, Italy). Patient characteristics were (means ± SD) as follows: age 62.00 ± 9.48 years; known diabetes duration 9.59 ± 8.21 years; BMI 29.04 ± 4.94 kg/m^2; therapy: diet 38.2%, oral hypoglycemic agents (OHAs) 44.4%, OHAs + insulin 7.2%, insulin alone 10.2%; actual/previous/never smokers: 21/24/55%; and normo-, micro- and macroalbuminuric subjects (i.e., AER <20, 20–200, and >200 μg/min): 72.2, 21.2, and 6.6%. WBC count (mean 7,028.8 ± 1,782.2/μl) was higher in microalbuminuric (7,359 ± 1,882/μl, $P = 0.0051$) and macroalbuminuric (7,574 ± 1981/μl, $P = 0.0143$) than in normoalbuminuric subjects (6,882 ± 1,713/μl) and correlated with AER by simple regression ($r = .180$, $P < 0.0001$). WBC count was higher in current (7,648 ± 2,108/μl) than in previous (6,929 ± 1,655/μl, $P = 0.0012$) and in never (6,844 ± 1,656/μl, $P < 0.0001$) smokers and was similar in men and women. The correlation between WBC count and AER remained significant when subjects were divided according to sex ($r = 0.140$, $P = 0.0072$ in men; $r = 0.220$, $P = 0.0001$ in women) and smoking status ($r = 0.150$, $P = 0.0004$ in never and past smokers; $r = 0.230$, $P = 0.0072$ in current smokers) and when only the 544 patients without history of cardiovascular events were considered ($r = 0.230$, $P < 0.0001$). WBC count correlated with AER (Std coefficient = 0.102, $P = 0.0120$) also in a multiple regression model considering age, known diabetes duration, BMI, systolic and diastolic blood pressure, HbA_{1c}, total and HDL cholesterol, triglycerides, uric acid, creatinine, erythrocyte sedimentation rate, fibrinogen, and hematocrit. When subjects were divided in tertiles according to WBC count (<6,140/μl: i.e., 5,300 ± 645/μl; 6,140–7,500/μl: i.e., 6,778 ± 372/μl; and >7,500/μl: i.e., 9,001 ± 1,424/μl), they differed for BMI (28.6 ± 4.7, 28.8 ± 4.6, and 29.7 ± 5.4 kg/m^2, ANOVA $P = 0.0417$), HbA_{1c} (7.6 ± 1.3, 7.7 ± 1.3, and 8.0 ± 1.5%, ANOVA $P = 0.0026$), HDL cholesterol (1.33 ± 0.42, 1.31 ± 0.40, and 1.20 ± 0.37 mmol/l, ANOVA $P = 0.0016$), log triglycerides (0.023 ± 0.003, 0.024 ± 0.002, and 0.025 ± 0.003 mmol/l, ANOVA $P < 0.0001$), fibrinogen (324.8 ± 79.0, 325.8 ± 69.6, and 363.2 ± 84.6 mg/dl, ANOVA $P < 0.0001$), hematocrit (40.7 ± 3.2, 41.4 ± 3.8, and 42.0 ± 3.9 mg/dl, ANOVA $P = 0.0008$), and log AER (1.05 ± 0.56, 1.07 ± 0.57, and 1.28 ± 0.67 μg/min, ANOVA $P < 0.0001$). For all of the parameters, the first and the second tertiles did not differ, whereas statistical differences were observed between the third and the first and/or the third and the second tertiles ($P = 0.01–0.0001$). Thus, patients with highest WBC values differed for AER, markers of insulin resistance (BMI, HDL cholesterol, and triglycerides), and inflammation (fibrinogen). In conclusion, in type 2 diabetes, AER correlates with WBC count, one of the major components of the chronic subclinical inflammation associated with both insulin resistance and atherosclerosis (1,2).

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Early Detection of Small-Fiber Neuropathy in Diabetes

A laser-induced pain somatosensory-evoked potentials and pupillometric study

Over the last 15 years, many authors have pointed out that small nerve fibers may be selectively damaged in the early stages of diabetes, leading to an early impairment of pain and temperature sensations and to a decline in autonomic nervous function (1,2).

The CO_2 pain-induced laser somatosensory-evoked potential (pSEP) evaluation may represent a new quantitative and objective approach to evaluate pain and temperature sensation functions (3–8). Recently, Agostino et al. (9) reported small-fiber dysfunction assessed by pSEPs in diabetic patients with different degrees of peripheral nerve damage.

A total of 27 diabetic patients (12 type 1 [group 1] and 15 type 2 [group 2]) and 27 age-, sex-, and height-matched control subjects were included in the study. In group 1 (6 men and 6 women), the mean age was 33.6 ± 9 years, the duration of diabetes was 13.5 ± 6.7 years, and the glycated hemoglobin was 6.1 ± 1.3%; two patients had nephropathy and three had background retinopathy. In group 2 (7 men and 8 women), the mean age was 55.3 ± 8.8 years, the duration of diabetes was 8.2 ± 6.2 years, and the glycated hemoglobin was 6.8 ± 1.5%; three patients had nephropathy and four had background retinopathy. The patients reported no history of autonomic and somatic neuropathy and had negative clinical examinations (evaluated using the Neuropathy Symptom Score [NSS], the Neurological Symptom Profile [NSP], and

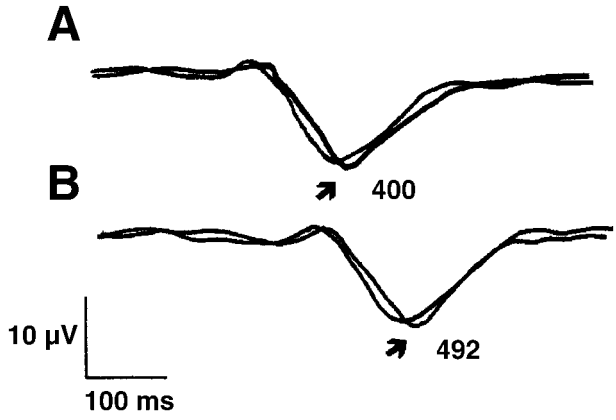

Figure 1—*Main positive component (arrows) of the pSEPs after foot stimulation (P400 wave) in a 52-year-old normal subject* (A) *and in an age- and sex-matched diabetic subject* (B). *Double traces represent averages over blocks of 30 artifact-free responses.*

the Neurologic Disability Score [NDS]) (10); normal nerve conduction velocity and P100 latency at visual-evoked potentials (VEPs) recording; no severe diabetic retinopathy or other opthalmological diseases; no recurrent ketoacidosis, ketonuria, or hypoglycemia; no psychiatric disorders or alcohol consumption; no cognitive impairment; and no other diseases or treatment with medications known to influence nervous system function. Two control groups (groups 3 and 4) were included in the study. Group 3 (6 men and 6 women, aged 34.1 ± 8 years) comprised healthy subjects age-, sex-, and height-matched with patient group 1, and group 4 (7 men and 8 women, aged 55.5 ± 8.7 years) comprised healthy subjects age-, sex-, and height-matched with patient group 2. All subjects were informed about the aim of the study and gave their consent. The experimental design of the study was approved by the ethics committee of "la Sapienza" University of Rome. Diabetic- and control-subject findings were compared with those obtained in our laboratories in a large number (60) of normal subjects acting as a normal reference control group.

