

OBSERVATIONS

Role of Triglyceride Levels in Identifying Insulin Resistance in Nonobese Type 2 Diabetic Japanese Patients With Hypertension

Although it is generally accepted that hypertension is associated with insulin resistance, not all patients with hypertension have insulin resistance or hyperinsulinemia (1–3). This suggests that factors other than high blood pressure cause insulin resistance or hyperinsulinemia in the patients with hypertension. Lind et al. (2) were the first to show that insulin-resistant patients with hypertension had not only a higher BMI but also more disturbances of glucose and lipid metabolism compared with insulin-sensitive patients with hypertension. Agata et al. (3) later demonstrated that disturbances of glucose and lipid metabolism might be related to insulin resistance in BMI-matched patients with hypertension. Irrespective of the existence of diabetes in the majority of hypertensive subjects (4), diabetic subjects were excluded from the evaluation in these 2 reports. We therefore recruited type 2 diabetic patients with essential hypertension ($n = 31$) and normotension ($n = 81$) and compared the clinical characteristics among these patients. They were all nonobese but were treated with an oral hypoglycemic agent (glibenclamide). Hypertension was diagnosed when blood pressure was $>160/95$ mmHg or when antihypertensive treatment was in progress (5). Insulin action was measured with homeostasis model assessment-insulin resistance index (HOMA-IR) and validated against a minimal model approach (6,7).

Data were expressed as means \pm SEM. The statistical analysis was performed with the StatView 5 system. Although no significant difference was observed in age, BMI, total cholesterol, HDL-cholesterol, fasting glucose, or HbA_{1c} levels between the patients with hypertension and normotension, fasting insulin (7.7 ± 0.7 vs. 5.9 ± 0.2 μ U/ml, $P = 0.002$), serum triglycerides (142

± 19 vs. 97 ± 3 mg/dl, $P < 0.001$) and HOMA-IR levels (2.5 ± 0.3 vs. 1.9 ± 0.1 , $P = 0.008$) were significantly higher in subjects with hypertension compared with those with normotension. Our present data for HOMA-IR in the hypertensive group indicate that the subjects with hypertension were divided into 2 distinct populations. Of the 31 patients, 19 (61%) had normal HOMA-IR (<2.5) (1.6 ± 0.1 , $P > 0.05$ vs. normotensives) and this group was referred to as the normal insulin sensitivity (N-SI) subset (8). Of 31 patients, 12 (39%) had increased HOMA-IR (>2.5) (4.0 ± 0.4 , $P < 0.001$ vs. normotensives) and this group was called the reduced insulin sensitivity subgroup (R-SI). When the subjects with hypertension were divided into N-SI and R-SI subgroups, fasting glucose (144 ± 7 vs. 125 ± 5 mg/dl, $P = 0.008$), serum triglycerides (225 ± 36 vs. 90 ± 6 mg/dl, $P < 0.001$), and serum insulin (11.4 ± 1.2 vs. 5.4 ± 0.4 uU/ml, $P < 0.001$) levels were significantly higher in R-SI subgroups ($n = 12$) than in N-SI subgroups ($n = 19$). There was, however, no significant difference in fasting glucose, serum triglycerides, and insulin levels between N-SI subgroups and normotensives. No significant difference was observed in BMI, HbA_{1c}, total cholesterol, and HDL-cholesterol levels among the 3 groups.

The reason our type 2 diabetic patients with hypertension are not all insulin resistant is not known, since type 2 diabetes is another disease that is associated with insulin resistance. One possible explanation may be because of the difference in populations studied. Japanese and African-American type 2 diabetic patients are divided into 2 distinct variants; one being insulin resistant and the other being insulin sensitive (9–11). On the other hand, 92% of type 2 diabetic patients are reported to be insulin resistant among the Caucasian population (12). Another possible explanation for the results may be because of the degree of BMI studied. Our patients with hypertension had a BMI <27.0 kg/m² (i.e., nonobese). It is well recognized that being overweight causes insulin resistance in humans (13). Cabezas-Cerrato et al. (14) previously showed that obese type 2 diabetic patients had a similar degree of insulin resistance irrespective of hypertension and suggested that hypertension is not generally associated with any significant increase in insulin resistance. Therefore, the prevalence of insulin resistance among hypertensive diabetic individuals would probably be higher

in a population-based study in which obese groups were included. One might argue that antihypertensive medications might affect insulin action in our present study. However, it seems unlikely, since the antihypertensive drugs used were either ACE inhibitors or calcium channel blockers and because no difference was found in the frequency of the use of the ACE inhibitors (10 of 19 in N-SI and 7 of 12 in R-SI) or calcium channel blockers (9 of 19 in N-SI and 5 of 12 in R-SI) between the 2 groups. From these results, it can be hypothesized that nonobese type 2 diabetic Japanese patients with hypertension can be subdivided into at least 2 subpopulations: one with insulin resistance and higher triglyceride levels and the other with normal insulin sensitivity and a normal lipid profile. In this regard, the previous study by Groop et al. (15), which showed that type 2 diabetic patients with hypertension had not only insulin resistance but also hypertriglyceridemia, supports our hypothesis.

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Effect of Physical Training on Insulin Sensitivity in Japanese Type 2 Diabetic Patients

Role of serum triglyceride levels

Type 2 diabetes is a complex disorder characterized by insulin resistance and/or impaired insulin secretion (1). Along with dietary therapy, exercise has been advocated for the management of type 2 diabetes. Although the potential role of exercise in diabetic subjects is primarily to increase insulin sensitivity, information concerning the mechanism is limited in type 2 diabetic patients (2–4). Exercise has been found to reduce serum triglyceride levels in patients with type IV hyperlipoproteinemia (5). However, to the best of our knowledge, it is not clear whether exercise training-induced reduction in triglyceride levels enhances insulin sensitivity in type 2 diabetic patients. In this context, a major issue is that not only exercise training but also body weight reduction might lower triglyceride levels and improve insulin sensitivity in type 2 diabetic patients. To separate the effects of exercise from those of weight reduction, short-term exercise protocol was performed in weight-stable patients during the study. Therefore, the purpose of this study was to determine whether short-term physical training of type 2 diabetic patients could reduce serum triglyceride levels and insulin resistance without affecting body weight.

Fifteen sedentary Japanese type 2 diabetic patients participated in the study after the provision of informed consent. Their age and BMI were 59.9 ± 2.6 years and 25.4 ± 1.7 kg/m², respectively. HbA_{1c} levels were $9.3 \pm 0.4\%$ (range 6.3–11.3%).

Type 2 diabetes was diagnosed based on the criteria of the World Health Organization (6). None of the subjects had a physical finding suggestive of cardiovascular or renal disease. Eight patients were taking sulfonylureas (glibenclamide), but their medication was not changed during the course of the study. None of them took any medications affecting lipid metabolism. All patients were hospitalized and ingested the following diet under supervision: $1,488 \pm 43$ kcal/day (range 1,200–1840 kcal/day) (50–58% carbohydrate, 18–20% protein, and 24–30% fat).

The patients engaged in an exercise program that consisted of aerobic and resistance exercise for 12 days. They were instructed to walk at least 7,000 steps per day as an aerobic exercise and perform dumbbell exercise described previously (7). The foot count was monitored by a pedometer (Calorie Counter; Suzuken, Nagoya, Japan). During the course of the study, they walked a mean of $11,398 \pm 1,435$ steps per day. After an overnight fast, the blood was drawn from an antecubital vein for the determination of glucose, insulin, and lipid profile before and after the exercise training. The estimate of insulin resistance by homeostasis model assessment (HOMA-IR) was calculated with the fasting serum insulin formula ($\mu\text{U/ml}$) \times fasting plasma glucose (mmol/l)/22.5, as described by Matthews et al. (8). Statistical analysis was performed by Student's *t* test, taking a value of $P < 0.05$ as significant.

After exercise, BMI decreased from 25.4 ± 1.7 to 24.9 ± 1.6 kg/m², but was not statistically significant ($P = 0.425$). Serum triglyceride level significantly fell from 175 ± 21 to 119 ± 9 mg/dl ($P = 0.013$), after exercise. In contrast, no significant changes in total cholesterol or HDL cholesterol concentrations occurred after exercise. Plasma glucose and serum insulin levels significantly decreased from 163 ± 11 to 128 ± 9 mg/dl ($P = 0.011$) and 7.2 ± 0.9 to 5.2 ± 0.6 $\mu\text{U/ml}$ ($P = 0.042$), respectively. The HOMA-IR value was significantly lower after exercise (2.97 ± 0.40) than before exercise (1.62 ± 0.19 , $P = 0.003$).

Exercise had long been considered in the treatment regimen for patients with type 2 diabetes. In the present study, we investigated the effect of exercise on insulin sensitivity and glucose levels in Japanese type 2 diabetic patients and found that exercise not only improved insulin sensitivity but also glycemic control. The beneficial effect of exercise on insulin sensitivity and

Plasma Brain Natriuretic Peptide Levels in Normotensive Type 2 Diabetic Patients Without Cardiac Disease

Brain natriuretic peptide (BNP) plays an important role in the regulation of body fluid and blood pressure (1,2). BNP is produced mainly by cardiac tissue and is secreted from the ventricle in humans. It has been reported that plasma concentrations of BNP in patients with congestive heart failure, chronic renal failure, and essential hypertension are greater than normal (3,4). Although abnormally high levels of BNP are sometimes found in patients with diabetes, there have been very few studies on the plasma levels of BNP in diabetic patients without cardiac dysfunction (5). We studied plasma BNP levels in normotensive diabetic patients without cardiac disease.

This study was comprised of 100 type 2 diabetic patients (62 men and 38 women, aged 57.4 ± 9.9 years, HbA_{1c} $7.6 \pm 1.6\%$, serum creatinine level 65 ± 18 $\mu\text{mol/l}$). Data obtained from 386 healthy volunteers involved plasma levels of BNP observed no more than twice. The first-time BNP value is called BNP1 and the second-time value is called BNP2. BNP1 (273 men and 21 women, aged 55.8 ± 3.3 years) and BNP2 (305 men and 23 women, aged 54.7 ± 3.4), with normal glucose tolerance, were available for comparison. The diabetic patients had no history of ischemic heart disease and had normal blood pressure ($<140/90$ mmHg), normal cardiothoracic ratio (on plain radiography of the chest), and no left ventricular hypertrophy (on electrocardiography). Fourteen of the 100 patients showed proteinuria, and 28 had retinopathy. None of the patients was receiving antihypertensive drugs, including ACE inhibitors, before beginning the study. Plasma BNP levels were measured with an immunoradiometric assay kit (Shionoria BNP kit; Shionogi, Osaka, Japan), following a method previously described (6). The detection limit of the assay was 2.0 pg/ml.

