OBSERVATIONS

Blood Catalase Deficiency and Diabetes in Hungary

Diabetes is a group of metabolic diseases characterized by hyperglycemia. Clinical expression of diabetes is dependent on both genetic and acquired factors (1).

The metabolic effects of oxidants, which are believed to contribute to many diseases, may influence the development of some forms of diabetes. The oxidant hydrogen peroxide (H_2O_2) is a by-product of normal cellular respiration and is also formed from superoxide anion by the action of superoxide dismutase. H_2O_2 has been reported to damage pancreatic β-cells (2–4) and inhibit insulin signaling (5).

The enzyme catalase (E.C. 1.11.1.6) has a predominant role in controlling the concentration of H_2O_2 (6,7), and consequently, catalase protects pancreatic β-cells from damage by H_2O_2 (3,8). Low catalase activities, which have been reported in patients with schizophrenia and atherosclerosis (9), are consistent with the hypothesis that long-term oxidative stress may contribute to the development of a variety of late-onset disorders, such as type 2 diabetes (10,11). Two categories of genetic deficiencies of erythrocyte catalase, which were reviewed in 1995 (12), are acatalasemia (<10% of normal activity) and hypocatalasemia (~50% of normal activity). In Hungary, 1 acatalasemic and 12 hypocatalasemic families have been described (10,13,14). These families include 2 acatalasemic, 61 hypocatalasemic, and 66 normocatalasemic individuals. Diabetes was diagnosed in eight members of these families. Both acatalasemic individuals were women with type 2 diabetes; five of the hypocatalasemic women had type 2 diabetes, and the only man with diabetes among the eight was hypocatalasemic. Therefore, for this cohort with inherited catalase deficiency, the incidence of diabetes was 12.7%. None of the 66 normocatalasemic members of the families had diabetes. Thus, it was suggested that deficiency of catalase and oxidant damage may contribute to the development of diabetes (10,11).

In this study, we report 1) the frequency of catalase deficiency observed among Hungarian subjects who do not have other family members with low catalase activity, 2) the blood catalase activity of randomly selected diabetic subjects, 3) data from laboratory indicators of glycemic control as well as insulin and C-peptide concentrations in nondiabetic hypocatalasemic and normocatalasemic members of five of the Hungarian families with catalase deficiency, and 4) insulin and C-peptide concentrations in diabetic patients with acatalasemia and hypocatalasemia.

Samples from 21,750 hospital patients, 1,630 clinic patients, and 3,300 healthy control subjects between the ages of 14 and 93 years were screened for catalase activity during a 3-year study program in the Sümeg region of Western Hungary. The criteria for diagnosis of diabetes were fasting plasma glucose >7 mmol/l, oral glucose tolerance test maximum glucose concentrations >11.1 mmol/l, and HbA$_{1c}$ >6.1%. Glucose was determined by a glucose oxidase-peroxidase method (Glucose test; Reanal, Budapest). The reference range for fasting glucose was 3.5–6.0 mmol/l.

Blood catalase activity was measured with a spectrophotometric assay (9,13). The mean ± 1 SD of the reference range for blood catalase activity was 113.3 ± 16.5 MU/l (14). Decreased blood catalase activity was defined as <80.3 MU/l, i.e., below the mean − 2 SD. Serum fructosamine was measured with the Roche Fructosamine Test (Roche Diagnostics, Basel). The reference range was ≤283 μmol/l. HbA$_{1c}$ was determined with a high-performance liquid chromatography method (Diamat; BioRad, Richmond, CA). The reference range was 4.2–6.1%. Serum insulin concentrations were determined using the radioimmunoassay method from Izotóp Intézet (Budapest). The reference range for fasting serum insulin was 5–35 mU/l (1 mU/l = 5.99 pmol/l). Serum C-peptide was determined using the C-PEP-CTZ radioimmunoassay kit from CIS Bio International (Cedex, France). The reference range was 280–1,320 pmol/l. Student's t test was used to evaluate the statistical significance of the differences between mean values.

The screening of a large population (26,680 subjects) of individuals from Hungarian hospitals and clinics as well as control subjects resulted in 174 nonanemic subjects with blood catalase activities below the reference range. Among these individuals, 13.8% had diabetes. This frequency is higher than that observed in hospitals (7.8%) or in the general population (1.7%) in Hungary (10).

Blood catalase activities among 137 randomly selected diabetic patients had a mean activity of 94.4 ± 19.2 MU/l. This mean value was significantly lower (P < 0.001) than the mean for the reference range. These diabetic patients were not members of families with established familial catalase deficiency. There was no significant difference (P > 0.7) between the mean catalase activities of the 45 patients with type 1 diabetes and the 92 patients with type 2 diabetes in this group.

Samples from five women with type 2 diabetes and inherited catalase deficiency (one acatalasemic subject with 7.6 MU/l and four hypocatalasemic subjects with 66.3, 24.4, 30.9, and 66.1 MU/l, respectively) were analyzed for insulin and C-peptide concentrations. In three patients (one acatalasemic and two hypocatalasemic subjects), insulin levels (1.1, 4.1, and 4.4 mU/l, respectively) were below the reference range (5–35 mU/l); the insulin level in the fourth hypocatalasemic patient was in the reference range (26.3 mU/l), and the insulin level in the fifth patient was above the reference range (40.0 mU/l).

In these patients, the C-peptide values (40.3, 189.1, 15.9, and 8.6 pmol/l, respectively) were below the reference range (280–1,320 pmol/l), and in one hypocatalasemic patient, the C-peptide value (1,185 pmol/l) was near the upper end of the reference range. The C-peptide versus insulin ratios in these patients were 0.36, 1.04, 0.52, 1.12, and 4.3 pmol/l, respectively, all of which were lower than the generally accepted ratio of >5:1 (15).

The indicators of hypoglycemia and β-cell secretion in nondiabetic catalase-deficient (n = 7) and age-matched (45.1 ± 10.5 vs. 44.7 ± 17.9 years) normocatalasemic relatives (n = 12) were for catalase (mean ± SD) 62.4 ± 23.5 vs. 114.3 ± 17.0 MU/l (P < 0.001), glucose 5.25 ± 0.72 vs. 4.94 ± 0.40 mmol/l (P = 0.241), HbA$_{1c}$ 5.38 ± 0.49 vs. 4.98 ± 0.41% (P = 0.078), fructosamine 235.6 ± 43.8l vs. 224.8 ± 26.2 μmol/l (P = 0.508), insulin 8.0 ± 3.4 vs. 18.8 ± 7.9 mU/l (P < 0.001), and C-peptide

173.7 ± 85.4 vs. 336.8 ± 189.8 pmol/l ($P = 0.046$).

The elevated concentrations of H_2O_2 that must exist in individuals with catalase deficiency may damage pancreatic cells, influence insulin release and signaling, and alter glucose metabolic processes, as discussed elsewhere (2–5,8,9,16,17). The increased frequency of diabetes associated with catalase deficiency and the observed low catalase activity among diabetic patients are consistent with the hypothesis that long-term oxidative stress due to lower-than-normal catalase activity may be a risk factor for diabetes.

In model systems that have a normal expression of catalase, the activity of this antioxidant enzyme has been observed to increase in response to oxidative stress (3,18) and protect pancreatic β-cells. Our data are consistent with the hypothesis that individuals with inherited catalase deficiency lack this ability to protect their β-cells and are consequently at risk of developing diabetes. It is clear from the small fraction of catalase-deficient subjects who have diabetes that this enzyme deficiency alone cannot be used to predict whether an individual will develop diabetes. It is also clear that catalase deficiency may be a risk factor for diabetes, at least in the Hungarian population that we analyzed.

