

## OBSERVATIONS

## Status of Research Funded by the American Diabetes Association: Year 2

In the Presidential Address in June of 1998 (1), I proudly announced that the recently adopted Five Year Plan of the American Diabetes Association (ADA) contained the goal of allocating one of every three dollars of total public support to research awards and grants by the end of five years. I pledged to keep the members of the Professional Section apprised of our progress toward that goal. The general approach envisaged gradual increases in funding during the first three years, with more steep increases during the final two years.

During the baseline fiscal year (FY) of 1998, before the initiation of the Five Year Plan, total public support was 90.8 million dollars, of which 15.5 million dollars, or 17.1%, was devoted to research awards and grants. During FY 1999, the first year of the Five Year Plan, total public support was 101.6 million dollars, of which 18.2 million dollars, or 17.9%, went to research awards and grants. During the past year, FY 2000, the second year of the Five Year Plan, total public support was 117.8 million dollars, of which 22.4 million dollars, or 19.0%, was allocated to research awards and grants.

Although the largest increases are due in the final two years, we still have a long way to go. Members of the Professional Section need to use their influence (with both their patients and the ADA) to ensure that this ambitious goal is reached.

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## Scintigraphic Evidence of Poor Salivary Function in Type 2 Diabetes

In type 2 diabetes, a high prevalence of oral problems has been detected. These problems include mouth dryness, an increase in periodontal disease, oral *Candida*, and swelling of the parotid glands (1). Salivary flow rates have often been found to be significantly reduced in type 2 diabetic patients (2), although these findings have not been confirmed (3). Scintigraphic changes correlate well with salivary gland abnormalities (4) and histopathological changes (5). This technique has gained widespread acceptance in evaluating a variety of salivary glandular disorders (6,7). The aim of the present study was to test whether patients with type 2 diabetes suffer from impaired salivary function using objective and quantitative salivary scintigraphy.

A total of 40 patients with type 2 diabetes for 10 years and 36 healthy age- and sex-matched control subjects (13 women and 23 men aged  $56.2 \pm 13.3$  years) were enrolled in the study. All of the type 2 diabetic patients had good

blood glucose control by analysis of HbA<sub>1c</sub> (3–8%) concentration (8). None had other systemic diseases or presented with autonomic neuropathy during orthostatic testing (9). In accordance with a previously established questionnaire, each type 2 diabetic patient was asked 10 specific questions related to oral dryness or oral health (10). These type 2 diabetic patients were separated into two subgroups. Group 1 consisted of 20 patients (13 men and 7 women aged  $54.2 \pm 14.5$  years) with xerostomia, and group 2 consisted of 20 patients (13 men and 7 women aged  $55.2 \pm 13.4$  years) without xerostomia. All of the study and control subjects were asked to refrain from drugs known to affect salivary secretion for at least 1 week and to fast overnight before the salivary scintigraphy. After intravenous injection of 5 mCi Tc-99m pertechnetate, sequential images at 1 min/frame were acquired for 30 min. The 1st- and 15th-min uptake ratios (URs) were calculated from the tracer uptakes in the four major salivary glands over the background regions of interest. Saliva excretion was stimulated by one tablet of 200 mg ascorbic acid given orally 15 min postinjection of the tracer. Then, the maximal excretion ratios (ERs) of the four major salivary glands after sialogogue stimulation were calculated. In all of the four major salivary glands, type 2 diabetic patients with xerostomia had significantly lower URs and ERs the 1st- and 15th-min compared with control subjects and type 2 diabetic patients without xerostomia (all  $P < 0.01$ ). However, no significant differences in the 1st- and 15th-min URs and ERs were found between control subjects and type 2 diabetic patients without xerostomia (all  $P > 0.05$ ) (Table 1).

Salivary scintigraphy is a readily available minimally invasive diagnostic test used to evaluate salivary gland function. Scintigraphy is a particularly valu-

Table 1—Scintigraphic data from type 2 diabetic patients and healthy control subjects

|                  | 1st-min UR values |           |           |           | 15th-min UR values |           |            |            | ER values   |             |             |             |
|------------------|-------------------|-----------|-----------|-----------|--------------------|-----------|------------|------------|-------------|-------------|-------------|-------------|
|                  | RPG               | LPG       | RSG       | LSG       | RPG                | LPG       | RSG        | LSG        | RPG         | LPG         | RSG         | LSG         |
| Group 1          | 2.0 ± 0.6         | 2.0 ± 0.8 | 3.3 ± 0.9 | 3.8 ± 1.2 | 4.5 ± 1.2          | 4.3 ± 1.3 | 5.5 ± 1.8  | 5.7 ± 1.8  | 0.50 ± 0.10 | 0.46 ± 0.09 | 0.46 ± 0.13 | 0.39 ± 0.09 |
| Group 2          | 5.2 ± 1.1         | 4.8 ± 0.8 | 5.6 ± 1.4 | 6.3 ± 1.4 | 8.5 ± 1.9          | 9.2 ± 2.1 | 10.3 ± 3.3 | 10.6 ± 2.8 | 0.60 ± 0.14 | 0.60 ± 0.14 | 0.54 ± 0.08 | 0.47 ± 0.11 |
| Control subjects | 4.6 ± 1.1         | 4.7 ± 1.0 | 5.7 ± 1.8 | 5.9 ± 1.6 | 8.8 ± 2.3          | 8.7 ± 2.1 | 10.3 ± 3.0 | 10.5 ± 2.8 | 0.64 ± 0.10 | 0.62 ± 0.10 | 0.55 ± 0.06 | 0.51 ± 0.10 |

Data are means ± SE. RPG, right parotid gland; LPG, left parotid gland; RSG, right submandibular gland; LSG, left submandibular gland.

able tool because it produces a dynamic, objective, and quantitative measurement of the major salivary gland function and allows for differentiation of abnormalities in saliva production (UR) and secretion (ER). From a review of the literature, no published studies have used salivary scintigraphy to evaluate salivary function of type 2 diabetic patients. Xerostomia is a subjective sensation of dryness of the mouth and is believed to be a common complaint among type 2 diabetic patients (8). It is usually associated with salivary gland hypofunction (1). In our study, significantly impaired salivary function was found in type 2 diabetic patients with xerostomia on salivary scintigraphy. Our results suggest that this discrepancy can be resolved by not classifying patients according to presence of xerostomia. In this study, impaired salivary function (represented by significantly decreased URs and ERs), in type 2 diabetic patients with xerostomia was demonstrated on objective and quantitative salivary scintigraphy. However, a larger series of type 2 diabetic patients is necessary to confirm our findings.

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## The Oral Insulin Sensitizer, Thiazolidinedione, Increases Plasma Vascular Endothelial Growth Factor in Type 2 Diabetic Patients

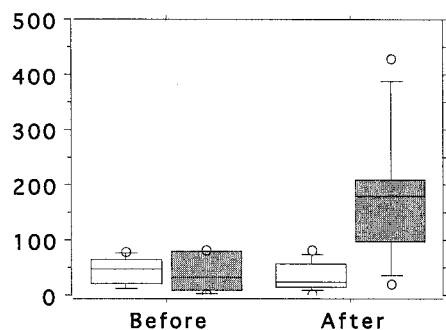
Thiazolidinediones, insulin sensitizers, are widely used for the treatment of insulin resistance in type 2 diabetic patients. One of the adverse effects is fluid retention. We evaluated the effect of pioglitazone, a thiazolidinedione, on plasma vascular endothelial growth factor (VEGF) in comparison with that of metformin in type 2 diabetic patients. A 4-week administration of pioglitazone significantly increased plasma VEGF levels, whereas levels were unchanged by metformin. The results suggest that thiazolidinediones may increase plasma VEGF levels, possibly one of the causes of the drug-induced fluid retention and edema. Type 2 diabetes is characterized by fasting hyperglycemia caused by increased insulin

resistance with or without decreased insulin secretion (1). Appropriate weight reduction and physical exercise in obese type 2 diabetic patients are useful in reducing the insulin resistance in these patients. Thiazolidinediones are a widely used class of drugs for the treatment of insulin resistance in type 2 diabetic patients (2). Two of the known adverse effects of this class are fluid retention and edema (2), especially in female patients. The pathogenic mechanism(s) behind the fluid retention is not yet clear.

VEGF is a cytokine that acts as an endothelial cell mitogen and also induces microvascular permeability (3), thus named a vascular permeability factor (VPF). Recent findings in in vivo studies suggest that thiazolidinediones may increase VEGF production (4,5). Therefore, we studied the effect of pioglitazone, a potent thiazolidinedione, on plasma VEGF levels in type 2 diabetic patients to elucidate whether a clinical dose of thiazolidinediones would increase VEGF secretion in diabetic patients. The effect was compared with that of metformin, another class of drug that is known to improve insulin resistance in type 2 diabetic patients (6). The study was approved by the Ethical Committee of Fukushima Medical University.