A portable CO_2 laser stimulator (Neurolas, Florence, Italy) was used in this study for the pSEP evaluation (11,12). The most consistent and prominent components of the response to laser stimulation are seen as a negative-positive complex (Fig. 1). Positive potentials, P340 in the hand pSEPs and P400 in the foot pSEPs, were evaluated in all subjects. The upper limit of the normal range of P340 and P400 was set to mean ±2.5 SD of the values obtained in the reference group. For the pupillometric monocular evaluation (ISCAN; sample rate 50 Hz), dark diameter (DD) for sympathetic function, and pupillary constriction latency (PCL) for parasympathetic autonomic function, respectively, were measured (13–16). Measurements were made in the right arm and left leg using a standard surface-stimulating and recording technique, maintaining skin temperature at 32°C. Motor nerve conduction velocity, sensory nerve conduction velocity, and sensory and motor action potential amplitude results were considered normal if the values were not >2.5 SD different from the data obtained in the reference group. In the VEP evaluation (13), P100 latency was considered abnormal if the value exceeded the mean ±2.5 SD of the control population. A blood sample to measure HbA_{1c} levels was collected from each fasting patient in the morning (HPLC; Menarini, Firenze, Italy; upper limit of the normal range 6%). Blood glucose levels measured before each session were >120<150 mg/dl, and no hypoglycemic episodes were recorded during the neurophysiological assessment.

The Student's *t* test, the Wilcoxon's rank-sum test, and the Fisher's exact test were used to study differences between groups. Univariate and multivariate logistic regression analyses were used to evaluate the correlation between parameters. Moreover, to measure the extent of agreement between pSEP and pupillary autonomic function tests, the McNemar test was computed. Neurophysiological data from diabetic patients and control subjects are shown in Table 1. The P340 latency was not significantly different between diabetic patients and control subjects. One patient in group 1 and one patient in group 2 showed a bilateral increase of P340 latency above the mean ±2.5 SD of normal reference values (maximal P340 value 384 m/sec). Mean peak latency of P400 was significantly prolonged in diabetic patients compared with control subjects. Individual analysis showed abnormally prolonged P400 values in 3 of 12 patients in group 1 and in 4 of 14 patients in group 2 (maximal P400 value 438 m/sec). Abnormalities were bilateral in each case. The correlation analysis indicated that duration of disease was independently associated with P400 latency in both type 1 ($r = 0.63$, $P < 0.05$) and type 2 ($r = 0.56$, $P < 0.05$) diabetic patients. DD was significantly reduced in both diabetic groups (group 1 and 2) compared with control groups. Four patients in group 1 and five patients in group 2 presented abnormally reduced bilateral DD values. No significant PCL difference was found between diabetic patients and control subjects. A prolonged latency was found bilaterally in two patients of group 1 and in two patients of group 2. Duration of disease was independently correlated with DD in group 1 ($r = -0.55$, $P < 0.05$) and in group 2 ($r = -0.53$, $P < 0.05$). Among the patients in group 1, 58.3% presented

Table 1—*Neurophysiological data*

Pupillometry	Group 1	Group 3	Group 2	Group 4
n	12	12	15*	15*
DD (m/m)	5.95 ± 0.9	6.6 ± 0.5†	5.48 ± 0.8	6.1 ± 0.4†
PCL (m/s)	278 ± 24	270 ± 17	300.1 ± 20	275 ± 17
PSEPs				
P340 (m/s)	342.6 ± 27	341 ± 16	344.7 ± 23	341.5 ± 19
P400 (m/s)	432.7 ± 40	404 ± 23‡	440 ± 42	410 ± 16‡

Data are means ± SD. *pSEPs were absent in one patient in group 2 and one subject in group 4; †Wilcoxon's Test, $P < 0.05$; and ‡Student's *t* test, $P < 0.05$.

one or more abnormalities in the neurophysiological recordings. One patient showed abnormality of P400, DD, and PCL, and two patients presented only abnormal pSEPs (in one both P340 and P400 were prolonged). No significant correlation was found between pSEPs and pupillometric parameters. One or more abnormal value of pSEPs and pupillometric parameters were found in 57.2% of group-2 patients: one patient presented abnormal values of P340, P400, and PCL and two patients showed abnormal DD and P400 results. No significant correlation was found between pSEPs and pupillometric parameters. Age, sex, and hypoglycemic treatment were not significantly different in patients with and without neurophysiological abnormalities. An early, subclinical, and selective damage of small nerve fibers, regarding both autonomic and somatic functions, in diabetic patients without clinical sign of diabetic neuropathy and electrophysiological evidence of large-fiber dysfunction, has been demonstrated in our study. We found a prolonged P400 latency in both type 1 and type 2 diabetic patients, whereas P340 latency was not significantly affected. Our results are partially contrasting with those obtained by other studies (9), but pSEP amplitude was not considered in our study. P400 latency was bilaterally abnormal in 25% of type 1 diabetic patients and 28.5% of type 2 diabetic patients, respectively.

These data indicate the presence of a selective subclinical symmetrical hypoalgesia involving first the longest pathways of the lower limbs. This pattern of small nerve fiber dysfunction may represent the result of a length-related degeneration of peripheral fibers. In our study, this hypothesis is also supported by the pupillometric results showing a predominant dysfunction of the longest sympathetic fibers. As for quantitative sensory testing findings, the latency of cortical-evoked response reflects both central nervous system (CNS) and peripheral nervous system function. Previous studies assessing central somatosensory pathways by means of the conventional electric pSEPs have shown that the CNS may be affected with a low frequency at an early stage of diabetic disease, before the appearance of overt neurological complications (17,18). Thus, in our study, the possibility of some CNS involvement cannot be dismissed. Interestingly, in electrical pSEP studies, the CNS parameter abnormalities were frequently monolateral (17). In our patients the abnormalities were always bilateral. This symmetry of pathological findings suggests a subclinical peripheral rather than a central neurological dysfunction.