The kind of diabetes involved with catalase deficiency may not be classic type 2 diabetes in which individuals progress from impaired glucose tolerance with modest hyperglycemia and hyperinsulinemia to overt diabetes. The low insulin and C-peptide values in most of the nondiabetic hypocatalasemic subjects and in the diabetic subjects with catalase deficiency indicate that most diabetic and diabetes-susceptible individuals with catalase deficiency may not have hyperinsulinemia of classic type 2 diabetes.

In conclusion, a mechanism may be that an elevated concentration of hydrogen peroxide, due to decreased catalase activity, could contribute to oxidative destruction of pancreatic β cells, to decreased insulin secretion and insulin effectiveness, and to the onset of diabetes.

LÁSZLÓ GÓTH, PHD[1]
ÁGOTA LENKEY, PHD[1]
WILLIAM N. BIGLER, PHD[2]

From the [1]Departments of Clinical Biochemistry, Molecular Pathology, and Clinical Analytical Chemistry, University of Debrecen, Debrecen, Hungary; and the [2]Center for Biomedical Laboratory Science, San Francisco State University, San Francisco, California.
Address correspondence to László Góth, Department of Clinical Biochemistry and Molecular Pathology, University of Debrecen, Debrecen PO Box 40, Hungary H-4012. E-mail: goth@jaguar.dote.hu.

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Don't Ignore the Patients

Over 90% of the daily long-term treatment of most chronic conditions is carried out by patients themselves in their own homes. Consequently, patients must be recognized and accepted as legitimate members of the health care team, with all of the associated rights and responsibilities. Chronic illnesses will, both now and in the foreseeable future, continue to be our nation's leading causes of death, disability, and medical expenditures, and as such, they will demand a delivery system responsive to their unique needs and to patient involvement.

The Institute of Medicine's (IOM's) Committee on Quality of Health Care in America recently published *Crossing the Quality Chasm: A New Health System for the 21st Century* (1). This publication will undoubtedly have a tremendous impact on the future of health care in this nation. The conclusions reached indicate the need for "fundamental changes" and declared "health care must be: safe, effective, patient-centered (providing care that is respectful of, and responsive to, individual preferences), timely, efficient and equitable." Unfortunately, the role and responsibilities of patients in this report were grossly neglected, and references were repeatedly made to "patients receiving care" or to the system "serving" or "providing patients with." These infer-

ences tend to perpetuate the existing paternalistic acute care system, which relies on activated health professionals providing care to a passive patient population.

Health maintenance, chronic disease prevention, and long-term treatment are largely the responsibility of patients themselves, and it is imperative that this concept be acknowledged, developed, and implemented as soon as possible. The IOM publication failed to advocate this concept. Currently, there is considerable interest in "patients' rights," which is a step in the right direction, but this must be accompanied simultaneously by an effort to define and legislate "patient responsibilities."

Health care for the 21st century must use a systems-approach directed at the prevention and treatment of chronic illnesses that is scientifically based, cost-effective, and patient-oriented (2). These demands vary widely from those society currently expects, demands, and rewards. It will require decades for society and the medical profession to develop, adapt, and implement these fundamental changes, but we must begin NOW. The basis for any successful "New Health System for the 21st Century" must be patient-led.

DONNELL D. ETZWILER, MD

Address correspondence to Donnell D. Etzwiler, MD, 7611 Bush Lake Rd., Minneapolis, MN 55438. E-mail: dretzwiler@aol.com.

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The American Diabetes Association Should Be a Leader in Reducing Medication Errors

The 1999 publication of the Institute of Medicine's (IOM's) review of medical errors, *To Err is Human: Building a Safer Health System* (1), along with the publication of Michael Cohen's book, *Medical Errors* (2), highlighted the impact that medical errors have on the U.S. health care system and the potential for high morbidity and possible fatal outcome for the individual patient (7,000+ deaths per year from medication errors). Throughout the country, health care providers, hospitals, and delivery systems are making elimination of medication-related errors a top priority, with strong support from major employers and the federal government. A cultural shift must occur, moving from "blame the individual," to an approach looking for the root cause of errors and focusing on system-wide changes to prevent recurrence. Until universal availability of physician order-entry, bar-coding, and other innovative methods to reduce errors are a reality (acknowledging that nothing is foolproof), errors will continue to plague our dysfunctional health care system.

By looking at the stages of the medication process, physicians' orders have been identified as the most common source of preventable errors. Insulin has been singled-out repeatedly as one of the medications most frequently involved in medication errors. Both the IOM's 1999 report and Cohen's book identify insulin as the medication with the greatest likelihood for harm when errors occur. Handwritten orders for insulin using the letter "U" for "units" leads to errors with 10-fold or more increases in insulin dosages occurring as the letter "U" is mistaken for a 0, 4, or 6. By establishing a task force to address this error in our hospital and making insulin order sheets with the word "units" preprinted in the order area, we have significantly reduced insulin-related errors.

The American Diabetes Association (ADA) is in the unique position to greatly reinforce the use of "units" for writing insulin orders throughout the U.S. and world health care systems. Eliminating the use of "U" in ADA publications and adopting an aggressive stance on this will make a tremendous impact on the health of diabetic patients by protecting them from insulin overdosage. Please incorporate these changes and help lead the world in the elimination of medical errors.

DANIEL J. CROWE, MD, CDE

From the Diabetes Resource Center, Portsmouth Regional Hospital, Portsmouth, New Hampshire.

Address correspondence to Daniel J. Crowe, Medical Director, Diabetes Resource Center, Portsmouth Regional Hospital, 333 Borthwick Ave., Suite 104, Portsmouth, NH 03802. E-mail: daniel.crowe@hcahealthcare.com.

Note from the Editor: When Dr. Crowe brought this problem to the attention of the ADA professional publications staff in May of this year, they immediately began spelling out "units" in text and revising figures whenever possible. The change has been in effect since the August issues of Diabetes *and* Diabetes Care *and the summer issues of* Diabetes Spectrum *and* Clinical Diabetes. *Although the use of "units" may take a little longer to become apparent in ADA's books and pamphlets, ADA staff have made a commitment to promoting this practice.*

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Immune Responses to Hepatitis B Vaccine With and Without the Pre-S2 Antigen in Children With Type 1 Diabetes

This study involved 20 patients (10 males and 10 females) with type 1 diabetes who were seronegative for hepatitis B surface-antigen (HBsAg) and other hepatitis B (HB) viral markers and had no diabetic complications. As patients were recruited over a period of several months, each patient was alternatively assigned to one of two vaccination groups (A or B). There was no significant difference between groups A and B in terms of age, sex, duration of diabetes, and levels of HbA_{1c}. Written informed parental consent was obtained for all patients. Patients in groups A (mean age 12.7 ± 2.6 years, mean duration of diabetes 5.1 ± 2.6 years, mean HbA_{1c} level 8.6 ± 0.7%) received three doses of 10 or 20 μg (the lower dose was given to patients aged <10 years, as recommended by the manufacterer) of a recombinant DNA HB vaccine (Engerix B; Smith-Kline Beecham, Rixensart, Belgium) by intramuscular injections in the left thigh at 0,

Table 1—*MTAs against HB virus (anti-HBs mIU/ml) of children in groups A and B 4–5 weeks after the administration of the first, second, and third dose of HB vaccine*

	n	First dose	Second dose	Third dose
Group A (Engerix-B)	10	0 (0–3.19)	15.44 (0–1,000)	157.5 (4.54–1,630)
Group B (GenHevac-B)	10	8 (0–150)	128.5 (0–1,000)	1,000 (170–2,796)

Data are median (range) unless otherwise indicated. For first and second dose, P = NS; for third dose, P = 0.014.