In the regression analysis with age as an explanatory variable, the regression of BNP levels (BNP1 and BNP2) with loga-

rithmic transformation in healthy subjects was not significant. According to the results of the Jonckheere's test, the values of BNP1 (10.8 ± 11.7 pg/ml, $P = 0.5117$) and BNP2 (10.1 ± 10.2 pg/ml, $P = 0.1022$) weren't significantly related to age. However, plasma levels of BNP that estimated the regression coefficient of age were positive (coefficient = 0.0125, $P = 0.0176$), and plasma HbA_{1c} levels were negative (coefficient = -0.0691 , $P = 0.0273$) in all diabetic subjects. Plasma BNP levels that estimated the regression coefficient of the other clinical findings, such as serum creatinine levels and presence of proteinuria and retinopathy, were not significant. The values of plasma BNP levels (14.7 ± 9.2 vs. 11.1 ± 6.2 pg/ml, $P = 0.1520$) were not significantly different between patients with proteinuria and those without proteinuria by Wilcoxon's rank-sum test. Also, the relationship of BNP plasma levels to the presence of retinopathy did not differ significantly between patients without retinopathy and with retinopathy (14.0 ± 9.1 vs. 14.6 ± 8.3 pg/ml, $P = 0.4678$).

Although the plasma BNP levels have not been well documented in diabetic patients, it was only reported that the plasma levels of BNP are significantly correlated with the degree of microalbuminuria in patients with diabetic nephropathy (5). On the contrary, the present findings show that there was no relationship between the plasma BNP levels and the presence of proteinuria, but the plasma levels of BNP were significantly correlated with HbA_{1c} and age in diabetic patients without cardiac disease. The reason for the negative correlation found between the plasma levels of BNP and HbA_{1c} remains unclear. One possible explanation is that the presence of hyperglycemia increases plasma osmotic diuresis and may suppress BNP secretion in the regulation of body fluid. It was reported that even in elderly inpatients without overt heart failure, the plasma BNP concentration tended to be greater in association with systolic dysfunction, cardiac hypertrophy, and renal dysfunction, and also with diastolic dysfunction (7). The plasma BNP levels in our diabetic patients tended to be greater in association with age. We speculated that the increase in plasma BNP levels may be attributable to some unknown factors related to diabetic duration. These factors include diabetic cardiomyopathy probably due to microangiopathy and the increased mass of myocardium, which cannot be estimated by ordinary routine examinations (8).

In conclusion, the results of our study suggest that the determination of plasma BNP levels is not an indicator of diabetic complications without cardiac disease, which can be estimated through routine examinations. Therefore, it is important to point out, as a clinical manager, that increased plasma level of BNP is a sign of cardiac dysfunction in diabetic patients and is the main indication for further cardiac examination.

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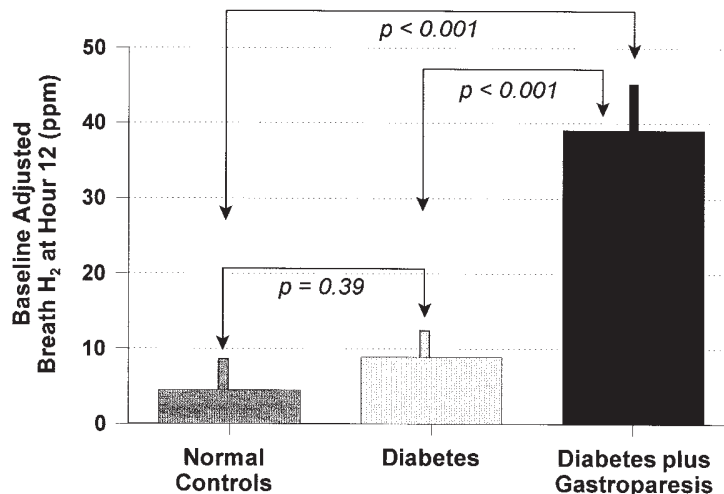
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Breath Hydrogen Testing Identifies Patients With Diabetic Gastroparesis

Delayed gastric emptying is a common and debilitating complication of long-standing diabetes (1). Unfortunately, diagnosis of this condition with current scintigraphic techniques is limited by high cost, wide inter- and intraindividual variability, and exposure of the patient to low levels of ionizing radiation. Because the ingestion of lactulose results in the colonic production of lactate, carbon dioxide, and hydrogen (H_2) gas, and because colonic H_2 is excreted by the lungs in direct proportion to the amount of lactulose being metabolized in the colon, lactulose-breath hydrogen testing can be used for the quantitative assessment of oral-cecal transit time in both diabetic and nondiabetic subjects (2,3). Ingestion of complex carbohydrates, such as potato starch, also results in breath H_2 excretion (4). We, therefore, hypothesized that breath hydrogen excretion would be abnormally prolonged in patients with previously diagnosed diabetic gastroparesis after ingestion of a potato-lactulose test meal compared with subjects without gastroparesis.

This 24-h pilot study consisted of 10 healthy nondiabetic control subjects, 10 diabetic subjects without gastroparesis (gastric emptying $T_{1/2} < 90$ min), and 10 diabetic subjects with previously diagnosed gastroparesis (gastric emptying $T_{1/2} > 90$ min). Radionuclide determination of gastric emptying was performed as previously described (5). Gastric motility agents were withheld 24 h before study, and euglycemia was established and maintained overnight in diabetic subjects with a continuous intravenous insulin infusion. At 6:00 A.M., all subjects ingested a breakfast containing 100 g dry-cooked potato starch and 20 g lactulose. Breath H_2 excretion was monitored at baseline and for 12 h after ingestion of the

Figure 1—Mean baseline-adjusted breath H_2 concentrations 12 h after ingestion of 100 g of potato starch and 20 g of lactulose among the 3 study groups.



test meal. All subjects provided written informed consent before the study as approved by the University of New Mexico Human Research Review Committee.

Breath H_2 samples were analyzed in duplicate by gas chromatography using the QuinTron SC Hydrogen Breath Analyzer (QuinTron Instruments, Milwaukee, WI). Study data were analyzed using a repeated measures analysis of variance with posthoc application of Fisher's least significant difference method of multiple pairwise comparisons, using SAS (SAS Institute, Cary, NC).

There was no significant difference in plasma glucose concentrations during the 12-h study period between the diabetic group and the gastroparetic group ($P = 0.79$). Baseline breath H_2 levels were elevated in the diabetic plus gastroparetic group (normal control group = 3.6 ± 5.2 , diabetic group = 4.1 ± 5.9 , diabetic plus gastroparetic group = 13.1 ± 12.4 ppm; $P = 0.06$ compared with the groups with no gastroparesis). There was no significant difference between the groups in peak breath H_2 concentrations or in time-to-peak breath H_2 . However, the time course of breath H_2 concentrations was significantly different in the gastroparetic group compared with those of the other 2 groups by repeated measures analysis of variance ($P < 0.01$). Figure 1 shows that baseline-adjusted breath H_2 concentrations were significantly elevated in the diabetic plus gastroparetic group compared with the diabetic and normal control groups 12 h after ingestion of the test meal.

Although current standards of practice call for a radionuclide gastric-emptying

study to aid in the diagnosis of gastrointestinal motility disorders in diabetic patients, the development of a functional test of upper gastrointestinal function after the ingestion of a solid meal may prove to be a valuable diagnostic aid for patients affected with this problem (1,6–10). This pilot study suggests that the presence of an elevated baseline-adjusted breath H_2 concentration, 12 h after the ingestion of an easily prepared test meal containing potato starch and lactulose, accurately identifies those patients with previously diagnosed diabetic gastroparesis. Such a test may prove to be a useful outpatient screening test for the identification of those symptomatic patients who should receive a more definitive (but expensive) scintigraphic study and those in whom the presence of gastroparesis can be excluded.

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Insulin-Induced Hypoglycemia Induces a Rise in C-Reactive Protein

The regulatory responses to hypoglycemia result in diverse physiological and biochemical changes. In a study to address physiological changes in lipids and blood viscosity after hypoglycemia, we measured C-reactive protein (CRP) before and 4 and 24 h after the hypoglycemic

nadir. We used a sensitive enzyme-linked immunosorbent assay capable of measuring levels between 1 and 8 mg/l with an intra-assay coefficient of variation <7% (1).

We matched 6 male patients with type 1 diabetes (median age 36.5 years, range 28-38; median duration of diabetes 15 years, range 10-17) with 6 nondiabetic male control subjects (median age 31.5 years, range 24-39) recruited from within the hospital staff. All patients had good glycemic control, median HbA_{1c} 7% (+4SD), range 6-8.3% (+1.5 to 7.3 SD), laboratory mean 5.4 ± 0.4%. No diabetic patient had a history of antecedent hypoglycemic episodes in the preceding 6 weeks, suffered diabetic complications, smoked, drank >10 U/week, or was on any drug treatment other than insulin.

After an overnight fast, acute hypoglycemia was induced by the intravenous administration of a soluble insulin bolus 0.15 U/kg. Hypoglycemia was confirmed by plasma measurement (Beckman Synchro CX3, Brea, CA), the physiological changes of tachycardia, and typical autonomic symptoms (2). The depth of the hypoglycemic stimulus and the subsequent hemodynamic changes are similar to those described in other studies (3), and they suggest that activation of the autonomic nervous system took place with epinephrine release.

Basal median plasma glucose fell to a nadir of 1.4 ± 0.2 and 1.5 ± 0.3 mmol/l in control subjects and diabetic patients, respectively. In the diabetic patients, the median basal CRP was 0.77 mg/l (range 0.26-2.1); at 4 h, it was 0.84 mg/l (range 0.26-2.06), before rising to 2.31 mg/l (range 1.01-2.79) 24 h after the hypoglycemic episode. Similar rises in the control subjects were observed from a median initial level of 0.32 mg/l (range 0.13-2.52) to 0.96 mg/l (range 0.49-6.38) at 24 h. No significant difference was present by 4 h, but the levels increased by 24 h ($P < 0.04$, $P < 0.04$) in both groups. The data were analyzed using a nonparametric statistical sign test on Minitab statistical software standard version 7.2 (State College, PA).