A total of 16 type 2 diabetic patients with normal kidney function (serum creatinine level <100  $\mu\text{mol/l}$ ) and an elevated HbA<sub>1c</sub> level (>7.5%), despite ongoing diabetic treatment, participated in the study after informed consent was given (3 men and 13 women, mean age 52 years [range 30–75], and known diabetes duration 13 years [5–21]). The patients were randomly allocated to receive either pioglitazone (15 mg once daily) or metformin (500 mg twice daily) for 4 weeks. Blood samples for the measurement of plasma VEGF were obtained from each patient before and after the 4-week administration of pioglitazone or metformin. The plasma VEGF level was determined by using a commercial enzyme-linked immunosorbent assay kit (Quantikine Human VEGF Immunoassay; R&D System). Comparison of the values between the two treatment groups and the values before and after each treatment was made by Wilcoxon's rank-sum and signed-rank tests, respectively.

The plasma VEGF level increased significantly with the administration of pioglitazone (from 33 pg/ml [3–81] to 180



**Figure 1**—VEGF levels before and after 4-week treatment with metformin (500 mg/day) (□) and pioglitazone (15 mg/day) (■). Plasma VEGF after pioglitazone administration was significantly higher than its basal level ( $P = 0.0117$ ) and the value after metformin treatment ( $P = 0.0070$ ), respectively. Data are medians, 75th and 90th percentiles.

pg/ml [22–430],  $P = 0.0117$ ;  $n = 7$ ), whereas the level remained unchanged with metformin (from 47 pg/ml [12–80] to 25 pg/ml [4–82];  $n = 9$ ). The post-treatment VEGF level was significantly higher in the pioglitazone group than in the metformin group ( $P = 0.0070$ ). The result is summarized in Fig. 1.

This preliminary observation suggests that pioglitazone may increase plasma VEGF levels. The assumption can be further supported by recent experimental findings that troglitazone, a thiazolidinedione, increased VEGF secretion and VEGF mRNA levels in vascular smooth muscle cells (4) and adipocytes (5) in a time- and dose-dependent manner. The results seem to be consistent with a clinical observation that troglitazone-treated patients had higher VEGF levels compared with those treated with diet alone, sulfonylurea, or insulin (5).

Our study implies the possibility that VEGF may have some role in developing thiazolidinedione-induced edema and fluid retention in type 2 diabetic patients. Meanwhile, VEGF is likely to contribute to the development and progression of diabetic retinopathy (7). Nevertheless, we do not yet know whether pioglitazone-induced increases in plasma VEGF adversely influence existing diabetic retinopathy (7) and nephropathy (8) or whether these increases can be useful for therapeutic angiogenesis for ischemic artery disease (4), which is often seen in type 2 diabetic patients. However, the possibility that VEGF is a potential tumor angiogenesis factor in human tumors (9)

must be taken into account. For clarification, these speculations may deserve further study.

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## Could Fasting Plasma Glucose Be Used for Screening High-Risk Outpatients for Gestational Diabetes Mellitus?

The simplified oral glucose tolerance test (sOGTT) with 50 g is recommended for the screening of gestational diabetes mellitus (GDM) (1). However, because of the complexity of the test, the investigation of less expensive and easier methods to perform the screening has been extensively studied. Recently, in agreement with other studies (3), a population-based prospective study conducted on the general population of pregnant women in Brazil showed that fasting plasma glucose (FPG) was a good screening method for GDM (2).

With the purpose of determining an FPG value with good sensitivity and specificity that could identify pregnant women at risk for GDM, we conducted a study in 1994 with high-risk outpatient pregnant women in the State University Hospital of Rio de Janeiro, Brazil. During that year, 269 pregnant outpatients with at least one risk factor for GDM were included in the study. A screening test was performed on 261 pregnant women with 50 g of glucose load at fasting state. Positivity occurred at a 1-h plasma glucose (PG)  $\geq 130$  mg/dl. GDM was diagnosed according to Third International Workshop cutoff values (4). A receiver operating characteristics (ROC) curve was constructed to test the ability of the FPG at the OGTT to discriminate patients with a diagnosis of GDM and to determine the best cutoff value in predicting a GDM diagnosis in our population.

A positive sOGTT was obtained for 100 patients (38.3% [32.6–44.3]), and an OGTT was performed for 77 (77% [68.0–84.4]) pregnant women. A total of 23 patients (23% [15.5–32.0]) did not return to test OGTT. GDM was diagnosed in 25 pregnant women (9.6% [6.4–13.6])



Table 2—Risk factors for GDM and their predictive value

|                                 | GDM<br>(n = 258) | NonGDM<br>(n = 2,539) | Odds<br>ratio |
|---------------------------------|------------------|-----------------------|---------------|
| Age (years)                     | 32.5 ± 4.7       | 29.8 ± 4.9            | 1.1*          |
| BMI (kg/m <sup>2</sup> )        | 23.3 ± 3.8       | 22.0 ± 2.9            | 1.1*          |
| Gravida                         | 2.4 ± 1.4        | 2.2 ± 1.2             | 1.1*          |
| Parity                          | 0.8 ± 1.1        | 0.7 ± 0.8             | 1.2*          |
| Family history of diabetes      | 24.3             | 12.5                  | 2.2*          |
| Previous GDM                    | 14.3             | 1.1                   | 14.5*         |
| Previous macrosomia             | 5.0              | 2.1                   | 2.5*          |
| Previous PIH                    | 6.6              | 2.0                   | 3.4*          |
| Previous miscarriage/stillbirth | 0.4 ± 0.8        | 0.2 ± 0.5             | 1.6*          |
| Previous fetal malformation     | 5.1              | 1.6                   | 3.3*          |

Data are means ± SD or % population. \*P < 0.001.

these women received a nonfasting 50-g glucose challenge at 24–26 weeks. If the 1-h capillary blood glucose level was ≥7.8 mmol/l, then the patient returned for a formal 75-g glucose tolerance test (GTT) and was classified as having GDM if on a 75-g GTT there was one blood glucose level ≥6.0, 10, or 8.0 mmol/l at 0, 1, or 2 h, respectively. GDM status and risk factors, including age, weight at first antenatal visit, BMI, gravida, parity, family history of diabetes, previous macrosomia, previous pregnancy-induced hypertension, previous miscarriage or stillbirth, and previous fetal malformation, were recorded for each subject.

The records of 2,797 completed pregnancies in 2,139 Asian women were examined. Twin pregnancies, miscarriages, terminations, and women with pre-existing diabetes were excluded. As a whole, the incidence of GDM was 9.2%; among women born in China, it was 8.6% (66 of 764), the Philippines 6.7% (40 of 599), Sri Lanka 10.5% (31 of 293), and Vietnam 10.6% (121 of 1,141). These incidences are comparable with those found in studies of Asian women in developed countries but are higher than those found in studies conducted in Asia.

Data were examined to determine the sensitivity of using clinical and historical risk factors to select patients for screening. Table 1 displays the effect of applying different BMI and age thresholds for screening. By changing the thresholds for testing, the sensitivity of using clinical risk factors for selecting patients to be tested for GDM as well as the number of women required to be tested vary. If 97% of GDM cases were detected (using an age threshold of 25 years and BMI of 30 kg/m<sup>2</sup>), then 91% of all pregnant women need to be tested.

Using logistic regression analysis, all risk factors examined were predictive for GDM (Table 2). There were differences between the four groups, but in some cases, this may have been caused by sample size. The only risk factor significant in all four populations was a previous history of GDM with an odds ratio of 14.5 for the entire group.

Our data indicate that although the historical or clinical risk factors for GDM are valid in Asians, using risk factors alone to select such patients for testing for GDM is inadequate. Many Asian women who develop GDM have no risk factors at all. To avoid overlooking significant numbers of women with GDM, one may lower the specificity of the criteria, but this requires that the majority of patients be tested. Logistically, it is much simpler to conduct universal screening for all Asian women in Western countries, rather than to apply selective testing in order to spare a small percentage of women from being tested. Therefore, our findings strongly support recommendations for universal screening for GDM in pregnant women of Asian origin in Western countries. However, in places where the incidence of GDM is low, such as in some developing countries, the selection of patients for testing by the risk factors may be reasonable.

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## Elevated Levels of Interleukin-6 in Young Adults With Type 1 Diabetes Without Clinical Evidence of Microvascular and Macrovascular Complications

The past decade has been characterized by growing interest in the idea that atherosclerosis is an inflammatory disease, and by the finding that serum levels of markers of inflammation can be used to predict the risk of cardiovascular events (1). Elevated concentrations of acute-phase reactants, such as C-reactive protein, soluble intercellular adhesion molecule-1, and fibrinogen, are found in patients with acute coronary syndromes and are predictors of future risk in apparently healthy individuals (1–3). The inflammatory cytokine interleukin-6 (IL-6) is a powerful inducer of the hepatic acute-phase response, and it has been proposed to be a central mediator in the pathogenesis of coronary heart disease through a combination of autocrine, paracrine, and endocrine mechanisms (4). In fact, in a recent study, serum levels of IL-6 were predictive of the risk of myocardial infarction in apparently healthy individuals, and although the levels of IL-6 were strongly correlated with the levels of C-reactive protein, the association between IL-6 and the risk of myocardial infarction remained significant, even after adjustment for the C-reactive protein level (5).