SEBASTIANO BIANCA, PHD
GIULIANA GRANATA, PR
GIOVANNI LI VOLTI, PHD
SALVATORE LI VOLTI, AP

From the Department of Pediatrics, University of Catania, Catania, Italy.
Address correspondence to Salvatore Li Volti, Dipartimento di Pediatria, Via S. Sofia 78, 95123 Catania, Italy. E-mail: livolti@mbox.unict.it.

1, and 2 months. Patients in group B (mean age 13.8 ± 5.7 years, mean duration of diabetes 6.3 ± 3.6 years, mean HbA$_{1c}$ level 8.4 ± 0.5%) were given three doses of 20-µg recombinant DNA HB vaccine that included pre-S1 and -S2 proteins encoded by the HB viral genome (GenHevac B; Pasteur Vaccins, Pasteur-Mérieux, Marcy l'Etoile, France). We adopted this schedule of vaccinations to induce early immunization because children with type 1 diabetes are considered to be at increased risk of infection by HB virus (1). A sample of venous blood (10 ml) was taken from each child in group A and group B at 4–5 weeks after the first, second, and third injection of HB vaccine to determine the serum concentrations of HBsAg, HBsAg–specific antibodies (HBsAb), and other common markers of infection by HB virus (Ausria II, Corab and Ausab; Abbott Laboratories, Chicago). Moreover, HLA class I antigens were analyzed by serological typing, whereas HLA class II antigens (DR3, DR4, DR7, and DQ2) were analyzed by polymerase chain reaction (Amplicor HLA DRB test; Roche, Monza, Italy). The HLA class II antigens DR3, DR4, DR7, and DQ2 were equally distributed in both groups of children. Both vaccines were well tolerated, and no immediate adverse reactions were observed in any of the 20 patients.

Table 1 shows the means of the titers of antibodies (MTAs) against HB virus in the children of groups A and B after each of the three doses of HB vaccine. The MTAs against HB virus in the patients of group B were higher than those of group A after each dose of vaccine. The difference between the groups was statistically significant (P = 0.014) after the administration of the third dose of the vaccine. However, the MTAs against HB virus of the patients in group B were lower than those determined in other young patients with type 1 diabetes who received three doses of HB vaccine at 0, 1, and 6 months (2). This difference was probably attrib-

utable to the schedule of vaccinations, which induced earlier immunization (3), albeit with lower titers of antibodies. Douvin et al. (4) found that the choice of the HB vaccine has little influence on the immune response to HB vaccine administered at 0, 1, 2, 4, and 12 months in adults with type 1 or type 2 diabetes. However, in patients vaccinated with GenHevac B, HBsAbs appeared earlier than in patients vaccinated with Engerix B. The different results in the two studies could be due to both the age of the patients and the schedule of vaccinations. It seems that the immune response to HB vaccine is poorer in adults (5); this could explain the low HBsAb titers found by Douvin et al. (4) after three doses of both vaccines administered at 0, 1, and 2 months. As we did not give a fourth dose at 12 months, we cannot exclude that we could have found a similar immune response to both vaccines after a fourth dose at 12 months.

The hyporesponsiveness and poorer immune response of the children in group A did not seem to correlate with the distribution of HLA class II antigens, nor did they appear to be due to age, sex, level of HbA$_{1c}$, or duration of diabetes. The better immune response of children who were given three doses of GenHevac B was probably a result of the formulation of the vaccine (6). It has been demonstrated that in mice, the pre-S2 region of HBsAg is more immunogenic than the S region in regard to induction of antibodies (7), probably because the pre-S2 region of HbsAg is able to recruit helper T-cells that circumvent nonresponsiveness to the S region. We suggest that when HB vaccine is administered to children with type 1 diabetes, a vaccine that includes the pre-S2 antigen is preferable because it may guarantee a more adequate and longer-lasting protection against infection by HB virus.

MICHELE MANCUSO, PHD
MANUELA CARUSO-NICOLETTI, AP

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Proinsulin-Specific Autoantibodies Are Relatively Infrequent in Young Offspring With Pre–Type 1 Diabetes

Insulin is a β-cell–specific and pivotal autoantigen in type 1 diabetes (1) and is produced after proteolysis of its prohormone proinsulin, which is a potential target of autoimmunity. T-cell studies in the nonobese diabetic (NOD) mouse (2,3) and in prediabetic relatives of patients with type 1 diabetes (4,5) indicate the existence of proinsulin-specific immunodominant epitopes localized in the region between the C-peptide and the A-chain, whereas immune intervention with proinsulin in the NOD mouse can prevent diabetes onset. These studies have led to the proposal of proinsulin as a primary target of autoimmunity associated with type 1 diabetes. At the humoral level, proinsulin-specific autoantibodies have also been reported in humans (6,7). Our own data show, however, that proinsulin au-

toantibody (PAA) and insulin autoantibody (IAA) levels correlate well in newly diagnosed patients, but IAAs are more sensitive and specific for type 1 diabetes (7). Furthermore, IAA or PAA could be completely displaced with either insulin or proinsulin in the majority of patients, suggesting that the main epitopes recognized by these antibodies at diabetes onset are on the insulin molecule. In the present study, we investigated whether antibodies to proinsulin-specific epitopes either preceded or were a more relevant finding early in the development of diabetes-associated autoimmunity.

We took advantage of the German BABYDIAB Study, in which samples from the offspring of parents with type 1 diabetes were obtained at birth, 9 months, and 2 and 5 years of age and where 62 (4.2%) offspring developed IAA by 2 years of age (8). Samples from 48 offspring (median age 2.6 years) were studied. All had islet autoantibodies that included IAA. Antibodies to insulin and proinsulin were measured by microradiobinding assays, incorporating displacement with unlabelled insulin or proinsulin, respectively, as previously de-

scribed (7,9). To identify proinsulin-specific antibodies, displacement of binding to radiolabeled proinsulin was also performed with excess cold insulin (52 μmol/l).

There was a strong correlation between IAA and PAA levels ($r = 0.88$, $P < 0.01$) (Fig. 1). Only 3 of the 48 IAA-positive offspring showed no binding to proinsulin; 1 of these 3 was positive for another diabetes-associated antibody in the index sample, but all 3 children had further IAA-positive samples after the index sample, and 1 of these subsequently developed islet cell antibody, GAD antibody, and antibody to IA2. Binding to insulin in the remaining 45 sera could be completely inhibited with cold proinsulin. Binding to proinsulin in 2 of the 45 PAA-positive sera could not be completely inhibited with cold insulin, suggesting that PAA in these 2 offspring were directed against both epitopes that are shared by proinsulin and insulin and epitopes found only on the proinsulin molecule. Both had relatively high PAA and IAA (case 1: 14.9 units IAA, 6.9 units PAA; case 2: 14.6 units IAA, 44.1 units PAA). Proinsulin-specific antibodies (not displaceable by insulin) accounted for 49% of the binding to proinsulin in case 1 and for 48% of the binding to proinsulin in case 2. Previous and follow-up samples were analyzed in these two subjects. Neither had PAA in a sample obtained before the first detection of IAA. In case 1, both IAA and PAA declined in follow-up samples, but the proportion of proinsulin-specific binding remained >30%. Case 2 developed type 1 diabetes 1 year after the measurement of IAA and PAA. Insulin antibodies increased (32 units) and PAA decreased (12.3 units) after the commencement of insulin therapy in this subject. The proportion of proinsulin-specific antibodies remaining were markedly reduced (4%), indicating that insulin therapy did not promote the production of proinsulin-specific autoimmunity. Type 1 diabetes genotyping identified HLA DR*11/0401 DQA*05/03 DQB* 0301/0302 in case 1 and HLA DR*11/12 DQA*05/0102 DQB*0301/0502 in case 2.