Studies have demonstrated that increased CRP concentrations are associated with preexisting peripheral vascular disease (4), increased atherosclerotic complications (5), and increased fibrinogen concentrations (5). In patients admitted with unstable angina, those with increased CRP levels have a worse prognosis (6). The reported CRP levels are similar to those found in this study.

Hypoglycemia has been postulated to aggravate diabetic microvascular disease (7), and recurrent hypoglycemic episodes may provoke changes in hemostatic factors and viscosity (7), resulting in reduced perfusion. It is possible that recurrent hypoglycemia in an individual contributes to such hemostatic perturbations by provoking significant subclinical inflammatory response, since an association between inflammation and thrombosis is established (8). Future studies are needed to elucidate the changes in inflammatory markers after hypoglycemia with particular respect to endothelial function and thrombosis.

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Trp64Arg Polymorphism of the β_3 -Adrenergic Receptor Is Not Associated With Diabetic Nephropathy in Japanese Patients With Type 2 Diabetes

Diabetic nephropathy is a clinical syndrome that is characterized by persistent albuminuria, an increase in blood pressure, a relentless decline in kidney function, and increased cardiovascular morbidity and mortality. Of type 2 diabetic patients, 15–60% develop diabetic nephropathy (1,2). Nevertheless, the precise mechanisms of development and progression of nephropathy are not fully understood. Diabetic nephropathy has been shown to cluster in the families of both in type 1 (3,4) and type 2 (5) diabetic patients to an extent that cannot be explained by shared environmental factors, suggesting that 1 or more genetic factors may be involved.

The Trp64Arg mutation of the β_3 -adrenergic receptor gene has been considered to be underlying in some forms of obesity and insulin resistance (6). It was recently suggested that Trp64Arg mutation of the β_3 -adrenergic receptor gene is associated with diabetic nephropathy in Japanese type 2 diabetic patients (7), whereas the association was not observed in Caucasian (Polish) patients with type 2 diabetes (8). On this basis, we conducted a cross-sectional association study and a 4-year follow-up study to test the contribution of the β_3 -adrenergic receptor gene polymorphism as a candidate gene for diabetic nephropathy in Japanese type 2 diabetic patients.

Table 1—Genotype distribution of β_3 -adrenergic receptor gene and frequency of Trp64 and Arg64 alleles in normoalbuminuric, microalbuminuric, and proteinuric type 2 diabetic patients

| | Type 2 diabetic patients | | |
|-------------|--------------------------|------------------|-------------|
| | Normoalbuminuric | Microalbuminuric | Proteinuric |
| <i>n</i> | 41 | 47 | 52 |
| Genotype | | | |
| Trp64/Trp64 | 26 (63.4) | 34 (72.3) | 40 (76.9) |
| Trp64/Arg64 | 13 (31.7) | 13 (27.7) | 11 (21.2) |
| Arg64/Arg64 | 2 (4.9) | 0 (0.0) | 1 (1.9) |
| Allele | | | |
| Trp64 | 65 (79.3) | 81 (86.2) | 91 (87.5) |
| Arg64 | 17 (20.7) | 13 (13.8) | 13 (12.5) |

Data are *n* or *n* (%).

In the study, there were 140 patients with type 2 diabetes (88 men and 52 women, aged 56 ± 6 years) from the out-patient diabetes clinic at Kitasato University Hospital: 41 normoalbuminuric patients (54.6 ± 6 years of age, diabetes duration 14.1 ± 5.4 years), 47 microalbuminuric patients (56.7 ± 7.9 years of age, 12.7 ± 5.9 years), and 52 proteinuric patients (55.8 ± 6.7 years of age, 14.9 ± 5.2 years). The patients were chosen on the basis of age (40–69 years old) and known duration of diabetes (>5 years). Normoalbuminuria was defined as having all 3 of the measurements of the urinary albumin-to-creatinine ratio (ACR) <30 mg/g creatinine during the last 6 months, and microalbuminuria as having all 3 of the measurements of ACR >30 mg/g creatinine in protein dipstick–test negative patients. Proteinuria was diagnosed when patients showed persistent proteinuria together with the presence of retinopathy.

Genomic DNA was extracted from peripheral blood leukocytes of each patient. The Trp64Arg mutation in the β_3 -adrenergic receptor gene was detected by the polymerase chain reaction–restriction fragment length polymorphism method with BstNI, as reported by Widén et al. (6).

The difference in the measured variables among the 3 groups was tested by the Kruskal-Wallis test, and the genotype distribution and allele frequency among the groups were analyzed by the χ^2 test. A *P* value <0.05 was considered to be significant. In addition, a backward stepwise multiple regression analysis ($F > 4.0$ to enter) was performed to assess the influence of independent variables (i.e., sex, age, known duration of diabetes, current BMI, current HbA_{1c}, presence of hypertension, and genotype of the β_3 -adrenergic receptor gene) on diabetic nephropathy.

The genotype distributions and allele frequency of the β_3 -adrenergic receptor gene are summarized in Table 1. There was no difference among the genotype distribution ($P = 0.857$) in the 3 groups. The frequency of mutated allele was even slightly, but not significantly ($P = 0.130$), lower in the microalbuminuric (13.6%) and proteinuric patients (12.5%) as compared with the normoalbuminuric patients (20.7%). A 4-year follow-up revealed that 25% of patients with wild homozygotes and 33.3% of patients with mutated alleles progressed from normoalbuminuria to microalbuminuria ($P = 0.750$). Similarly, 22.2 and 16.7% of microalbuminuric

Table 2—Progression of nephropathy during 4-year follow-up in type 2 diabetic patients with and without ARG64 allele

| Genotype | Normoalbuminuria to microalbuminuria | Microalbuminuria to proteinuria | Proteinuria to hemodialysis |
|--------------|--------------------------------------|---------------------------------|-----------------------------|
| Wild type | | | |
| Trp64/Trp64 | 4/16 (25.0) | 6/27 (22.2) | 11/36 (30.6) |
| Mutated type | | | |
| Trp64/Arg64 | 4/12 (33.3) | 2/12 (16.7) | 4/12 (33.3) |
| Arg64/Arg64 | | | |

Data are *n* of progressor/total (%).

patients with wild homozygotes and with mutated alleles progressed from microalbuminuria to persistent proteinuria, respectively ($P = 0.745$). The progression rate from persistent proteinuria to end-stage renal failure (i.e., introduction to hemodialysis) was similar in the 2 groups (30.6% in those with wild homozygotes vs. 33.3% in those with mutated alleles, respectively, $P = 0.917$) (Table 2). The result of multiple regression analysis did not suggest a significant contribution of the mutation to nephropathy ($F = 0.928$), whereas the presence of hypertension was the most relevant variable ($F = 25.340$).

We could not confirm the suggested association (7) between the β_3 -adrenergic receptor gene polymorphism and nephropathy in Japanese type 2 diabetic patients. Our observations, however, are in accordance with those of Grzeszczak et al. (8), who also failed to find the association in Polish type 2 diabetic patients. Therefore, the difference between the findings of Sakane et al. (7) and the observations of Grzeszczak et al. (8) is not likely to be caused by the type of diabetes or the ethnic origin of the study subjects. In addition, there was no significant difference in the progression of nephropathy between the patients with and without a mutated allele, although we are aware that the follow-up period might be too short and the number of patients studied is too small to draw any definite conclusions. Nevertheless, these results imply that the association between the Trp64Arg mutation in the β_3 -adrenergic receptor gene and nephropathy, even if it exists, would be modest, and is not a clinically useful marker for the predisposition to diabetic nephropathy in type 2 diabetic patients.

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Telemedicine in the Management of Pregnancy in Type 1 Diabetic Women

The intensive care and treatment of pregnant women with type 1 diabetes, which aims for normoglycemia during the entire time of pregnancy, is very

important to prevent congenital malformations, macrosomia, and peripartur complications in the children (1). We studied whether the diabetological care of pregnant women can be improved through the use of telemedicine, which offers facilitated communication between clinicians and patients (2,3). For this purpose, we used the prototype of a remote data management system (CareLink; Abbott-MedSense, New Bedford, MA) and are reporting our preliminary results.

Eleven pregnant women with type 1 diabetes (all treated with an intensified insulin therapy, either with multiple daily injections or continuous subcutaneous insulin infusion, mean age 30.5 ± 3.4 years, mean diabetes duration 13.5 ± 7.7 years) were controlled by the CareLink system from the 15th gestational week on. This was in addition to the usual diabetological care, which consists of regular ambulatory visits with consultations and examinations every 2–3 weeks in our diabetes center. The appointments made for the visits depended partially on the achieved metabolic control and on the personal situation of the patients (e.g., distance between home and hospital, mothers with babies or young children).

The telemedicine system we used enabled the patients to easily transmit their blood glucose values from the memory of the glucose meter (storage capacity 125 values), by means of a modified modem via phone line, directly to a personal computer in our diabetes center. There the data were stored and evaluated by means of appropriate software. The patients performed blood glucose self-monitoring at least 4–6 times a day and usually transmitted their glucose values once a week. We aimed for fasting and preprandial blood glucose values between 60 and 90 mg/dl and postprandial 2-h blood glucose values ≤ 120 mg/dl (4). The patients were advised to make additional transmissions when values outside these targets occurred frequently. In this case, we provided advice over the telephone to make appropriate corrections of the insulin doses. Besides different graphs showing the development of the blood glucose values over time, mean values (calculated monthly) and standard deviations of all glucose values and of the fasting glucose values separately were taken for evaluation.

A control group was formed of 10 pregnant women with type 1 diabetes with

comparable age, diabetes duration, self-monitoring practice, and insulin regimen, who received standard diabetological care during the same time period as the CareLink group, but without the addition of telemedicine. Since these patients used different glucose meters, their glucose values could not be automatically evaluated by the applied computer software. The patients of both groups also kept a conventional diabetes diary.

The mean time between 2 visits was 3.3 weeks for the CareLink group and 2.9 weeks for the control group. HbA_{1c} (assessed by high-pressure liquid chromatography, normal range 4.3–6.1%) was improved in the CareLink group (mean duration of care 22.5 ± 5.9 weeks) from 6.1 ± 1.0 to 5.4 ± 0.3% and in the control group (mean duration of care 26.8 ± 4.5 weeks) from 6.2 ± 0.8 to 5.7 ± 0.6% (the difference between the groups in the 2-sided independent-samples Student's *t* test was not significant). The mean blood glucose (all values) in the CareLink group was reduced from 141 ± 90 to 110 ± 18 mg/dl, the mean fasting glucose from 111 ± 17 to 101 ± 23 mg/dl (*P* < 0.05, 2-sided paired-samples Student's *t* test). The variation of blood glucose was markedly reduced, too: the standard deviation in the individual patients fell from 51.6 to 44.4 mg/dl (*P* < 0.01) for the mean blood glucose and from 41.4 to 31.0 mg/dl for the mean fasting glucose. There was no significant difference in the number of instances of severe hypoglycemia in both groups.