There is little and somewhat conflicting information regarding the impact of the diabetic state per se on serum IL-6, particularly in type 1 diabetic patients. Serum levels of IL-6 have been found to be normal (6) or higher (7) in type 1 diabetic individuals compared with those in control subjects. However, in these studies no information was available on macrovas-

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cular complications, smoking habit, blood pressure, or plasma lipids of the participants, which are all factors known to adversely affect serum IL-6 levels (1,4,7). In the present study, we endeavored to evaluate a selected group of lean, nonsmoking, normotensive, normolipidemic young type 1 diabetic patients who were without any clinical evidence of microvascular and macrovascular complications.

We compared serum levels of IL-6 and fibrinogen in 20 young adults with type 1 diabetes who regularly attended our diabetes clinic with those of 20 healthy volunteers who were matched for age ( $30 \pm 1.8$  vs.  $31 \pm 1.7$  years), sex (M/F = 11/9 vs. 11/9), BMI ( $23.4 \pm 0.7$  vs.  $23.5 \pm 0.8$  kg/m<sup>2</sup>), systolic ( $127 \pm 2$  vs.  $126 \pm 2$  mmHg) and diastolic blood pressure ( $80 \pm 1$  vs.  $81 \pm 2$  mmHg), and lipids (total cholesterol  $4.6 \pm 0.1$  vs.  $4.8 \pm 0.2$  mmol/l, triglycerides  $1.03 \pm 0.2$  vs.  $1.04 \pm 0.1$  mmol/l). All of the participants were nonsmokers. The average glycometabolic control of diabetic patients was fairly good (HbA<sub>1c</sub>  $6.2 \pm 0.2\%$ ); the diabetes duration was  $12.2 \pm 2$  years. None of the patients had diabetic retinopathy (by ophthalmoscopy), sensorimotor neuropathy (by biothesiometer), or nephropathy (by urinary albumin excretion rate). To exclude the presence of clinical macrovascular complications, a 12-lead resting electrocardiogram, measurement of ankle brachial pressure index, and carotid ultrasonography were performed in all of the diabetic patients.

Serum levels of IL-6 (ELISA-kit; Bender MedSystems Diagnostics, Vienna) and fibrinogen (IL-test-PT-fibrinogen HS; Instrumentation Laboratory, Lexington, MA) were markedly elevated in type 1 diabetic patients versus healthy control subjects ( $2.81 \pm 0.5$  vs.  $1.36 \pm 0.6$  pg/ml,  $P < 0.001$ , and  $3.24 \pm 0.2$  g/l vs.  $2.41 \pm 0.1$  g/l,  $P < 0.05$ , respectively). Although the strongest correlate of IL-6 in these data was fibrinogen concentration ( $r = 0.58$ ,  $P < 0.01$ ), the serum levels of IL-6 remained markedly higher in diabetic patients (by  $\sim 106\%$ ) than in healthy control subjects, even after adjustment for this factor. On the contrary, the difference in fibrinogen concentration observed between the two groups disappeared when allowance was made for serum IL-6 levels. This finding probably reflects the fact that IL-6, along with other cytokines, is a primary stimulant for the hepatic production

of acute-phase proteins, such as fibrinogen and C-reactive protein (1,4). Plasma HbA<sub>1c</sub> concentration had a marginal, but not significant, correlation with IL-6 levels ( $r = 0.39$ ,  $P = 0.08$ ).

Overall, therefore, the present results indicate that in young type 1 diabetic individuals with a good glycometabolic control and without any clinical evidence of microvascular and macrovascular complications, there is a marked increase of serum levels of IL-6 when compared with a matched-group of lean, nonsmoking, normotensive, and normolipidemic healthy control subjects. The present findings suggest that the increase of serum IL-6 levels observed in type 1 diabetic patients may be at least partly independent of the presence of clinical microvascular and macrovascular complications, cigarette smoking, or other traditional coronary risk factors and that other specific and diabetes-related mechanisms may be involved, such as advanced glycosylation end products (8). The evidence from this and other studies further supports the possibility that in young adults with type 1 diabetes, there is a low-level chronic inflammatory state, as reflected by levels of serum IL-6, that may play a key role in the early stages of atherogenesis and in the development of microvascular disorders. This hypothesis lends itself to testing the use of interventions to influence IL-6 secretion and actions.

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## Rate and Predictors of Glycemic Testing of Nondiabetic Patients in a Managed Care Population

Unrecognized diabetes is a major public health problem. Approximately one-third of all patients with diabetes are undiagnosed (1). Because treatment of diabetes improves outcomes by reducing complications (2–4), screening for diabetes is an attractive intervention. The American Diabetes Association therefore recommends screening all adults over 45 years of age every 3 years (and younger if at high risk) (5). One presumption underlying a recommendation for doctors to screen for diabetes is that such surveillance is not part of routine medical care. However, there is a certain level of glucose testing of patients without diabetes that occurs in medical practice, which may or may not represent intentional diabetes screening. The frequency of this ad hoc diabetes screening in med-



type II diabetes among men enrolled in the usual care group of the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:1331–1339, 1993

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## Association of Metabolic Control With Problem-Solving Skills

**P**roblem solving has been identified as a skill necessary for effective diabetes self-management (1) and as a training paradigm incorporated into diabetes education (2,3). However, relatively little has been reported about problem-solving skills among patients with diabetes. Self-management models describe steps in the problem-solving process that rely on reasoning, conceptualization, planning, mental flexibility, and sequencing/ability to proceed in an ordered fashion. In this study, a sample of adults with type 1 diabetes was examined to assess 1) performance on neuropsychological measures of problem solving and related skills relative to normative data and 2) the association between metabolic control and problem solving.

A total of 23 adults with type 1 diabetes (ages 18–45 years, 78% with 13–18+ years education) were administered the

Comprehension, Similarities, Block Design, and Picture Arrangement subtests from the Wechsler Adult Intelligence Scale-Revised (WAIS-R), the Trail Making Test Part B (Trails B), the Wisconsin Card Sorting Test (WCST), and the Porteus Maze Test (PMT). Disease variables investigated were disease duration (mean  $13.4 \pm 7.9$  years); number of episodes of severe hypoglycemia requiring help, including hospitalization and coma (mean  $2.6 \pm 2.9$ ); number of episodes of severe hyperglycemia, including hospitalizations, ketoacidosis, and coma (mean  $1.2 \pm 1.6$ ); hypoglycemic coma duration (mean  $17.9 \pm 37.9$  min); hyperglycemic coma duration (mean  $2.8 \pm 7.8$  h); and HbA<sub>1c</sub> ( $n = 17$ , mean  $8.0 \pm 1.3\%$ ). The mean blood glucose level at the time of testing was  $201 \pm 81$  mg/dl.

Individual performance on problem-solving measures was unimpaired relative to adjusted (age, sex, education) normative data (4–7). Significant Pearson's and partial correlations were found with inverse relationships between severe hypoglycemia and both block design ( $r = -0.69$ ,  $P < 0.001$ ) and Trails B ( $r = -0.50$ ,  $P = 0.01$ ) performance; between severe hyperglycemia and picture arrangement ( $r = -0.51$ ,  $P = 0.01$ ); and between HbA<sub>1c</sub> and both PMT ( $r = -0.50$ ,  $P < 0.05$ ) and WCST loss of set after five consecutive correct sortings ( $r = 0.58$ ,  $P < 0.05$ ). A Pearson's correlation between severe hyperglycemia and WCST categories ( $r = -0.42$ ,  $P = 0.05$ ) approached significance. Glucose at time of testing, disease duration, and coma duration were not significantly related to test scores.

Complex thinking and problem solving have been reported as significant challenges in diabetes education (8). Our findings suggest that problem-solving skills may be differentially associated with metabolic control. We observed that history of severe hypoglycemia was associated with reduced performance on timed tasks that required visuospatial skills, which may reflect a slowed and deliberate pattern of problem solving (9). In contrast, history of severe hyperglycemia and recent metabolic control were associated with social reasoning, planning, and complex problem solving, all of which are required for real-world application of decision making in diabetes self-management. Deficits in these skills, relative to normal control subjects, have been re-

ported in various diabetes populations (10,11). Results need to be replicated in large-scale prospective studies in which consistent measures of problem-solving skills (neuropsychological and applied) are investigated.

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## Progression of Cardiac Dysfunction in a Case of Mitochondrial Diabetes

### A case report

**A**lthough there are some reports that have evaluated the correlation between mitochondrial tRNA(Leu)(UUR) mutation at position 3243 and cardiomyopathy, the degree of cardiac involvement in mitochondrial diabetes is still largely unknown. Here we report the case of a patient with mitochondrial diabetes who developed cardiac dysfunction, evaluated by echocardiography, along with worsening of glycemic control over a relatively short period, within a year.

