

## OBSERVATIONS

## Acceptability of Pig Xenografts by Patients With Type 1 Diabetes and the General Population

During xenografts, the barrier between species would be transgressed, raising the issue of the “social” acceptability of xenografts, particularly those from pigs (1). A few studies, mainly carried out in the U.S. and Australia, have considered this aspect relative to the general population (2,3), nurses (4), and patients awaiting renal or heart allografts (5). No study of this type has been published in Europe, and no effort has been made to compare acceptability by patients who may receive xenografts with that of the general population. Type 1 diabetes is potentially curable through the use of a graft of pig islets, which has been performed in preliminary fashion (6). Our group is involved in the study of xenografts of pig islets (7–11), and we developed a survey on the acceptability and main reasons for reluctance to receive xenografts in 377 adult type 1 diabetic patients according to World Health Organization criteria (169 men, 208 women; age  $47.2 \pm 0.8$  years; duration of diabetes  $18.2 \pm 0.6$  years) and to determine whether acceptability and reasons for reluctance differed from 697 adult subjects (298 men, 399 women; age  $42.0 \pm 0.6$  years) representative of the French general population according to data from the National Institute of Economic and Statistical Information. We are grateful to Michel Marre (Diabetology Department, Angers Hospital, Angers, France) and the Association Française des Diabétiques for recruiting the patients. Individuals were assured of confidentiality and were requested to give their voluntary consent. All solicited subjects answered the oral questionnaire. The oral questionnaire, consisting of 12 items approved by the ethics committee, aimed to assess the acceptability of the general concept of a graft, acceptability of a xenograft, evaluation of the different animal species considered as potential donors, and specific evaluation of the pig. The questions were asked without comment from the investigator and in the absence of a diabetologist. The replies were recorded in the order of

the questions without any possibility of reconsidering a previous reply. Most questions had five possible answers (“yes,” “probably,” “no,” “probably not,” or “no opinion”), allowing quantification of the percentage of individuals accepting (“yes” or “probably”) or refusing (“no” or “probably not”) the proposal and comparison of percentages in the general population and diabetic patients using  $\chi^2$  analysis. Questions about the rating of animal species preferred for xenografts consisted of replies scored from 0 to 5, which provided a mean score  $\pm$  SEM in the population studied, allowing comparison of scores in the general population and diabetic patients with use of the Student’s *t* test. Most of the participants (97%) in our survey were Catholic. Those from other religions were too few (Muslims, 1.9%; Jews, 1.0%) to allow analysis of differences in replies relative to religion (2).

In the general population, 96% of subjects were willing to accept an organ graft before any allusion was made to xenografts. Of these individuals, 54% would accept an animal graft (Fig. 1A). Among subjects who had initially expressed reluctance to accept xenografts, the main reasons for reluctance to accept a xenograft were compared with those for reluctance to accept a graft in general. The percentage of individuals who expressed reluctance because of the risk of disease transmission was very similar for a xenograft (23.5%) and a graft in general (23.0%). However, more subjects considered risks “not yet identified” more dissuasive for a xenograft than for a graft in general (31.4 vs. 12.0%, respectively;  $P < 0.001$ ). The subjective notion of “living with an organ from another being” was more frequently mentioned for a xenograft than for a graft in general (24.3 vs. 9.9%, respectively;  $P < 0.0001$ ). Figure 1B indicates what subjects among the general population willing to accept xenografts said when asked, “If you were to receive an organ graft, rate the donor species by order of preference.” After human, the monkey, pig, dog, and cow were ranked from most to least desirable. Two other independent complementary questions were intended to clarify the evaluation of the pig as potential donor. Figure 1C shows that 34% of subjects specified the pig in replying to the question, “Would you refuse a graft from any of the species?” Finally, 84% of subjects willing to accept an animal graft replied favorably to the question, “Are you willing to accept a graft from a pig?”

The percentage of diabetic patients willing to accept an organ graft (90%) was not statistically different from that for the general population. Of these patients, 64% were favorable to receiving a xenograft (Fig. 1A), which was significantly higher than the percentage in the general population ( $P < 0.001$ ). Among those subjects who had initially expressed reluctance to accept xenografts, the percentage of diabetic patients who directly expressed reluctance because of the risk of disease transmission by an organ graft was higher for a xenograft than for a graft in general (30 vs. 23%, respectively;  $P < 0.03$ ). The risk of disease transmission by a xenograft was thus more frequently recognized by diabetic subjects than by the general population ( $P < 0.02$ ), whereas both populations reacted similarly to the risk of disease transmission by a graft in general. Risks “not yet identified” were considered by more patients for a xenograft than for a graft in general (34.0 vs. 23.8%, respectively;  $P < 0.002$ ). The objection to “living with the organ of another being” was less frequent ( $P < 0.01$ ) for a xenograft among diabetic patients (17.5%) than the general population (24.3%). This notion was more frequently regarded by diabetic patients as a reason for reluctance to accept a xenograft than a graft in general (17.5 vs. 8.6%, respectively;  $P < 0.0001$ ). Diabetic patients ranked the pig in second position as donor species (Fig. 1B)—before the monkey—so that the mean rank of classification for the pig was significantly higher than in the general population ( $3.34 \pm 0.12$  vs.  $2.69 \pm 0.07$ ,  $P < 0.0001$ ). Consistently, in replying to the question, “Would you refuse a graft from any of the species?” (Fig. 1C), diabetic patients were less insistent than the general population in refusing a pig graft (21 vs. 34%, respectively;  $P < 0.0001$ ). When people were asked, “What sort of reluctance would you have to accept a pig graft?” the notion that the pig is “too different from man” was less often indicated by diabetic patients than by the general population (17.9 vs. 29.7%, respectively;  $P < 0.002$ ). The absence of reluctance to accept a pig graft was more frequently cited by diabetic patients ( $P < 0.002$ ). No correlation was found between the duration of diabetes or the presence of complications to diabetes and acceptability of a xenograft in general or from a pig in particular. The percentage of individuals indicating that they had already heard about the possibility of xenografts did not differ significantly for diabetic patients

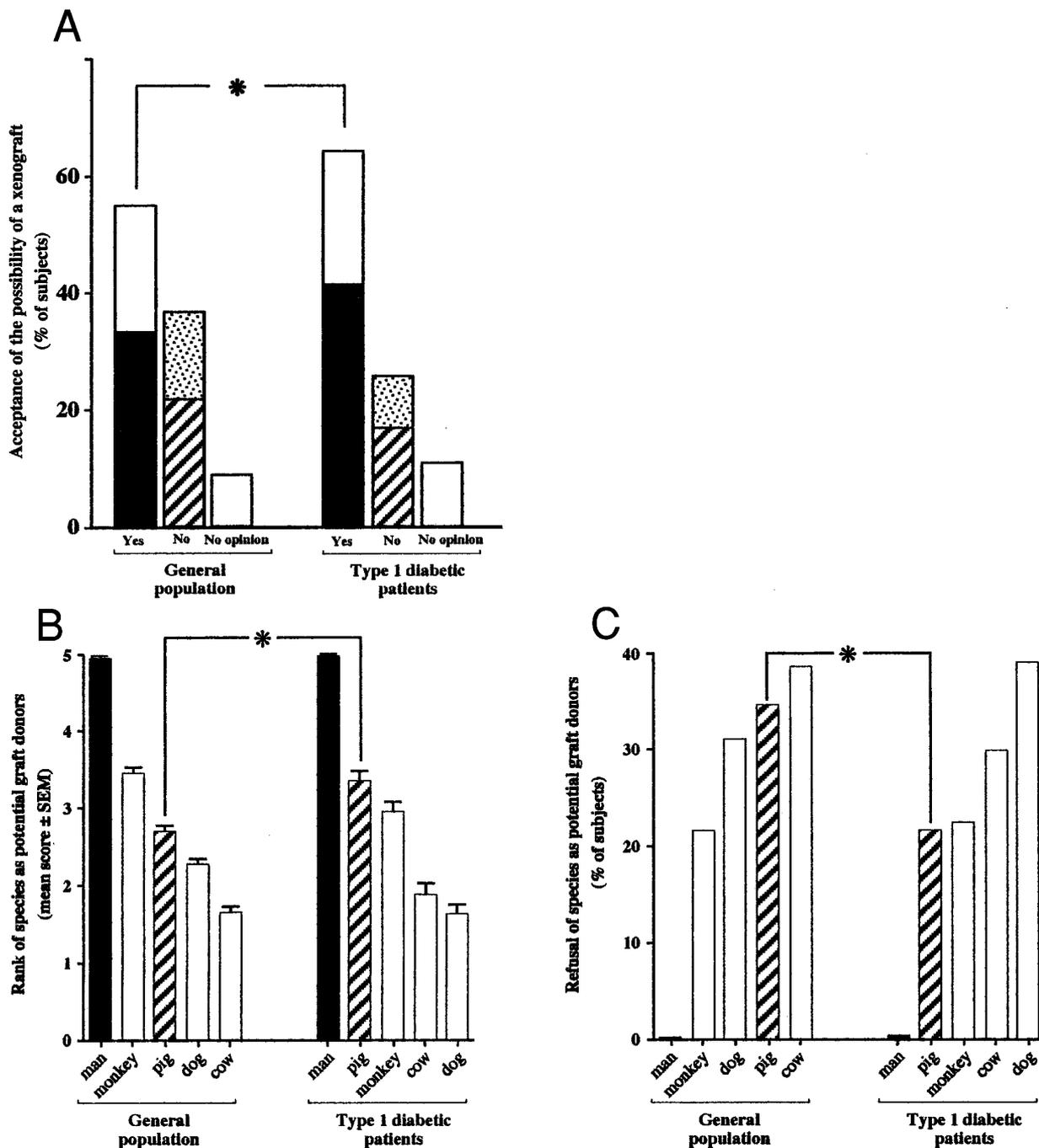


Figure 1—A: Replies of 697 individuals from the general population and 377 type 1 diabetic patients to the question, “Would you be willing to accept a graft from an animal?” Results indicate the percentages of subjects accepting (“yes” or “probably”) or refusing (“no” or “probably not”) the possibility of receiving a xenograft, or undecided (“no opinion”). \* $P < 0.001$ , acceptability greater among diabetic patients. B: Replies to the question, “If you were to receive an organ graft, rate the donor species by order of preference.” Results represent the means  $\pm$  SEM of individual acceptability scores. \* $P < 0.0001$ , with higher pig acceptability for diabetic patients. C: Replies to the question, “Would you refuse a graft from any of the species?” Results are the percentages of individuals who refuse each species. \* $P < 0.0001$ , with lower refusal of pig by diabetic patients.

(67%) and the general population (63%) and was higher for those willing to accept than refuse an animal graft ( $P < 0.01$ ). Diabetic patients and the general population were similarly convinced (63 vs. 69%,

respectively) that additional information might change their replies.

In conclusion, the possibility of receiving xenografts of pig tissues is rather well accepted by the general French population.

The data of our study also indicate that the information currently available to the public is limited, and that more information might increase xenograft acceptability. The level of acceptability in our survey is lower

than that reported (66%) in a previous study (4) that comprised selected subjects (health professionals). A study comprising a less narrowly defined population (2) found results (51%) comparable to ours. Among the reasons for reluctance in the general population, the risk of disease transmission to a recipient was not considered to be higher for a xenograft than for a graft in general, whereas the risk of transmitting an infectious agent from the donor animal to the human recipient is the major objection among the scientific community. However, risks "not yet identified" (which, for some individuals, may have included unidentified infectious risks) were more frequently cited for a xenograft than for a graft in general. This fear of unidentified risks related to the use of products of animal origin could have been recently accentuated by the possible transmission to man of the prions responsible for mad cow disease. Among the animal species proposed as potential graft donors, the general population ranked the monkey just after man. However, for ethical and sanitary reasons, scientific consensus excludes the monkey as a donor, preferring the pig. In the general population, the pig was ranked after the monkey as a potential donor. The acceptability of pig grafts is thus satisfactory, considering the lack of information on this subject and the negative image of the pig in the human subconscious.