From our experience, we conclude that the described remote data management system for glucose monitoring is easy to use and helpful for tight and efficient care of pregnant diabetic women, even when the number of personal ambulatory visits in the diabetes center is decreased. Thus, this tool of telemedicine is suitable especially for women who have difficulties adhering to the regular visits at a diabetes center.

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Gly82Ser Polymorphism of the Receptor of Advanced Glycation End Product Gene Is Not Associated With Coronary Heart Disease in Finnish Nondiabetic Subjects or in Patients With Type 2 Diabetes

Long-lasting exposure to hyperglycemia leads to the accumulation of advanced glycation end products (AGEs), which could lead to diabetic complications (1). The effects of AGEs are mediated via the cellular receptor of AGE (RAGE), and variants in the RAGE gene could potentially enhance the development of coronary heart disease (CHD). Recently, Hudson et al. (2) detected 4 functional amino acid variants in the RAGE gene. The relatively common Gly82Ser polymorphism in exon 3 was not associated with myocardial infarction in type 2 diabetic patients of different ethnic origin. In another study, Liu and Xiang (3) found no association of the Gly82Ser polymorphism with diabetic microangiopathy in Chinese type 2 diabetic patients.

We screened the Gly82Ser polymorphism of the RAGE gene among 308 unrelated Finnish nondiabetic subjects with CHD (221 men and 87 women, aged 60 ± 1 years), 206 unrelated type 2 diabetic patients with CHD (141 men and 65 women, aged 64 ± 1 years), and in 82 randomly selected healthy men (aged 54 ± 1 years). Patients with CHD had to have stenosis >50% in at least 2 coronary vessels in a coronary angiogram. Genotyping was performed according to the method of Hudson et al. (2). There were no differences in genotype frequencies (12 vs. 15 vs. 7%

Gly82Ser), (no Ser82Ser homozygotes) or Ser allele frequencies in codon 82 of the RAGE gene (6 vs. 7 vs. 4%) among the study groups. The Ser allele frequency (6%) was as frequent as that in Caucasian patients (6%) (2), but lower than that in Chinese type 2 diabetic patients (23%) (3). In the present study, the Ser allele was not associated with any of the CHD risk factors (data not shown).

Our study indicates that the Gly82Ser polymorphism in exon 3 of the RAGE gene is not associated with CHD in Finnish nondiabetic subjects or in patients with type 2 diabetes.

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Relationship Between Plasma Adrenomedullin Levels and Metabolic Control, Risk Factors, and Diabetic Microangiopathy in Patients With Type 2 Diabetes

Well-controlled diabetes prevents the occurrence of microvascular complications and decreases the formation of macrovascular complications (1,2).

Table 1—Plasma ADM levels and general features of the treatment and control group

| Group | Treatment | n | Sex | | Age | BMI (kg/m ²) | Disease duration | Adrenomedullin (pg/ml) |
|---------|-----------------|----|-----|----|------------|-----------------------------|---------------------|---------------------------|
| | | | F | M | | | | |
| I | Diet | 14 | 9 | 5 | 46.9 ± 9.0 | 29.2 | 2.9 | 63.59 ± 11.71 |
| II | Diet + OAD | 11 | 8 | 3 | 47.4 ± 6.6 | 27.5 | 6.7 | 64.94 ± 9.11 |
| III | Diet + OAD + HT | 21 | 16 | 5 | 55.6 ± 9.2 | 28.5 | 6.6 | 64.55 ± 9.53 |
| IV | Diet + insulin | 18 | 12 | 6 | 55.7 ± 8.1 | 28.4 | 10.5 | 65.43 ± 10.89 |
| Control | — | 20 | 8 | 12 | 50.2 ± 6.0 | 27.6 | — | 55.90 ± 11.12 |

Data are n and means ± SEM, unless otherwise indicated. HT, hypertension.

Studies concerning the pathogenesis, complications, and metabolic control of diabetes are still continuing. Adrenomedullin (ADM) is a vasorelaxing peptide produced from endothelium and smooth muscle cells. ADM is known to decrease the levels of insulin and delay the insulin response to oral glucose (3,4). In this study, we investigated plasma ADM levels in patients with type 2 diabetes and healthy subjects.

Enrolled in the study were 64 type 2 diabetic patients (19 men and 45 women) aged 52 ± 9 years. The mean disease duration was 6.9 ± 5 years and the mean BMI was 28.5 ± 4.5 kg/m². These patients were grouped according to age, sex, type of treatment, presence of microangiopathy, fasting blood glucose levels, HbA_{1c} levels, hypertension, hyperlipidemia, BMI, and disease duration. Patients with liver disease, renal failure, or congestive heart failure were excluded from the study. The control group consisted of 20 healthy subjects (12 women and 8 men), aged 50 ± 6 years with a mean average BMI of 27.6 ± 4.8 kg/m².

Fasting venous blood samples were immediately transferred into a chilled polypropylene tube containing EDTA (1 mg/ml blood) and aprotinin (500 kU/ml blood). Plasma ADM was measured by radioimmunoassay using the kit supplied by Phoenix Pharmaceutical (Mountain View, CA). Glucose was measured by the hexokinase method (Olympus AU 600; Olympus Diagnostica GmbH, Hamburg, Germany). HbA_{1c} was measured by micro-column chromatographic spectrophotometry (Poli Industria Chimica, Milan, Italy).

Mean plasma ADM levels were found to be 64.6 ± 10.1 pg/ml in patients with type 2 diabetes. In the control group, it was 55.9 ± 11.1 pg/ml. The difference was statistically significant ($P = 0.0036$). The 64 patients with type 2 diabetes were divided into 4 groups according to the type of treatment as follows: group I ($n = 14$) was on a diet; group II ($n = 11$) was on

a diet and oral antidiabetic drugs (OAD), e.g., gliclazide, glipizide, glibornuride, metformin, and glimepirid acarbose; group III ($n = 21$) hypertensive diabetic patients were on a diet and OAD; and group IV ($n = 18$) consisted of patients on a diet and insulin treatment. The plasma ADM levels in the 4 groups were 63.5 ± 11.7, 64.9 ± 9.1, 64.5 ± 9.5, and 65.4 ± 10.8 pg/ml, respectively. The differences were statistically significant when compared with the control group (all P values <0.05). However, the difference between the 4 groups was not significant (all P values >0.05). Table 1 shows the general characteristics and ADM levels of the control group and patients with type 2 diabetes.

When the effects of microangiopathy on ADM levels were investigated, the mean ADM level was found to be 65.3 ± 10.6 pg/ml in patients with only nephropathy ($n = 14$), 62.4 ± 9.3 pg/ml in patients with only neuropathy ($n = 7$), 62.2 ± 11.7 in patients with only retinopathy ($n = 3$), 67.1 ± 10.1 pg/ml in patients with nephropathy and neuropathy ($n = 6$), 71.1 ± 10.9 pg/ml in patients with nephropathy and retinopathy ($n = 4$), 62.5 ± 10.8 pg/ml in patients with neuropathy and retinopathy ($n = 4$),

and 64.9 ± 9.5 pg/ml in patients with neuropathy, retinopathy, and nephropathy ($n = 10$). The mean ADM level was found to be 63.2 ± 10.2 pg/ml in patients having no diabetic microvascular complications ($n = 16$) (Table 2). There were statistically significant differences in all groups with diabetic microangiopathy when compared with the control group (all P values <0.05). However, the difference between the groups with and without microangiopathy was not statistically significant (all P values >0.05). There were also no significant differences in patients with or without nephropathy, neuropathy, or retinopathy (all P values >0.05) (Table 3). Plasma ADM levels were found to be 65.4 ± 7.1 pg/ml in patients with HbA_{1c} levels ≤8% ($n = 21$) and 64.9 ± 11.6 pg/ml in patients with levels >8% ($n = 43$) ($P > 0.05$). When fasting blood glucose levels were compared, plasma ADM levels were found to be 61.5 ± 11.3 pg/ml in patients with fasting blood glucose levels ≤140 mg/dl ($n = 14$) and 65.5 ± 9.7 pg/ml in patients with fasting blood glucose levels >140 mg/dl ($n = 50$) ($P > 0.05$). When hypertensive diabetic patients ($n = 28$) were compared with normotensive

Table 2—Mean plasma ADM levels of patients with diabetic microangiopathy compared with the control group

| Diabetic microangiopathy | n | ADM (pg/ml) |
|--|----|-------------|
| Nephropathy | 14 | 65.37 |
| Neuropathy | 7 | 62.41 |
| Retinopathy | 3 | 62.23 |
| Nephropathy + neuropathy | 6 | 67.13 |
| Nephropathy + retinopathy | 4 | 71.10 |
| Neuropathy + retinopathy | 4 | 62.55 |
| Nephropathy + neuropathy + retinopathy | 10 | 64.94 |
| Without complication | 16 | 63.28 |
| Control | 20 | 55.90 |

Data are n, unless otherwise indicated. All P values are <0.05.

Table 3—Plasma ADM levels of patients with and without nephropathy, neuropathy, or retinopathy

| Diabetic microangiopathy | n | ADM (pg/ml) |
|--------------------------|----|---------------|
| With nephropathy | 34 | 66.23 ± 9.99 |
| Without nephropathy | 30 | 66.10 ± 11.06 |
| With neuropathy | 27 | 64.41 ± 10.63 |
| Without neuropathy | 37 | 64.41 ± 11.29 |
| With retinopathy | 21 | 65.27 ± 10.10 |
| Without retinopathy | 43 | 64.32 ± 11.24 |

Data are n and means ± SEM. All P values are >0.05.

patients (n = 36), ADM levels were 64.3 ± 9.0 and 65.0 ± 11.1 pg/ml, respectively, with no statistically significant difference (P > 0.05). When BMI values were compared, they were 59.8 ± 12.4 pg/ml in patients with >28 kg/m² (n = 29) and 64.0 ± 10.0 pg/ml in patients with ≤28 kg/m² (n = 35) with no statistically significant difference (P > 0.05). There were also no statistically significant differences between men and women (P > 0.05). Patients having a diabetes duration of <5 years (n = 25) were compared with those having a duration of ≥5 years (n = 39). Duration of diabetes did not influence the ADM levels significantly (P > 0.05). The effects of age (patients <50 years of age, n = 27 and >50 years of age, n = 37) had no significant influence on ADM levels (P > 0.05). Patients having hypercholesterolemia (>200 mg/dl) or hypertriglyceridemia (>160 mg/dl) were compared with patients having normal lipid levels, and no statistically significant difference existed (all P values >0.05). Patients having risk factors for atherosclerosis such as hypertension, smoking, hyperlipidemia, low HDL levels, increased age, sex, and family history of diabetes were divided into 2 groups. Group A consisted of patients having 1 or 2 risk factors and group B consisted of patients with ≥3 risk factors for atherosclerosis. The plasma ADM levels were 64.3 ± 10.3 pg/ml in group A and 65.0 ± 10.1 pg/ml in group B with no statistically significant difference (P > 0.05).