A further issue to be discussed regards the type of cortical responses recorded after laser stimulation, since the extent to which cortical pSEPs reflect sensory or cognitive processing of nociceptive inputs is still under debate (6,19). At present, data from previous studies support the notions that cortical pSEPs *1*) are unlikely to represent a purely endogenous potential and *2*) measure an exogenous potential, exploring the function of pain and temperature sensitive pathways (6,7,20). In our study, we have carefully kept all factors influencing pSEPs constant; moreover, we used a standardized distraction task to separate the exogenous component of the late responses. We also found a prolongation of P400 latency in the presence of normal P340 values. These data do not reflect a cognitive dysfunction, the effect of which should not have been limited to lower limb stimulation, and are consistent with the conclusion that abnormalities of pSEP latency reflect a small-fiber dysfunction. Investigating the parasympathetic and sympathetic pupillary function we found in both type 1 and type 2 diabetic patients, a significantly reduced DD with no significant difference for latency was found. These data suggest that sympathetic dysfunction precedes parasympathetic damage, leading to a diminished size in darkness of pupils normally responding to light stimuli. No significant correlation was found between pupillometric parameters and pSEP latencies. These data indicate that somatic and autonomic nerve fibers are differently affected in preclinical stages of diabetes and are consistent with the view that cranial autonomic fibers and somatic peripheral fibers may not be damaged at the same time, confirming the necessity of a simultaneous neurophysiological assessment of different nerve fibers (21). Additionally, these data suggest that abnormal pupillary parameters may be considered a simple and useful marker of subclinical diabetic neuropathy, relatively independent of other neurological abnormalities. Duration of diabetes was significantly correlated with both autonomic and somatic parameters, whereas we failed to find any correlation between pupillometric parameters, pSEP latency, and metabolic control.

In conclusion, this study evaluated for the first time the impairment of pain sensation by measuring pain pSEP latency in diabetic patients with no clinical or electrophysiological evidence of large nerve-fiber dysfunction. The abnormalities of pSEP latencies mainly affected the lower limbs resembling a length-related pattern of neuropathy. Pupillary study showed a contemporary but unrelated damage of small sympathetic autonomic fibers. These findings confirm that small fibers may be selectively involved and more prone to damage in diabetic patients, strengthening the necessity for an accurate, quantitative, and noninvasive simultaneous assessment of different nerve fibers. In this view, the cortical pain pSEPs may be used to evaluate the electrophysiological integrity of A-Δ fibers in diabetic patients, because no other accurate objective examinations are available to study the impairment of nociceptive sensitivity.

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Early Improvement of Unstable Diabetic Retinopathy After Solitary Pancreas Transplantation

Solitary pancreas transplantation (SPT) can significantly improve the quality of life of diabetic patients by eliminating the need for exogenous insulin, frequent home glucose monitoring, and many of the dietary restrictions imposed by the disease. In addition, successful SPT is able to eliminate acute diabetes complications, such as hypoglycemic and/or hyperglycemic episodes (1).

The effects of SPT on long-term complications of diabetes is less clear, but restoration of long-lasting normoglycemia seems to have several beneficial actions, including improvement of neuropathy and nephropathy (2). Little information is currently available on the role of SPT on the evolution of diabetic retinopathy. Ramsay et al. (3) studied the progression of retinopathy in solitary pancreas recipients and found some beneficial effect at 3 years after transplant.

An SPT program in type 1 diabetic patients has recently started in our center. Inclusion criteria are as recommended (1). In a group of nine patients (four men and five women who were aged 35 ± 10 years, had duration of diabetes of 24 ± 11 years, and received SPT with portal drainage), we evaluated the early effects of the transplant on unstable diabetic retinopathy, a condition characterized by a drop in visual acuity of at least 2 Snellen lines, and/or an episode of vitreous hemorrhage, and/or laser treatment and/or vitrectomy in the 2 years before transplantation (4).

Normalization of glucose levels with no exogenous insulin administration was quickly achieved and maintained by SPT. Fasting plasma glucose concentrations, HbA_{1c} levels, and C-peptide concentrations pretransplant and at 6 months post-grafting were 13.9 ± 0.4 and 4.6 ± 0.2 mmol/l, 9.1 ± 0.3 and 5.8 ± 0.1%, and 0.02 ± 0.00 vs. 3.2 ± 0.2 ng/ml, respectively (all $P < 0.01$). All of the patients were examined with corrected visual acuity, slit lamp examination, measurement of intraocular pressure, indirect and direct retinoscopy, and two nonstereoscopic 45° retinal photographs for each eye (4,5). Compared with pretransplant examination, at 6 months from SPT, two patients showed an improvement of visual acuity of >2 Snellen lines. The number of microaneurisms and microhemorrhages decreased, respectively, from 17 ± 4 to 9 ± 3 and from 12 ± 3 to 5 ± 2 per field (both $P < 0.05$). The number of hard hessudates did not change significantly (8 ± 2 vs. 7 ± 2 per field). Macular lesions of varying degree were present in six eyes (four patients) before transplantation and disappeared in five eyes (three patients) after 6 months from grafting. Intraocular pressure remained unchanged. No negative ocular event occurred in this early post-transplant follow-up.

The effects of pancreas transplantation on diabetic retinopathy are still controversial. In recent work (2,4), improvement or stabilization of advanced diabetic retinopathy was shown when the pancreas was transplanted together with a kidney to also treat renal insufficiency. Scant information is available as for the effects of solitary pancreas grafting on retinopathy in type 1 diabetic patients. In a previous article, a group of patients (most of them with proliferative retinopathy) were followed up to 3 years from SPT (3). At 2 years after SPT, the incidence of progression to a higher grade of retinopathy was the same in the eyes of recipients with versus without pancreas function. However, after 3 years, no further progression occurred in the patients with surviving

graft, and 70% with failed pancreas transplants advanced to a higher grade of retinopathy by 5 years (2). In the present letter, we provide evidence of stabilization or improvement of pretransplant unstable retinopathy, early after pancreas transplantation. Although this finding needs to be confirmed in larger series, and long-term follow-up is obviously required, this is the first reported evidence that normalization of blood glucose levels in type 1 diabetic patients, as achieved by successful SPT, can quickly improve some lesions of diabetic retinopathy.

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Efficacy of Glimepiride for the Treatment of Diabetes Occurring During Glucocorticoid Therapy

Approximately 5–25% of patients receiving glucocorticoids exhibit overt diabetes (1). Glucocorticoids may precipitate diabetes in individuals with impaired insulin secretion by reducing insulin sensitivity (2). The relative importance of β-cell dysfunction and insulin resistance for the pathophysiology of the glucocorticoid-induced diabetes is not well defined. Therefore, there is no consensus treatment for glucocorticoid-induced diabetes. Glimepiride is a sulfonylurea that lowers blood glucose levels by stimulating insulin secretion from pancreatic β-cells and secondarily by increasing glucose uptake in peripheral tissues (3). Such action mechanisms might be suitable for the treatment of glucocorticoid-induced diabetes. Here we examined the effects of glimepiride on patients with newly diagnosed diabetes during glucocorticoid therapy.