It is noteworthy that diabetic patients were more receptive to the possibility of a xenograft than the general population was. However, diabetic patients were more aware than the general population of the specific risks inherent in a xenograft. They more frequently considered risks of disease transmission or risks "not yet identified" in the case of a xenograft than of a graft in general. Thus, the existence of a chronic disease makes type 1 diabetic patients more willing to overcome their reluctance, even with respect to sanitary and unidentified risks, and accept a xenograft. This may be explained, at least in part, by a higher familiarity and acceptance of medical procedures in general by diabetic patients. It is also noteworthy that diabetic patients were more favorable to the possibility of receiving a pig xenograft. Among the species proposed as potential donors, the pig was more often chosen by diabetic patients than by the general population. Consistently, diabetic patients indicated less frequently that they would refuse an organ from a pig. Diabetic patients were thus also

more willing than the general population to overcome their reluctance in relation to a pig graft. They may have been more accustomed to the idea of using products of porcine origin for medical purposes because of their familiarity with pig insulin. However, patients with a longer history of diabetes or complications were not more "desperate" to receive a xenograft in general or a pig graft in particular than those with a short history of diabetes or no complications. It would seem advisable to consider the opinions of the patients who may receive xenografts. However, the decision to perform a xenograft, particularly of pig islets for type 1 diabetes (for which the benefit/risk ratio is not particularly favorable), should not be decided simply on the basis of the expectations of possible recipients, even though they are more aware than the general population (but certainly not more aware than the scientific community) of the sanitary risks relating to a xenograft. A serious effort is needed to ensure international coordination in assessing the infectious risks associated with xenografts.

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## Can Enterovirus Infections Explain the Increasing Incidence of Type 1 Diabetes?

An increasing incidence of type 1 diabetes has been reported from many countries, but the reason for this is not known. In Finland, the incidence is now twice as high as it was 30 years ago, and it is currently the highest in the world (1,2). We introduce here a new hypothesis implying that this increase could be caused by changing epidemiology of enterovirus infections, one of the major candidates for environmental triggers of  $\beta$ -cell damage (3-8). We tested our hypothesis by comparing the temporal trends in the incidence of type 1 diabetes in Finland with those observed in the frequency of enterovirus infections in the background population.

Incidence data for type 1 diabetes were obtained from the central drug register of Social Insurance Institution as previously described (1). The incidence of enterovirus meningitis was analyzed in

children aged 0–14 years living in the hospital district of the Tampere University Hospital. Incidence cases were identified from the hospital case records according to the following criteria: typical symptoms of aseptic meningitis (headache, fever, nausea, meningeal signs, or anorexia as well as irritability in infants) together with  $\geq 10$  leukocytes/mm<sup>3</sup> of cerebrospinal fluid. Cases with confirmed bacterial or other nonenteroviral infections (like parotitis) were excluded. These criteria were based on traditional clinical and laboratory techniques, which have not changed during the study period (like bacterial and viral cultures). The rate of enterovirus exposure in the background population was evaluated by measuring IgG class antibodies against two enterovirus antigens (purified Coxsackievirus B4 and a synthetic peptide) from serum samples taken from pregnant women during one day in July in the years 1983, 1989, and 1995 as a part of the national infectious screening program as previously described (6). The number of women was 232, 240, and 243, and their mean age ( $\pm$  SD) was  $27 \pm 5.5$ ,  $28 \pm 5.1$ , and  $29 \pm 5.6$  years, respectively.

The incidence of type 1 diabetes increased linearly during the study period from 21.9 per 100,000 in 1966–1969 to 43.0 in 1995–1997 ( $p$  for trend  $P < 0.001$ ). This increase was evident in all age-groups, and it was strongest in the youngest children (from 11.7 to 29.7 in children 0–4 years old) as described earlier (2).

An opposite trend was observed in the incidence of enterovirus meningitis. It decreased linearly from 16.8 in 1980–1984 to 12.0 in 1985–1989 and 8.7 in 1990–1994 ( $P < 0.001$ ). However, in the very young infants (<6 months old), whose protection against infections is based on maternal transplacentally acquired antibodies, no decrease was found. In fact, their proportion increased from 12% (8 of 65) in 1980–1984 to 29% (10 of 34) in 1990–1994 ( $P < 0.05$ ). The seasonal distribution of cases (peak between July and November) and the predominance of boys (male-to-female ratio 1:8) were both typical for enterovirus meningitis.

The same kind of decrease was found in enterovirus antibody levels in pregnant women ( $P < 0.001$  in analysis of variance). The proportion of women lacking antibodies to purified Coxsackievirus B4 increased from 6% in 1983 to 10% in

1989 and 17% in 1995 ( $P < 0.001$ ) and that lacking enterovirus peptide antibodies increased from 13 to 28 and 42%, respectively ( $P < 0.001$ ).

The results suggest that the frequency of enterovirus infections has rapidly decreased in the Finnish population. We propose that this epidemiological change could explain the increasing incidence of type 1 diabetes. The reason is that an increasing proportion of infants and fetuses is infected when they lack protective maternal antibodies and are therefore prone to complications like meningitis and  $\beta$ -cell damage. This could also explain the observations indicating higher incidence of type 1 diabetes in rural than in urban areas as low population density is associated with low transmission rate of enteroviruses. This was found also in the present study, since the frequency of seronegative mothers in rural areas (20 inhabitants per km<sup>2</sup> or less) was 29% (61 of 213) compared with 21% (106 of 502) in suburban or urban areas ( $P < 0.05$ ).

Interestingly, the epidemiology of paralytic poliomyelitis, a well-known enterovirus disease, has followed the same kind of epidemiological pattern. Poliovirus infections used to be endemic, but their transmission (fecal-oral route) decreased rapidly toward the end of the 19th century as the standard of living improved. At the same time, the incidence of paralytic complications paradoxically increased. The basis for this phenomenon was a delay in the age of the initial infection; some children were not exposed to polioviruses until later in childhood when they were protected no more by maternal antibodies and thus were more severely affected by the infection (9).

Accordingly, we propose that there is an analogy between the epidemiology of poliomyelitis and type 1 diabetes. In both instances, a dramatic decrease in the frequency of enterovirus infections has led to a new epidemiological situation favorable for the frequent occurrence of complications, such as poliomyelitis or  $\beta$ -cell autoimmunity. This may have happened by two mechanisms which do not exclude each other: 1) decreased antibody levels in pregnant mothers can no longer protect the fetus or newborn; and 2) infants acquire their first enterovirus infections at an older age when protective maternal antibodies have already disappeared. This, in turn, would lead to the observed increase in the incidence of type 1 dia-

betes, which is particularly acute in the youngest age groups. This hypothesis is testable and should be evaluated in other countries than Finland.

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## The Insulin-Plus-Sugar Method for Controlling Recently Diagnosed Type 2 Diabetes Without Hypoglycemia

The blood glucose test accurately defines the glycemic level. In recently diagnosed type 2 diabetes, the 2-h postmeal urine glucose (2hug) and the bedtime urine glucose (btug) tests, when negative or 0.1% for glucose, indicate the possibility of hypoglycemia developing before the next meal or during the night, respectively, while the concurrent blood glucose values may still be hyperglycemic or normoglycemic (1).

The insulin-plus-sugar method for treatment of recently diagnosed type 2 diabetes uses the 2hug test to determine the need for both sugar supplementation and insulin dose adjustment and the concurrent blood glucose values to determine the amount of such adjustments. The premeal blood and urine glucose values are not used in insulin dose adjustments and may serve as guides in the sugar dose adjustment.

Insulin is administered below estimated requirements as NPH and regular before breakfast, regular before lunch, and NPH and regular before supper. Other insulins can be substituted. Subsequently, each insulin dose is increased daily until one negative or 0.1% 2hug value mani-

fest, which usually occurs within 1 week. This development may also occur while the concurrent blood glucose level is still hyperglycemic. The negative or 0.1% 2hug value signals that a maximal insulin requirement will be reached within 1-3 days and that insulin dose adjustments should be lessened.

A negative or 0.1% 2hug value occurs at a time when both the glucose-raising component of the meal is already used and the previous premeal dose of regular insulin is near its peak level of activity. Thus, a negative or 0.1% 2hug value calls for the following two concurrent actions, even when in the presence of concurrent hyperglycemia: 1) oral sugar, which is substituted for all snacks, is administered, usually in doses of 8 g for a negative test and 4 g for a 0.1% test; and 2) the insulin doses that cover that particular test period are reduced, usually by 1-2 U, on the next day. The combination of the two actions prevents hypoglycemia and iatrogenic hyperinsulinemia; yet, these changes are too small to cause hyperglycemia (2). In early type 2 diabetes, negative or 0.1% 2hug and btug values will continue, predictably, on succeeding days, and the development of normoglycemia will ensue. Thus, daily insulin reductions will continue until a zero insulin requirement is reached within 2 months.

The patients are allowed their usual diet except for a reduction in refined sugars and total and saturated fats. Weight loss through reduced caloric intake and increased exercise is encouraged throughout the treatment period, but weight loss is most emphatically encouraged after a zero insulin requirement is reached. These actions cannot, by themselves and at the zero insulin stage, cause either hypoglycemia or hyperglycemia, although they can increase insulin reserve capacity (3,4).

### Case reports

**Case 1.** A 57-year-old African-American woman was hospitalized for diabetes 6 months before the initiation of treatment with the insulin-plus-sugar method. After the patient was discharged from the hospital, her insulin requirements were 23 U of NPH insulin before breakfast and 10 U of NPH insulin before dinner. She was also taking an ACE inhibitor and a diuretic for the previous 2 years for hypertension. Her weight was 230 lbs.; her height was 5'4½"; her BMI was 41.4; and her blood pressure was 150 over 90. She was able to read four lines without glasses and nine

lines with glasses on the Snellen's eye chart. The insulin schedule of the patient was changed to 8 U of NPH insulin and 7 U of regular insulin before breakfast, 4 U of regular insulin before lunch, and 7 U of NPH insulin and 7 U of regular insulin before dinner for the same total of 33 U of insulin per day. When the 2hug or btug value was negative, 8 g of sugar was prescribed; when the 2hug or btug value was 0.1%, 4 g of sugar was prescribed. Her urine tests and premeal and 2-h postmeal levels became consistently negative, which necessitated glucose supplementation and daily reductions in her insulin dosage. At 3 weeks, she was down to a zero insulin requirement. Her blood pressure was 110 over 70 without the diuretic, and she could read all 11 lines on the Snellen's eye chart. Her blood glucose levels ranged from 85 to 123 mg/dl.

**Case 2.** A 42-year-old African-American man had a body weight of 203 lbs., a height of 5'10", and a BMI of 30. He was highly symptomatic for the previous 2 weeks before treatment with the insulin-plus-sugar method. His blood glucose level was 400 mg/dl. His eyesight was blurry, and he could not see well enough to drive. He was started on 32 U of regular insulin and NPH insulin in three divided doses, with sugar supplementation for negative or 0.1% 2hug and btug values. All of his urine tests quickly turned negative, and his insulin doses were reduced daily, despite fasting blood sugars that ranged from 124 to 211 mg/dl during the first week of treatment. At 2 weeks, he was down to a zero insulin requirement. His spot blood sugar was 85 mg/dl, his eyesight was 20/20, and his weight was 202 lbs. Over the course of the next year, he lost 12 lbs. in weight and his spot blood glucose values ranged from 79 to 94 mg/dl.

**Case 3.** A 25-year-old morbidly obese Caucasian man weighed 500 lbs. His height was 5'7" and his BMI was 78.5. He was very symptomatic for the 3 weeks before the initiation of treatment with the insulin-plus-sugar method. He had heavy ketonuria, and he refused hospitalization. On day 1 of treatment, he was started on 120 U of regular insulin and NPH insulin in three divided doses. He reached a peak insulin requirement of 247 U on the 11th day. After 45 days, he reached a zero insulin requirement. After 1 year, he underwent open heart surgery for a congenital abnormality and still did not require any medication for his diabetes. The initial increases in the insulin dosage were 5 U per dose and were

1 U per dose on the 11th day. Similarly, the initial dose reductions were 5 U per dose, and they were gradually brought to 1 U per dose. His initial doses of sugar supplements were 32 g per dose. These were gradually reduced to zero when he was taking 2 U of insulin per dose, because hypoglycemia does not occur in early type 2 diabetes at individual doses of 2–3 U.