It was reported that plasma ADM levels, in patients with type 2 diabetes having poor metabolic control, were increased when compared with control subjects (5). In a Spanish study, there was no statistically significant difference in hypoglycemic or hyperglycemic patients and no correlation between plasma ADM and HbA_{1c} levels was found (4). It may be commented that well- or poorly controlled diabetes did

not change the plasma levels of ADM. However, a local increase in ADM levels in the vascular endothelium may not greatly affect the plasma levels. Because ADM is thought to be produced by many of the tissues, local vasodilatory effects are more prominent than the systemic effects (6).

ADM may also affect and play a role in the neovascularization process, which occurs after retinal ischemia. ADM controls proliferation, differentiation, and migration of cell functions and stimulates organ development, normal epithelial turnover, and tissue development (5,7). ADM may take part in the pathogenesis of nephropathy. The use of ACE inhibitors in diabetic patients with microalbuminuria may be the reason for the insignificant ADM levels, since these drugs improve endothelial function and elevated ADM levels may be normalized.

In conclusion, plasma ADM levels were significantly elevated in patients with type 2 diabetes when compared with the control group. However, plasma ADM levels were not affected by poor metabolic control, type of microangiopathy, or other risk factors that can cause endothelial injury. Elevated plasma ADM levels in patients with type 2 diabetes may be related to endothelial damage or leukocyte activation, which is thought to be important in the formation of diabetic micro- and macrovascular complications. Because the source of circulating ADM could not be shown exactly, it could have only a local vasodilatory role (6,8,9). That is why an increase in plasma levels of ADM may not be correlated with the risk factors.

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Cautionary Note Regarding HbA_{1c} Methods Predicting the Clinical Status of Diabetic Patients

Our laboratories (Elmhurst Memorial Hospital and Evanston Northwestern Healthcare) were independently involved in discussions with physicians that quickly progressed to clinical complaints about the lack of correlation between HbA_{1c} results and the clinical status of the patient. Physicians complained that the current laboratory method (Abbott's affinity ion capture method correlated to HbA_{1c}) was giving low values compared with the daily glucose values being reported by the patients. The assessment of clinical status was performed, in part, by review of daily self-monitored glucose values logged by the patient and brought to the office visit. Values for HbA_{1c} <7.0% indicate successful glycemic control based on the results of the Diabetes Control and Complications Trial (DCCT) (2). This study demonstrated that intensive treatment of patients with type 1 diabetes reduces the risk of the development or the progression of retinopathy, nephropathy, and neuropathy (50–75% reduction). HbA_{1c} values between 7.0 and 8.0% are acceptable, but the patient is advised to watch his or her regimen closely and try to lower the values even further. Values >8.0% indicate the need for a change in treatment and/or behavior.

Both laboratories investigated the performance of the Abbott method but found no explanation for the discrepancy between the HbA_{1c} values and the physician's assessment of the patient status. Therefore, a collaborative retrospective study was designed to evaluate the correlation of patient status with 3 commercially available methods for measuring HbA_{1c}. The total patient population consisted of 63 diabetic outpatients (35 type 1 and 28 type 2) treated by physicians at Elmhurst Memorial Hospital. To be enrolled in the study, patients had to have a complete record of their daily glucose values determined by home glucose monitors over the last 60 days. The methods evaluated were a high-performance liquid chromatography

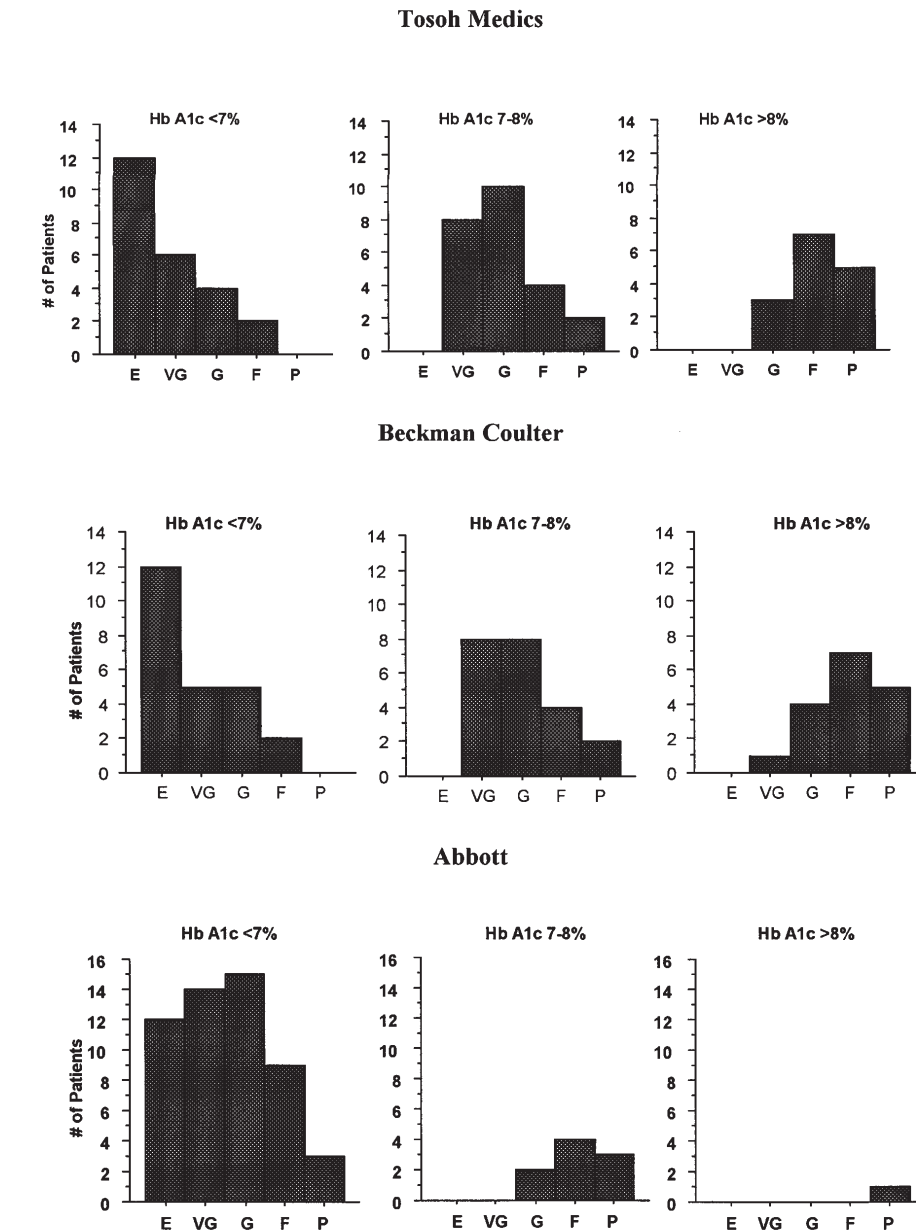


Figure 1—The number of patients in each category of glycemic control for each HbA_{1c} method separated into HbA_{1c} values of <7, 7–8, and >8%. Categories of glycemic control are as follows: excellent (E), very good (VG), good (G), fair (F), and poor (P), based on mean fasting morning glucose values of the previous 60 days.

(HPLC) method by Tosoh Medics (South San Francisco, CA), the Beckman Coulter Synchron turbidimetric immunoinhibition assay (Beckman, Brea, CA), and the Abbott IMX affinity ion capture method (Abbott Diagnostics, Abbott Park, IL). All HbA_{1c} methods were performed according to the manufacturer's written specifications.

The mean morning fasting daily serum glucose level was used to assign each patient a specific glycemic control category.

Using their experiences in treating diabetic patients, 2 endocrinologists created the categories. The categories were as follows: poor (7 patients, mean morning fasting glucose >200 mg/dl, 11.1 mmol/l), fair (13 patients, 160–200 mg/dl, 8.9–11.1 mmol/l), good (17 patients, 135–160 mg/dl, 7.5–8.8 mmol/l), very good (14 patients, 110–135 mg/dl, 6.2–7.4 mmol/l), and excellent (12 patients, 80–110 mg/dl, 4.4–6.1 mmol/l). We assessed the validity