Three Japanese female patients who had been taking oral glucocorticoids were newly diagnosed with diabetes. Patient 1 (aged 68 years) had systemic lupus erythematodes, patient 2 (aged 65 years) had Behcet's disease, and patient 3 (aged 48 years) had angiolymphoid hyperplasia with eosinophilia. They had been initially given 20–40 mg/day prednisolone. The dosage of prednisolone was tapered and maintained at 5–10 mg/day. The status of these diseases was well controlled with the glucocorticoid treatment. At 1–2 years after starting the glucocorticoid therapy, they showed overt diabetes, with mean fasting blood glucose 12.6 ± 0.7 mmol/l and HbA_{1c} 9.5 ± 1.5% (means ± SE). Their index for pancreatic β-cell function (HOMA-%β), as determined by the correct homeostasis model assessment evaluation (4), was 27 ± 8%, significantly ($P < 0.001$) lower than that (72 ± 4% [range 44–111%]) in 24 healthy Japanese control subjects (mean age 47 ± 2 years) who had normal glucose tolerance by 75-g oral glucose tolerance test. The index for insulin sensitivity (HOMA-%S) (4) was 56 ± 15% in the patients, significantly ($P < 0.01$) lower than in healthy control subjects (144 ± 10% [81–273%]). We administered glimepiride to these patients (1 mg/day for patient 1 and 3 mg/day for patients 2 and 3). The dosage of prednisolone was unchanged throughout the observation period (24 weeks). Fasting blood glucose declined 4 weeks after the glimepiride administration and was kept below 7 mmol/l until 24 weeks. HbA_{1c} significantly decreased 4 weeks after the treatment, decreasing to 6.7 ± 0.6% after 8 weeks and maintaining that level until 24 weeks. HOMA-%β and HOMA-%S increased to the control levels (76 ± 9% and 108 ± 54%, respectively) 8 weeks after the treatment, remaining within the control ranges at 24 weeks (60 ± 3% and 99 ± 55%).

In three patients with glucocorticoid-induced diabetes, HOMA-%β and HOMA-%S were lower than in healthy control subjects. After the treatment with glimepiride, HOMA-%β and HOMA-%S increased to control ranges, in association with remarkable improvement of glycemic controls persisting until 24 weeks. This is suggested to be attributable to the dual effects of glimepiride on β-cell function and insulin sensitivity. It has been shown that a thiazolidinedione has a potential for the glucocorticoid-induced diabetes (5). From our study, glimepiride is also a strong candidate for the treatment of the glucocorticoid-induced diabetes.

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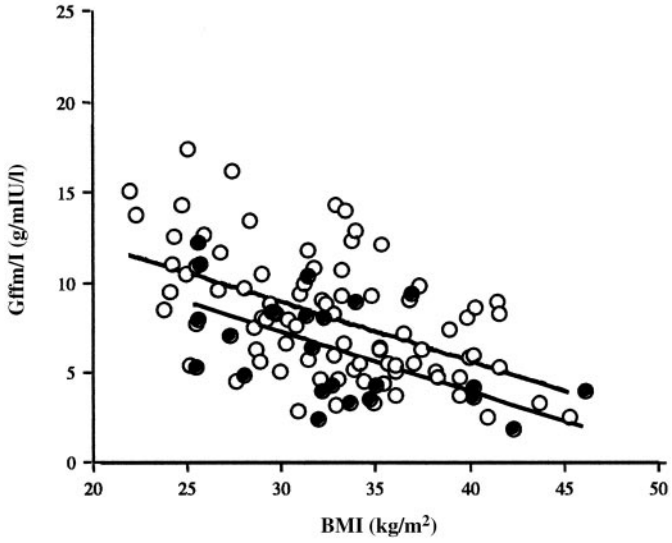

Figure 1—*The figure shows the relationship between the observed (○ European; ●, Maori) and predicted values (top line for European and bottom line for Maori) of glucose infused for fat-free mass divided by the average plasma insulin (Gffm/I) and BMI.*

Increased Risk of Type 2 Diabetes Despite Same Degree of Adiposity in Different Racial Groups

As indigenous people of the South Pacific have rates of type 2 diabetes and obesity among the highest in the world (1,2), diabetes risk reduction strategies include identification of high-risk individuals. Different cutoffs to define overweight and obesity have been suggested for some indigenous people because they have more lean mass and less adipose tissue than Europeans with comparable BMIs (3). However, cutoffs for BMI should be based on risk of comorbidities associated with a given BMI, not simply with lean body mass. To further assist the process of defining appropriate BMI levels for South Pacific people, we have investigated insulin sensitivity in New Zealand women of Maori and European descent who have similar levels of BMI, fat, and lean mass.

A total of 88 European and 23 Maori women consented to participate in an ethically approved study to measure body composition (using dual-energy absorptiometry) and insulin sensitivity (using the euglycemic insulin clamp). Smoking history, weight, height, blood pressure, BMI, and waist circumference were recorded, and fasting plasma glucose and insulin levels were measured. The methodologies are described elsewhere (4). The significance of differences between the two groups was tested by regression analysis.

The Maori women were on average 7 years (95% CI 3–11) younger, had a higher prevalence of smoking (39 vs. 7%), and had higher fasting glucose levels (difference = 0.7 [95% CI 0.3–1.1]) than the European women. The fasting insulin level was 1.5 (1.1–2.0) times higher in Maori women compared with European women. There was no significant difference between Maori and Europeans for weight, BMI, waist circumference, blood pressure, total fat, truncal fat, or lean mass. There was no evidence of reduced adiposity or increased lean mass for a given BMI in Maori women.

Maori had lower levels of insulin sensitivity than Europeans, despite similar BMI levels and total and truncal fat levels. The difference was 1.8 $G \cdot mIU^{-1} \cdot l^{-1}$ (0.3–3.3) (expressed for fat-free mass, where G is glucose infused during the euglycemic in $mg \cdot kg^{-1} \cdot min^{-1}$). After adjusting for age, BMI, fasting glucose levels, and smoking, the difference in insulin sensitivity was 2.0 $G \cdot mIU^{-1} \cdot l^{-1}$ (0.5–3.6). The relationship between insulin resistance and BMI for Maori and European women is illustrated in Fig. 1.

The early report suggesting that Maori and Pacific people in New Zealand have more lean mass and less adipose tissue than Europeans with comparable BMIs (5) did not describe measures of comorbidity. Furthermore, their European group was significantly lighter than the Maori and Samoan groups; therefore, comparisons of lean mass at higher BMI levels may not be valid. The results of our study suggest that for any given level of BMI or total or truncal fat, Maori are more likely than Europeans to have insulin resistance and are therefore at greater risk of type 2 diabetes and cardiovascular disease. After adjusting for age, smoking, and glucose levels, as well as BMI, the difference in insulin sensitivity remained significant. Therefore, there is no evidence to support the use of higher cutoffs for BMI in Maori and, perhaps, other indigenous people.

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Hyperhomocysteinemia and Increased Risk of Retinopathy

A cross-sectional, case-control study in patients with type 2 diabetes

Increased total plasma homocysteine (tHcy) level—secondary to excessive alcohol intake, cigarette smoking, or deficiency of methylene-tetrahydrofolate reductase—is one new identified risk factor for atherosclerotic cardiovascular diseases and for macrovascular complications of diabetes (1,2). Homocysteine (Hcy)-induced vascular damage is probably initiated by reactive oxygen species formed during Hcy auto-oxidation in plasma and results in decreased nitric oxide production, platelet activation, impaired arterial vasodilatation, and isolated systolic hypertension (1).