The insulin-plus-sugar method, when used to control hyperglycemia while avoiding hypoglycemia, attains glycemic control and a zero insulin requirement in recently diagnosed type 2 diabetes.

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## Increased Spontaneous Adherence of Neutrophils From Type 2 Diabetic Patients With Overt Proteinuria

Possible role of the progression of diabetic nephropathy

It has been reported that diabetic patients show various neutrophil dysfunctions. Increased susceptibility to bacterial infections observed in diabetic patients may be attributed to impaired phagocytosis

or bactericidal activity, whereas increased release of oxygen radicals, such as superoxide anion ( $O_2^-$ ), might damage endothelial cells and accelerate the progression of diabetic microangiopathy (1–3). In fact, it has been reported that neutrophils from diabetic patients with retinopathy release a greater amount of  $O_2^-$  in response to phorbol myristate acetate (PMA) than do neutrophils from diabetic patients without retinopathy (4). Here we report that the magnitude of spontaneous adherence of neutrophils from diabetic patients with overt proteinuria is significantly greater than that of neutrophils from diabetic patients with normoalbuminuria as well as healthy control subjects. In addition, we found that these neutrophils responded normally to granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor (TNF)- $\alpha$  with increased adherence and  $O_2^-$  release. Normal  $O_2^-$ -releasing capacity of neutrophils in response to proinflammatory cytokines (GM-CSF and TNF- $\alpha$ ) and increased spontaneous adherence might play a role in the progression of diabetic nephropathy.

A total of 36 type 2 diabetic patients and 14 age-matched healthy control subjects were studied. The mean age of the patients was 47.1 years (range: 22–68 years), and the mean duration of diabetes was 11.8 years (range: 1 month to 25

years). Patients were classified into four groups according to the degree of nephropathy: 1) normoalbuminuria (<20 mg/day, n = 17); 2) microalbuminuria (20–200 mg/day, n = 6); 3) macroalbuminuria (200–1,000 mg/day, n = 5); and 4) overt proteinuria (>1 g/day, n = 8). Patients were also classified into three groups according to the degree of diabetic retinopathy: 1) no retinopathy (n = 13), 2) simple retinopathy (n = 8), and 3) proliferative retinopathy (n = 15). The release of  $O_2^-$  was assayed by superoxide dismutase (SOD)-inhibitable reduction of ferricytochrome C (5). Neutrophil suspension ( $3 \times 10^5$  cells) in Hanks' balanced salt solution was added to each well of a 48-well plate (Falcon #3078) containing 100  $\mu$ mol/l ferricytochrome C with or without SOD (200 U/ml), and the reaction mixture was incubated for 3 h at 37°C. When required, 50 ng/ml granulocyte colony-stimulating factor (G-CSF), 5 ng/ml GM-CSF, or 100 U/ml TNF- $\alpha$  was added to the wells precoated with fetal calf serum (FCS). Using the same plate, neutrophil adherence was assayed by measuring the protein content (Lowry's method) of adherent neutrophils in each well after washing each well thoroughly and was expressed as the percentage of applied cells.

Neutrophils from healthy control subjects adhered to the plastic surface (21.90  $\pm$  9.76% of applied cells) and released  $O_2^-$

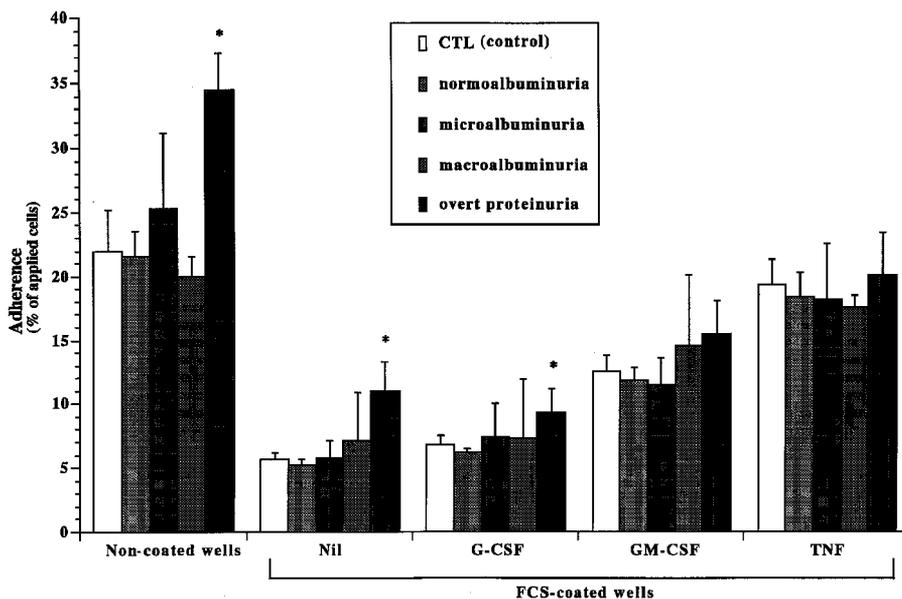


Figure 1—Spontaneous and cytokine-induced adherence of neutrophils from control subjects (n = 14) and diabetic patients (n = 36). Data are means  $\pm$  SEM. \*P < 0.05, significantly increased compared with the values obtained from normal control subjects.

( $6.16 \pm 0.79 \text{ nmol} \cdot 3 \times 10^5 \text{ cells}^{-1} \cdot 3 \text{ h}^{-1}$ ) when placed in untreated wells of the plate. Both responses were markedly inhibited ( $P < 0.01$ ) when the wells were coated with FCS (adherence:  $5.62 \pm 0.58\%$  of applied cells;  $\text{O}_2^-$  release:  $0.97 \pm 0.17 \text{ nmol} \cdot 3 \times 10^5 \text{ cells}^{-1} \cdot 3 \text{ h}^{-1}$ ). These findings indicate that the release of  $\text{O}_2^-$  from neutrophils placed in untreated wells reflects adherence-induced  $\text{O}_2^-$  release, and that spontaneous release of  $\text{O}_2^-$  and adherence may be assessed by placing cells in FCS-coated wells. Stimulus-induced  $\text{O}_2^-$  release and adherence could be assessed by incubating cells in the presence of G-CSF, GM-CSF or TNF- $\alpha$  in FCS-coated wells. Neutrophils from healthy control subjects released  $\text{O}_2^-$  in response to GM-CSF or TNF- $\alpha$  but not G-CSF ( $0.94 \pm 0.56$ ,  $3.19 \pm 1.40$ , and  $6.15 \pm 1.03 \text{ nmol} \cdot 3 \times 10^5 \text{ cells}^{-1} \cdot 3 \text{ h}^{-1}$  for G-CSF, GM-CSF, and TNF- $\alpha$ , respectively). The amounts of spontaneous, adherence-induced, or stimulus-induced  $\text{O}_2^-$  release in neutrophils from diabetic patients were essentially identical to that in neutrophils from healthy control subjects. These findings indicate that neutrophil functions from diabetic patients respond normally to proinflammatory cytokines such as GM-CSF and TNF- $\alpha$ . In contrast to  $\text{O}_2^-$  release, the magnitude of neutrophil adherence to the plastic surface was significantly greater in patients with overt proteinuria than in patients with normoalbuminuria ( $P < 0.01$ ) or in healthy control subjects ( $P < 0.01$ ) (Fig. 1). The magnitude of neutrophil adherence to FCS-coated surface was also significantly greater in patients with overt proteinuria than in patients with normoalbuminuria ( $P < 0.05$ ) or in healthy control subjects ( $P < 0.05$ ). However, GM-CSF- or TNF- $\alpha$ -induced neutrophil adherence to the FCS-coated surface in patients with overt proteinuria was essentially identical to that in patients with normoalbuminuria or in healthy control subjects. Moreover, no significant difference in neutrophil  $\text{O}_2^-$  release and adherence was observed between each retinopathy group and healthy control subjects.

Multiple regression analysis revealed the close relationship between increased spontaneous adherence of neutrophils and the level of serum creatinine ( $r = 0.738$ ,  $P = 0.001$ ), but not disease duration, gender, BMI, age, and the level of HbA<sub>1c</sub>. There are some conflicting reports concerning adherence of neutrophils in diabetic patients. Wierusz-Wysocka et al. (6) reported that in

diabetic patients, neutrophil adherence to a human albumin-coated petri dish was enhanced in the presence of autologous plasma. Andersen et al. (7) reported that in diabetic patients, both basal and PMA-induced adherence of neutrophils to bovine aortic endothelial cells were impaired. These differences may be attributed to the difference of the assay method and the heterogeneity of patients.

In conclusion, the present experiments show that spontaneous adherence of neutrophils to the plastic surface and FCS-coated surface are enhanced in patients with overt proteinuria and that enhanced adherence is closely associated with the level of serum creatinine. These findings and the unimpaired  $\text{O}_2^-$ -releasing response to proinflammatory cytokines suggest that enhanced neutrophil adherence in these diabetic patients may play a role in further progression of diabetic nephropathy as well as microangiopathy.

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## Erythrocyte Sodium/Hydrogen Exchange Activity and Albuminuria in Type 1 Diabetic Families

Enhanced Na/Li countertransport (NLCT) and Na/H exchange (NHE) have been suggested to predict diabetic nephropathy (1-3). Nevertheless, other studies have excluded this hypothesis (4). We found NHE activity increased in type 1 diabetes (5), yet we could not detect any relationship between NHE and urinary albumin excretion rate (UAER) either in essential hypertension (6) or in type 1 diabetes. Therefore, we measured erythrocyte NHE activity and UAER in healthy nondiabetic family members of type 1 diabetic patients with or without diabetic nephropathy.

We recruited 20 siblings and 18 parents of 18 type 1 diabetic patients with nephropathy, 22 siblings and 20 parents of 18 type 1 diabetic patients with normoalbuminuria, and 60 healthy subjects without a family history of type 1 diabetes. Venous blood and 24-h urine collections were sampled to assay glucose, creatinine, and albumin. Erythrocyte NHE activity, HbA<sub>1c</sub>, and other biochemical measurements were determined as described previously (5,6).

The patients with and without nephropathy were of similar age ( $35 \pm 11$ ,  $33 \pm 8$ ,  $35 \pm 10$  years, respectively) but had higher BMI ( $24 \pm 2$ ,  $24 \pm 2$ ,  $23 \pm 3 \text{ kg/m}^2$ ;  $P < 0.05$ ) than healthy control subjects. The two groups of patients with or without nephropathy had similar durations of dia-

betes ( $20 \pm 6$  and  $19 \pm 7$  years, respectively) and daily insulin requirements ( $0.7 \pm 0.1$  and  $0.7 \pm 0.1$  U/kg, respectively). Patients with nephropathy had poorer metabolic control than patients with normoalbuminuria (glycemia, geometric mean,  $16.4$  vs.  $9.9$  mmol/L,  $P < 0.001$ , both values vs. control subjects'  $4.9$  mmol/L,  $P < 0.001$ ; HbA<sub>1c</sub>  $8.9 \pm 1.5$  vs.  $7.8 \pm 1.2\%$ ,  $P < 0.05$ , both values vs. control subjects'  $5.3 \pm 0.4\%$ ,  $P < 0.001$ ). In addition, they had higher systolic blood pressure than control subjects ( $130 \pm 23$  in nephropathy vs.  $117 \pm 6$  mmHg in control subjects,  $P < 0.05$ ;  $121 \pm 17$  mmHg in normoalbuminuria). NHE activity was significantly elevated in both groups of patients as compared with that in the control group; there was no difference between the two diabetic groups (Tables 1–3). Siblings of diabetic patients were similar to the control group with respect to age, sex, BMI, blood pressure, blood glucose, HbA<sub>1c</sub>, and serum creatinine. NHE activity was significantly higher in both groups of siblings as compared with

that in control subjects, but no difference was detectable between siblings of diabetic probands with nephropathy and siblings of normoalbuminuric patients. Siblings of diabetic patients with nephropathy had higher

UAER than control subjects and siblings of normoalbuminuric probands. The two groups of parents differed from the well matched control subjects only with respect to erythrocyte NHE activity, which was elevated in both groups. Parents of diabetic patients with nephropathy had higher UAER than healthy control subjects and parents of diabetic probands with normoalbuminuria. Simple regression analysis showed a significant correlation ( $R 0.4$ ,  $P < 0.001$ ) between antiport activities of diabetic probands and their relatives, both parents and siblings. In all groups, the velocity of NHE was weakly associated with diastolic pressure ( $R 0.2$ ,  $P < 0.05$ , Fig. 1).