The data indicate that proinsulin-specific humoral autoimmunity is relatively uncommon in the initial stages of diabetes-associated autoimmunity. The relative absence of proinsulin-specific autoantibodies during the disease process
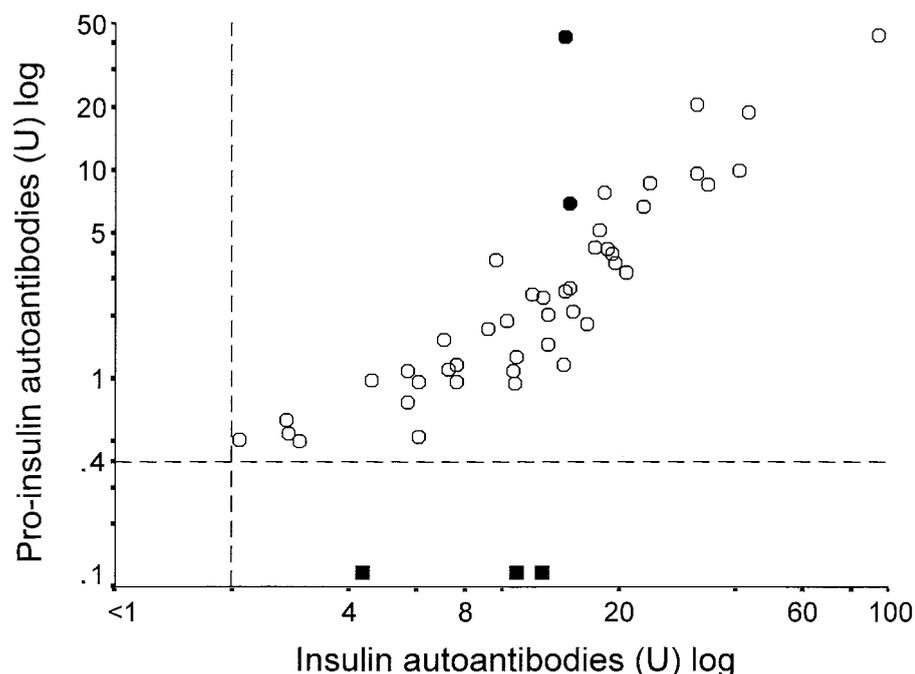
Figure 1—*Correlation between the insulin (abscissa) and proinsulin autoantibodies (ordinate) in 48 young offspring with positive IAA. – – –, the threshold for positivity for each of the antibodies; ●, offspring with proinsulin-specific autoantibodies; ■, offspring with insulin-specific autoantibodies. The majority of samples react with insulin and proinsulin, indicating that the relevant epitopes are on the insulin molecule.*

does not suggest that screening for PAA rather than IAA would be more effective in early identification of young children at risk and does not provide evidence for proinsulin rather than insulin as a primary target in diabetes-associated autoimmunity.

MICHAEL HUMMEL, MD[1]
ALISTAIR J.K. WILLIAMS, PHD[1]
ALASTAIR NORCROSS, PHD[1]
EBERHARD STANDL, MD[2]
EZIO BONIFACIO, PHD[2]
ANETTE G. ZIEGLER, MD[2]
POLLY J. BINGLEY, MD[1]

From the [1]Division of Medicine, University of Bristol, Bristol, U.K., and the [2]Diabetes Research Institute and Third Medical Department, Academic Hospital München-Schwabing, Munich, Germany.

Address correspondence to Dr. Michael Hummel, Diabetes Research Institute, Kölner Platz 1, D 80804 Munich, Germany. E-mail: michael.hummel@lrz.uni-muenchen.de.

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Effect of Repaglinide Addition to NPH Insulin Monotherapy on Glycemic Control in Patients With Type 2 Diabetes

Repaglinide is a new type of oral hypoglycemic agent. Its rapid absorption, short metabolic half-time (1 h), and novel insulin release profile are all characteristics desired for treating elderly people with type 2 diabetes. The aim of this study was to investigate the efficacy and safety of repaglinide in combination with NPH insulin in subjects with type 2 diabetes on insulin monotherapy with suboptimal glycemic control.

The prospective, no-blind study was performed in our clinical research unit. We selected 27 (7 men and 20 women) type 2 diabetic patients who were nonsmokers and whose glycemic control was unsatisfactory (HbA$_{1c}$ >7.1%) with two doses of NPH insulin for >6 months. Additional inclusion criteria were age 45–75 years (mean 65 ± 9.6) and BMI >21 kg/m^2 (mean 27.2 ± 3.9). For the purpose of this study, we followed the American Diabetes Association's standards for medical care, which considers an HbA$_{1c}$ level <1% above the upper reference range for a nondiabetic population as a goal for good glycemic control and an HbA$_{1c}$ level >7.1% as unsatisfactory glycemic control. All subjects were required to be able to comply with the protocol and to carry out home blood glucose monitoring. The initial exclusion criteria included any clinically significant elevation of liver transaminases, previous rapid insulin treatment, alcohol or drug abuse, and unawareness of hypoglycemia. All subjects gave informed consent to participate in the study. The study was conducted in accordance with the 1964 Declaration of Helsinki and Guidelines for Good Clinical Research Practice in Spain.

After recruitment, prestudy insulin doses for each patient were maintained during a 4-week baseline period. Subjects who still were not under optimal control after that period were maintained on their prestudy insulin doses, and a fixed dose of repaglinide (2 mg three times a day) was added to the treatment until the end of the study. Insulin doses were adjusted only during the first 4 weeks and were then kept constant for 3 months. Blood pressure, HbA$_{1c}$, number of ambulatory hypoglycemic events, mean of premeal and 2-h postprandial glucose profiles, and total, LDL, and HDL cholesterol and triglycerides were measured at the start of the study and after 3 months of treatment with repaglinide. Changes in insulin dosage from the initial treatment as well as hypoglycemic events compared with the previous 3 months of the study were also assessed. During treatment, patients followed the same dietary intake (1,500 calories/day) and physical activity regimen that they followed before the study. If antihypertensive and antilipid agents were being taken at recruitment, administration was continued during treatment. Blood pressure was measured twice after a 10-min rest with a random zero mercury sphygmomanometer, and the two measurements were averaged.

Demographic and baseline characteristics were mean age 65 ± 9.6, duration of diabetes 12.2 ± 8.4 years, and BMI 27.2 ± 3.9 kg/m^2. HbA$_{1c}$ decreased significantly from 8.2 to 7.0% ($P = 0.0001$) (14.7% decrease from baseline), and 2-h postprandial glucose decreased significantly by 3.61 mmol/l (from 11.4 ± 2.5 to 7.8 ± 2.7 mmol/l, [$P = 0.01$]). No significant decrease was detected in preprandial glucose levels (9.44 ± 3.2 to 9.17 ± 3.2 mmol/l, P = NS). No significant changes were observed in other cardiovascular functions, BMI, or blood pressure. Taken together, the patients' insulin

dose did not change significantly (33.1 ± 17.5 to 29 ± 27 units/day, P = NS); although 66.7% of our patients needed less insulin while on repaglinide than before, 29.6% maintained the same dose, and 3.7% increased their dosage. The number of hypoglycemic events during 3 months of treatment with repaglinide was similar to that before treatment (2.7 ± 2.4 to 1.45 ± 2.6 events, P = NS). None of the subjects were in optimal glycemic control at entry. By the end of our study, 37.5% of subjects were in optimal control (HbA$_{1c}$ <7.1%).