In April 1997, a 22-year-old Japanese man presented to a medical practice because of thirst, polyuria, and body weight loss of ~8 kg over a few months. Because blood testing revealed a high plasma glucose level (23.6 mmol/l), and because urine was positive for ketone bodies, he was immediately admitted to the hospital. After initial therapy with insulin for 5 months, he was mainly treated with oral hypoglycemic agents. In July 1998, his glycemic control worsened and he presented to our hospital; insulin therapy was restarted. Because his maternal grandmother and paternal uncle had diabetes and he complained of hearing impairment, he was thought to have "mitochondrial diabetes." After obtaining informed consent from the patient, mutation of the mitochondrial gene was analyzed. In leukocytes, A→G transition at the nucleotide pair 3243 of the mitochondrial gene was detected. Although he had no cardiac symptoms, to rule out cardiac involvement, further investigations (i.e., electrocardiography, chest X-ray, and echocardiography) were performed. No abnormal findings were found in all of these tests. Subsequently, his glycemic control worsened again (HbA<sub>1c</sub> 9.5%) because of poor compliance with insulin injections. Therefore, he was admitted to our hospital in May 1999. On admission, his BMI was 18 kg/m<sup>2</sup>, his blood pressure was 103/64 mmHg, and his pulse rate was 70/min. Physical examination revealed no sign of neuropathy, skeletal myopathy, or

heart failure. The postprandial plasma glucose level was 8.3 mmol/l, HbA<sub>1c</sub> 8.6% (normal range 4.2–5.5%), urine ketone bodies negative, serum lactate level normal, serum pyruvate level slightly elevated, GAD65 antibody negative (cutoff <1.3 U/ml; 100% sensitivity and 100% specificity of the assay in GAD antibody proficiency test [Immunology of Diabetes Workshop]; lab ID no. 305), urine C-peptide level 25.8 μg/day (normal range 18.3–124.4 μg/day), fasting C-peptide level 0.13 nmol/l (0.17–0.76 nmol/l), and glucagon-stimulated serum C-peptide level 0.56 nmol/l (6 min after intravenous injection of 1 mg glucagon). He did not consume alcohol, and his thyroid function was normal. Although he had no cardiac symptoms and his electrocardiography and chest X-ray were normal, we performed follow-up echocardiography to evaluate "latent" mitochondrial cardiomyopathy. To our surprise, echocardiography revealed diffuse hypokinesis of the left ventricle, especially in the apex, which was not apparent in his former study performed the previous year. Left ventricular hypertrophy was not observed. Left ventricular ejection fraction calculated by automated quantification by modified Simpson method using an Agilent Sonos 5500 was decreased to 37% (normal >65%). Myocardial perfusion scintigraphy using 99mTc-tetrofosmin showed no perfusion defect. Radioisotope multigated ventriculography also showed diffuse hypokinesis of the left ventricle, with a low ejection fraction (41% [normal >65%]). Ambulatory Holter recording showed no abnormality. To rule out ischemic heart disease, coronary angiography was performed, and no significant stenosis was found. Transvenous endomyocardial biopsy was performed after obtaining informed consent from the patient. Histological examination of right ventricular myocardial specimens revealed mild hypertrophy and a disarray of myofibrils with marked vacuolar degeneration, which was considered to be the swelling of mitochondria. These findings are compatible with mitochondrial cardiomyopathy. Moreover, using a ventricular septal endomyocardial specimen, A→G transition at nucleotide pair 3243 of the mitochondrial gene was detected; heteroplasmic concentration was 82.4%, whereas that in leukocytes was 10.5%. Interestingly, the proportion of heteroplasmy was markedly higher in the heart, which may

reflect the pathological heterogeneity of organ involvement.

In Japan, nearly 1% of patients with diabetes possess a mitochondrial tRNA(Leu)(UUR) mutation at position 3243 (1), which was originally found in MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) (2). Some recent articles have reported that this mutation may be a cause of cardiomyopathy in diabetes (3–6). The degree of cardiac involvement in mitochondrial diabetes, however, is still largely unknown. In our case, even though the patient had no cardiac symptoms and electrocardiography and chest X-ray showed no obvious change within a year, echocardiography revealed diffuse hypokinesis of the left ventricle, which was not apparent a year previously. It has been reported that left ventricular hypertrophy is a clinical feature of patients with MELAS (7–10). Generally, in mitochondrial disease, both hypertrophic and dilated cardiomyopathy can be observed (5–7). Because this case cannot be diagnosed as MELAS because of the lack of abnormality in neurological findings, and given that the clinical features of cardiac involvement in mitochondrial disease vary among different subgroups (7), left ventricular systolic dysfunction without left ventricular hypertrophy, as seen in our patient's case, is not a surprising feature of this disorder. Presently, this patient is clinically asymptomatic (NYHA functional class I). Left ventricular thickness might increase in the future as the disease progresses; therefore, strict follow-up with echocardiography is considered to be essential. Regarding the progression of cardiomyopathy in mitochondrial diabetes, Momiya et al. (5) reported a patient with left ventricular hypertrophy who developed left ventricular systolic dysfunction and dilatation over 8 years of observation, as compared with progression of cardiac dysfunction within 1 year in our patient. Our search of the literature revealed no report of change of cardiac function using echocardiography over such a short period. We would like to emphasize that such a change in left ventricular function could occur in a short period of time in mitochondrial diabetes. Yoshida et al. (3) reported that patients with mitochondrial diabetes should be examined by echocardiography periodically, even if their electrocardiogram and chest X-ray are normal. Our report con-

firmed the importance of serial echocardiography to evaluate the cardiac involvement in mitochondrial diabetes.

Then, what is the cause of left ventricular deterioration over a relatively short period? In this case, coronary artery disease; hypertensive heart disease; alcoholic cardiomyopathy; congenital heart disorders, including glycogen storage disease, thyroid disorders, and pericardial disease; and myocarditis were excluded. We speculate that poor glycemic control may have been responsible for the progression of cardiac dysfunction in this case, because no factor other than worsening of glycemic control seemed to have changed. Therefore, we suggest that it may be worth trying to evaluate cardiac function by echocardiography once a year in patients with mitochondrial diabetes, especially in those with poor glycemic control. Although more cases should be accumulated to reach a conclusion, we propose that recognition of cases like this is clinically important for the proper management of mitochondrial diabetes.

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## Is Altered Body Fat Distribution Responsible for Reduced Pulmonary Function in Obese Type 2 Diabetic Adult Women?

Most patients with type 2 diabetes are overweight (1), and their fat-distribution pattern shows more truncal and less peripheral subcutaneous fat. Particularly, adult women with type 2 diabetes have been reported to have significantly reduced lower-body fat compared with normoglycemic women matched for age and BMI (2). With regard to the association between diabetes and pulmonary function, only a few studies have described pulmonary function in subjects with type 2 diabetes, and their results are conflicting. Lange et al. (3) reported reductions in forced vital capacity (FVC) and forced expiratory volume in

one second (FEV1) among type 2 diabetic patients of different ages. The Rancho Bernardo Study (4), on the other hand, found no relationship between type 2 diabetes and pulmonary function in normal-weight adults.

The effects of obesity on ventilatory parameters may depend on both the distribution and size of excess adipose tissue, and may also change according to the specific pattern of body fat deposition. In fact, decreased ventilatory parameters have been reported to be associated with the accumulation of fat in the abdominal region, which impedes the descent of the diaphragm into the abdominal cavity. Dual-energy X-ray absorptiometry (DEXA) is an accurate and precise method of measuring body composition (i.e., fat mass and lean body mass). When measuring body fat distribution by DEXA, it has been shown that a decrease in the upper-body fat of obese subjects, without any decrease in upper-lean body mass, is associated with improvements in ventilatory function (5). Although the role of central obesity in ventilatory impairment is well known, few studies have evaluated this association among individuals with type 2 diabetes. Our objective was to evaluate this association between pulmonary dysfunctional restrictive pattern and body fat distribution, as measured by DEXA, and anthropometry in type 2 diabetes obese women.

We recruited seven nonsmoking mildly obese adult women affected with type 2 diabetes (mean age  $50.29 \pm 2.87$  years) and seven normoglycemic nonsmoking women matched for age and BMI. After physical examination, patients with respiratory symptoms, lung diseases, FEV1/FVC  $< 76\%$  of the predicted value, or limited joint mobility were excluded. Dynamic spirometric tests were performed for all participants according to the guidelines of the American Thoracic Society. Maximum voluntary ventilation (MVV) was determined by fast deep breathing for 12 s. Total fat mass, total lean body mass (LBMtot), trunk fat mass (FMtrunk), and trunk lean body mass (LBMtrunk) were measured using DEXA.

Dynamic respiratory indexes FVC, VC, and FEV1 in the type 2 diabetes women had significantly lower ( $P \leq 0.05$ ) values ( $2.68 \pm 0.30$ ,  $2.59 \pm 0.40$ , and  $2.17 \pm 0.20$  l, respectively) compared with control subjects ( $3.23 \pm 0.30$ ,  $3.15 \pm 0.46$ , and  $2.59 \pm 0.31$  l, respec-



type 1 diabetes or offspring of the hospital staff. The sample size was calculated using the power analysis aimed at detecting an anticipated difference in the prevalence of GI symptoms  $\geq 15\%$  ( $\alpha = 0.05$  and power = 0.8). The study was approved by the Ethics Committee of the P & A Kyriakou Children's Hospital, Athens, Greece.