Our findings, as well as the confirmation that NHE activity is increased in type 1 diabetic patients, show for the first time that enhanced NHE activity is also a common feature of nondiabetic first-degree relatives of patients with type 1 diabetes. We found no difference in the NHE activity among the two groups of diabetic patients with and without nephropathy as we found no difference among the corresponding groups of relatives. Unlike NHE, abnormal albumin excretion was a distinctive feature of nondiabetic first-degree relatives of diabetic patients with nephropathy. Thus, NHE activity is significantly higher in nondiabetic family members of type 1 diabetic probands, but it is of no use as a genetic marker for the development of diabetic nephropathy in type 1 diabetes. The consistency of association between antiport activities of diabetic probands and their relatives excludes an effect of the diabetic state in itself and suggests that the altered activity of the proton exchanger is deter-

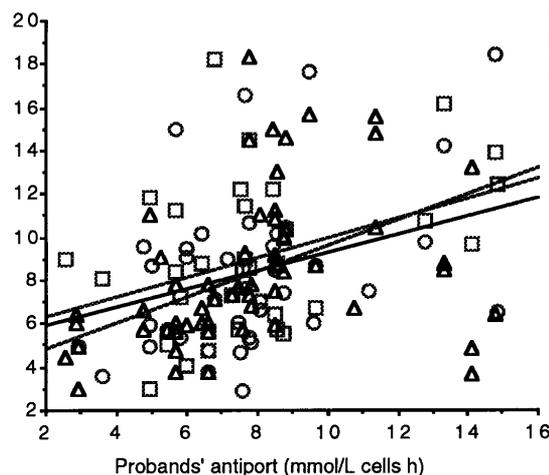


Figure 1—Relationship between sodium-hydrogen exchange activities of type 1 diabetic probands and their relatives. ○, Mothers' antiport; □, fathers' antiport; △, siblings' antiport.

Table 1—Clinical data of type 1 diabetic patients and healthy control subjects

Clinical characteristic	Control subjects	Type 1 diabetic patients	
		With nephropathy	Without nephropathy
NHE ( $\mu\text{mol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ )	$5.66 \pm 2.16$	$7.70 \pm 2.69^*$	$8.13 \pm 2.41^\dagger$
UAER (mg/24 h)	12.5	$100.7^\ddagger$	9.7§

Data are means  $\pm$  SD for NHE and geometric means for UAER. \* $P < 0.05$  vs. control subjects,  $^\dagger P < 0.01$  vs. control subjects,  $^\ddagger P < 0.001$  vs. control subjects,  $^\S P < 0.001$  vs. probands with nephropathy (or their relatives).

Table 2—Clinical data of siblings of type 1 diabetic patients both with and without nephropathy, and healthy control subjects

Clinical characteristic	Control subjects	Siblings of type 1 diabetic patients	
		Patient with nephropathy	Patient without nephropathy
NHE ( $\mu\text{mol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ )	$5.46 \pm 2.12$	$8.89 \pm 3.68^*$	$9.14 \pm 3.67^*$
UAER (mg/24 h)	10.8	$23.3^*$	$8.3^\dagger$

Data are means  $\pm$  SD for NHE and geometric means for UAER. \* $P < 0.001$  vs. control subjects,  $^\dagger P < 0.001$  vs. probands with nephropathy (or their relatives).

Table 3—Clinical data of parents of type 1 diabetic patients both with and without nephropathy, and healthy control subjects

Clinical characteristic	Control subjects	Parents of type 1 diabetic patients	
		Patient with nephropathy	Patient without nephropathy
NHE ( $\mu\text{mol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ )	$5.87 \pm 1.59$	$8.11 \pm 3.22^*$	$8.78 \pm 2.46^\dagger$
UAER (mg/24 h)	10.4	$26.5^*$	$10.6^\ddagger$

Data are means  $\pm$  SD for NHE and geometric means for UAER. \* $P < 0.01$  vs. control subjects,  $^\dagger P < 0.001$  vs. control subjects,  $^\ddagger P < 0.01$  vs. probands with nephropathy (or their relatives).

mined primarily by familial factors. Whether familial abnormality of erythrocyte NHE can be explained by familial aggregation of environmental or genetic factors remains to be elucidated.

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## Factors Influencing Plasma Homocysteine Levels in Type 2 Diabetes

Mild hyperhomocysteinemia is detected in 6-8% of the general population and appears to be a stronger cardiovascular risk factor in type 2 diabetic subjects than in those with normal

or impaired glucose tolerance (1). Few data are available concerning factors affecting plasma homocysteine concentration (Hcy) in diabetic patients; even less is known about the link between Hcy and microvascular complications of diabetes with the exception of nephropathy (2). To assess whether Hcy is an independent risk factor for vascular disease in diabetic patients, we evaluated the effect of clinical characteristics, such as metabolic control and duration of the disease, on Hcy in type 2 diabetic patients with normal renal function. We recruited 318 type 2 diabetic patients with a broad range of duration of disease (0-41 years) for the study. None of them was taking medications potentially influencing Hcy. Fasting plasma glucose, HbA<sub>1c</sub>, serum lipids (total and HDL cholesterol and triglycerides), blood urea nitrogen, creatinine, vitamin B<sub>12</sub>, and folate were determined in all patients. Plasma Hcy concentrations were determined by high-performance liquid chromatography and fluorescence detection (3). The albumin excretion rate (AER) was measured by radioimmunoassay technique in two consecutive 24-h urine collections. People with AER >20 µg/min were excluded from the study. Estimated creatinine clearance was calculated by the Cockcroft-Gault formula (4). An electrocardiogram (ECG) was recorded during the same day of the blood sampling. Data were analyzed with the Statistical Analysis System program (SAS Institute, Cary, NC). Differences between groups were evaluated by use of the Student's t test or Kruskal-Wallis test. Spearman's rank correlation and multiple regression analysis, as well as logistic regression analysis, were used to determine relationships between variables of interest.

In diabetic patients, Hcy levels (12.3 ± 1.8 µmol/l, mean ± SD) were significantly higher in comparison with those in nondiabetic subjects matched for age, sex, and prevalence of hypertension (10.8 ± 1.2 µmol/l, *t* = 8.069, *P* < 0.001). All patients had plasma B<sub>12</sub> and folate concentrations within the normal range. By Spearman's correlation analysis, Hcy was strongly related to fasting plasma glucose, HbA<sub>1c</sub>, and duration of disease (all *P* < 0.001, Table 1). Hcy was also related to systolic blood pressure and plasma triglycerides, although to a lower level of statistical significance (Table 1). In a multiple regression analysis, Hcy ended up being independently related to HbA<sub>1c</sub>, duration of disease, and systolic blood

pressure (*R*<sup>2</sup> for the entire model: 0.44, *P* < 0.005).

To define the role of plasma Hcy as a cardiovascular risk factor in our population, we divided the patients into two subgroups according to the presence or absence of coronary heart disease (CHD), identifying 82 subjects with a history of myocardial infarction, coronary artery bypass grafting, or Minnesota codes 1-1 or 1-2 on the ECG. Mean Hcy was higher in patients with CHD than in patients without CHD (13.3 ± 0.9 vs. 12.0 ± 1.3 µmol/l, *P* < 0.001). By logistic regression analysis, plasma Hcy emerged as the strongest independent variable related to CHD. Each µmol/l of increment of plasma Hcy concentration was associated to an odds ratio of 1.455 (95% CI 1.142-1.855, *P* = 0.002). Smoking status and presence of hypertension were independently associated to the presence of CHD as well (*P* = 0.03 and 0.02, respectively).

Our data show that in type 2 diabetes, Hcy may be affected by both metabolic control and duration of disease; however, it remains strongly associated to the presence of CHD, independently from age, sex, BMI, smoking status, hypertension, and lipid pattern. We therefore conclude that Hcy can be seen as a major cardiovascular risk factor in type 2 diabetic patients with CHD.

There are conflicting reports in the literature concerning the impact of type 2 diabetes on Hcy. Some authors have described higher Hcy in type 2 diabetic patients with macrovascular disease with respect to control subjects (5), whereas in an article by Hoogeveen et al. (1), Hcy appears to be a stronger (1.6-fold) risk factor for cardiovascular disease in patients with type 2 diabetes than in subjects with normal or impaired glucose tolerance, but without any correlation with the degree of metabolic control or the duration of disease. Our observations do not allow us to conclude that type 2 diabetes is always characterized by hyperhomocysteinemia, but they suggest that Hcy might be one of the factors underlying the link between hyperglycemia and cardiovascular risk in diabetic patients. The strong relationship between Hcy and HbA<sub>1c</sub> may well support a role for day-long hyperglycemia in affecting Hcy, although increased levels of Hcy are likely to represent a further independent cardiovascular risk for type 2 diabetic patients. This role of Hcy is well documented in our study, where it stands as a variable associated to CHD as strong as smoking or hypertension.

Table 1—Relationship between Hcy levels and other variables in 318 type 2 diabetic patients

Variable	Spearman's coefficient	P
Age	0.094	0.094
BMI	0.012	0.832
Blood pressure		
Systolic	0.154	0.007
Diastolic	0.045	0.433
Cholesterol		
Total	0.074	0.190
LDL	0.047	0.404
HDL	-0.040	0.491
Triglycerides	0.166	0.003
Folate	0.085	0.139
Vitamin B <sub>12</sub>	0.029	0.618
Fasting plasma glucose	0.401	<0.001
HbA <sub>1c</sub>	0.627	<0.001
Duration of diabetes	0.511	<0.001

The mechanisms by which hyperhomocysteinemia may promote atherosclerosis in diabetic patients are poorly understood. Both conditions are characterized by a number of similar abnormalities in hemostasis, platelet aggregation, and fibrinolysis. More recently, an alteration of endothelial function after increased Hcy has been reported in humans. With respect to this last mechanism, our observation that Hcy is a strong independent variable related to the presence of CHD in normoalbuminuric type 2 diabetic patients becomes of interest. Impaired kidney function is a well-recognized cause of hyperhomocysteinemia, and glomerular filtration rate has been recently identified as rate limiting for renal clearance of Hcy (6). In the present study, patients were carefully selected, and only subjects with type 2 diabetes, normal AER, and normal creatinine clearance were included. If we accept microalbuminuria as a marker for widespread endothelial damage (7), our results in normoalbuminuric patients may suggest that Hcy could be a useful index of impaired endothelial function, even earlier than altered albumin excretion rate.

In conclusion, the present study suggests that in type 2 diabetes, plasma homocysteine levels are influenced by both the duration of disease and metabolic control; nevertheless, mild hyperhomocysteinemia still emerges as a major risk factor for CHD, in agreement with previous observations. The reduction of Hcy should be therefore attempted in type 2 diabetic patients. If improvement of meta-

bolic control is a sufficient measure to do so, however, remains to be investigated.

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## Acute Rhabdomyolysis Associated With Troglitazone

Although troglitazone is effective in reducing hyperglycemia, several reports on the side effects of hepatic dysfunction have raised concerns (1). We report here, for the first time, the appearance of acute rhabdomyolysis as a side effect of troglitazone.