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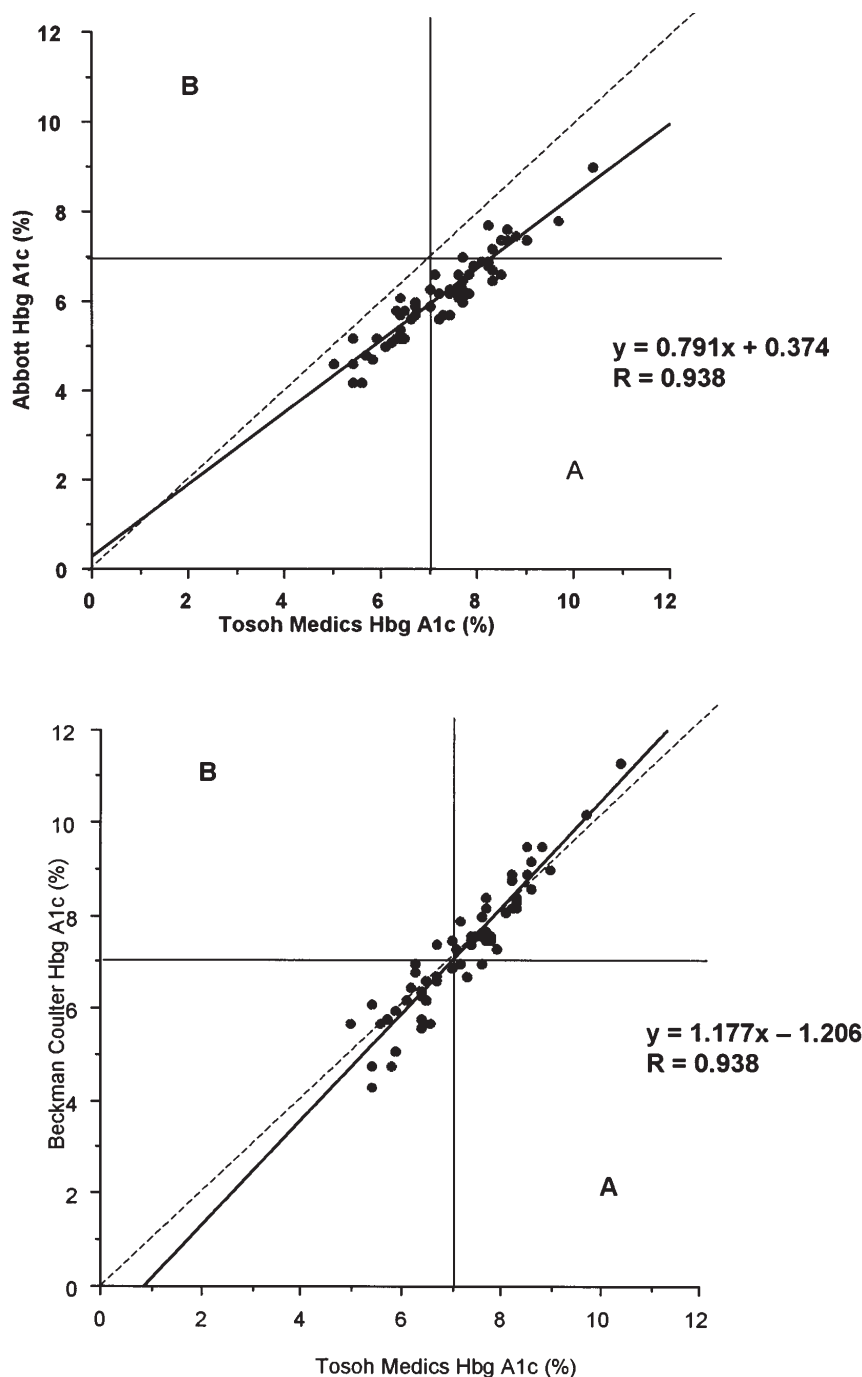


Figure 2—Linear regression plots of Tosoh Medics versus Beckman (A) and Tosoh Medics versus Abbott (B). Single vertical and horizontal lines mark an HbA_{1c} of 7.0%, the target value used in current practice guidelines. The dashed line is the line of perfect regression. The method of least-square regression was used to generate plots.

of using mean morning fasting glucose values from the previous 60 days as compared with the mean of all daily glucose values used in the DCCT trial (3). The results were remarkably similar as shown by the following data: 1) The target glucose means were very similar at the same HbA_{1c} levels (at HbA_{1c} = 7.0%, DCCT trial glucose = 8.3

mmol/l, current study = 8.3 mmol/l). 2) The ranges of glucose means were similar (HbA_{1c} = 7.0%, DCCT trial range = 3.6–13.3 mmol/l, current study = 3.6–12.8 mmol/l). 3) The linear regression statistics were comparable (DCCT trial: $r = 0.80$, slope = 36.0; current study: $r = 0.75$, slope = 31.0, $P < 0.005$). The similarity of the

study data and the original DCCT trial data indicates that using fasting morning glucose values to predict the expected HbA_{1c} level is acceptable. The fasting morning glucose value has the advantage of being easier to use because calculating the mean of all of the glucose results brought to the office visit is often impractical.

Figure 1 shows the number of patients for each method sorted by HbA_{1c} values of <7.0, 7.0–8.0, and >8.0%. The distribution of patients across the 3 HbA_{1c} groups is almost identical for the Tosoh Medics and Beckman Coulter methods, whereas most patients (53) have values of <7.0% with the Abbott method. Overall, 20 of the 63 patients were categorized as having poor or fair control based on mean fasting morning glucose values. In this group of 20, the HbA_{1c} values suggested that improved treatment was needed for 18 patients by both the Tosoh Medics and Beckman Coulter methods and for 8 patients by the Abbott method.

Figure 2A and 2B shows the linear regression graphs for Tosoh Medics versus Beckman Coulter and Tosoh Medics versus Abbott. Each graph is divided into 4 quadrants by a single vertical and horizontal line marking an HbA_{1c} value of 7.0%. Data points that fall in quadrant A represent patients for whom the method on the x-axis suggests that improvement is needed, whereas the method on the y-axis suggests that treatment is appropriate. The opposite is true for quadrant B. When the Tosoh Medics versus Beckman Coulter data are compared, a single patient falls into quadrant B and 3 patients fall into quadrant A. For the Tosoh Medics versus Abbott data, 22 patients fall into quadrant A and none are in quadrant B. More significant to the clinical status and treatment of the diabetic patients are any data points >8% for 1 method and <7% with the other method. There were no occurrences in Fig. 2A; however, Fig. 2B showed 6 data points where the Tosoh Medics method had values >8% and the corresponding Abbott value was <7%.

Implementation of the American Diabetes Association recommendations concerning the utilization of HbA_{1c} assays in patient management requires that the assays used perform in a manner similar to the DCCT method (HPLC). The Beckman Coulter method, in this study, demonstrates comparability with the Tosoh Medics method in assessing the clinical status of the diabetic patient. The Abbott

method demonstrates a significant difference in performance, indicating a better degree of metabolic control than what is actually being achieved. In Fig. 2B, 22 patients fell into quadrant A, indicating discrepancies in clinical control by the Abbott method compared with the Tosoh Medics method. Even more serious, 6 patients had values $>8\%$ with the Tosoh Medics method and $<7\%$ for the Abbott method. The importance of these discrepancies resides with the guideline to alter treatment when HbA_{1c} levels are $>8.0\%$. The outcome of this performance variance has the potential of leading to serious errors in treatment plans, thus placing the patient at increased risk for the complications associated with diabetes.

Physicians and laboratory scientists should be aware of this potential problem because the Abbott method is widely used; $\sim 45\%$ of laboratories reporting results in the College of American Pathologist Glycohemoglobin Survey are using this method. Review of the performance of the Abbott method on survey samples reveals that the method shows a low recovery with moderately and markedly elevated specimens when compared with target concentrations. This finding has been the subject of comment in the discussion section of multiple survey reviews (5,6). This is comparable with the data presented in this study, suggesting that the performance on survey samples is not because of a matrix effect. The data in Fig. 2B suggest that the problem with the Abbott method may be caused by method calibration because there is good correlation with the Tosoh Medics method with a proportional bias throughout the analytical range. Abbott presented an abstract at the 1999 American Association for Clinical Chemistry National Meeting describing a reagent reformulation of the method. In the abstract (7), Abbott indicated that the major goal for the revised method was to achieve a total coefficient of variation of $<5\%$. The problem described in our study is not directly addressed by the Abbott abstract. Therefore, until proven otherwise, physicians and laboratory scientists should assume that the problem still exists with the reformulated reagent.

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COMMENTS AND RESPONSES

Where Is the Evidence That Radial Artery Tonometry Can Be Used to Accurately and Noninvasively Predict Central Aortic Blood Pressure in Patients With Diabetes?

Brooks et al. (1) describe a study of patients with type 1 diabetes and control subjects, during which they applied the blood pressure analysis (BPA) system (PWV Medical, Sydney, Australia)

to derive what they claim to be central aortic blood pressure. Three important issues need to be highlighted.

First, none of the 14 patients who provided the data used to originally develop the radial artery generalized transfer function (GTF) incorporated within the BPA system were reported to have diabetes (2). Given the early onset of arterial disease and pathological differences in diabetic individuals, it remains to be proven whether a GTF developed with data from nondiabetic subjects is equally applicable to patients with diabetes.

Second, no validation studies of the GTF approach in diabetes have been described. Therefore, once again the reliability of this GTF in type 1 diabetes remains completely unproven. In addition, there is an absolute paucity of evidence that radial artery tonometric blood pressure waveform signals, calibrated with brachial artery sphygmomanometry and passed through a GTF, actually give central aortic blood pressure data accurately, least of all in patients with diabetes.

Third, the authors' statement that "the validity of such an approach was recently independently verified by Chen et al. (3)" is simply not correct. Chen et al. (3), from Johns Hopkins, never tested the BPA system from PWV Medical, and certainly have never established the validity of the GTF approach.

Chen et al. (3) have developed their own GTF, using a completely different computational technique, based on data from 20 patients. They then reverse-tested the predictive accuracy of their GTF in those same 20 patients, calibrating their radial artery tonometric waveform signals, not using brachial artery sphygmomanometry as Brooks et al. (1) did, but rather using data from a transducer cited within the aorta (3). Therefore, the work by Chen et al. in no way serves as a validation of the noninvasive technique, which Brooks et al. have used. In this respect, for a validation study to be valid, it must test the methodology in a way that is identical to that which is applied in practice.

Some of the authors (4) from the earlier study by Chen et al. (3) have tried to test their own system prospectively, in a separate study (4), by calibrating their radial artery tonometric waveform signals using noninvasively measured systolic and diastolic blood pressures. However, they found that "since estimated mean pressure by this method varied >10 mmHg from the simultaneously invasive data in 67% of

cases...[they] recalibrated the tonometer signal assuming equal mean and diastolic pressures between aortic and radial pressure" (4). That is to say, there were such big differences between the mean blood pressure measured in the aorta and that estimated noninvasively using radial artery tonometry with noninvasive brachial artery calibration, that those authors scrapped the noninvasive calibration and relied instead on invasive data from the aorta to calibrate their signals.

It is baffling how researchers can rely on intra-aortic blood pressure data to calibrate blood pressure waveform signals obtained noninvasively from tonometry of the radial artery, and then claim to be able to use such radial artery signals to predict central aortic blood pressure (5).

In addition, those authors have written "there is no argument that individual patient TF [transfer function] differences exist, and for some estimation parameters, such as augmentation index...these differences likely preclude reliable use of a GTF" (6), which rather throws into doubt the reliability of the whole approach used by Brooks et al. to assess central aortic augmentation.

Other researchers have also independently raised substantial concerns about the reliability of the GTF approach for estimating the augmentation index in the aorta (7–9).