In short-term placebo-controlled trials, HbA$_{1c}$ was reduced from 8.5% at baseline to 7.8% after only 12 weeks with repaglinide monotherapy (1). In diabetic patients not previously treated with other drugs, repaglinide resulted in a 30% decrease in HbA$_{1c}$, from 6.98 to 4.87%, with fasting and postprandial blood glucose levels decreased by 70 and 112 mg/dl, respectively (2). A long-term trial (3) compared repaglinide with glipizide at doses of 5–15 mg twice a day. This study (3) demonstrated the significant superiority of repaglinide in lowering HbA$_{1c}$ (0.6%, P < 0.05) and fasting glucose (16 mg/dl, P < 0.05) over a period of 1 year. In two direct comparison studies with glyburide, the degree of glycemic control was similar between repaglinide and glyburide after 14 months. In patients naive to oral treatment, repaglinide resulted in a decrease in HbA$_{1c}$ from 9.4 to 7.6% within the first 3 months, and the effect was maintained for 1 year (4–5). In another study (6), repaglinide and glibenclamide were compared, and the decrease in blood glucose levels and HbA$_{1c}$ values were similar in both groups (0.3% of HbA$_{1c}$). When repaglinide was used in combination with metformin for the treatment of type 2 diabetes, more improvements in glycemic control were observed than with metformin therapy alone (HbA$_{1c}$ 6.9 vs. 8.3%, respectively) (7). With the combination of repaglinide and troglitazone, Raskin et al. (8) showed a significant reduction in mean HbA$_{1c}$ values (−1.7%) that was greater than that with either therapy alone.

Our study is the first prospective evaluation specifically designed to examine the efficacy of administering repaglinide to patients on insulin monotherapy with inadequate glycemic control. In conclusion, insulin therapy combined with repaglinide resulted in a decrease in HbA$_{1c}$ and postprandial glucose levels, with no increase in the number of hypoglycemic episodes.

DANIEL ANTONIO DE LUIS, MD
ROCÍO ALLER, MD
LUIS CUELLAR, PHD
CONCEPCION TERROBA, PHD
HILDA OVALLE, PHD
OLATZ IZAOLA, PHD
ENRIQUE ROMERO, MD

From the Instituto de Endocrinología y Nutrición, Facultad de Medicina y Hospital Rio Hortega, Universidad de Valladolid, Valladolid, Spain.
Address correspondence to Dr. D.A. de Luis, Associate Professor of Endocrinology and Nutrition, Executive Director of Instituto de Endocrinología y Nutrición, Universidad de Valladolid, C/Caamaño 51 Bis 3° C, Valladolid 47013, Spain. E-mail: dadluis@yahoo.es.

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Loss of Awareness of Hypoglycemia Temporally Associated With Selective Serotonin Reuptake Inhibitors

Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed for the treatment of depression in patients with diabetes. We previously reported a 17-year-old patient with type 1 diabetes who developed loss of hypoglycemic awareness while on fluoxetine hydrochloride for depression (1). He regained hypoglycemic awareness after discontinuing fluoxetine. We hereby present two other cases of loss of hypoglycemic awareness temporally associated with the use of SSRIs.

CASE 1
This Caucasian female was diagnosed with type 1 diabetes at 10 years of age. She was treated with an intensive subcutaneous insulin program (total 1.1 units · kg^{-1} · day^{-1}) and a 1,600-kcal diabetic diet. Her HbA$_{1c}$ was 9.7%. Blood glucose levels <70 mg/dl occurred approximately once per week and were always accompanied by warning symptoms (hunger, diaphoresis, and generalized weakness). All low blood-glucose levels were self-treated, and there was no history of sustained hypoglycemia.

At 17 years of age, the patient became depressed and was treated with sertraline hydrochloride (200 mg orally daily). While on therapy with sertraline, she experienced decreased awareness of hypoglycemia; hypoglycemic episodes increased in frequency to two to three times per week. She had two episodes of severe hypoglycemia complicated by unconsciousness. Insulin dosages, prescribed diet, weight, and HbA$_{1c}$ were similar before and during treatment with sertraline. Sertraline was discontinued 6 months later, and awareness of hypoglycemia returned.

CASE 2
Our second patient, a Caucasian female with cystic fibrosis, had diabetes since 14

years of age, when she presented with diabetic ketoacidosis. During treatment with an intensive subcutaneous insulin program (total 0.77 units · kg^{-1} · day^{-1}) and a 2,300-kcal diabetic diet, HbA$_{1c}$ was 8.1%. Blood glucose levels <70 mg/dl occurred three to four times per week and were typically accompanied by warning symptoms. The patient had two severe hypoglycemic episodes (manifested by lack of consciousness) over a period of 5 years.

At 21 years of age, she became depressed and was treated with paroxetine hydrochloride (30 mg once daily). During 3 months of paroxetine treatment, she experienced sedation, fatigue, and decreased awareness of hypoglycemia. Severity of hypoglycemic episodes worsened (external assistance was required five times), as the frequency of hypoglycemia increased to four to five hypoglycemic episodes per week. Insulin dosage was decreased to a total of 0.6 units · kg^{-1} · day^{-1}, and the prescribed meal plan remained unchanged. Her weight increased by 10 kg. The HbA$_{1c}$ was 7.9%. After discontinuation of paroxetine, awareness of hypoglycemia improved dramatically; no severe hypoglycemic reactions recurred.

The mechanism of hypoglycemic unawareness in our patients is unknown. SSRIs do not influence plasma insulin levels (2) or augment hypoglycemic action of injected insulin (3). SSRIs do not seem to affect ACTH, cortisol, growth hormone (4), or sympatho-adrenal responses to insulin-induced hypoglycemia (5). SSRI-associated hypoglycemic unawareness may result from autonomic dysfunction, comprising a forme fruste of serotonin syndrome. A similar mechanism may cause some anorgasmia in SSRI-treated patients.

In conclusion, SSRIs are effective antidepressants commonly used in patients with diabetes. However, we advise close monitoring for potential loss of hypoglycemic awareness in diabetic patients treated with SSRIs.

ANNA M. SAWKA, MD[1]
VIRGINIA BURGART, RN[2]
DONALD ZIMMERMAN, MD[1,2]

From the Departments of [1]Endocrinology, Metabolism, and Nutrition and [2]Pediatrics, Mayo Clinic, Rochester, Minnesota.

Address correspondence to D. Zimmerman, MD, Mayo Clinic, 200 First St. SW, Rochester, MN 55905.

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Genetic and Environmental Factors for Type 1 Diabetes

Data from the province of Oristano, Sardinia, Italy

The exact role of genetic susceptibility and environmental factors in determining type 1 diabetes has not been clarified. The island of Sardinia has one of the highest rates of type 1 diabetes in the world (1). The contribution of genetic susceptibility to the etiology of type 1 diabetes has been investigated in several studies of migrants of Sardinian origin who live in various regions of Italy (2–4). However, few data are available on the risk of type 1 diabetes among immigrants of mainland origin who live in Sardinia (5).

To further examine the contribution of genetic and environmental factors to type 1 diabetes in Sardinia, we enrolled all incident cases of type 1 diabetes diagnosed in the province of Oristano aged 0–29 years between 1993 and 2000 (5). Data sources included the local pharmaceutical service, which provides patients with insulin syringes and other material needed for daily therapy (primary source), a register of individuals with chronic conditions that are exempt from health service fees, and the pediatric department of Oristano's provincial hospital. Finally, our data were checked with the Eurodiab Ace Sardinian Registry. We used the capture-recapture method to assess the completeness of our sources, and we used the population data supplied annually to the Public Health Service by the 78 municipalities of the province to calculate rates.

The primary source completeness was 100%. As of 31 December 2000, 303 patients aged 0–29 years who were residents of the Oristano province had been identified, including 10 with secondary diabetes who were excluded from further analysis. The point prevalence was 5.4 per 1,000 (95% CI 4.8–6.0). The incidence rate, standardized by age (direct method; reference population with a uniform age distribution) from 1993 to 2000, was 52.7 per 100,000 person-years (95% CI 42.4–63.0) in the 0- to 14-year age-group, 21.5 (16.0–26.9) in the 15- to 29-year age-group, and 37.1 (31.2–42.7) in the overall 0- to 29-year age-group.