A 59-year-old man who had been on a medication regimen of glibenclamide (7.5 mg/day) for type 2 diabetes with complications (diabetic retinopathy and neuropathy) since 1992 was admitted to our hospital in July 1999 because of malaise, marked weakness, and muscle aches throughout his body. Although mild alcoholic liver injury was detected (serum alanine transferase [ALT] 55 IU/l [upper normal limit: 40 IU/l]), his HbA<sub>1c</sub> level was 9.0%. Troglitazone (400 mg/day) was prescribed in addition to sulfonylureas for 6 months before his admission to our hospital. During physical examination, all muscle groups were extremely tender. Laboratory examinations revealed marked increases in the patient's level of creatinine phosphokinase (CK) (10,570 IU/l vs. upper normal limit of 150 IU/l), serum myoglobin, aldolase, and aspartate aminotransferase (AST) levels. The CK isozyme pattern was consistent with skeletal muscle origin. Serum creatinine level and elec-



a previous study (15), and was expressed as the M index (glucose disposal rate during the final 40 min of the euglycemic insulin clamp; glucose utilization [ $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ]). The euglycemic-hyperinsulinemic clamp was performed also during the follicular phase of the menstrual cycle in a control group of 10 healthy premenopausal women aged  $32 \pm 4$  years with a BMI of  $24 \pm 3.2$ . In each study subject, before and after treatment, levels of plasma total cholesterol, triglycerides (by use of enzymatic methods [Roche Diagnostics, Milan, Italy]), and HDL cholesterol (after plasma precipitation with dextran-magnesium) also were measured. LDL cholesterol was indirectly estimated by means of the Friedewald formula (16).

In the control group of premenopausal women, the M index was  $8.6 \pm 1.2$ , which was significantly higher than that in group 1 (estrogen only) ( $M 6.2 \pm 1.5$ ,  $P < 0.001$ ) and in group 2 (estrogen and progestin) ( $M 6.4 \pm 2.1$ ,  $P < 0.001$ ); however, the difference between these two groups was not significant. The M index did not correlate with age or with amenorrhea duration in the overall study group. At the end of the treatment period, the M index in both groups was not modified ( $6.7 \pm 1.5$  and  $6.9 \pm 2$ , respectively). In group 1, the total and LDL cholesterol levels significantly ( $P < 0.0001$ ) decreased (from  $6.03 \pm 0.36$  to  $5.01 \pm 0.36$  and from  $4.07 \pm 0.47$  to  $2.63 \pm 0.34$  mmol/l, respectively), and the HDL cholesterol levels increased (from  $1.42 \pm 0.26$  to  $1.67 \pm 0.2$  mmol/l,  $P < 0.05$ ). In addition, levels of triglycerides increased (from  $2.79 \pm 0.92$  to  $3.57 \pm 0.76$  mmol/l), but this increase was not significant. In group 2, no differences were observed before and after treatment in levels of total cholesterol ( $6.42 \pm 0.93$  and  $6.3 \pm 0.72$  mmol/l), LDL cholesterol ( $4.49 \pm 1.08$  and  $4.33 \pm 0.87$  mmol/l), HDL cholesterol ( $1.39 \pm 0.34$  and  $1.47 \pm 0.33$  mmol/l), and triglycerides ( $2.71 \pm 1$  and  $2.46 \pm 0.78$  mmol/l). No significant modifications in BMI, glucose tolerance during an OGTT, and systolic and diastolic blood pressure were observed during the study period.

It has been previously demonstrated that oral estrogen replacement therapy with estradiol-17 $\beta$  or with low-dose conjugated equine estrogens improves insulin sensitivity, whereas no effects have been observed when high-dose equine estrogens or a potent alkylated estrogen, such as ethinyl-estradiol, are used (11). In regard to transdermic estradiol, a previous study has demonstrated that it is able to modify

insulin metabolism but does not affect insulin sensitivity (12). In all of these studies, however, insulin sensitivity was calculated by means of mathematical models or was indirectly estimated from fasting glucose and insulin levels; no data on direct measurements of insulin sensitivity with the reference method (i.e., the euglycemic-hyperinsulinemic clamp) are available. By using this reference method, we can confirm that transdermal estradiol is unable to improve insulin sensitivity in healthy postmenopausal subjects, even after prolonged administration of transdermal estradiol. This discrepancy between administration of transdermal and oral estradiol can be explained by the lower estrogen plasma levels reached by treatment with the former, with respect to the oral route. These levels are probably not efficient enough to modify insulin resistance, but they are still able to positively affect plasma cholesterol profiles. It is well established that estrogens, regardless of the type or route of administration (9,10), lower total cholesterol levels; this effect is maintained with long-term treatment (17). Our results confirm the findings of these previous reports and those reports that demonstrate an increase in HDL cholesterol levels with administration of oral and transdermal estradiol (18). In regard to triglycerides, there are conflicting reports of increased levels during oral treatment and reduced levels with transdermal estradiol treatment (18). Our results show a modest and insignificant increase of triglyceride levels during estradiol treatment. Consequently, these findings do not allow us to draw any conclusion concerning this parameter. Depending on their dose or their androgenicity, progestogens have different effects on lipids and lipoproteins. Androgenic progestogens, such as norgestrel, reverse the HDL-raising effect of estrogen and can reduce VLDL secretion and thereby lower triglyceride levels (18). Our results demonstrate that a pregnant progestogen, such as medroxyprogesterone, when used in combination with transdermal estradiol, shows similar effects, such as a blunted estradiol-induced lipid modification.

In conclusion, our data do not support the possibilities that long-term treatment with transdermal estradiol has beneficial effects on insulin resistance and that it can contribute to the reduction of cardiovascular risk observed during replacement therapy after menopause. On the contrary, they give further evidence of a

positive effect, which is lost when medroxyprogesterone is added, on plasma cholesterol levels. This last finding suggests that better hormone replacement regimens are needed to combine the most favorable changes in cardiovascular risk factors with the lowest risk of side effects.

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## A Severe Clinical Phenotype Results From the Co-inheritance of Type 2 Susceptibility Genes and a Hepatocyte Nuclear Factor-1 $\alpha$ Mutation

**M**aturity-onset diabetes of the young (MODY) is a monogenic autosomal dominant form of diabetes. To date, five different genes are

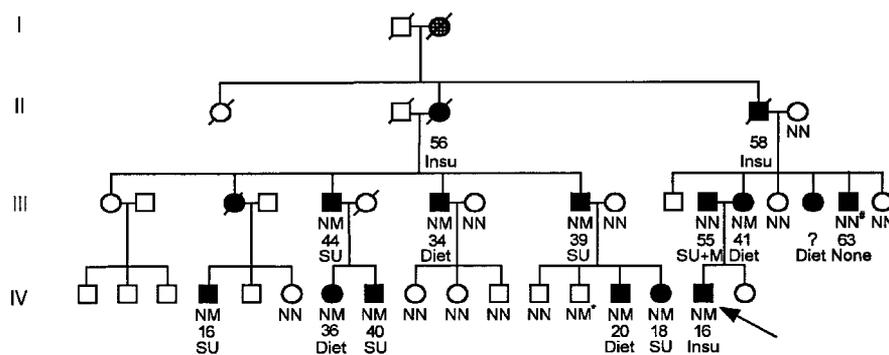


Figure 1—Family tree of a MODY3-affected family. ●, Female with diabetes; ○, female with no diabetes known; ■, male with diabetes; □, male with no diabetes known. Arrow indicates index patient, details about whom are in the text. NN and NM indicate MODY3 alleles: N = normal, M = mutation. Age of onset of diabetes and treatment are indicated. \*This patient inherited the mutation, but diabetes has not yet been diagnosed. #This patient was diagnosed with diabetes but does not carry the mutation. His phenotype is different from that of the rest of the family.

known to cause MODY; hepatocyte nuclear factor (HNF)-1 $\alpha$  mutations that cause MODY3 are the most prevalent in Western Europe (1,2). The majority of patients with HNF-1 $\alpha$  mutations present with mild symptoms and are recognized as having type 2 diabetes. A number of reports have found HNF-1 $\alpha$  mutations in patients who had previously been classified as having type 1 diabetes (3–6). In most of these cases, it appears that the subjects misclassified as having type 1 diabetes had a phenotype similar to that of the subjects diagnosed with MODY. We propose that in some patients, genes predisposing to type 2 diabetes increase the severity of the phenotype resulting from an HNF-1 $\alpha$  mutation. This results in early diagnosis and more severe hyperglycemia, so the subject is more likely to be classified as having type 1 diabetes. O’Rahilly et al. (7) proposed that early-onset type 2 diabetes could be caused by a “double gene dose” from two affected parents (diabetes or impaired glucose tolerance [IGT]), leading to a more severe clinical course with a rapid transfer to insulin treatment and a high prevalence of microvascular complications. The co-inheritance of an HNF-1 $\alpha$  mutation with predisposing type 2 diabetes genes is likely to occur when one parent has diabetes as a result of an HNF-1 $\alpha$  mutation and the other has non-MODY type 2 diabetes or IGT. Here we report on a case that illustrates this potential mechanism.

We have recently studied a family with MODY3 resulting from a nonsense mutation at position 171 (R171X) (8)

where there was striking heterogeneity in the clinical phenotype associated with the HNF-1 $\alpha$  mutation (Fig. 1). One family member (IV-16) was diagnosed with type 1 diabetes at 16 years of age because of severe hyperglycemia and classical symptoms for a few weeks, and he was treated immediately with insulin. This clinical presentation was in marked contrast to the other nine mutation carriers, one of whom (aged 42 years) was not diabetic, and most mutation carriers with diabetes were diagnosed over the age of 25 years (n = 6) and easily treated with sulfonylurea derivatives (n = 5) or diet alone (n = 4) (Fig. 1). In IV-16, HbA<sub>1c</sub> was 12.8%, compared with a mean HbA<sub>1c</sub> level of 6.8  $\pm$  1.2% for the other family members who carried the mutation. In IV-16, severe diabetic complications endured over time: proliferative retinopathy treated with panretinal laser coagulation, severe peripheral neuropathy with recurrent foot ulcers, autonomic neuropathy with orthostatic hypotension, impotence and gustatory sweating, and diabetic nephropathy. However, none of his other family members had manifest diabetic complications. Evidence that this patient was not insulin dependent was provided by the patient’s admission that he would omit insulin for periods up to a week without developing ketoacidosis and by a maximum C-peptide concentration of 0.30 nmol/l after intravenous glucagon stimulation. GAD antibodies were not detected.

Both of IV-16’s parents had diabetes (Fig. 1). His mother also had the same R171X mutation and had mild uncom-

plicated diabetes, diagnosed at age 41, for 20 years treated with diet only. His father, being unrelated to IV-16's mother, did not carry the mutation, but 13 years after his son's diagnosis, he was diagnosed with "classical" type 2 diabetes, age of onset 55 years. He was overweight and treated with a combination of glibenclamide and metformin. He had no diabetes complications 6 years after diagnosis.

We propose that the severe phenotype in patient IV-16 may be explained by co-inheritance of the type 2 susceptibility gene or genes from his father in addition to the inheritance of an HNF-1 $\alpha$  mutation. This is a further example of the "double gene dose" proposed by O'Rahilly et al. (7). Because of the high prevalence of type 2 diabetes in the general population, this situation may occur not infrequently in MODY-affected families. This may only become apparent later on, because the parent may present with non-MODY type 2 diabetes many years after the diagnosis of his or her child, as illustrated in our case. Obesity did not modify the phenotype in our subject (BMI = 24.2 vs. 25.4  $\pm$  2.0 kg/m<sup>2</sup> in the other mutation carriers), but in a large Finnish study, nondiabetic HNF-1 $\alpha$  mutation carriers were slimmer than diabetic mutation carriers (9). This result and our case imply that even in HNF-1 $\alpha$  mutations, a monogenic condition with high penetrance, the phenotype will be modified by environmental and genetic factors that are also important in type 2 diabetes. The identification of major predisposing genes in type 2 diabetes will allow the relative role of these modifying factors to be studied in greater detail.

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## Gestational Diabetes Alters the Male Bias for Cesarean Section

There are various maternal and fetal risk factors for a cesarean section. One of the lesser known of these is pregnancy with a male fetus. In 1982, Hall and Carr-Hill (1) reported that for singleton births, the male-to-female ratio for emergency cesarean section after spontaneous labor was 1.20 and after induced labor was 1.47. In 1995, Harlow et al. (2) reported similar findings. For maternal, fetal, and combined indications for section, the male-to-female ratios were 1.33, 1.56, and 1.39, respectively (P < 0.001).