To assess whether hyperhomocysteinemia is also an independent risk factor for microvascular complications, we cross-sectionally studied the relations among tHcy levels, diabetic retinopathy (diagnosed by indirect ophtalmoscopy and retinal photography), insulin sensitivity and glomerular filtration rate (measured by euglycemic-hyperinsulinemic clamp and unlabeled iohexol plasma clearance, respectively), lipid profile, overnight urinary albumin excretion rate, and other clinical and laboratory parameters in 11 consecutive type 2 diabetic subjects with retinopathy (case subjects) and 26 age- and sex-matched type 2 diabetic subjects without retinopathy (control subjects). To limit the confounding effect of decreased tHcy clearance associated with advanced renal insufficiency, only subjects with serum creatinine ≤2.0 mg/dl were considered. Patients gave written informed consent. The Ethical Committee of the Clinical Research Center, "Aldo & Cele Daccò," Mario Negri Institute, Italy, approved the protocol.

Altogether, 1 case subject had proliferative and 10 subjects had nonproliferative diabetic retinopathy. Prevalence of hyperhomocysteinemia (54.5 vs. 26.9%) and tHcy levels (17.2 ± 6.6 vs. 12.3 ± 4.1 μmol/l, $P < 0.05$) were higher in case than in control subjects. Case subjects also had longer diabetes duration (171 ± 96 vs. 73 ± 75 months), lower BMI (27 ± 5 vs. 31 ± 5 kg/m^2), higher systolic blood pressure (155 ± 15 vs. 145 ± 12 mmHg) and albumin excretion rate (412 ± 600 vs. 212 ± 385 μg/min), and were more frequently micro- or macroalbuminuric ($P < 0.05$ for all comparisons). Age, sex distribution (with a male prevalence in both groups), smoking habit, HbA_{1c} level, diastolic blood pressure, insulin sensitivity, lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides), serum creatinine, glomerular filtration rate, and antidiabetic and antihypertensive treatments were comparable in both groups. At univariate logistic regression analysis, only longer duration of diabetes, higher systolic blood pressure, and increased tHcy levels were associated with diabetic retinopathy. Glomerular filtration rate was not related to diabetic retinopathy ($P > 0.05$). At multivariate forward stepwise logistic regression, tHcy ($\beta = 4.47$, $P = 0.019$), diabetes duration ($\beta = 2.10$, $P = 0.009$), and systolic blood pressure ($\beta = 0.09$, $P = 0.020$) were independent markers of diabetic retinopathy. Altogether, they predicted the disease with 86.5% accuracy (model $\chi^2 = 23.19$, $P = 0.0001$).

Thus, hyperhomocysteinemia is associated with an increased risk of diabetic retinopathy and clusters with longer diabetes duration and systolic hypertension as an independent marker. The finding that case subjects had higher albumin excretion rates than control subjects and were more frequently micro- or macroalbuminuric suggests that hyperhomocysteinemia is also an indicator of overall microvascular damage in type 2 diabetes.

Hcy evaluation may serve to identify diabetic patients predisposed to sight-threatening complications who may benefit from intensified screening and treatment strategies, including folic acid and vitamin B_6 and B_{12} supplements, aimed to limit or prevent the incidence and progression of micro- and macrovascular complications.

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COMMENTS AND RESPONSES

Comment on study by Perkins et al.

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The results of the study by Perkins et al. (1) are surprising since it is usually possible to make an electrophysiological diagnosis of carpal tunnel syndrome (CTS) even in the presence of a peripheral neuropathy. It is not clear from the report whether they made an electrophysiological diagnosis of CTS in any of their cases.

When routine antidromic sensory nerve conduction studies are nondiagnostic median/ulnar palmar latencies at 8 cm, median/radial antidromic latencies to the thumb at the same distance or median/ulnar motor to lumbrical/second interosseous muscle (2) recordings at the same distance should be used. Segmental sensory nerve conduction studies can localize the slowing of a prolonged median antidromic latency to the carpal tunnel (3).

Perhaps the criteria used for the clinical diagnosis of CTS in diabetic patients with a peripheral neuropathy did not discriminate well. The sensory symptoms experienced by patients with CTS are quite varied (4), and the addition of a diabetic neuropathy can further obscure the picture. The study required any four of six criteria to make the diagnosis. Patients with a condition other than CTS, such as ulnar neuropathy, cervical radiculopathy, diffuse peripheral neuropathy, or even thoracic outlet syndrome, might have been included as having CTS. Since their patients may have hand paresthesias from neuropathy, do we need to rely on more specific symptoms such as nocturnal awakening, relief by shaking the hand, and aggravation by reading, driving, etc.? Thenar muscle weakness is a late sign and not helpful in most cases. Perhaps the CTS cases of Perkins et al. were mostly mild. Subclinical slowing of median nerve conduction at the wrist is very common in diabetic subjects, and it's surprising that this did not show up in their data.

Repeating the study using one of the much more appropriate nerve conduction techniques listed above and with different clinical inclusion criteria would be of considerable interest.

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Response to Stevens and Harper

We thank Stevens and Harper (1) for their response to our study and for identifying citations that help to highlight the important issues associated with research in this field. The aim of our study (2) was to determine the best standard nerve conduction study (NCS) marker of clinical carpal tunnel syndrome (CTS), in diabetic subjects, under the hypothesis that at least one parameter would reliably identify CTS. Surprisingly, the results indicated that standard NCS techniques fail to reliably distinguish the presence or absence of CTS in subjects with diabetes. From the results, we were able to infer that the electrophysiological changes at the wrist most likely arise from diffuse nerve injury associated with diabetes rather than signifying the specific symptomatic entrapment of the median nerve. Stevens and Harper express two main concerns with the study: the methods of electrophysiological evaluation and the potential for misclassification bias in clinical CTS cases.

Stevens and Harper recommend and cite specific methods for which they suggest exclusion from the NCS evaluation invalidates our study results. We strongly feel, however, that the recommended methods either do not diverge significantly from our protocol or do not have reasonable support from the literature. Further ratio testing such as median-to-radial latencies may be of interest, although similar techniques using median-to-ulnar and median-to-sural ratios were conducted in our study and were nondiscriminatory for symptomatic CTS. In this regard, neither were median-to-ulnar motor latency ratios. The authors cite the study of Vogt et al. (3), in which the results appear contradictory: the difference in abductor pollicis brevis and abductor digiti minimi (ADM) latencies, which might be expected to be consistent with the more divergent difference in lumbrical and interosseous latencies (L-I DIFF), were minor. Furthermore, it appeared that a diffuse increase in ADM and lumbrical latencies existed across groups whether CTS or diabetic polyneuropathy (DPN) were present exclusively or in combination. L-I DIFF values are associated with large variance, and statistical comparisons were not provided. Furthermore, the comparison relevant to this discussion was underpowered (only 30 subjects with DPN and 22 subjects with DPN and coexistent symptomatic CTS were studied). Although this observation may be a very important one, the study from which it arose is not sufficient to suggest that a study excluding this technique is invalid or inappropriate. The study by Hansson (4) compared segmental median nerve conduction measurements between three mutually exclusive groups: those with CTS and without diabetes, those with diabetes and CTS and without DPN, and those with DPN excluding coexistent CTS. Although we partially evaluated segmental conductions (at the elbow and the wrist) in our study, we feel it necessary to highlight this article as one that we strongly considered in our study design—we aimed to achieve a method that would discriminate CTS regardless of diabetes or DPN status and thus made no effort to create an artificial case-control comparison in which conditions were mutually exclusive. We feel that the studies by Vogt et al. and Hansson have provided very important contributions, but they did not offer convincing evidence for us to undertake methodology that diverges from standard tech-

niques. Furthermore, our study aimed to answer a separate question altogether: in a population of diabetic patients with varying degrees of DPN, can NCS discriminate CTS findings? Excluding or minimizing those with both conditions serves to limit the generalizability of the clinical method we aimed to identify. We do, however, encourage investigators to consider nonstandard techniques in the context of clinical studies that are similarly generalizable, but, based on our study results, remain skeptical.