In this respect, despite the obvious commercial claims that are being made about the approach (10), to date there has been no published independent peer-reviewed study of a proper validation of the BPA system (11,12). A proper validation study would have had peripheral waveform data collected noninvasively and calibrated noninvasively with sphygmomanometric brachial artery blood pressure readings. These calibrated peripheral waveform data would then have been passed through the GTF, and the computed central aortic blood pressure values and waveform shapes would have been compared with those measured simultaneously in the aorta using a catheter-tipped pressure transducer (12).

The closest to such a study that can be found in the literature involves the use of a brachial artery cuff oscillometric method (instead of a sphygmomanometer) for calibration of the radial artery waveform signals. In this study involving 20 patients, there was a mean difference of 11 mmHg between measured and esti-

mated aortic systolic blood pressure, and a mean difference of 8 mmHg between measured and estimated aortic diastolic blood pressure (13).

According to the Association for the Advancement of Medical Instrumentation, in guidelines endorsed by the U.S. Food and Drug Administration, when the accuracy of any new blood pressure measurement method is compared with that of an intra-arterial catheter, the maximal mean allowable difference both for systolic and diastolic readings is 5 mmHg (14). Therefore, far from the BPA system being validated, it appears that it does not actually comply with these guidelines.

Given all of the above, Brooks et al. should exercise caution in making claims about the validity of the approach they have adopted, which at present remains completely unproven with noninvasive sphygmomanometric calibration, especially in diabetic individuals.

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Response to Lehmann

Dr. Lehmann's negative views on the derivation of central arterial pressure waveform by the mathematical transformation of radial tonometry pressure data are well known, as evidenced by the several debates he has entered into in the correspondence columns of various journals. These issues have been addressed by Kass et al. in one letter by Lehmann (1) and Wilkinson et al. in another letter (2). The debate largely concerns the accuracies of indirect methods for measuring arterial pressure (3). There is no argument that the most accurate measurement of aortic pressure is by direct catheterization. However, this is obviously not possible for studying a large group of individuals. Therefore, we will address only the question of whether this method is applicable to various disease

states, including diabetes. Studies by Gal- lagher of 439 subjects showed that there is no significant difference in transfer function between normal subjects and patients with a variety of cardiac and vascular diseases (4). At the moment, there is no evidence that diabetic patients behave differently from nondiabetic individuals in this regard. Our findings of the abnormal results in dia- betic patients (5), especially the male cohort, indicate that further study in this area would provide interesting information. Our reference to the validation work of Chen et al. (6) referred to their approach of using a transfer function to derive central arterial pressure from peripheral waveform. We neither stated nor implied that identical methods were used.

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Hospital Management of Diabetic Ketoacidosis in the U.K.

We note with interest the article by Levetan et al. (1) discussing the care of patients with diabetic ketoacidosis (DKA) in the U.S. They found that the length of stay and the hospital costs incurred were greater when the patient was treated by a nonspecialist rather than an endocrinologist. There is limited evidence in the literature comparing nonspecialist physicians' care with diabetologists' care of patients with DKA. A comparison of the management of DKA provided by general physicians with that of diabetologists was made in Glasgow Royal Infirmary (a large city-center teaching hospital) in 1989-1990. Episodes of DKA were identified from the hospital's computerized discharge coding list. A total of 58 episodes of DKA in 44 patients were included in the review. Patients were included if they had an initial blood glucose >17 mmol/l, pH <7.25, and ketonuria. Nondiabetologists treated 25 and diabetologists treated 32 of the episodes (in 1 case, no physician was recorded).

There was no significant difference between the 2 groups with respect to age, duration of diabetes, or admission biochemistry. Fluid and potassium replacement and insulin dose were assessed in the first 4 h after admission and also from 4 to 24 h after admission. The amount of insulin and fluids given in both time periods did not differ significantly between the 2 groups. The only significant difference between the groups was in the amount of potassium given in the first 4 h, mean 37.2 mmol (CI 27.7-46.7) in the diabetologists' group and 13.9 mmol (5-22.8) in the non-diabetologists' group, $P < 0.0001$. This difference had disappeared by 24 h. The patient outcomes were identical in the 2 groups.

Patients treated by nondiabetologists showed a trend toward longer hospital stays, mean 8.2 days vs. 5.6 days, which was not, however, statistically significant. This may reflect the general tendency for specialist diabetes physicians to manage problems on an outpatient basis.

In the U.K., it would be unrealistic, especially in smaller hospitals, to expect that all cases of DKA would be managed by a specialist. Based on our results, we feel that well informed nonspecialist physicians

can manage DKA adequately. However, resources may be used more efficiently by specialist diabetes physicians.

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Response to Buchanan and Paterson

We thank Buchanan and Paterson (1) for their thoughtful comments and appreciate their presentation of data from the Glasgow Royal Infirmary. Their cohort confirms our findings of a difference in length of stay (LOS) between generalists' and specialists' care. The Glasgow investigators found a 2.6-day (46.4%) reduction in LOS when care is provided by specialists compared with a 1.6-day (48.5%) reduction in our series (1).

Our population of 260 patients achieved statistical significance between the generalists and specialists, whereas their study was underpowered, despite a similar difference in LOS. We agree with their conclusions that well informed nonspecialist physicians can manage diabetic ketoacidosis (DKA) adequately, but specialists may use resources more efficiently. We also agree that it may be unrealistic, especially in smaller hospitals, to expect a specialist to manage all cases of DKA. At a \$4,646 reduction in hospital charges per admission for DKA cared for by endocrinologists, a hospital would find it cost effective to hire a full-time endocrinologist (at an average annual salary of \$160,000) (2), if the hospital treats at least 35 DKA patients per year.

Regardless of their primary diagnosis, patients with diabetes have longer hospital stays than those without diabetes hospitalized for similar conditions (3). Given that there are 4.2 million annual U.S. hospital-

izations for patients with diabetes, inpatient care by specialists may be an extremely cost-effective measure if LOS is reduced by as little as 1 day (2).

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Hemophilus Vaccine Associated With Increased Risk of Diabetes

Causality likely

Graves et al. (1) recently published a study that cited our research several times and questioned the ability of the hemophilus vaccine to cause type 1 diabetes. We recently published our findings from a prospective clinical trial on the hemophilus influenza B vaccine (2). The data indicate that the hemophilus vaccine is likely to cause type 1 diabetes and the risk of the vaccine exceeds the benefit multifold. The study by Graves et al. did find the hemophilus vaccine associated an odds ratio of 1.18 with a mean follow-up of 6.2 years, and we found the vaccine associated with a relative risk of 1.19 at age 5.

The differences between our finding and that of Graves et al. can be explained by several factors. We measured an increase of 58 cases of type 1 diabetes per 100,000 patients when the vaccinated cases were

studied from immunization at 3 months until the age of 10. The additional cases did not begin to occur until ~3.5 years postimmunization. In contrast, the authors rely on a single autoantibody to predict the development of type 1 diabetes, and it is well known that a single autoantibody has very low specificity for predicting the development of type 1 diabetes. Our analysis involved studying more than 100,000 vaccinated children and an equal number of control subjects. The authors, in contrast, studied only 25 individuals with an autoantibody and 292 control subjects. Their study group has found only 5 antibody-positive children who developed diabetes, whereas our study involved 886 cases of type 1 diabetes. In summary, their study is too small, their follow-up too short, and their markers too nonspecific to obtain the findings we made. However, even with all of these limitations, the authors found the hemophilus vaccine associated with an odds ratio of 1.18 (72/62, Graves et al. Table 1), which was similar to the relative risk of 1.19 (166/140) that we found in the hemophilus vaccinated children by age 5.

We would like to clarify several points pertaining to our research to which Graves et al. elude. We propose that immunization starting in the first month of life will lead to a decreased risk of type 1 diabetes when compared with immunization starting after 2 months of life (3,4). We are not proposing that immunization be delayed until 2 or 5 years of age but instead be administered earlier. There is now a large amount of data to support an association between immunization starting after 2 months and an increased risk of type 1 diabetes (3,5). The data include the hemophilus, pertussis, measles, mumps, rubella, hepatitis B, and Bacillus Calmette-Guérin vaccines. We are aware that vaccine manufacturers and public health officials do not want to alarm the public. However, we believe that the public has the right to know that data indicate that the increased risk of diabetes associated with the hemophilus and other vaccines appears to exceed the benefit of these vaccines (2), and efforts to deny this cause many children to forgo the needed financial compensation to which they are entitled.

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Hemophilus Vaccine and Diabetes

In our recent case-control study of the association between immunizations and β -cell autoimmunity (1), the odds ratio for exposure to *Hemophilus* vaccine before the age of 9 months was 1.64, not 1.18 as calculated by Classen and Classen (2). However, it was not statistically significant, since the 95% CI of 0.62–4.5 included 1. In reporting their analysis of data from a Finnish randomized trial of different *Hemophilus* vaccine schedules (3), Classen and Classen do not mention that a separate published analysis of the data has concluded that there is no evidence of a significant increase in incidence of type 1 diabetes in children given a 4-dose schedule starting at age 2 months compared with 1 dose at age 2 years (4). There is no evidence from this Finnish trial or any other study in humans that vaccination with *Hemophilus* at birth would reduce the risk of diabetes.

Graves et al. agree that a larger sample size would be desirable to increase the power of the study, but feel that the importance of a possible harmful or protective effect of immunizations precluded waiting for more cases to accrue in the Diabetes Autoimmunity Study in the Young.

Although Classen and Classen imply that the cases in our study had transient autoantibodies, in fact the case definition required persistent autoimmunity and the majority of cases had multiple autoantibodies, which are predictive of a high risk of developing type 1 diabetes (5). Nevertheless, a larger case-control study to further investigate this issue in children who have type 1 diabetes is in progress.

Prospective studies of the effect of immunizations on diabetes incidence, such as that performed in Finland (4), will be extremely difficult because of the large number of participants and the very long follow-up time required. Case-control studies are a more productive way of investigating this important topic. Studies to date have not produced any evidence that change in the childhood immunization schedule would prevent β -cell autoimmunity or lower the risk of type 1 diabetes.