We have recently reported the rate and indications for section in a group of women with gestational diabetes mellitus (GDM). Data were available for 216 women with GDM who had had a section and were compared with data from an equal number of glucose-tolerant control women (3). The numbers of male and female babies delivered with the mean

Table 1—Mean birth weight and gestational age of delivery for male and female babies of 216 women with GDM and 216 glucose-tolerant control women who had a section

	n	Birth weight (g)	Gestational age (weeks)
GDM			
Males	113	3,469 $\pm$ 595*	38.7 $\pm$ 1.53
Females	103	3,233 $\pm$ 500	38.5 $\pm$ 1.40
Control subjects			
Males	137	3,462 $\pm$ 648*	38.9 $\pm$ 1.86
Females	79	3,257 $\pm$ 603	38.7 $\pm$ 1.73

Data are n or means  $\pm$  SD. \*The male babies were significantly heavier than the female babies in both the GDM and control groups (P < 0.01). There were no significant differences between the gestational weeks of delivery. There were no significant differences between the birth weights of male and female babies in the GDM group compared with male and female babies in the control group. With a  $\chi^2$  test, there was a significantly higher proportion of male babies born to control women than to women with GDM (P = 0.0215).

birth weight and gestational week of delivery are shown in Table 1.

Thus, in glucose-tolerant women, we have confirmed the previous observation that a section is more likely if there is a male fetus, with a male-to-female ratio of 1.73. However, this observation does not apply to a group of women with treated GDM, with a ratio of 1.10. Whereas it has been suggested (1,2) that a plausible reason for the increased rate of section could be the higher weight of male fetuses, this is not substantiated from our data. Although male babies were heavier than female babies in both groups, the male babies of women with GDM were the same weight as the male babies of control women. A recent report by Remsberg et al. (4) has also shown that birth weight had little effect on the association with cesarean section for either diabetic or nondiabetic women.

It has been speculated that a higher male ratio for section may come about because the fetal hormone contribution of male fetuses to labor may be less effective and therefore be an exacerbating factor for any uterine dysfunction (1,2). Why this should not be the case for women with GDM is not clear but is undoubtedly worthy of further examination.

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## Diabetic Gastroparesis Improved by Percutaneous Endoscopic Jejunostomy

Diabetic gastroparesis (delayed gastric emptying) is found in ~50% of patients with type 1 and type 2 diabetes (1) and is clinically important, because it may be associated with gastrointestinal symptoms, alterations in glycemic control, and changes in oral drug absorption (2). Both symptomatic and asymptomatic gastroparesis may be associated with poor glycemic control by causing mismatch between the action of insulin (or oral hypoglycemic agents) and the absorption of nutrients. We have studied two patients who presented with extremely difficult management problems with severe diabetic gastroparesis and who have had a significant improvement in lifestyle, diabetic control, and gastric emptying with percutaneous endoscopic jejunostomy (PEJ). The first patient, a 42-year-old woman with type 1 diabetes since 1992, presented in early 1995 with frequent hypoglycemic attacks. No cause could be detected for these frequent hypoglycemic episodes. In mid-1996, she presented with catastrophic hypoglycemia that caused cerebral damage and amnesia. Early in 1999, she presented with recurrent vomiting and an average of two hypoglycemic events per day. She had lost 9 kg in weight in 3 months.

A radioisotope study using 99m Tc-labeled scrambled egg revealed no gastric emptying after 80 min, thus confirming gastroparesis. Despite the use of cisapride, domperidone, metoclopramide, and erythromycin (both alone and in combination), she did not improve. At this stage, she had a PEJ inserted and we managed to keep her glycemic control stable and prevent her from having hypoglycemic events. After 3 months, a repeat radiolabeled gastric-emptying study revealed that she had evidence of 80% gastric emptying at 60 min. A further study 3 months later confirmed the improvement. The second patient, a 38-year-old woman with type 1 diabetes since 1980, had established diabetic nephropathy and proliferative retinopathy. She presented in 1995 with recurrent episodes of

ketoacidosis secondary to severe vomiting. She experienced daily hypoglycemic events. A diagnosis of gastroparesis was made after the absence of 99m Tc-labeled scrambled egg from the stomach at 90 min. High-dose prokinetic therapy was wholly unsuccessful. A PEJ was inserted and it stabilized the patient's condition. After 4 months, radionucleotide imaging showed 85% gastric emptying within 60 min. The PEJ was removed. She presented with further episodes of profuse vomiting 2 years later, and the diagnosis of recurrent gastroparesis was made after radionucleotide imaging. Despite the insertion of another PEJ for 6 months, her symptoms did not improve. She underwent a partial gastrectomy with a Roux-en-Y procedure. She remains very well with a regular dietary intake with marked improvement in her diabetic control.

The treatment of diabetic patients suffering from gastroparesis is far from satisfactory. Conventional management involves nutritional support, antiemetics, prokinetics, and, rarely, surgery. Dietary changes, such as the addition of high fiber and complex carbohydrates, prolong postprandial food absorption. Prokinetic agents, such as cisapride, domperidone, or metoclopramide, may alleviate symptoms, but not cure the problem. These drugs have a high prevalence of central nervous system side effects, such as somnolence, akathisia, or depression (3). Gastric pacing provides some hope in the future management of these patients who might otherwise require surgery for this disorder (4). In the past, surgical jejunostomy has been reported to be successful in patients with diabetic gastroparesis (5). These two cases show that gastroparesis may be reversible with the advent of endoscopic insertion of a jejunostomy feeding tube. We advocate early intervention in patients with gastroparesis who have poor response to prokinetic agents: This improves their glycemic control, prevents frequent hypoglycemic events, and improves their nutritional status.

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## Severe Nonproductive Cough and Cough-Induced Stress Urinary Incontinence in Diabetic Postmenopausal Women Treated With ACE Inhibitor

A frequently recommended class of antihypertensive agents in the treatment of diabetes is the ACE inhibitors (ACEIs) (1). Coughing that results from ACEI therapy is a well-recognized complication. The frequency of cough reported in the literature varies from 0.7% to as high as 48% (2). A high incidence of cough has been noted in the Chinese population (3) and is more common in nonsmokers, women, and patients with renal failure (4,5).

Angiotensin II receptor antagonist (ARA) blocks the angiotensin-renin system at a different level compared with ACEI (6). Preliminary experience has confirmed its efficacy in lowering blood pressure and decreasing urinary protein excretion in animal models and in both diabetic and nondiabetic individuals (7). Reports have also indicated that ARA has a lower incidence of cough associated with treatment compared with that of ACEI (8).

We studied 173 postmenopausal type 2 diabetic patients with hypertension or proteinuria who consecutively attended the outpatient diabetes clinic of our hospital. Hypertension was defined as patients having an office diastolic blood pressure of >90 mmHg on a mercury sphygmomanometer. Most of the hypertensive patients were untreated, and the 12 hypertensive patients who were receiving antihypertensive agents were asked to discontinue treatment for 2 weeks before the study. Patients who had had a diagnosis of urinary tract infection, urolithiasis, liver cirrhosis, bronchitis, emphysema, congestive heart failure, or chronic lung disease were excluded. Cough was considered to be present if the study subjects had been bothered by a cough during the treatment and if they had symptoms for at least 2 weeks without an identifiable cause, such as acute respiratory infection.

The current study was a prospective double-blind parallel study, approved by the human research review committee of our hospital. Informed consent was obtained from each patient. Postmenopausal women were selected for this study. Patients were randomly chosen to receive a single 4-mg dose of perindopril (Acertil; Les Laboratoires Servier Industrie, Gidy, France) or 50 mg losartan (Cozaar; Merck Sharp and Dhome, West Point, PA) daily. Patient visits were scheduled every 2 weeks during the study period. The dosage of the each patient's usual antihypertensive therapy could be increased or other non-ACEI and non-ARA antihypertensive therapy could be added if necessary to achieve goal blood pressure. The primary end point for the study was to compare the incidence of cough associated with ACEI and ARA therapy.

There were 165 patients who completed the study. The groups of patients randomly allocated to receive losartan or perindopril had similar demographic and clinical characteristics at baseline. After 8 weeks of therapy, significant changes in blood pressure were found, yet there was no statistical difference found between the perindopril and losartan groups. The visual analog scale (VAS) was used to quantify the patients' perception of frequency of cough. A very low VAS score (0.2 cm) was found at the beginning of the study, with changes in VAS score being significantly higher in the perindopril group ( $3.3 \pm 2.5$  cm) than in the losartan group ( $1.3 \pm 1.3$  cm,  $P < 0.01$ ) at the end

of the study. The severity of cough, as assessed by a symptom-assessment questionnaire (SAQ), was also higher in the perindopril group than in the losartan group ( $7.2 \pm 4.5$  vs.  $4.5 \pm 3.5$ ,  $P < 0.01$ ). There was a significant positive correlation between the frequency and severity of cough; higher VAS scores were generally associated with a more severe cough on the SAQ ( $r = 0.88$ ,  $P < 0.01$ ). The time of coughing associated with ACEI varied among patients, ranging from 2 days to 1 month (mean  $7.5 \pm 5.5$  days). The percentage of patients responding positively was significantly higher in the perindopril group than in the losartan group (60.0 vs. 25.9%,  $P < 0.01$ ). Patients with advanced renal disorders had a higher incidence of cough. There were 20 patients who reported suffering from intolerable cough and cough-related urinary stress incontinence. These 20 patients were found to belong to the perindopril treatment group and were thus withdrawn from perindopril. When these 20 patients with severe intolerable cough were changed to losartan treatments, the symptoms of 19 patients (95%) subsided within 1 week (3–7 days) and no further attacks were found to occur during the follow-up period of 6 months.

The current study shows that postmenopausal Chinese patients with type 2 diabetes who are receiving perindopril have a higher prevalence (60%) of cough than those using an ARA. Furthermore, severity of cough was similarly worse with more than 10% having intractable cough with stress urinary incontinence. This was largely relieved with conversion to therapy with losartan. The substitution of an ARA for an ACEI therapy associated with intractable cough is likely to be well tolerated in a population at high risk for drug-induced cough.

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## Can Mobile Cellular Phones Affect Functioning of Implantable Insulin Pumps?

The mobile telephone network has rapidly expanded, and a large part of the world is now served by several network operators working in parallel. This proliferation of communication with cellular phones leads to more and more questions regarding possible interactions with biological tissues (1) and interferences with electronically controlled medical equipment (2). During the last few years, several investigations have been carried out concerning the disturbance of medical devices such as pacemakers (3,4) or external insulin pumps (5) exposed to

electromagnetic field radiated by cellular phones. Some in vivo trials have detected intermittent dysfunction of implanted pacemakers caused by digital mobile phones (6,7). Darmon et al. (5) tested the performance of two different models of the external insulin pump while exposed to various types of cellular phone emission. The old-fashioned pump used during specific conditions of testing showed inadequate electronic protection against such radiofrequency interference. But the electromagnetic field radiated by cellular phones did not cause any disturbance of the tested new-fashioned portable insulin pump and would not induce harmful effects in clinical practice, according to the manufacturer (Minimed Technology, Sylmar, CA). In this respect, the Minimed external pump has been designed and tested to demonstrate immunity to radiofrequency emission at a level of 3 V/m in the 26-1,000 MHz frequency range, according to the current international regulations for radiofrequency immunity of medical devices.

In regard to implantable insulin pumps, the manufacturer's recommendations advise against exposure to strong magnetic fields, such as diagnosis procedures like magnetic resonance imaging or industrial magnetic fields. So we were interested in verifying, in a collaborative work with the Research and Development Center of France Telecom, whether radiofrequency emission from mobile cellular phones may cause disturbance of this implantable device.

The current mobile telephone systems operate with radio signals that have a frequency of ~900 MHz and 1,800 MHz and a usual output power transmission of 1 or 2 W. So we tested ex vivo two implantable insulin pumps of the same model (Minimed MIP 2001 [pump A and pump B] with two types of cellular phones (respectively, DCS Flare B 300 [1,800 MHz, 1 W] with pump A and GSM Lisa P 9026 [900 MHz, 2 W] with pump B). Telephones were held, ex vivo, in contact with the pumps and used first, in test mode with maximum output power; secondly in actual use during the ringing phase; and thirdly, in use during an actual conversation call. We searched for an effect on alarm activation, pump programming, and volumes delivered by the pump. Because the insulin currently used in implantable pumps has a concentration of 400 U/ml, the volumes of insulin deliv-

ered by the pump are very small. For instance, an insulin bolus of 10 U corresponds to a volume of 25  $\mu$ l and a basal rate of 1 U/h to a rate of 2.5  $\mu$ l/h. So a special protocol was designed to permit a quantitative measurement. Pumps were filled with Actrapid HMG U40 (Novo Nordisk, Bagsvaerd, Denmark) instead of insulin currently used, HOE 21 PH U40 (Hoechst, Frankfurt, Germany). A maximal basal rate was programmed during 1 h (~25  $\mu$ l/h) and a maximal bolus over 30 min (~150  $\mu$ l). The insulin delivered by the pump was collected in 1 ml of buffer. The quantitative measurement of the insulin in these experimental samples used a particular enzyme immunoassay designed to be sensitive enough to detect 2  $\mu$ U/ml. The intra- and interassay coefficients of variation were 3.2 and 4.8%, respectively.