Drs. Stevens and Harper very appropriately raise the issue of misclassification bias. The inclusion criteria in our report are based on those recommended by the American Academy of Neurology (5) for diagnosis of CTS, and thus were considered appropriate. The requirement that four of six clinical criteria be present to establish a diagnosis of clinical CTS, with the requirement in all subjects for a discrepancy between lower limb and upper limb paresthesiae, are reasonable in this clinical context. As Stevens and Harper note, symptoms of CTS, even in those without diabetes, vary widely (6), and thus a diagnostic instrument for clinical CTS requires the ability to select from a list of criteria. Our criteria included the predominance of radial digit involvement, the presence of nocturnal awakening, and the precipitation of paresthesiae by activity as suggested. The presence of cervical or shoulder pain with radiation and of ulnar nerve palsies were excluded based on clinical neurological evaluation. Although we agree that misclassification bias is a potential factor in the negative study results, we feel comfortable that its contribution is minor for the reasons stated, and we note further that the criteria we used compare favorably with the criteria used in the references cited by Stevens and Harper. Furthermore, increasing the stringency for case definition may serve to create misclassification of control subjects (and thus a biased and deceptively elevated specificity), and restriction of case and control subjects would have even further limited the generalizability of a potential diagnostic method.

In summary, the criticisms and citations raised by Stevens and Harper highlight the difficulty in designing a diagnostic study for a syndromic diagnosis without an unequivocal gold standard. The usual selection of subjects limits generalizability of results and thus fails to answer broad clinical questions. We therefore remain comfortable with and stress the importance of our inferences: the ubiquitous nature of nerve injury, and not the absence of distal median nerve changes, defeated the diagnostic ability of NCS to identify symptomatic CTS in this diabetic population.

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Clinical Heterogeneity of Patients With Adult-Onset Diabetes and GAD Autoantibodies

We read with great interest the recent article by Takeda et al. (1) reporting that GAD autoantibody (GADab)-positive non–insulin-deficient patients differ from GADab-positive insulin-deficient patients with respect to clinical characteristics, humoral autoimmunity to other organ-specific autoantibodies, and HLA class II genes.

The prevalence of GADab in Takeda's study was lower than that in our study (3.8 vs. 6.8%; $P = 0.0009$ by χ^2 analysis), even in patients who were initially diagnosed as having type 2 diabetes (2). The authors should present the number of patients with insulin-deficient and non–insulin-deficient diabetes and the prevalence of GADab in both groups of patients. We speculate that the lower prevalence of GADab might have been due to a higher number of non–insulin-deficient patients in their study than in our study because the prevalence of GADab is reported to be higher in insulin-deficient patients than in non–insulin-deficient patients (3). The authors defined insulin-deficient patients as those patients with typical type 1 diabetes and slowly progressive type 1 diabetes. The authors stated that there are distinct differences between typical type 1 diabetes (rapid progression to insulin dependency) and slowly progressive type 1 diabetes (slowly progressive deterioration of β-cell function through the non–insulin-dependent state and ultimately to insulin dependency). Therefore, the authors should have further divided these phenotypes into two subgroups in order to investigate the differences in clinical characteristics, humoral autoimmunity to other organ-specific autoantibodies, and HLA class II genes.

The results of this study are in accordance with those of our study in that both studies demonstrate that HLA-DRB1 alleles contributed to the prognosis of Japanese patients with diabetes who are positive for GADab (4). The prevalence of HLA-DRB1*0405, which is one of the susceptible alleles to typical type 1 diabetes, was significantly lower, and the prevalence of HLA-DRB1*1502, which is one of the protective alleles to typical type 1 diabetes, was significantly higher in non–insulin-deficient diabetic patients positive for GADab than in typical type 1 diabetic patients positive for GADab.

Furthermore, we demonstrated a significantly greater proliferative response of peripheral blood mononuclear cells to GAD in type 2 diabetes positive for GADab (5), especially in those with alleles susceptible to typical type 1 diabetes; the

responses were useful markers for the later development of insulin deficiency in type 2 diabetes positive for GADab. Interestingly, low GADab levels declined to negative levels during a few years in our study (5). Low levels of GADab in non–insulin-deficient diabetic patients may decline to negative levels, which demonstrates the possibility of pseudopositive GADab. Higher levels of GADab (6), seropositivity for GADab with humoral autoimmunity to other organ-specific autoantibodies, HLA class II genes, or cellular response to GAD may be useful for predicting the clinical course of diabetic patients positive for GADab.

Finally, Kobayashi et al. (7) reported that small doses of subcutaneous insulin in islet cell antibody–positive patients with type 2 diabetes resulted in improved serum C-peptide response. Non–insulin-deficient diabetic patients with GADab without insulin therapy in Takeda's study may prefer to be treated with insulin to reserve residual β-cell function.

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The Clinical Heterogeneity of Adult-Onset Diabetic Patients With GAD Autoantibodies in Japan

Response to Fukui et al.

We would like to thank Fukui et al. (1) for the interest in our article (2) and the editor for the opportunity to clarify the several points raised. As stated several times in our article, this is a cross-sectional and hospital-based study to investigate the clinical, autoimmune, and genetic characteristics in adult-onset diabetic patients with GAD antibodies (GADab). All diabetic patients who visited our hospital during 1998 and 1999, including those with typical type 1 diabetes (a slowly progressive form of type 1 diabetes [SPIDDM]) and type 2 diabetes, regardless of their therapy, were recruited into this study. In our Ehime Study, the overall prevalence of GADab in patients with adult-onset diabetes was 3.8%. Among them, the prevalence of GADab in patients with type 1 diabetes was 61% (72 of 118). The prevalence of GADab in patients with non–insulin-dependent diabetes was 2.0%, which is similar to that reported by Abiru et al. (2.4% in patients with non–insulin-deficient diabetes) (3). The lower prevalence of GADab in our study compared with the study by Fukui et al. (4) is ascribable to several points.