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Consensus Development Conference on Diabetic Foot Wound Care

A randomized controlled trial does exist supporting use of adjunctive hyperbaric oxygen therapy

I read with interest the Consensus Development Conference Report published in the August 1999 issue of *Diabetes Care* (1). As an attendee and participant in that conference, I am puzzled and concerned about the statement in that report regarding the use of adjunctive hyperbaric oxygen (HBO) therapy: "There are no randomized controlled trials supporting the use of hyperbaric oxygen therapy to treat neuropathic foot wounds." I discussed the work of Faglia et al. (2) in which 70 diabetic patients were randomized to undergo either adjunctive HBO therapy or more traditional care. The groups were well matched and very carefully investigated and monitored. Sensory motor neuropathy was present in all 35 of the HBO-treated patients and in 31 of the control subjects ($P = 0.23$); thus, neuropathy was a prominent feature in this study. Additionally, both groups were well matched for vasculopathy and corrective angioplasty or bypass surgery. This study clearly demonstrates an improvement in salvage in the HBO-treated group in whom Faglia et al. demonstrated a 9.1% (2 of 22 patients) amputation rate from Class IV Wagner lesions in the HBO-treated patients vs. a 55% (11 of 20 patients) amputation rate in the control group ($P = 0.02$). These findings support, in a randomized and controlled trial, the benefit of HBO therapy as an adjunct in the treatment of the ischemic and/or infected neuropathic foot.

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Response to Cianci

Thank you for the opportunity to comment on the letter from Dr. Cianci (1) regarding the consensus statement "Consensus Development Conference on Diabetic Foot Wound Care" (2). As Dr. Cianci correctly points out, there is indeed a single randomized controlled trial in the literature describing the use of hyperbaric oxygen (HBO) therapy for diabetic patients with primarily ischemic lesions of the lower extremity (3). Although the mean values of vibration perception in the patients studied were within age-adjusted norms (4), the mean transcutaneous oxygen tensions were significantly below normal limits, >60% of the patients had gangrene, and the mean ankle brachial indexes were 0.65. Thus, the patients had primarily ischemic ulcers.

The consensus statement correctly stated that there were no trials of HBO in neuropathic patients, referring to patients whose lesions had a primarily neuropathic etiology and who were without significant lower-extremity vascular disease. Until evidence exists for the efficacy of HBO in healing primarily neuropathic foot ulcers, this treatment modality cannot be recommended for such patients, and relief of mechanical stress must be considered the primary approach to treatment.

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Response to Bazzigaluppi et al.

Capillary whole-blood measurement of islet autoantibodies

We read with interest the article by Bazzigaluppi et al. (1). They reported that initial screenings for diabetes risk with islet autoantibodies could be performed with 1 drop of capillary blood. The authors used a 50- μ l sample from relatives of patients with type 1 diabetes who were islet cell antibody-positive. To further extend the idea of Bazzigaluppi et al., our study aimed to elucidate whether a smaller venous blood spot on a filter paper kept at room temperature (RT) could be used to measure GAD65 antibodies in newly diagnosed type 1 diabetic subjects.

GAD65 was assessed from dried venous blood spot samples obtained from 57 newly diagnosed type 1 diabetic patients (0–6 months after diagnosis). A 10- μ l drop of anticoagulated blood was put on a 6-mm diameter filter and air-dried for 5 min. It was kept in a polypropylene tube at RT until a GAD65 measurement was determined a week later. A serum sample was also obtained to measure GAD65 as usual. The antibodies were measured with a commercial kit (CIS Biointernational, Gif-sur-Yvette, France). The intra- and interassay coefficients of variation were <10%. Values greater than the mean \pm 3 SD of the values observed in a control group were considered positive (>2 U/ml).

Of the 57 newly diagnosed type 1 diabetic patients, 37 (64.9%) had elevated GAD65 antibodies in the serum. Of the 37 subjects who tested positive by serum, 31 tested positive with the 10- μ l dried blood spot. There were 2 subjects who were neg-

ative in the serum (1.4 and 1.5 U/ml) but were positive with the blood spot. The discrepancy samples were those with the lowest serum antibody levels. Sensitivity, specificity, and positive predictive value (PPV) of the blood spot method were 0.84, 0.90, and 0.94, respectively. The results obtained for GAD65 antibodies in blood spot were generally lower than those in corresponding serum samples; however, the correlation between both methods was 0.851, $P < 0.001$. The paired Student's t test (exactitude) showed weak statistical differences ($P = 0.042$).

The prevalence of GAD65 positivity was higher when it was studied in serum samples compared with blood spot samples. However, GAD65 antibodies measured in a 10- μ l venous dried blood spot demonstrated a good sensitivity, specificity, and PPV, as well as a good correlation with the GAD65 antibodies measured in serum. These data are in concordance with the results previously found by Bazzigaluppi et al. (1), suggesting that the blood spot sample can be collected equally from either capillary or total venous blood and kept at RT for almost a week.

Our results support that a dried venous blood spot could be used to measure GAD65 antibodies in newly diagnosed type 1 diabetic subjects, assuming a lower prevalence of positivity. Taking into account that the identification of disease-associated autoantibodies in type 1 diabetes is not only a matter of a correct classification (2) but also of clinical relevance, and considering that many health centers have to send out serum samples to measure antibodies, the dried blood spot could be an option to facilitate the GAD65 test.

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HbA_{1c} Determination With High-Performance Liquid Chromatography

We read with interest the recent letter from Herranz et al. (1) reporting on a patient with mild immunohemolytic anemia causing low HbA_{1c} values measured with high-performance liquid chromatography (HPLC). In an effort to explain the clinically low HbA_{1c} values in the patient described, several possibilities were excluded. The authors state that Hb variants may lower HbA_{1c} values, but this was ruled out since the assay used (HPLC; Bio-Rad, Richmond, CA) is not affected by them (1).

HbA_{1c} was originally a term for an ion exchange chromatographic peak and is now defined as irreversibly glycated Hb molecules at 1 or both NH₂-terminal valines of the β -chains. Glycohemoglobin is a marker of long-term glycemic control and has been shown to correlate with mean blood glucose concentrations. Methods to determine HbA_{1c} results include boronate affinity and cation exchange HPLC, electrophoresis, and immunoassays.

HPLC methods for HbA_{1c} determination usually indicate the presence of a hemoglobinopathy, but they lack the resolution necessary to differentiate Hb variants. Chromatograms may demonstrate additional peaks combined with abnormally low or high HbA_{1c} results (2). Even normal chromatograms, with the HPLC methods for HbA_{1c} determination, do not prove that there are no Hb variants present. In general, HPLC methods are not adequate for the measurement of HbA_{1c} in samples that contain Hb variants (2), despite manufacturers' claims to the contrary. An increas-

ing number of Hb variants can falsify HbA_{1c} results with immunoassays. Only boronate affinity methods measure glycohemoglobin, regardless of the glycation site and may be more useful to reflect glycemic control in samples with Hb variants.

Shortened erythrocyte life span, as a reason for these low HbA_{1c} results, makes sense in the patient reported (1), but we would suggest that Hb electrophoresis be performed to exclude a hemoglobin variant.

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The 12-Item Well-Being Questionnaire

Origins, current stage of development, and availability

Pouwer et al. (1) reported on the psychometric properties of a Dutch translation of the 12-Item Well-Being Questionnaire (W-BQ12) in a recent article in *Diabetes Care*. However, in their article,

no mention was made of the source of the original W-BQ12 and no information was given about other translations of the W-BQ12 or how to obtain permission to use the instrument. This information is provided below.

The W-BQ12 was first developed by members of the Diabetes Research Group at Royal Holloway, University of London, in collaboration with Dr. Ishii and his colleagues at the Tenri Hospital, Nara, Japan. A Japanese version of the Well-Being Questionnaire was used with a sample of people with diabetes attending the Tenri Hospital diabetes clinic. This work, sponsored by Eli Lilly Japan, was documented in a 1996 internal report to Eli Lilly (2) and in a published abstract by Riazi et al. (3). The W-BQ12 consists of selected items from the longer parent instrument, the 22-Item Well-Being Questionnaire (W-BQ22) (4,5). The W-BQ12 achieves a balance of positively-worded versus negatively-worded items with subscales of equal length, thereby improving on the structure of the W-BQ22, as well as providing a welcome short form.

The W-BQ22 is available in >20 translations, in addition to Japanese (2,3) and Dutch (1). In work sponsored by Hoechst Marion Roussel, Germany (now Aventis Pharma Deutschland), the psychometric properties of 8 translations of the W-BQ12 (including English for use in the U.S.) have now been examined, and the factor structure and reliability were shown to be excellent for all but 1 language, in which further investigation with a larger sample size was needed (6). Thus, it would appear that the selection of items made to produce the W-BQ12 in the Japanese translation is also producing a psychometrically sound instrument in other translations, at least in terms of internal consistency, reliability, and factor structure. Reanalysis of previous data sets using the W-BQ22 and further use of the W-BQ22/12 in clinical trials and other intervention studies are now needed to establish the new W-BQ12's sensitivity to change in comparison with that of the parent instrument.

As the copyright holder of the W-BQ22 and W-BQ12 and their translations, potential users of these instruments may contact me for permission to use them in any of the translations currently available. Readers wishing to reanalyze existing data sets from the W-BQ22 to further investigate the properties of the new W-BQ12 are welcome to contact me for further details.

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Errata

Rohlfing CL, Little RR, Wiedmeyer H-M, England JD, Madsen R, Harris MI, Flegal KM, Eberhardt MS, Goldstein DE: Use of GHb (HbA_{1c}) in screening for undiagnosed diabetes in the U.S. population. *Diabetes Care* 23:187–191, 2000

The authors have asked that 2 sentences in column 3 on page 188 be corrected. The sentence starting on line 10, which reads “Sensitivity at each possible HbA_{1c} cutoff level was calculated as $[TP/(TP + FN)] \times 100$, where TP = true positive (diabetic fasting plasma glucose and HbA_{1c} cutoff level) and FN = false negative (diabetic fasting plasma glucose, \leq cutoff level HbA_{1c}),” should instead read, “Sensitivity at each possible HbA_{1c} cutoff level was calculated as $[TP/(TP + FN)] \times 100$, where TP = true positive (diabetic fasting plasma glucose and $>$ cutoff level HbA_{1c}) and FN = false negative (diabetic fasting plasma glucose, \leq cutoff level HbA_{1c}).”

The sentence that begins on line 16, which reads, “The sensitivity represents the percentage of those with fasting plasma glucose <7.0 mmol/l who are classified as positive according to HbA_{1c},” should read, “The sensitivity represents the percentage of those with fasting plasma glucose >7.0 mmol/l who are classified as positive according to HbA_{1c}.”

Fujimoto WY: Background and recruitment data for the U.S. Diabetes Prevention Program. *Diabetes Care* 23 (Suppl. 2):B11–B13, 2000

The author of the above paper should have been listed as the Diabetes Prevention Program Research Group. Dr. Wilfred Y. Fujimoto prepared the paper on behalf of the DPP Research Group.