Results did not show any alarm activation or change in programmed parameters while phones were used in the test mode with maximal output power and then during the ringing phase. In actual transmission, the amounts of insulin delivered by the pump were measured twice in basal conditions without phone as control measure, and then twice while cellular phones were calling in actual transmission during the whole test as simulating conditions. For pump A tested with phone DCS, bolus was measured at 5.3 and 5.4 U for the first and second testings under basal conditions vs. 5 and 5.4 U for the first and second measures during simulating tests. Basal rates were 0.9 and 0.86 U/h vs. 0.86 and 0.85 U/h. For pump B tested with phone GSM, bolus was 5.26 and 4.96 U vs. 5.33 and 5.06 U. Basal rates were 0.84 and 0.84 U/h vs. 0.86 and 0.86 U/h. So whatever the type of cellular phone coming in contact with the implantable pump, we did not observe any significant modification of the amount of insulin delivered during bolus and basal rate.

When placed in close proximity to the implantable insulin pump Minimed MIP 2001, neither type of tested cellular phone (1 or 2 W) affects the programming and functioning of this medical device. Our results confirm that the design of the electronic system of the implantable pump Minimed MIP 2001 ensures protection against radiofrequency field currently used by typical cellular phones.

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## Usual Delay in Sample Processing Can Modify Gestational Diabetes Screening

**B**lood cell glycolysis decreases glucose concentration in blood samples depending on temperature, time, and hematocrit and can result in clinically significant errors (1,2). The addition of sodium fluoride (NaF) is the most usual method to preserve blood glucose (3) but is currently not included in the recommendations for

gestational diabetes (GD) screening (4). We aimed to assess if ordinary delay in sample processing influences GD screening results.

In 158 consecutive pregnant women undergoing GD screening, 5 ml of blood was drawn as two consecutive samples (S0, S1) using the usual tubes for glucose measurement in our center (evacuated tubes containing 2.5 g/l NaF Vacutainer [ref. 606659]; Beckton-Dickinson, Rutherford, NJ). The S0 samples were immediately centrifuged and plasma glucose analyzed with a polarographic method (Beckman Synchron CX-3 analyzer, Fullerton, CA) with an interassay coefficient of variation <2.5% for the range 0.5-10.0 mmol/l. The S1 samples were kept at room temperature and analyzed after the usual time delay. Leukocyte and erythrocyte counts (Coulter T-540; Coulter Electronics, Hialeah, FL) were performed in the last 93 samples.

Mean glucose concentrations were  $6.75 \pm 1.52$  mmol/l in S0 samples and  $6.50 \pm 1.52$  mmol/l in S1 samples ( $P < 0.001$ , Wilcoxon's rank-sum test), measured after  $65.9 \pm 33$  min. Plasma glucose decrease was significant in 21 samples measured with a processing delay of <30 min ( $-0.18 \pm 0.26$  mmol/l,  $P < 0.01$ , Wilcoxon's rank-sum test), whereas further decreases were not significant. Both in the whole group and in samples showing discordant results, the percent decrease in plasma glucose was higher than the interassay coefficient of variation. Of 35 women with a positive screening test in S0 samples, 6 (17%) were misclassified as negative in S1 samples, resulting in a lower rate of positive screening tests in the latter (22.2 vs. 18.4%,  $P < 0.05$ , McNemar's test). In the subset of 93 samples in which blood cell counts were performed, erythrocytes were  $3.82 \times 10^6 \pm 0.66 \times 10^6/\mu\text{l}$  and leukocytes were  $9,298 \pm 2,261/\mu\text{l}$ , and there was no relationship between cell count and blood glucose decrease (data not shown).

The overall rate of GD screening misclassification (3.8%) is similar to that described for oral glucose tolerance tests (2). Our results may have been influenced by the concentration of NaF but the absolute plasma glucose decrease in this report is quite similar to the  $-0.27$  mmol/l reported at 60 min with the use of more concentrated preparations of NaF (5).

In conclusion, in samples kept at room temperature, we have corroborated

that 2.5 g/l NaF is not sufficient to prevent plasma glucose decrease in the first 30 min after sample drawing, and this can modify GD screening categorization. Because immediate sample processing is not feasible in current practice, additional measures should be taken, such as keeping blood at low temperature.

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## Operational Errors Cause Inaccurate Glucose Results

**W**e report here on a case in which a common glucose meter reported falsely high readings on patient blood samples. The incident occurred in a busy intensive-care unit (ICU). The glucose meter (Accu-Chek Advantage GTS; Roche Diagnostics, Indianapolis, IN), test strips (Advantage H; Roche Diagnostics),



Table 1—Concordance between the 1997 ADA criteria to assess IFG and the 1998 WHO criteria to assess IFG and IGT in 932 women with previous GD

	1998 WHO criteria			Prevalence by 1997 ADA criteria (%)
	Normal	IFG and IGT	Diabetes	
1997 ADA criteria (n)				
Normal	772	77	3	91.4
IFG	—	55	5	6.4
Diabetes	—	—	20	2.1
Prevalence by 1998 WHO criteria (%)	82.8	14.2	3.0	100

ria in women with prior GD, rates of diabetes were reported to be similar, but estimates of impaired glucose homeostasis significantly differed (31.5% IFG vs. 10.9% IGT, respectively).

Our aim was to evaluate the concordance between the 1997 ADA criteria and the 1998 WHO criteria in 932 women with previous GD (based on the Third Workshop-Conference criteria) who underwent an OGTT 4.96 ± 2.96 months after delivery. Prepregnancy BMI was 24.06 ± 3.91 kg/m<sup>2</sup>, mean age was 32.48 ± 4.49 years, 99.8% were Caucasian, and 59% had received insulin therapy during pregnancy. The diagnostic results according to the two sets of criteria are shown in Table 1. Overall concordance was good (kappa 0.609, 95% CI 0.536–0.682), but 50% of abnormal tests that were based on the 1998 WHO criteria were classified as normal by the 1997 ADA criteria. Discordance was higher in those women who were diagnosed as having IFG and IGT according to the 1998 WHO criteria (58.3% had normal glucose homeostasis according to the 1997 ADA criteria) than in those who were diagnosed as having diabetes according to the 1998 WHO criteria (28.6% did not have diabetes according to the 1997 ADA criteria [*P* < 0.01]).

This report and that of Kousta et al. (7) have found that use of the ADA criteria grossly underestimates the frequency of AGH in women with previous GD. The discordance is easy to understand if we consider that FPG-derived criteria do not take sex into account (8). Given our present knowledge of the relationships between postchallenge blood glucose and later diabetes, cardiovascular disease, and mortality, we must aim at the diagnoses of IGT and postchallenge diabetes. Therefore, we must consider OGTT-derived criteria as the “gold standard”; the 1997 ADA criteria categorize as normal and skip meaningful diagnoses in 50% of

women with previous GD and current AGH, as diagnosed according to the 1998 WHO criteria.

Our opinion is that women with previous GD should be tested with an OGTT.

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## A Role for Gestational Diabetes in the Excess Maternal Transmission of Type 2 Diabetes?

By analyzing a large multiethnic study sample, Karter et al. (1) recently gave further support to the hypothesis that type 2 diabetes is predominantly transmitted through one's maternal side. As discussed by the authors, exposure to a diabetic intrauterine environment has been proposed to be a possible mechanism responsible for this excess maternal transmission (2,3). Interestingly, maternal aggregation was more pronounced in female type 2 diabetic patients than in males (1). It is known that in a considerable number of type 2 diabetic women, gestational diabetes (GD) precedes the onset of type 2 diabetes for years (4). On the other hand, GD might reflect the diabetic intrauterine environment as mentioned above. Consequently, one could expect to find a maternal aggregation of type 2 diabetes in the family history of women with GD. There is only one study that investigated the family history of diabetes in GD pregnancies. Martin et al. (5) observed a maternal aggregation of diabetes in women with GD. However, since the type of parental diabetes was not considered in this study, it is still unclear whether there is a predominance of type 2 diabetes in mothers of

patients with GD, as compared with fathers. Furthermore, maternal excess transmission was also observed on the maternal-grandmaternal side of patients with type 2 diabetes (6). On the other hand, animal studies suggest an epigenetic transmission of diabetes over several generations of the maternal side, accompanied by spontaneous GD in each generation (7,8). Therefore, it would be of interest to extend the investigation on maternal and paternal grandparents.

The family history of type 1 and type 2 diabetes was evaluated in 106 unselected patients (mean age  $27 \pm 5.6$  years) with GD attending the Clinic of Obstetrics and Gynecology Berlin-Kaulsdorf, Berlin, Germany. GD was diagnosed during the 26th–28th week of gestation by means of a 50-g oral glucose tolerance test using the criteria of Fuhrmann (9,10). For all patients, a standardized interview on the history and type of diabetes in their parents and grandparents was performed by a physician during the first visit at the clinic. If family members were said to have diabetes, patients were asked for the age of onset, medication (insulin or oral), and diabetes complications to verify the information about the type of diabetes of the affected relative. For further analysis, all cases with an uncertain classification of the type of parental diabetes were excluded from evaluation ( $n = 5$ ). Comparison of proportions was performed by  $\chi^2$  statistics.

In patients with GD, the prevalence of diabetes (all types) was significantly greater in mothers than in fathers (20.7% [22 of 106] vs. 9.4% [10 of 106];  $\chi^2 = 4.45$ ;  $P = 0.03$ ). Diabetes was significantly more prevalent on the maternal-grandmaternal side than on the paternal-grandpaternal side of women with GD (23.6% [25 of 106] vs. 9.4% [10 of 106];  $\chi^2 = 6.71$ ;  $P = 0.009$ ). After exclusion of all cases with uncertain classification ( $n = 5$ ), a greater prevalence of type 2 diabetes was observed in mothers than in fathers of women with GD (17.8% [18 of 101] vs. 7.9% [8 of 101];  $\chi^2 = 3.58$ ;  $P = 0.05$ ). A significant aggregation of type 2 diabetes was also observed on the maternal-grandmaternal side as compared with the paternal-grandpaternal side of patients with GD (19.8% [20 of 101] vs. 7.9% [8 of 101];  $\chi^2 = 5.02$ ;  $P = 0.02$ ). Concerning the prevalence of type 1 diabetes, no significant difference between the maternal and paternal side of patients with GD

was observed (1% [1 of 101] vs. 1% [1 of 101];  $\chi^2 < 0.001$ ; NS).

In conclusion, for the first time, an aggregation of type 2 diabetes in mothers and grandmothers of women with GD is reported here. The data seem to favor the hypothesis that maternal excess transmission of type 2 diabetes over successive generations includes the occurrence of gestational diabetes, i.e., of a diabetic intrauterine environment, intergeneratively. History of type 2 diabetes on the maternal side of pregnant women should be considered as a particular risk factor for intergenerative transmission of type 2 diabetes, which therefore might be prevented, at least in part, by strict avoidance of GD.

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## Recent Commencement of Dialysis Is a Risk Factor for Lower-Extremity Amputation in a High-Risk Diabetic Population

We read with interest the recent study by Moss et al. (1) that describes several risk factors for lower-extremity amputations (LEA). During the last 2 years, we have observed a high number of diabetic patients on renal dialysis requiring LEA in Northland, New Zealand (NZ). We have retrospectively reviewed this group plus all diabetic patients requiring amputation in our region. We identified diabetic patients who had had any type of limb amputation between 1 January 1997 and 31 December 1998 and reviewed their hospital files.