Incidence was not stable in the 8-year period of the study. In particular, we observed a peak in the 0- to 14-year age-group in 1998, with 24 cases observed (incidence rate 105.2/100,000, 95% CI 70.5–157.0), as compared with 13 cases expected, resulting in a standardized incidence ratio (SIR) of 1.92 (95% CI 1.23–3.01, $P = 0.004$; Wald test). In 1994, five cases were observed (incidence rate 19.6/100,000, 8.2–47.1), far lower than the 14.5 cases that were expected, resulting in a SIR of 0.35 (0.14–0.88, $P = 0.03$).

When the overall 0–29 year age-group was considered, annual fluctuations were less dramatic, with SIRs of 1.56 (95% CI 1.06–2.30, $P = 0.02$) in 1998 and 0.44 (0.26–0.74, $P = 0.002$) in 1994. These data suggest that there is an interaction between type 1 diabetes and age.

When we examined geographic variations, no cases were reported in the municipality of Arborea among the population aged 0–29 years (1,571 inhabitants, 8.5 cases expected, $P = 0.0005$; Fisher's exact test). According to previous observations (5), this is likely explained by the fact that ~65% of the Arborea population is of non-Sardinian origin; most were originally from the Veneto Region of Italy. Also, data on the incidence of type 1 dia-

betes in the previous 12 years (our data plus data for 1989–1992, published by the Regional Department of Epidemiology) (6) support our findings of a lower-than-expected rate in Arborea (0 cases of 20,896 person-years in the 0- to 29-year age-group vs. 234 cases of 718,739 person-years in the remaining province, $P = 0.002$; Fisher's exact test).

In conclusion, our data further support the findings of others that type 1 diabetes is determined by genetic and environmental factors. The rapid fluctuations in incidence during the study period suggest a role of environmental factors, whereas the absence of observed cases among a population mainly of non-Sardinian origin suggests the role of genetic risk factors.

ONORATO FRONGIA, MD[1]
CRISTIANA PASCUTTO, PHD[2]
GIUSEPPE M. SECHI, MD[3]
MIRIAM SORO, MD[4]
ROSA M. ANGIOI, PHARM D[5]

From the [1]Epidemiology Unit, Oristano, Italy; the [2]University of Pavia, Pavia, Italy; the [3]Regional Department of Epidemiology, Cagliari, Italy; the [4]Pediatrics Department, S. Martino Hospital, Oristano, Italy; and the [5]Pharmaceutical Service, Oristano, Italy.

Address correspondence to Dr. Onorato Frongia, Osservatorio Epidemiologico ASL 5, Via Carducci 35, 09170 Oristano, Italy. E-mail: onoratof@tin.it.

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Serum Nonesterified Fatty Acids Are Increased in Nonobese Japanese Type 2 Diabetic Patients With Microalbuminuria

The major clinical consequence of type 2 diabetes is mortality and morbidity from atherosclerotic vascular disease. The degree of atherosclerosis can be evaluated by high-resolution B-mode ultrasound scan (1). Using high-resolution B-mode ultrasound scan, we recently demonstrated that serum nonesterified fatty acids (NEFAs) are independently associated with carotid atherosclerosis in nonobese nonhypertensive Japanese type 2 diabetic patients (2). Michaud et al. (3) recently reported that fatty acids enhance lipoprotein lipase (LPL) production in human macrophages. LPL secreted from macrophages is known to contribute to the development and progression of atherosclerosis (4). Thus, fatty acids are considered to be associated with atherosclerotic vascular disease in type 2 diabetic patients.

Slightly increased urinary albumin excretion rate, microalbuminuria, is considered as an index of diabetic nephropathy. Microalbuminuria has also been identified as an independent risk factor for atherosclerosis in type 2 diabetic patients. An association between microalbuminuria and cardiac disease in type 2 diabetic patients has been demonstrated (5). To the best of our knowledge, however, it remains to be clarified whether type 2 diabetic patients with microalbuminuria have higher concentrations of serum NEFAs than those with normoalbuminuria. In this context, a major problem is that microalbuminuria has been associated with atherosclerotic risk factors, such as high blood pressure, hypertriglyceridemia, and low HDL cholesterol, thus complicating the relation of microalbuminuria with serum NEFAs (6,7). Moreover, it is well recognized that being overweight or hyperglycemic per se affects microalbuminuria and serum NEFA levels in humans. We therefore recruited nonobese type 2 diabetic patients with microalbuminuria who were carefully matched for BMI, blood pressure, serum lipid level, and fasting glucose to those with normoalbuminuria. This is the first description that serum NEFAs are increased in nonobese Japanese type 2 diabetic patients with microalbuminuria.

A total of 21 diabetic patients with microalbuminuria (15 men and 6 women) and 45 patients with normoalbuminuria (33 men and 12 women) were enrolled in the study. They all were nonobese (BMI <27 kg/m^2) Japanese type 2 diabetic patients (8). Type 2 diabetes was diagnosed based on the criteria of the World Health Organization (9). All subjects had ingested at least 150 g carbohydrate for the 3 days preceding the study. A total of 14 (67%) patients with microalbuminuria and 23 (51%) patients with normoalbuminuria were taking sulfonylureas. The rest were treated with diet alone. None had received insulin therapy. Ten (48%) patients with microalbuminuria and 15 (33%) patients with normoalbuminuria were treated with antihypertensive drugs. Four (19%) patients with microalbuminuria and seven (16%) patients with normoalbuminuria were treated with lipid-lowering agents. There was no significant difference in sex and medication status between the patients with microalbuminuria and those with normoalbuminuria. They did not consume alcohol or perform heavy exercise for at least 1 week before the study.

Blood was drawn on the morning after a 12-h fast. Plasma glucose was measured with the glucose oxidase method. Total, HDL, and LDL cholesterol, triglycerides, remnant-like particle cholesterol (RLP-C), and NEFAs were also measured. The LDL cholesterol level was calculated using the Friedewald formula (10). RLP-C level was measured by the method reported by Nakajima et al. (11). Serum NEFAs were measured in duplicate using the enzymatic method (NEFA HR kit; Wako Chemicals, Osaka, Japan), and the mean of the two values was used (2). The coefficient of variation for NEFAs was 2%.

Urinary albumin concentration was

assessed in a morning spot urine sample using the single radial immunodiffusion method (12). Urinary albumin concentration was measured in duplicate, and the mean of the two values was used for the study. Intra- and interassay coefficients of variations were <6% (Alb-Tia Seiken; Denka-Seiken, Tokyo, Japan). Several reports have indicated that early morning spot urine is usually sufficient for detecting the presence of microalbuminuria (13,14). In the present study, we calculated urinary albumin excretion rate as a ratio of urinary albumin and urinary creatinine, a ratio that markedly enhances the accuracy of the single spot urine sample in the assessment of microalbuminuria (15). Microalbumiuria was defined as a urinary albumin concentration >30 but <300 mg/g creatinine. Normoalbuminuria was defined as urinary albumin concentration <30 mg/g creatinine.

The statistical analysis was performed with the StatView 5.0 system (Statview, Berkeley, CA). The differences of mean were determined by Student's *t* test. Data were expressed as the means ± SEM.