First, the proportion of the patients treated with insulin in the two studies is quite different. The number of patients under insulin treatment in Japan has been estimated to be ~20% (5). However, the proportion of the insulin-treated patients in their study is extremely high (>50%), which probably resulted in the higher prevalence of GADab in their subjects. In fact, the prevalence of GADab in 392 type 2 diabetic patients was 3.1% in their study, which is not significantly different from our data. Second, the GADab assay used in their study is different from our radioassay, which can measure GAD65-specific autoantibodies. They used radioimmunoassay with purified rat brain GAD as antigen that contains both GAD65 and GAD67. It is well known that there are a certain number of patients with type 1 diabetes who are positive for only GAD67ab. Although the predictive power of GAD67ab on future insulin deficiency in GADab-positive non–insulin-dependent diabetes has not been established yet, Fukui et al. might have overestimated the prevalence of GADab in their patients.

In this Ehime Study, we are prospectively following the GADab$^+$ patients with non–insulin-dependent diabetes to clarify the factors that distinguish the nonprogressors from those who develop the insulin-dependent state. Therefore, with the successful completion of the study, the clinical characteristics, humoral autoimmunity to other organ-specific autoantibodies, and HLA class II genes in patients with slowly progressive form of type 1 diabetes will be clarified.

Fukui et al. misread in part our results on the frequency of HLA-DRB1 haplotypes shown in Table 3 of our article. They pointed out that the prevalence of HLA-DRB1*0405 in GADab$^+$ patients with non–insulin deficiency was signifi-

cantly lower than GADab$^+$ patients with insulin deficiency. However, as shown in Table 3 of our article, the frequency of DRB1*0405 was increased in both GADab$^+$ patients with insulin deficiency (28.5%) and non–insulin deficiency (21.0%) compared with healthy control subjects (11.3%), and no difference was observed between two groups. On the other hand, DRB1*0901 was decreased in frequency in GADab$^+$ patients with non–insulin deficiency compared with GADab$^+$ patients with insulin deficiency. Then only the DRB1*0405 was frequent in GADab$^+$ patients with non–insulin deficiency, which may be one of the characteristics in Japanese non–insulin-deficient patients with anti-islet autoantibodies. We agree that one of the features that distinguishes GADab$^+$ non–insulin-deficient patients from insulin-deficient patients is the frequencies of type 1 diabetes–protective HLA haplotypes DRB1*1501 and *1502. We kindly ask Fukui et al. to read our article again to resolve these misinterpretations.

Recently, the results of a randomized controlled clinical trial to prevent the onset of type 1 diabetes in islet cell antibody–positive relatives of patients with type 1 diabetes who were using prophylactic parenteral insulin has been reported; there was no effect on the development of diabetes (6). Although a pilot study in islet cell antibody–positive patients with type 2 diabetes has suggested that the small doses of subcutaneous insulin improve the serum C-peptide response (7), the large randomized controlled trials are necessary to prove whether prophylactic insulin administration can alter the course of development of insulin deficiency.

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The ACE Insertion/Deletion Polymorphism Is Not Associated With the Metabolic Syndrome (WHO Definition) in Brazilian Type 2 Diabetic Patients

The ACE gene has received substantial attention in recent years as candidate for a variety of diseases. The most common polymorphism in ACE gene is the insertion-deletion (I/D) polymorphism located in intron 16. The D allele is associated with higher levels of ACE, and it is considered to be a risk factor for development diseases such as coronary artery disease, hypertension, type 2 diabetes, or related complications (1). Recently, the presence of the metabolic syndrome (MS), according to World Health Organization (WHO) criteria (2), was associated with the D allele in Chinese patients with type 2 diabetes (3). The authors found that patients with MS were more often carriers of the D allele (DD/ID) than the patients without the MS (58 vs. 42%, $P < 0.01$). The prevalence of MS in this sample of Chinese diabetic patients was 75%. The observation that the D allele is associated with the MS is an interesting finding, suggesting that the MS components (glucose homeostasis abnormalities and/or insulin resistance, hypertension, dyslipidemia, obesity, and/or microalbuminuria) might share a common genetic predisposition. We genotyped the ACE I/D polymorphism in 643 type 2 diabetic patients originally from the state of Rio Grande do Sul in the southernmost part of Brazil. Of the 643 subjects, 86% of the state population is from European ancestry and self-classified as white (4); 4 and 8.4% are black and mixed-race, respectively (4). Type 2 diabetes and MS diagnosis followed WHO definitions (2). The ACE genotyping was done as previously reported (5). The prevalence of carriers (DD/ID) and noncarriers of the D allele (II) was compared among the patients with and without the MS.

The MS prevalence in our sample was 75% and did not differ between white subjects and nonwhite subjects. The genotypes were in Hardy-Weinberg equilibrium (DD 30%; ID 50%; and II 20%). In the total group, the frequency of the carriers of the D allele among patients with MS was similar to that of those without MS (81 vs. 76%, $P = 0.18$). It also did not differ among white subjects (81 vs. 76%, $P = 0.19$) and nonwhite subjects (80 vs. 77%, $P = 0.69$). The power of our study to detect an association of the same magnitude as described by Lee and Tsai (3) was >95%. There were also no differences regarding age, duration of diabetes, proportion of males, or any other feature of the MS between carriers or noncarriers of the D allele.

At first glance, an apparent explanation for the discrepancy between our re-

sults and those reported by Lee and Tsai (3) would be due to ethnic reasons, as a result of I/D polymorphism interaction with other DNA sequence changes or environmental factors. It is well known that the allele frequency of ACE I/D polymorphism varies according to the ethnic group (6). The D allele is less frequent among Asian subjects than Caucasian subjects. In fact, the allele frequency in our sample of white individuals was higher than the Chinese study (33 vs. 55%, $P = < 0.01$), and it was similar to that described among other Caucasian populations (6). We do not believe that ethnic factors explain the differences between the two studies. If the ACE gene had a major role in MS predisposition, we would expect to observe lower prevalence of MS among Chinese patients than among Caucasian patients. Nevertheless, the prevalence was almost identical in both studies. An alternative hypothesis, still too complex to be proved, would be that the association reported in the Chinese study was found by chance and not casually related to the development of the syndrome. Further studies in other populations will be very informative in this regard.

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Response to Costa

I am very grateful to Costa et al. (1) for their letter in this issue of *Diabetes Care*, because their findings give me the opportunity to clarify several important points. Our recent paper (2) associated ACE gene insertion/deletion (I/D) polymorphism with metabolic syndrome (MS) in Chinese type 2 diabetic patients. Although Costa et al. showed a similar prevalence of MS in Brazilian type 2 diabetic patients, they did not find any association between ACE gene I/D polymorphism and MS. While we believe their findings are interesting, we do not believe that they can be used to support their claim that our findings came about by chance. The prevalence of MS in patients with type 2 diabetes among different ethnic populations could not be evaluated and compared until MS was defined by the World Health Organization in 1998 (3). According to Zimmet et al. (4), the incidence of type 2 diabetes is changing and ethnic differences exist in global distribution. Furthermore, while the incidence of type 2 diabetes may be similar among Chinese, Caucasian, and Brazilian populations, the prevalence of individual components of MS are quite different. While the prevalence of obesity, hypertension, and dyslipidemia (WHO criteria) are similar in our study and were comparable to those of the Botnia study in Finland and Sweden (5), the prevalence of "microalbumiura-nephropathy" differed. The prevalence of nephropathy was higher in Chinese than Caucasians in the Botnia group (41 vs. 17%). In fact, ethnic differences in macrovascular and microvascular morbidity and mortality have already been reported in one WHO MSVDD study (6), which showed that the prevalence of proteinuria-albuminuria renal failure was especially higher in Asian Chinese than in other populations studied. That same WHO Multinational Study of Vascular Disease in Diabetes (MSVDD) study also revealed that the major cause of death in Asian type 2 diabetic subjects was stroke, while the major cause of death in Western type 2 diabetic populations was ischemic coronary disease. Thus, based on our study and those of others, it is clear that ethnic differences exist in the phenotype presentation of MS, and from these differences it may be implied that the genetic mechanism of type 2 diabetes is also different. It is important to note that Costa et al. mention that the allele frequency of ACE gene I/D polymorphism varies according to the ethnic group.