The existence, frequency, and nature of GI symptoms in the control subjects and patients with type 1 diabetes were recorded after a single researcher interviewed the patients and their parents using a standard questionnaire. Recurrent abdominal pain (RAP), chronic dyspepsia (CD), and chronic constipation (CC) were defined according to previously published definitions. Weight, height, insulin requirements, and HbA<sub>1c</sub> were recorded. HbA<sub>1c</sub> concentrations at the previous three visits were also recorded. Nutritional status was expressed as BMI. *Helicobacter pylori* serum antibodies were detected by enzyme-linked immunosorbent assay. All of the seropositive patients were further investigated with upper-GI endoscopy and biopsies.

A total of 63 (36.8%) control subjects and 53 (44.9%) patients with type 1 diabetes manifested GI symptoms ( $P = 0.17$ ). RAP, CD, and CC were present in 24 (20.8%), 14 (11.8%), and 19 (16.1%) of the type 1 diabetic patients compared with 23 (13.5%), 18 (10.5%), and 32 (18.7%) of the control subjects, respectively ( $P = 0.12$ , 0.52, and 0.57, respectively).

Patients with type 1 diabetes who manifested GI symptoms were comparable with patients who did not with respect to age, sex, duration of diabetes, BMI, insulin requirements, and mean HbA<sub>1c</sub> (last year) or current HbA<sub>1c</sub> levels (Table 1). The same was true for the various subgroups of patients with type 1 diabetes with and without RAP, CD, or CC.

Positive serum antibodies (both IgG and IgA) for *H. pylori* were detected in eight of the control subjects, all of whom had RAP, and in eight of the patients with type 1 diabetes (six with RAP and two with CD). The prevalence of antibody positivity was similar in control subjects and patients with type 1 diabetes ( $P = 1.00$ ). *H. pylori* gastritis was diagnosed in all of the above 16 patients, by endoscopy and biopsies. No differences were found between type 1 diabetic children with and those without *H. pylori* gastritis with respect to duration of type 1 diabetes, BMI, insulin requirement, fasting glucose level,

**Table 1—Characteristics of the patients with type 1 diabetes**

|  | Patients with GI symptoms | Patients without GI symptoms | P  |
|--|---------------------------|------------------------------|----|
| n (%)  | 53 (45)                   | 65 (55)                      |    |
| Males  | 22                        | 35                           |    |
| Age (years)  | 14.9 ± 4.5                | 14.1 ± 5.0                   | NS |
| Duration of diabetes (years)                                     | 5.6 ± 4.1                 | 6.43 ± 4.3                   | NS |
| HbA <sub>1c</sub> (%)  | 8.9 ± 2.0                 | 8.4 ± 1.7                    | NS |
| HbA <sub>1c</sub> of the previous year (%)                       | 8.7 ± 1.8                 | 8.3 ± 1.5                    | NS |
| Insulin requirements (U · kg <sup>-1</sup> · day <sup>-1</sup> ) | 0.8 ± 0.2                 | 0.8 ± 0.2                    | NS |
| BMI (kg/m <sup>2</sup> )   | 20.4 ± 3.4                | 20.5 ± 3.7                   | NS |

Data are n or means ± SD.

and HbA<sub>1c</sub> concentration. Furthermore, the 2-week treatment of *H. pylori* gastritis with clarithromycin (15 mg · kg<sup>-1</sup> · day<sup>-1</sup>), metronidazole (20 mg · kg<sup>-1</sup> · day<sup>-1</sup>), and omeprazole (1 mg · kg<sup>-1</sup> · day<sup>-1</sup>) did not affect HbA<sub>1c</sub> concentration: the mean ± SD HbA<sub>1c</sub> pre- and 3 months posttreatment were 8.6 ± 2.2 vs. 7.8 ± 1.5%, respectively ( $P = 0.1$ ).

The prevalence of CD and CC in adult patients with type 1 diabetes is controversial. Some researchers report increased prevalence of GI symptoms (1), whereas others do not (6). To our knowledge, this is the first controlled study to assess the prevalence of GI symptoms in children and adolescents with type 1 diabetes. Very few uncontrolled studies in children with type 1 diabetes focused on acid peptic disease (4,5).

In agreement with previous studies in adults, the current study showed that the prevalence of RAP was comparable between patients with type 1 diabetes and healthy control subjects (6). Furthermore, the frequency of *H. pylori* gastritis was similar in diabetic and nondiabetic children with RAP and/or CD, a finding in agreement with that previously reported in adult patients with diabetes (7). In an uncontrolled study, Burghen et al. (5) recently reported an increased (7%) prevalence of acid peptic disease in children with diabetes. According to the authors, children with peptic disease had poorer glycemic control and linear growth. We were unable to show any differences in nutritional status, insulin requirement, or HbA<sub>1c</sub> concentration between patients with type 1 diabetes who had *H. pylori* gastritis and those patients who did not. Furthermore, in agreement with Burghen's study (5), our study showed that the treatment of *H. pylori* gastritis did not improve HbA<sub>1c</sub> concentration.

Poorer glycemic control in patients with diabetes and chronic dyspepsia has been attributed to gastric emptying delay, causing mismatch between the onset of insulin action and the absorption of carbohydrates. It should be noted, however, that gastric emptying delay does not occur in all of the patients with RAP and/or CD. In a large study in dyspeptic nondiabetic adults, gastric emptying of a solid meal, assessed by gastric scintigraphy, was reported to be normal in all of the dyspeptic patients who presented with epigastric pain and impaired in only 42% of dyspeptic patients with postprandial fullness and early satiety (8). Therefore, further studies assessing both GI motility in type 1 diabetic children with individual GI symptoms and the impact of possible motility abnormalities on glycemic control are required.

In conclusion, GI symptoms are as frequent in children with type 1 diabetes as in the general population and have no impact on diabetes control. The treatment of *H. pylori* gastritis does not affect glycemic control. GI symptoms in patients with type 1 diabetes should be investigated and treated as indicated in the nondiabetic individuals.

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## Glucagon-Like Peptide-1 (7-37) Augments Insulin Release in Elderly Patients With Diabetes

Glucagon-like peptide-1 (GLP-1) is a potent stimulator of glucose-induced insulin release (1). Unlike glucose-dependent insulinotropic polypeptide (GIP), GLP-1 augments insulin release in middle-aged patients with type 2 diabetes (1) and is therefore a potentially promising agent for the treatment of

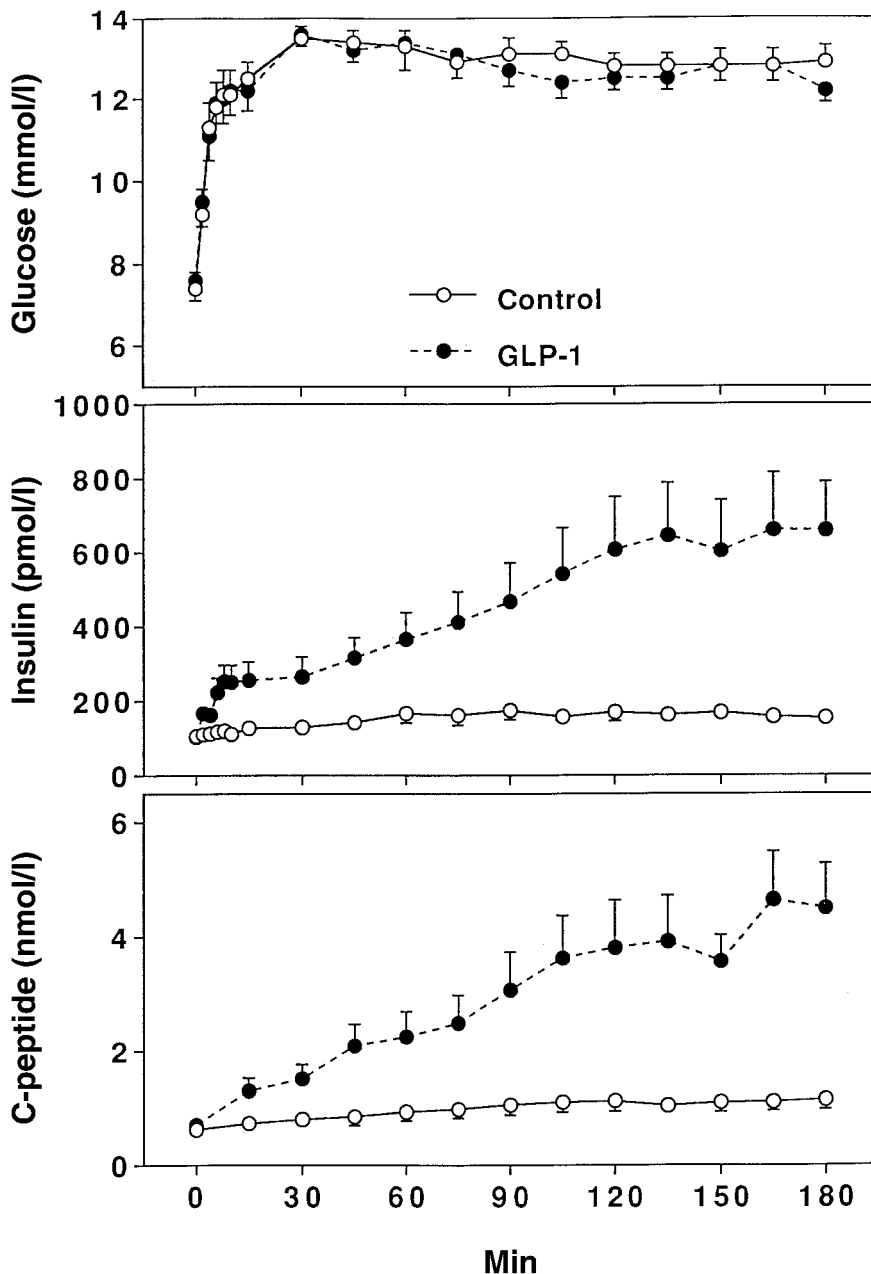


Figure 1—Plasma glucose, insulin, and C-peptide levels during the hyperglycemic clamp studies.

type 2 diabetes. We undertook the following experiments to determine whether the effects of GLP-1 on glucose-induced insulin release are preserved in elderly patients with diabetes.