The clinical characteristics and profile between the patients with microalbuminuria ($n = 21$) and normoalbuminuria ($n = 45$) were compared. Urinary albumin concentrations in the patients with microalbuminuria and normoalbuminuria were 89 ± 12 mg/g creatinine (range 44–236) and 5 ± 1 (range 0.1–17.0), respectively. No significant difference was observed in age (64.2 ± 2.2 vs. 61.8 ± 1.0 years, $P = 0.127$), BMI (22.7 ± 0.4 vs. 22.8 ± 0.3 kg/m^2, $P = 0.481$), or systolic (128 ± 2 vs. 126 ± 2 mmHg, $P = 0.288$) and diastolic (73 ± 2 vs. 73 ± 1 mmHg, $P = 0.448$) blood pressure between the two groups. Fasting glucose (154 ± 7 vs. 143 ± 5 mg/dl, $P = 0.109$) and HbA$_{1c}$ (7.5 ± 0.2 vs. 7.0 ± 0.2%, $P = 0.066$) levels were higher in patients with microalbuminuria than in those with normoalbuminuria, but the difference was not statistically significant. The patients with microalbuminuria had higher triglycerides (127 ± 13 vs. 110 ± 5 mg/dl, $P = 0.066$) and higher RLP-C (5.3 ± 0.5 vs. 4.9 ± 0.2 mg/dl, $P = 0.208$) concentrations than those with normoalbuminuria, but the difference was not statistically significant. There was no significant difference in serum total (200 ± 7 vs. 195 ± 5 mg/dl, $P = 0.290$), HDL (51 ± 3 vs. 50 ± 2 mg/dl, $P = 0.279$), and LDL cholesterol (124 ± 4 vs. 124 ± 6 mg/dl, $P =$ 0.478) levels between the two groups. In contrast, serum NEFAs were significantly higher in patients with microalbuminuria (0.64 ± 0.05 mEq/l) than in those with normoalbuminuria (0.51 ± 0.02 mEq/l, $P = 0.002$).

In the present study, we first confirmed the presence of elevated NEFAs in nonobese Japanese type 2 diabetic patients with microalbuminuria. Microalbuminuria has been shown to be not only an indicator of incipient nephropathy, but also a risk marker for early mortality, especially in type 2 diabetes, due to cardiovascular diseases.

Although the mechanisms underlying the relation between NEFAs and microalbuminuria are unclear, serum NEFAs seem to be associated with early atherosclerotic changes in nonobese Japanese type 2 diabetic patients. Using high-resolution B-mode ultrasound scan, we very recently demonstrated that serum NEFAs are independently associated with carotid atherosclerosis in nonobese, non-hypertensive, well-controlled (mean HbA$_{1c}$ 7.0%), unique type 2 diabetic patients (2). Interestingly, the degree of carotid stenosis was 8.1 ± 2.1%, suggesting that serum NEFAs are associated with the very early stages of carotid atherosclerosis. Microalbuminuria is also considered to be a risk marker for the early events in athrosclerosis. Thus, it may be hypothesized that serum NEFA level is reflective of the early stage of atherosclerosis in nonobese Japanese type 2 diabetic patients.

The mechanism by which serum NEFAs are associated with atherosclerosis in nonobese Japanese type 2 diabetic patients is unclear. In this respect, the recent report of Michaud et al. (3) that fatty acids enhance macrophage LPL production is very interesting. LPL secreted from macrophage is known to contribute to the development and progression of atherosclerosis (4). Alternatively, serum NEFAs might cause atherosclerosis by stimulating a coagulation cascade sequence. Didisheim et al. (16) previously showed that saturated long-chain fatty acids activate Hageman factor (factor XII). Hageman factor initiates the cascade sequence of enzymatic reactions, culminating in the production of thrombin and the conversion of fibrinogen to fibrin. Thrombin also induces an increase in fibrinogen biosynthesis. Pilgeram and Pickart (17) have shown that free fatty acids (FFAs) stimulate the rate of biosynthesis of fibrinogen in vitro. Schneider et al. (18) demonstrated that FFAs had synergistic effects on the insulin-stimulated increase in plasminogen activator inhibitor-1 (PAI-1) in the blood of type 2 diabetic patients. Enhanced coagulation cascade sequence in conjunction with decreased fibrinolytic capacity due to overexpression of PAI-1 might explain the reason why serum NEFA level is associated with the early stage of atherosclerosis and microalbuminuria in nonobese Japanese type 2 diabetic patients.

In summary, we first demonstrated that serum NEFAs are increased in nonobese Japanese type 2 diabetic patients with microalbuminuria. Further study should be undertaken to clarify whether serum NEFAs are reflective of the early stages of atherosclerosis, including microalbuminuria, in nonobese Japanese type 2 diabetic patients.

ATARU TANIGUCHI, MD[1]
MITSUO FUKUSHIMA, MD[3]
MASAHIKO SAKAI, MD[1]
KENICHI HAMA[2]
KENJI SAKAGUCHI[2]
NAOFUMI NEZUMI, MD[2]
HIROYUKI KISHIMOTO, MD[1]
TOSHIKI WATANABE, MD[1]
KAZUNARI MATSUMOTO, MD[4]
SHOICHIRO NAGASAKA, MD[5]
KUMPEI TOKUYAMA, PHD[6]
YOSHIKATSU NAKAI, MD[7]

From the [1]Division of Diabetes, Kansai-Denryoku Hospital, Osaka, Japan; the [2]Department of Biochemistry, Kansai-Denryoku Hospital, Osaka, Japan; the [3]Department of Metabolism and Clinical Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan; the [4]Department of Internal Medicine, Sasebo Chuou Hospital, Nagasaki, Japan; the [5]Division of Endocrinology and Metabolism, Jichi Medical School, Tochigi, Japan; the [6]Laboratory of Biochemistry of Exercise and Nutrition, Tsukuba University, Tsukuba, Japan; and the [7]College of Medical Technology, Kyoto University, Kyoto, Japan

Address correspondence to Ataru Taniguchi, MD, First Department of Internal Medicine, Kansai-Denryoku Hospital, 2-1-7 Fukushima, Fukushima-ku, Osaka-city, Osaka 553-0003 Japan. E-mail: K-58403@kepco.co.jp.

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Insulin Therapy Does Not Itself Induce Weight Gain in Patients With Type 2 Diabetes

Of obese individuals who lose weight by dieting, >90% eventually return to their original weight; one- to two-thirds of the lost weight is regained within 1 year, and almost all is regained within 5 years. This suggests that body weight is physiologically controlled and that weight changes, in either direction, elicit a potent counter response that resists the change (1). This should also hold true when weight loss is attributable to high blood glucose levels and/or insulin deficiency. Insulin is often first prescribed to type 2 diabetic patients after a period of poor metabolic control by oral agents; this period may be accompanied by weight loss due to insulin deficiency and/or the poor metabolic control itself. We postulated that the weight gain observed during insulin therapy in patients with type 2 diabetes may simply correspond to re-expression of their physiologically controlled body weight. We tested the hypothesis that insulin-treated type 2 diabetic patients might return to the previous maximal weight they reached before the onset of diabetes- and insulin deficiency–induced weight loss.

We conducted a retrospective cohort study of 58 patients with type 2 diabetes who required insulin because of poor metabolic control, despite dietary measures and maximal-dose oral agents (e.g., glibenclamide 15 mg/day and metformin 1,700 mg/day). Insulin-treated type 2 diabetic patients were included in the study if they met the following criteria: *1*) diagnosis of diabetes after 30 years of age (such patients were excluded if they had a family history suggestive of maturity-onset diabetes of the young, known islet autoimmunity, or diabetes secondary to endocrine or chronic pancreatic disease), *2*) an interval of at least 1 year between diagnosis and insulin therapy, *3*) maximal previous lifetime weight mentioned in the file, *4*) at least 2 years of follow-up after the beginning of insulin therapy, *5*) no use of oral antidiabetic drugs after insulin introduction, and *6*) no cancer or other progressive chronic disease and a serum creatinine level <150 μmol/l.