ACE gene I/D polymorphism is associated with MS in our study mostly because we associated it with nephropathy. We have found ACE gene I/D polymorphism to be strongly associated with nephropathy in our patients when only 300 cases were studied. The more cases we collected, the stronger the statistical significance. Using correlation analysis for each component of MS, we found the correlation between ACE gene I/D polymorphism and dyslipidemia to be weak, but the association between ACE gene I/D polymorphism and MS to be strong. In fact, the association of ACE gene I/D polymorphism with individual components of MS and major cardiovascular diseases has been extensively reviewed (7), and our results are quite similar to those of others. Our only novel finding was that there was a strong association between the ACE gene I/D polymorphism and nephropathy in Chinese subjects with type 2 diabetes, thus contributing to those subjects with MS. Although the prevalence of the individual components of MS are not actually shown by Costa et al., we believe that if their prevalence of individual components of MS were revealed, the differences between Chinese and Brazilian subjects with regard to MS would be found. Costa et al. found no association between ACE gene I/D polymorphism with individual components of MS, and no association with MS at all. Thus, the major difference between our article and the article by Costa et al. may be the prevalence of

nephropathy and the significance of association between the ACE gene I/D polymorphism and nephropathy. We conclude that ethnic and genetic differences do exist in the phenotype presentation of MS.

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Diabetes and the State Capital

Response to Lockwood

In a recent letter in *Diabetes Care*, Lockwood (1) pointed out that there was a strong correlation ($r = 0.54$, $P = 0.000057$) between the statewide self-reported diabetes prevalence in 2000 and the total statewide air toxic release inventory (TRI) in 1999 for the 50 states and Washington, D.C. He pointed out that "[although] [. . .] the correlation between air emissions and the prevalence of diabetes does not prove a cause-and-effect relationship, the significance of the relationship demands attention."

Table 1—*The Pearson correlations between statewide diabetes prevalence and selected state level variables, with associated significance levels*

	r	*P*
Latitude of the state capital	−0.54	<0.001
Longitude of the state capital	−0.31	<0.02
State population	+0.46	<0.001
Numerical position of the alphabetized state list (i.e. Alabama = 1, Wyoming = 51)	+0.49	<0.001

I agree that the correlation does not prove a cause-and-effect relationship, but the demand for attention is questionable. The demand for attention is based on the magnitude of the observed correlation, but to attribute possible cause requires at least a plausible mechanism and individual-level data (not statewide averages). Lockwood developed an impression that dioxins are the main culprit in the hypothetical exposure-response relationship, but it is difficult to understand how the reported correlation is useful for developing the relationship since dioxins are not, as he noted, one of the chemicals inventoried in the TRI.

As an example of how looking at statewide averages (group data) can lead to questionable results, the self-report diabetes data were downloaded from the CDC behaviorial risk factor surveillance systems Web site (2), as were the latitudes and longitudes of each of the state capitals and the state population sizes in 2000. Correlations were calculated among these variables using the same techniques as Lockwood (1) used.

The correlations were instructive. Table 1 shows the Pearson correlation between statewide diabetes prevalence and a selected state level variable in addition to the associated significance level of the correlation (*P* values available only to three significant figures).

The correlation between statewide diabetes prevalence and the latitude of the state capital is the same magnitude as that reported by Lockwood (1) for the correlation between statewide diabetes prevalence and statewide toxic air emissions. The correlations with the other variables are about the same size and are all statistically significant.

The conclusion is that to reduce the risk of diabetes a person should move to a northwestern state with a low population, whose state name is near the beginning of the alphabet—Alaska is a reasonable choice based on an unreasonable application of statistics. However, this application is not very different from the methods used by Lockwood.

I hope that this demonstrates that a highly significant correlation between two variables based on statewide data doesn't show anything.

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Response to Nicolich

In a previous letter (1), I identified a significant correlation between the statewide prevalence of diabetes and air releases of toxicants reported in the Toxics Release Inventory (TRI) report (available from www.epa.gov/tri). Although I suggested a possible mechanism for this link, related to dioxins, I was care-

ful to note that correlations do not prove cause-and-effect relationships. Dr. Nicolich expands on that point in his letter (2).

A coincidence is a "striking occurrence of two events at one time apparently by mere chance" (3). I would agree that it is a virtual certainty that the correlation between state names and diabetes is a coincidence. It is probably a coincidence that latitude is correlated with the prevalence of diabetes, although there are diseases, such as multiple sclerosis and malaria, where latitude is important. It is almost certainly not a coincidence that Dr. Nicolich's authorship is related to his employment history with ExxonMobil Biomedical Sciences, as shown by institutional affiliation listings on another letter like this one and publications retrieved via MEDLINE (4).

Perusal of the ExxonMobil web pages (www.exxonmobil.com) and TRI data show why this company might seek to dissociate itself from consequences of TRI-reported air releases. Affiliates of ExxonMobil mine and sell coal to electrical utilities; according to the 2000 TRI, electric utilities released 787.8 million pounds of toxicants into the air—more than any other industry. ExxonMobil produces a variety of chemicals: the chemical industry ranked 2nd in total TRI-reported air releases, discharging 277.5 million pounds of toxicants. The petroleum industry ranked 10th, discharging 10.5 million pounds. Together, these three industries accounted for ~57.6% of all TRI air releases in 2000. Dioxins released by electric utilities and chemical industries accounted for ~46% of all reportable air discharges.

Air releases tell only a small part of the story of toxicants. The TRI also provides data on waste disposal, where there is also a potential for human exposure. Production-related toxic waste reported by all TRI industries increased by 7.77 billion pounds or 26% between 1999 and 2000. In 2000, reporting industries listed a total of 37.9 billion pounds of TRI chemicals that were managed (recycled, treated, burned for energy, etc.). The chemical industry led all others in off-site waste transfers of >923 million pounds and off-site transfers of dioxins of >40,000 g. When managed wastes are added to air and other releases, the total shows that U.S. industries reported ~46 billion pounds of toxic waste in 2000 or ~160 pounds per U.S. citizen.

Is it a coincidence that the prevalence of diabetes is also rising? I don't know the answer. However, Dr. Nicolich's attempt to discourage rigorous efforts to find an answer makes the search seem more important.

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