These studies were conducted on 8 elderly patients with diabetes (age  $77 \pm 1$  years, BMI  $28 \pm 1$  kg/m<sup>2</sup>). The mean duration of diabetes was  $8 \pm 1$  years and mean HbA<sub>1c</sub> was  $7.1 \pm 0.2\%$ . All subjects were free of clinically apparent microvascular, macrovascular, or neuropathic complications. Patients with hyperten-

sion were not excluded. Three of the subjects were treated with sulfonylurea drugs, and four were being treated with angiotensin-converting enzyme inhibitors for hypertension. All medications were stopped at least 2 weeks before the test. This study was approved by the Committee on Human Investigation at the University of British Columbia. All subjects gave written informed consent before participation.

Each subject underwent two hyperglycemic clamp studies in random order,

separated by at least 4 weeks (2). From -20 to 0 min, three blood samples were taken at 10-min intervals to measure basal glucose, insulin, glucagon, C-peptide, and GLP-1. At time 0, glucose was acutely increased to 5.4 mmol/l above basal using the hyperglycemic clamp protocol and was kept at that level for 180 min. In one study, glucose alone was infused. In the other study, GLP-1 was infused in a primed continuous manner at a rate of 1.5 pmol/l · kg<sup>-1</sup> · min<sup>-1</sup> for the duration of the study, as previously described (3). Blood samples were taken every 2 min from 0 to 10 min to measure glucose and insulin values, every 5 min for the rest of the study to measure glucose, and every 15 min to measure hormone levels. Blood samples were collected in heparinized syringes. Plasma glucose was measured immediately at the bedside using a YSI Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH). The remaining blood was placed in prechilled test tubes containing diproten A (for measurement of GLP-1), aprotonin (400 KIU/ml), and EDTA (1.5 mg/ml) (for measurement of C-peptide, glucagon, and insulin) and centrifuged at 4°C. Samples were stored in a -70°C freezer until analysis. Insulin, glucagon, C-peptide, and total and active GLP-1 were measured by radioimmunoassays, as previously described (3,4). Differences between studies were evaluated with Student's paired *t* test. Except where otherwise indicated, results are presented as mean ± SEM; *P* < 0.05 was considered significant in all analyses.

Fasting glucose and hormone values did not differ between studies. Plasma glucose, insulin, and C-peptide levels for the hyperglycemic clamp studies are illustrated in Fig. 1. Steady-state (120–180 min) glucose values were similar in each study (control 12.8 ± 0.4 mmol/l and GLP-1 12.6 ± 0.3 mmol/l; *P* = NS). Steady-state plasma insulin (control 161 ± 22 pmol/l and GLP-1 643 ± 141 pmol/l, *P* < 0.01) and C-peptide (control 1.1 ± 0.2 nmol/l and GLP-1 4.2 ± 0.7 nmol/l, *P* < 0.0001) values were substantially higher during the GLP-1 study. Steady-state total (control 7 ± 2 pmol/l and GLP-1 226 ± 25 pmol/l, *P* < 0.0001) and active GLP-1 values (control 1 ± 1 pmol/l and GLP-1 17 ± 2 pmol/l, *P* < 0.0001) were substantially higher during the GLP-1 study, whereas glucagon values (control 20 ± 2 pmol/l and GLP-1 13 ± 1 pmol/l, *P* < 0.001) were lower.

In middle-aged patients with diabetes, glucose-induced insulin responses to GLP-1 are preserved. But GLP-1 does not lead to hypoglycemia, because little or no insulin is secreted at glucose levels < 4 mmol/l, even when large doses of GLP-1 are given (1). Because older-aged patients with diabetes have an increased risk of severe hypoglycemia when treated with oral hypoglycemic agents or insulin (5), GLP-1 could prove to be a particularly safe therapeutic agent in this patient population. However, although GLP-1 has a similar insulinotropic effect in healthy young and old rats (6,7), the effect of GLP-1 on insulin release is reduced in healthy elderly humans (8). For GLP-1 to be a useful therapy in the elderly, it must maintain its insulinotropic effectiveness in this patient population. In this study, we demonstrated for the first time that the insulin responses to GLP-1 are preserved in elderly patients with diabetes. We also showed that GLP-1 maintains its ability to suppress glucagon levels in elderly patients with diabetes. The levels of active GLP-1 were substantially lower than that of total GLP-1. The biological effect GLP-1 is partly regulated through metabolism by dipeptidyl peptidase IV (DPIV), an enzyme that cleaves the first two amino acids from both GIP and GLP-1 (9). Our data suggest that DPIV inhibitors have the potential to substantially enhance the therapeutic effect of GLP-1 in the elderly.

In conclusion, GLP-1 maintains significant insulinotropic potential in elderly patients with diabetes. The results of this study are the basis for ongoing clinical trials designed to evaluate the usefulness of GLP-1 as a therapeutic agent in elderly patients with diabetes.

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## Relationship of Regional Adiposity to Insulin Resistance in Nonobese Japanese Type 2 Diabetic Patients

Type 2 diabetes is a heterogeneous disorder characterized by insulin resistance and/or defective insulin secretion (1,2). The mechanisms underlying insulin resistance in type 2 diabetes are not fully understood, but the numerous studies in nondiabetic populations have addressed the importance of upper body fat distribution. A study by Vague (3) was the first to show that upper body fat distribution has particularly adverse metabolic abnormalities. Higher concentrations of plasma glucose, insulin, and triglycerides have been shown to be associated with increasing abdominal body fat in nondiabetic subjects (4–7). However, in diabetic patients, very little has been reported on the associations between insulin resistance and body fat distribution.

Banerji et al. (8) recently disclosed that visceral but not subcutaneous abdominal fat volume is associated with insulin resistance in black subjects with type 2 diabetes. In contrast, Abate et al. (9) recently demonstrated that subcutaneous but not intraperitoneal nor retroperitoneal fat volume is associated with insulin resistance in non-Hispanic whites with type 2 diabetes. The reason for the discrepant result between the two groups is unknown, but it can be speculated that there may be an ethnic difference in the relationship between insulin resistance and body fat distribution in type 2 diabetes.

Nonobese Japanese type 2 diabetic patients are unique in that they are divided into two variants: those with insulin resistance and those with normal insulin sensitivity (10–12). However, to the best of our knowledge, the relationship between insulin resistance and body fat distribution has not been investigated in nonobese Japanese type 2 diabetic populations. Thus, the aim of the present study was to investigate the relationships among insulin resistance and subcutaneous or visceral fat area in nonobese Japanese type 2 diabetic patients.

A total of 75 nonobese Japanese type 2 diabetic patients at Kansai-Denryoku Hospital were recruited for the present

study. Type 2 diabetes was diagnosed based on the criteria of the World Health Organization (13). Of the 75 patients, 36 were taking sulfonylureas; the rest were treated with diet alone. None of the patients has received insulin therapy. All subjects ingested  $\geq 150$  g of carbohydrate for the 3 days preceding the study. None of the subjects had significant renal, hepatic, or cardiovascular disease. Of the 75 patients, 18 had hypertension, and these patients were treated with ACE inhibitors ( $n = 10$ ), calcium channel blockers ( $n = 6$ ), or both ( $n = 2$ ). They did not consume alcohol or perform heavy exercise for  $\geq 1$  week before the study.

Blood was drawn in the morning after a 12-h fast. Plasma glucose was measured with the glucose oxidase method, and serum insulin was measured using a two-site immunoradiometric assay (Insulin Riabead II; Dainabot, Osaka, Japan). Triglycerides, total cholesterol, and HDL cholesterol were also measured. The LDL cholesterol level was calculated using the Friedewald formula (14).

The estimate of insulin resistance by the homeostasis model assessment (HOMA-IR) was calculated with the following formula: fasting serum insulin ( $\mu\text{U/ml}$ )  $\times$  fasting plasma glucose (mmol/l) / 22.5 (15). One might argue that the use of sulfonylureas in patients with diabetes might significantly affect the estimate of HOMA-IR, because these drugs are known to decrease fasting plasma glucose without substantially changing fasting plasma insulin (16). However, this seems unlikely, because Bonora et al. (17) and Emoto et al. (18) confirmed that in the validation studies of HOMA, the correlation of insulin sensitivity estimated by HOMA and that measured by the glucose clamp was not substantially different in diet-treated and sulfonylurea-treated type 2 diabetes. Therefore, we estimated HOMA-IR in diet-treated and sulfonylurea-treated diabetic patients.