As HbA_{1c} levels increased during follow-up, the best metabolic control obtained with insulin was expressed as the lowest HbA_{1c} value recorded during follow-up (at 6 months or 1, 2, or 3 years after insulin introduction). In the same way, the maximal daily insulin dose was the highest value recorded at 6 months or 1, 2, or 3 years.

There were 25 women and 33 men. Age at diagnosis was 52 ± 9 years and age at insulin introduction was 65 ± 9 years. BMI at diagnosis was 29.3 ± 5.6 kg/m², and the HbA_{1c} level at insulin introduction was 10.9 ± 1.8% (median 10.6%). A total of 11, 42, and 5 patients required 1, 2, and 3 daily insulin injections, respectively, at the end of follow-up. Follow-up after insulin introduction was 2 years in 10 patients and 3 years in 48 patients.

The HbA_{1c} level fell from 10.9 ± 1.8% at insulin introduction to 8.2 ± 1.8% at 6 months. It then increased at 1 year and subsequently stabilized (8.9 ± 2.1, 8.9 ± 1.5, and 8.3 ± 1.8% at 1, 2, and 3 years, respectively). The minimal HbA_{1c} level during follow-up was 7.5 ± 1.2%. The initial daily insulin dose was 0.6 ± 0.3 units · kg^{-1} · day^{-1}, and requirements remained stable during follow-up (0.5 ± 0.2, 0.5 ± 0.2, 0.5 ± 0.2, and 0.6 ± 0.2 units · kg^{-1} · day^{-1} at 6 months and 1, 2, and 3 years, respectively). The maximal daily insulin dose reached during follow-up was 0.7 ± 0.3 units · kg^{-1} · day^{-1}.
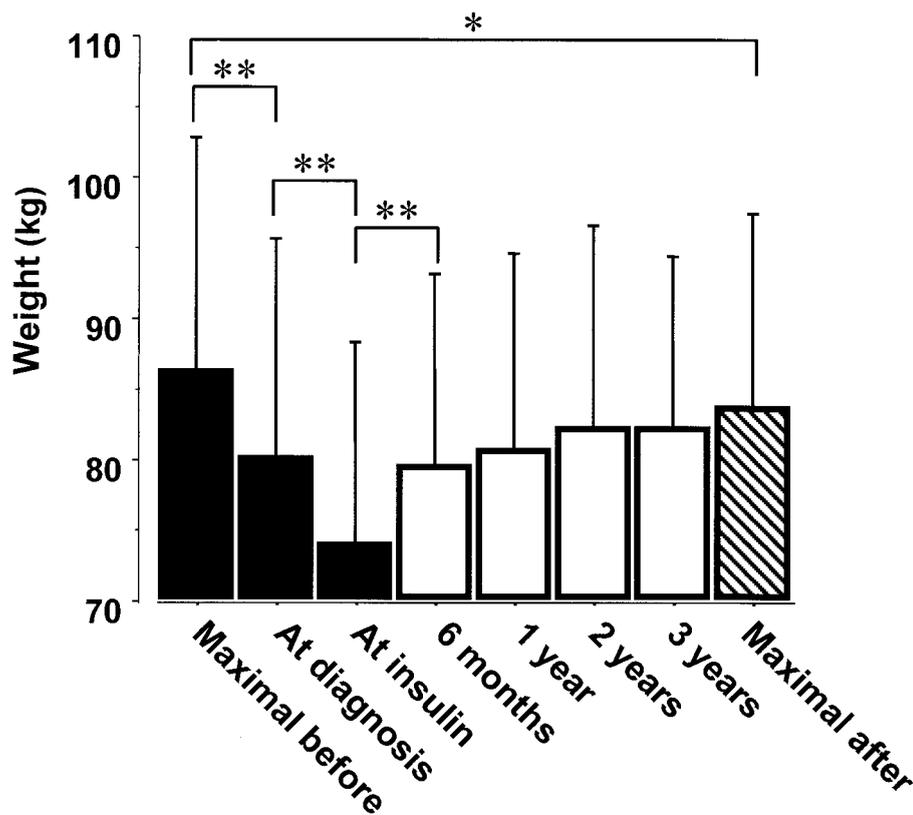
In 71% of patients, weight at diagno-

Figure 1—*Weight changes in 58 insulin-treated type 2 diabetic patients.* ■*, Weight before insulin therapy;* □*, weight during insulin therapy;* ▨*, maximal weight reached during insulin therapy.* *P = 0.01; **P < 10^{-4}.

betes Program (6) were unusual because the patients treated with insulin did not gain weight. In that study, the patients in the placebo group lost weight.

Our results need to be confirmed in a prospective study of early insulin introduction, i.e., before onset of weight loss, in patients with type 2 diabetes. Unfortunately, such a study may prove difficult, as half the weight loss in our population occurred before the diagnosis of diabetes. Our results have important practical implications, as they suggest that maximal previous weight may be predictive of the degree of subsequent weight gain.

ETIENNE LARGER, MD
PIERRE RUFAT, MD
DANIELE DUBOIS-LAFORGUE, MD
SEVERINE LEDOUX, MD

From the Service de Diabétologie-Endocrinologie, Hôpital Bichat, Paris, France.

Address correspondence to Dr. Etienne Larger, Service de Diabétologie-Endocrinologie, Hôpital Bichat, 46 rue H. Huchard, F-75877 Paris cedex 18, France. E-mail: etienne.larger@college-de-france.fr.

sis was below the maximal previous weight. Mean weight at diagnosis (80.0 ± 16.6 kg) was 6.1 ± 6.8 kg below the previous maximal weight (86.0 ± 17.0 kg) ($t = -6.8, P < 10^{-4}$). All of the patients lost weight between diagnosis and insulin introduction. Weight at insulin introduction (73.8 ± 13.5 kg) was 6.2 ± 9.7 kg below weight at diagnosis ($t = -4.8, P = 10^{-4}$) (Fig. 1).

Patients gained 8.0 ± 5.3 kg during the first 2 years of insulin therapy ($t = 11.1, P < 10^{-6}$) as follows: 5.0 ± 4.6 kg during the first 6 months, 1.3 ± 3.1 kg between 6 months and 1 year, and 1.3 ± 3.0 kg during the second year. Weight stabilized during the third year ($+0.15 \pm 3.1$ kg, $t = 0.3, P = 0.74$). Maximal weight during insulin therapy remained 2.6 ± 7.8 kg below maximal weight before insulin therapy ($t = 2.6, P = 0.012$) (Fig. 1) but was higher than weight at diagnosis ($t = -2.94, P = 0.005$). Maximal weight during insulin therapy correlated strongly with maximal weight before insulin: maximal weight during insulin =

$0.96 \times$ maximal weight before (95% CI $0.94–0.98, t = 13, P < 10^{-6}$, adjusted R^2 99.2%). Weight gain correlated with the maximal daily insulin dose (Student's t test = 2.86, $P = 0.03$, adjusted R^2 = 6.6%) but not with metabolic control in terms of the minimal HbA$_{1c}$ level (Student's t test = 1.62, $P > 0.10$, adjusted $R^2 = 4.4\%$).

In this cohort, weight loss had already started at the time of diagnosis and continued until insulin was introduced. The weight patients reached after the introduction of insulin was highly correlated with their maximal weight before diabetes. Onset of weight loss before the diagnosis of diabetes has rarely been observed, even in obese type 2 diabetic patients and even though type 2 diabetes may remain undiagnosed for as long as 9–12 years (2). Long-term studies of insulin-treated patients with type 2 diabetes suggest that the weight such patients reach is asymptotic, and that most weight gain occurs during the first 3 years (3–5). The results of the University Group Dia-

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