All subjects underwent computed tomography (TSX-012A, X-Vigor; Toshiba, Osaka, Japan) to measure cross-sectional abdominal subcutaneous and visceral fat areas, as described previously (6,19). Briefly, the subjects were examined in the supine position, and computed tomography scans were performed at the umbilical levels. Adipose tissue areas were determined using commercially available software (Fat Scan; N2 System Corporation, Osaka, Japan) (20). Subcutaneous

and visceral borders were defined using a manual cursor.

Data are presented as means  $\pm$  SEM. Statistical analyses were conducted using the StatView 5 system (Statview, Berkeley, CA). Simple (Spearman's rank) correlation coefficient and stepwise multiple regression analyses were used to examine the relationships between insulin resistance and subcutaneous or visceral abdominal fat area, BMI, or the measures of variables, including triglycerides.  $P < 0.05$  was considered as significant. In multivariate analysis,  $F$  values  $\geq 4$  were considered significant.

The subjects studied were all Japanese type 2 diabetic patients (49 men and 26 women) with an age range of 39–83 years ( $61.8 \pm 1.1$ ) and a BMI of 16.2–26.8 kg/m<sup>2</sup> ( $22.2 \pm 0.3$ ). They all were nonobese (21). The mean fasting plasma glucose was 144  $\pm$  3 mg/dl, and the HbA<sub>1c</sub> level was 6.9  $\pm$  0.1%. The mean fasting plasma insulin level was 6.2  $\pm$  0.4  $\mu\text{U/ml}$ . Serum triglycerides, total and HDL cholesterol levels were 108  $\pm$  7 mg/dl, 197  $\pm$  4 mg/dl, and 58  $\pm$  2 mg/dl, respectively. The mean serum LDL cholesterol level was 117  $\pm$  3 mg/dl.

There was a wide variation in insulin resistance values calculated from HOMA-IR in our diabetic patients (range 0.64–9.67, mean 2.21  $\pm$  0.16). Of the 75 patients, 23 (31%) had HOMA-IR values  $> 2.5$ , indicating that they were insulin-resistant (22). Similarly, there was also a wide variation in subcutaneous and visceral abdominal fat areas. Mean subcutaneous and visceral abdominal fat areas were 127.4  $\pm$  6.3 cm<sup>2</sup> (range 27.6–267.4) and 80.4  $\pm$  4.1 cm<sup>2</sup> (12.2–179.1), respectively.

Insulin resistance calculated from HOMA-IR was positively correlated to subcutaneous ( $r = 0.583$ ,  $P < 0.001$ ) and visceral ( $r = 0.490$ ,  $P < 0.001$ ) fat areas in our diabetic patients. Furthermore, insulin resistance was positively correlated to BMI ( $r = 0.458$ ,  $P < 0.001$ ) and levels of HbA<sub>1c</sub> ( $r = 0.253$ ,  $P = 0.026$ ) and serum triglycerides ( $r = 0.419$ ,  $P < 0.001$ ). In contrast, insulin resistance was negatively correlated to serum HDL cholesterol levels ( $r = -0.334$ ,  $P = 0.003$ ). However, there was no relationship between insulin resistance and measures of other variables, including total cholesterol.

Multiple regression analyses showed that insulin resistance was predicted by the area of subcutaneous ( $F = 6.7$ ) and





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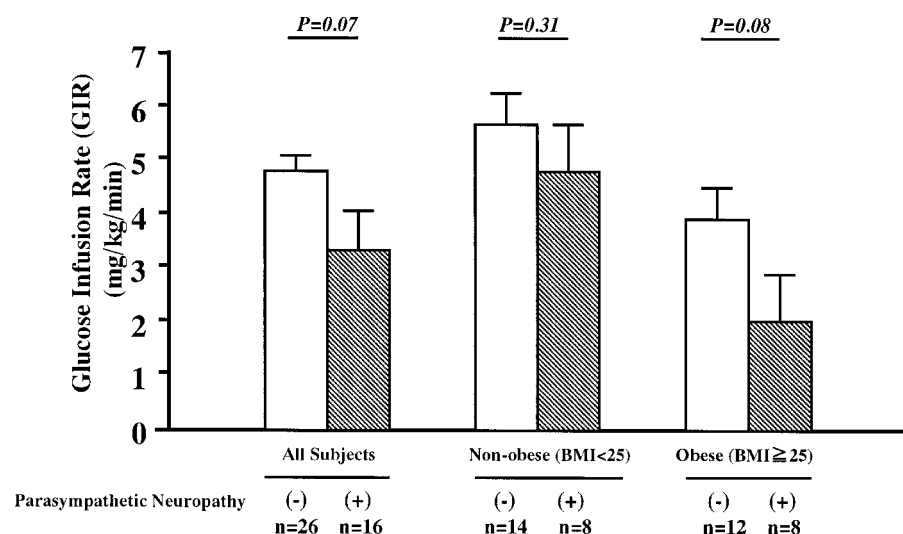
## A Possible Involvement of Parasympathetic Neuropathy on Insulin Resistance in Patients With Type 2 Diabetes

We reported that insulin sensitivity is inhibited by interruption of the hepatic parasympathetic reflex by surgical denervation of the liver or pharmacological blockade of the cholinergic neurotransmitter in rats (1–3). Therefore, we tested the hypothesis that parasympathetic neuropathy is associated with insulin resistance in type 2 diabetic patients.

At the present time, only one method

is available to directly measure autonomic nerve activity (4), and there are no methods available to measure hepatic parasympathetic nerve activity in humans. Thus, we used the deep breathing test to evaluate a cardiovascular parasympathetic nerve function. Hepatic parasympathetic neuropathy will often occur in parallel with cardiovascular parasympathetic neuropathy, because diabetic autonomic neuropathy is a systemic distal polyneuropathy.

A total of 42 patients with type 2 diabetes (age [means ± SD] 37 ± 9.7 years, HbA<sub>1c</sub> 9.8 ± 2.2%, duration of diabetes 7.1 ± 7 years) were divided into two groups: those with obesity (BMI ≥25 kg/m<sup>2</sup>) and those without obesity (BMI <25). Insulin sensitivity was tested using a hyperinsulinemic-euglycemic clamp (5,6). Parasympathetic nerve function was assessed by the deep breathing test, which assesses the heart rate response to deep breathing at 6 cycles/min. The results were expressed as the mean of the difference between the inspiratory heart rate and the expiratory heart rate in each respiratory cycle. Patients were grouped into two categories: those with parasympathetic neuropathy showing <15 beats/min in the deep breathing test, and those without parasympathetic neuropathy showing ≥15 in the test. There was no difference in age, HbA<sub>1c</sub>, or duration of diabetes between the patients with and



**Figure 1**—Comparison of GIR values between patients with and without parasympathetic neuropathy. Data are expressed as means + SEM. Because GIR values were affected by BMI, we compared the GIR values of patients with and without parasympathetic neuropathy after adjustment by BMI using minimum square average (analysis of covariance).

without parasympathetic neuropathy who were with and without obesity.

Because glucose infusion rates (GIR) correlated with BMI ( $r = -0.326$ ,  $P = 0.035$ ), we compared the GIR of patients with and without parasympathetic neuropathy, adjusted for BMI using minimum square average (analysis of covariance).

As shown in Fig. 1, the GIR of patients with parasympathetic neuropathy was marginally lower than that of those without parasympathetic neuropathy among the total subjects ( $3.39 \pm 0.64$  vs.  $4.72 \pm 0.43$   $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  [means  $\pm$  SEM],  $P = 0.07$ ). The GIR of patients with parasympathetic neuropathy was lower than that of those without parasympathetic neuropathy in obese subjects ( $1.99 \pm 0.7$  vs.  $3.71 \pm 0.56$   $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P = 0.08$ ). On the other hand, there was no difference in GIR between the patients with and without parasympathetic neuropathy in the nonobese subjects.

Gottsater et al. (7) reported that parasympathetic neuropathy is associated with hyperinsulinemia in type 2 diabetic patients. We reported that insulin sensitivity is affected by hepatic parasympathetic nerve (1–3). The results of this study support these reports, and we speculate that diabetic parasympathetic neuropathy affects the insulin resistance in type 2 diabetic patients with obesity.

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## COMMENTS AND RESPONSES

### Low Total Plasma Homocysteine Concentrations in Patients With Type 1 Diabetes

**W**e read with great interest the recent article by C. Pavia et al. (1) on total homocysteine (tHcy) and its modifying vitamin factors (folates and vitamins B<sub>12</sub> and B<sub>6</sub>) in adolescents with type 1 diabetes. The authors studied the possible relationship between hyperhomocysteinemia in type 1 diabetes and duration of disease, degree of metabolic control, presence of microalbuminuria, alterations in fundus oculi, and chronic lymphocytic thyroiditis.

Numerous studies clearly demon-

strate that mild hyperhomocysteinemia is strongly related to vascular diseases (2,3). Patients with diabetes often develop premature vascular disease. In type 2 diabetes, increased plasma concentrations of tHcy were found to be significantly associated with macroangiopathy, whereas there are only limited data on tHcy concentrations in patients with type 1 diabetes, especially children and/or adolescents (4,5). Increased plasma tHcy concentrations were found in diabetic patients with clinical signs of nephropathy (6), probably caused by reduced renal function (7), whereas plasma tHcy does not seem to play a major role in diabetic retinopathy (8).

C. Pavia et al. (1) reported no differences in tHcy concentrations between patients and control subjects; this observation suggests that the detection of no hyperhomocysteinemia in adolescents with type 1 diabetes has no predictive value for cardiovascular disease.

In our experience, we obtained similar results. We compared plasma tHcy concentrations in a group of type 1 diabetic patients attending the Genoa Pediatric Diabetologic Center and in 123 healthy control subjects matched for sex and age. Plasma tHcy concentrations ( $\mu\text{mol/l}$ ) were measured with the high-performance liquid chromatography method and fluorescence detection (9). The patient group consisted of 112 subjects (63 males and 49 females) aged 17.6 years (range 4.4–33.9) with diabetes duration ranging from 0.7 to 3.0 years (mean 9.4); diabetes duration was >10 years in 65 patients. Plasma tHcy concentrations in type 1 diabetic patients were significantly lower ( $P < 0.001$ ) than in sex- and age-matched control subjects (females <14 years, 3.24 vs. 7.90  $\mu\text{mol/l}$ ; males <14 years, 3.27 vs. 8.66  $\mu\text{mol/l}$ ; females >14 years, 4.27 vs. 7.10  $\mu\text{mol/l}$ ; males >14 years, 5.03 vs. 8.40  $\mu\text{mol/l}$ , respectively). Moreover, plasma tHcy levels increased with age ( $P < 0.001$ ) and with diabetes duration ( $P < 0.05$ ) in type 1 diabetic patients. No sex-related difference was found in plasma tHcy concentrations between type 1 diabetic patients >14 years of age with or without microvascular complications. Serum folate concentrations were higher in type 1 diabetic patients than in control subjects.

We confirm, as other authors have (4), that in children and adolescents with type 1 diabetes, hyperhomocysteinemia is not detected and therefore not predictive

of microvascular lesions. Further studies are needed to define the role of tHcy levels and of latent hyperhomocysteinemia in type 1 diabetic patients. Currently, the methionine loading test may identify >50% of subjects with hyperhomocysteinemia, and it is a valuable adjunct to fasting tHcy (10).

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## Plasma Homocysteine Levels in Type 1 Diabetic Patients

We appreciate the comments of Cotellessa et al. (1) as well as their interest in our work. We agree on their results, which confirm our findings. In patients with type 1 diabetes, in the absence of serious complications,

the plasmatic concentration of homocysteine is in the low range of the reference values for age-matched control subjects. For this reason, it does not seem to have a predictive role for angiopathy in these types of patients. On the other hand, in patients with type 2 diabetes, especially when signs of nephropathy or macroangiopathy coexist (2), hyperhomocysteinemia is a usual finding. In a study carried out in a group of 19 type 1 diabetic adolescents (aged 14 to 18 years), those patients with microalbuminuria (defined as an index albumine/creatinine >3 mg/mmol) (Fig. 1) had basal plasma values of total homocysteine at  $7.24 \pm 1.62 \mu\text{mol/l}$  (mean  $\pm$  SD). After a period of treatment (3–5 months) with captopril (50 mg/day), the microalbuminuria values returned to normal, whereas those of total homocysteine,  $7.06 \pm 1.56 \mu\text{mol/l}$ , remained similar to those found in basal conditions. These results suggest that only in cases of impaired renal function (3) would an increase of plasma homocysteine take place as an indicator of subclinical atherosclerosis (4).

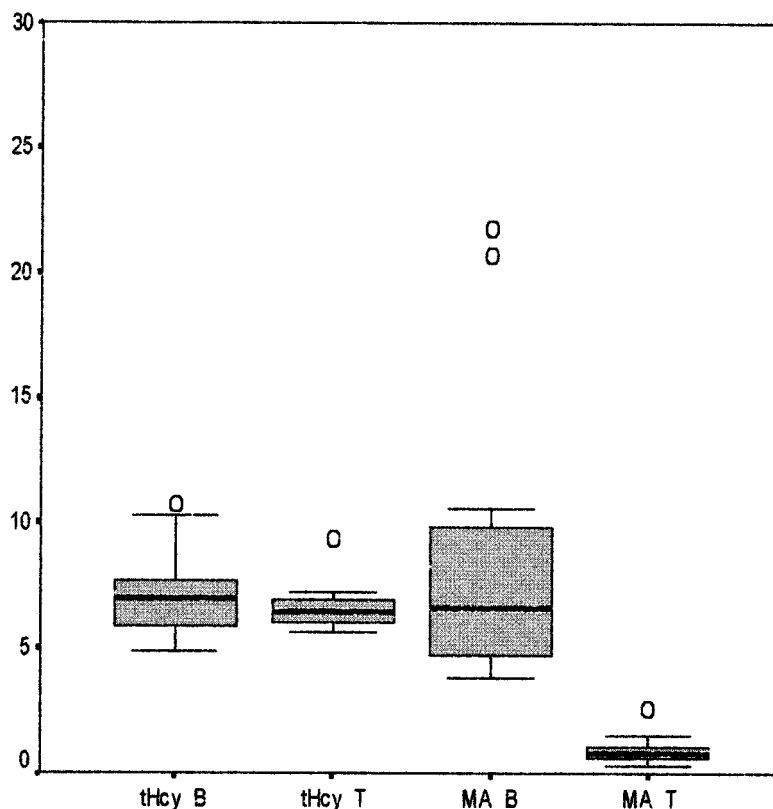


Figure 1—Multiple box-plot of plasma total homocysteine (tHcy,  $\mu\text{mol/l}$ ), microalbuminuria (MA, mg/mmol creatinine), basal (B) versus posttreatment (T). Significant differences (Mann-Whitney U test) were observed only in MA ( $P < 0.0001$ ).

The data obtained by Cotellessa et al. (1) as well as our results point to the fact that hyperhomocystinemia does not have a predictive value for the microangiopathy in patients with type 1 diabetes.

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## Lack of Effect of Pioglitazone on Postprandial Triglyceride Levels in Type 2 Diabetes

We read with interest an article by Aronoff et al. (1), which indicated that monotherapy of pioglitazone, a peroxisome proliferator-activated receptor (PPAR)- $\gamma$  agonist, can improve lipid profile as well as glycemic

control in type 2 diabetes. However, because they evaluated dyslipidemia in the fasted state, as most investigators do, it remains unknown whether pioglitazone may ameliorate postprandial dyslipidemia, which has recently been suggested to be an independent risk factor of atherosclerosis (4–6). We have previously indicated that troglitazone, another PPAR- $\gamma$  agonist, can cause a decrease in postprandial triglyceride levels in subjects with type 2 diabetes, although there is no significant relationship between a decrease in postprandial triglyceride levels and a decrease in intima-media thickness (IMT) of the common carotid artery (2,3).

In the present study, we investigated whether pioglitazone, another PPAR- $\gamma$  agonist, can decrease postprandial triglyceride in type 2 diabetes. Subjects included a total of 150 (87 men and 63 women, mean  $\pm$  SEM age  $61.1 \pm 0.8$  years) Japanese subjects with type 2 diabetes. HbA<sub>1c</sub>, total cholesterol, and postprandial triglycerides were measured 3 and 6 months after pioglitazone in the subjects receiving pioglitazone (30 mg/day) in combination with sulfonylurea drugs ( $n = 75$ ) and in the control subjects, who were only receiving sulfonylurea drugs ( $n = 75$ ). Postprandial triglyceride levels were examined 2 h after the conventional breakfast, as described previously (2). Although HbA<sub>1c</sub> showed a statistically significant decrease in comparison with control subjects ( $-0.885 \pm 0.118$  vs.  $0.072 \pm 0.130\%$ , respectively,  $P < 0.05$ ), there was no statistically significant change in total cholesterol ( $197.1 \pm 4.7$  vs.  $202.7 \pm 3.6$  mg/dl,  $P = 0.350$ ) or in postprandial triglycerides ( $150.3 \pm 11.6$  vs.  $152.0 \pm 12.4$  mg/dl,  $P = 0.921$ ).

Various PPAR target genes involved in fatty acid metabolism, such as lipoprotein lipase, have been identified to have PPAR response elements (7), which may, at least partly, result in an inhibitory effect of PPAR- $\gamma$  agonists on plasma triglyceride levels. This preliminary result, however, suggests that pioglitazone does not decrease postprandial dyslipidemia unlike troglitazone, suggesting a possibility that PPAR- $\gamma$  agonists may act differently on postprandial triglycerides. Taken together with our previous results, which showed that there is no relationship between a decrease in postprandial triglyceride and that in IMT after troglitazone (2,3), and with the fact that there is no

correlation between postprandial triglyceride and IMT in a total of 250 subjects with type 2 diabetes (H. Koshiyama, unpublished data), it appears that postprandial triglyceride has no great impact on early atherosclerotic process, at least, in Japanese subjects with type 2 diabetes.

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