There were 47 patients in total, 35 of which were men. Although NZ Maori account for only 25% of the Northland population, 32 (86%) of the study group were Maori, and the rest were of European descent. There was a wide age range (32–89 years), but most patients were in the 50- to 60-year age-group (median age 62). Most had had known diabetes for at least 7 years (range 1–63, median 12).

Of the group, 79% had a raised serum creatinine level ( $>0.12$  mmol/l) and pro-

teinuria (urinary protein >0.3 g/l) and 30% (14 patients) were on renal dialysis at the time of their amputations. Time from starting dialysis (either hemodialysis or chronic ambulatory peritoneal dialysis) to requiring amputation was <1 year in the majority of patients (range 2 weeks to 40 months, median 7 months, mean 11.1 months). Seven of these dialysis patients died within 1 year of undergoing amputation.

To put this in perspective, we have a total of 61 patients in Northland on dialysis, of whom 67% are diabetic and 50% are Maori. There was only one nondiabetic dialysis patient who required amputation during the time period studied.

Looking more closely at the 14 diabetic dialysis patients who required amputation, again the majority were Maori men with type 2 diabetes who were in their 50s or early 60s. Half of them continued to smoke and just under half had a history of heavy alcohol use. All had LEA, and five required bilateral amputations.

Peripheral vascular disease (PVD) was universal, but only four patients had had symptoms attributable to PVD, including previous amputation, before commencing dialysis. Most had documented peripheral neuropathy.

Although the members of this group were obviously at high-risk for foot pathology, only eight had actually seen a podiatrist and only two had had orthotic input, usually because of nonattendance to health care providers. Six patients underwent angioplasty or surgery, but they still came to need amputation within a few weeks or months.

All had increased blood pressure and diabetic retinopathy, and more than half had ischemic heart disease, but very few had abnormal lipids. Glycemic control was poor in the majority of the patients, but most were not overweight at the time of amputation.

Northland has the highest rate of amputation among renal dialysis units in NZ and this is virtually all attributable to the male Maori type 2 diabetic patients undergoing dialysis. There is a known increased prevalence of diabetes in the Maori compared with New Zealanders of European descent (2), and Northland has a high percentage of Maori in the general population compared with other regions in NZ. The Maori have an increased incidence of diabetic renal disease (2).

Most of the patients required amputation relatively soon after commencing

dialysis. The 50% 1-year mortality after LEA in this group parallels the recent observation that dialysis patients are more likely to have a myocardial infarction within 2 years of commencing dialysis and are less likely to survive compared with patients undergoing renal transplant or those with chronic renal failure not treated with dialysis (3). Does dialysis treatment cause acceleration of vascular disease? Or is it that patients undergoing transplant or not needing dialysis have less vascular disease? There has been a progressive lowering of the threshold for acceptance of patients with comorbidities in the dialysis program in NZ over the last 5 years and, as this study illustrates, diabetic patients with established peripheral vascular disease, even if asymptomatic before commencement of renal replacement therapy, are at particularly high risk of requiring LEA soon after starting dialysis.

Interestingly, in the patients not on dialysis in this group, the majority had abnormal renal function, indicated by a raised serum creatinine level and proteinuria. Proteinuria has previously been identified as a risk factor for LEA (1).

In conclusion, our findings support the concept of chronic renal failure as a "vasculopathic state" and highlight recent commencement of renal dialysis as another risk factor for LEA in a high-risk diabetic population.

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## Response to Editorial by Flegal

A recent editorial by Flegal (1) provided commentary on the methodologies that we used in a study in the same issue of *Diabetes Care* (2). This dealt with potential effects of dietary macronutrients on lipoprotein profile in people with type 2 diabetes. The study used methods commonly utilized in observational epidemiologic studies; the editorial discussed the relative merits of this approach compared with experimental designs, which are issues that are familiar to epidemiologists. Flegal noted that, despite several strengths, observational studies are subject to a variety of biases and that studies of dietary intake and health outcomes may be particularly prone to some of these.

Unlike the vast majority of studies of diet and health, results presented in our article were based on two very different dietary assessments: one relying nearly exclusively on generic or habitual memory (the food-frequency interview), and the other relying nearly exclusively on episodic short-term recall (24-h dietary recall). Although both methods have their shortcomings, it is widely assumed that the errors in each are fairly different from one another and therefore not highly correlated (3). To obtain fairly similar results across such different assessment methods contributes to one of the key criteria in judging causality (i.e., consistency) (4-6).

We agree that it is generally underappreciated that errors in dietary assessment can create serious problems in data analyses and interpretation of results from epidemiologic studies. It must be noted, however, that many conceivable measurement errors could beset a study, irrespective of its design. This point notwithstanding, we would like to note that experimental designs also suffer from a unique set of limitations that represent additional potential sources of inaccuracy and imprecision. These include the following:

1. Incomplete compliance to dietary interventions that may or may not be measured. (It is widely appreciated that people are poor at maintaining directed dietary change over periods of even a few months.)
2. Unmeasured or highly inaccurate assessment of (nonintervention) dietary and other potential confounders. There

is a general lack of appreciation of the need to measure and control for other aspects of diet and relevant aspects of lifestyle, such as physical activity, that may differentially impact control and experimental groups. Such measurements may not be necessary in highly controlled studies, such as those conducted in metabolic wards, but then issues about sample size, duration of exposure, and applicability to the real world must be faced.

3. Limited duration of the intervention, particularly when short-term effects may differ from long-term effects due to biologic accommodation (as may be the case with effects of carbohydrate on triglyceride concentration).
4. Sample sizes that are often limited for practical reasons of cost. This usually severely limits statistical power, particularly within subgroups of importance (e.g., sex, race or ethnicity, diabetes status, or weight-change status).

Such difficulties may explain, in part, inconsistencies within the clinical trial literature, as evidenced by the need for meta-analyses to understand overall trends, even across studies that were clearly well conducted in a highly consistent manner (e.g., randomized crossover designs with isocaloric interventions in subjects with diabetes, all with durations of 2–6 weeks) (7).

The process of science is one that allows for truth to emerge from numerous sources, each with its own unique set of strengths and weaknesses. This is especially true in studies of humans. It was with this in mind that Hill (6) developed the criteria for judging causality that became the centerpiece for the 1964 Surgeon General's Report on Smoking and Health (4). Each taken alone, observational and experimental designs are inadequate for elucidating the truth. There is a clear need for both types of studies to be conducted so that each approach informs the other. Consistency of results across well-conducted studies that differ in design, subject populations, overall settings, and other characteristics is a key tenant of establishing causal relationships. It was with this in mind that we included results from—not one—but two epidemiologic studies that used two different approaches to dietary assessment. The general consistency in findings between these two studies speaks to the potential for each to have yielded an estimate of

truth. Furthermore, within each study, although all participants included in the analyses had confirmed type 2 diabetes, subgroups of particular interest to the research questions were included. Again, general consistency between subgroups (with some exceptions, as discussed in the article) and across studies indicates that the results have scientific merit. Taken together, the findings contribute to the hypothesis that reducing dietary fat intake improves lipoprotein profile in most people with diabetes.

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## Controlled Trials of HbA<sub>1c</sub> Measurements

Thaler et al. (1) have studied a clinically relevant research question in their recently published article. Specifically, they ask: Does the availability of rapid HbA<sub>1c</sub> measurement technology lead to changes in diabetes management and later outcome? Because this is a question of

intervention effect, they appropriately proposed a randomized controlled trial design. This design is the “gold standard” for evidence of treatment effects because it is the method most likely to give results that are relatively free from confounding (2). However, it is unclear whether such a design was actually implemented. Thaler et al. (1) instituted a system of even and odd days for availability or not of the rapid HbA<sub>1c</sub> technology. This system has the potential to introduce bias if the choice of appointment day was influenced by patient characteristics (either known or unknown), unless the patients were individually randomized first and then booked according to their assignment without allowing changes from even to odd days, or vice versa.

Confounding leads to incorrect conclusions because the effects of the confounder on the outcome are wrongly considered as effects resulting from the intervention. Biased results due to confounding have been shown when observational studies of treatment effect that used either historical controls or concurrent controls were compared with results of the same intervention studied by randomized controlled trials (3,4). Random allocation of patients to the experimental or control condition prevents biased allocation while tending to balance groups for both known and unknown potential confounders (2). Appropriate study design is critical, second in importance only to the research question itself. Even though there do not seem to be large differences between the groups for the “bare-bones” demographic features listed in Table 1, a full description (as detailed in the CONSORT Statement [5]) of whether this study team (1) used randomization is important to the readers considering the results.

If individual randomization is impractical, group randomization can be considered. For example, here one could reveal on any given day whether that day had previously been randomized to rapid or conventional (if secrecy could be maintained). The appropriate sample size and statistical test would then be based on the number of success versus failure days under each system.

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## Response to Taback

We appreciate the comments of Dr. Taback (1) regarding randomization of patients for receiving a conventional or rapid HbA<sub>1c</sub> measurement in our study (2). His concern is whether the system of even versus odd days might have introduced a bias if the choice of appointment day was influenced by patient characteristics. He also suggests that randomization should have occurred first, then patients booked according to assignment.

We offer the following clarifications regarding the methodology that we used:

Patients were not actively randomized on an individual basis, which otherwise would have been the ideal situation, as suggested by Dr. Taback.

The patients' appointment dates were preset by front-desk clerks who were not involved or aware of the study before, during, or after it was completed. When patients check out, front-desk clerks normally receive a written request from providers to schedule a follow-up appointment for an individual patient in a specific period of time. The clerk (not the care provider) decides on the specific date, depending on availability, irrespective of whether the date is even or odd. In addition,

the clerk is not aware of a patient's characteristics, other than name and medical record number.

Therefore, we made use of the fact that patients were preassigned an odd or even follow-up appointment date at random, without interference of the provider, so that a crossover bias could not have occurred. Table 1 shows, as expected, that there were no significant differences in number, age, sex, race, BMI, diabetes duration, type of therapy, years of follow-up, and HbA<sub>1c</sub> between the two groups.

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## Cancer in a Patient Receiving IGF-I Therapy

We read with great concern the article by Thrailkill et al. (1) that appeared in the April 1999 issue of *Diabetes Care*. During the trial of IGF-I cotherapy with insulin described in this article, we were involved in the operations of the clinical center at Harper Hospital and Wayne State University School of Medicine in Detroit as principal investigator (G.G.) and as ophthalmic examiner and also as a study subject (R.N.F.). One serious adverse event that occurred during our center's participation in this trial that may have been related to this therapy was not described by the authors, even though this event was reported to the sponsor (Genentech) and to the U.S. Food and Drug Administration and therefore should have been known to Thrailkill et al.

One of our subjects, a 58-year-old man with a 42-year history of type 1 diabetes, developed pain in the right hip and experienced a 10-lb (4.5-kg) weight loss after 2 months of therapy in this study with what was later revealed to be the low-dose (40 µg twice daily) IGF regimen. The symptoms were initially ascribed to intertrochanteric bursitis because the physical examination, plain-film X-rays of the femurs and pelvis, complete blood count, and sedimentation rate were all within normal limits, and joint pain and weight loss were described in the investigators' brochure supplied by Genentech as previously observed side effects of IGF therapy. However, when the pain persisted after the conclusion of the 3-month therapeutic regimen and the weight loss increased to 16 lb (7.3 kg), further investigation including magnetic resonance imaging and biopsy revealed a primary non-Hodgkin's lymphoma in the proximal right femur with a secondary lesion in the distal left femur.

The occurrence of cancer in a patient related to his or her participation in a clinical trial is, by definition, a serious adverse event that must be reported, even if the relationship of the cancer to the study drug or other therapy is considered unlikely. For example, Thrailkill et al. (1) reported the single death in their study even though, as they pointed out, the individual who died was enrolled in the control group. They also described other possible adverse effects of IGF treatment even when only one study subject was involved. The malignancy that occurred in the case described above occurred in a patient in one of the treatment groups, making the reporting of this event even more essential. The occurrence of a malignancy in only 1 of 223 subjects enrolled in this study makes a relationship to IGF therapy uncertain, but there are at least some reasons for viewing it as plausible. The symptoms began during treatment with the study drug. The symptoms had been described in the investigators' brochure supplied by the sponsor as having been observed previously in individuals treated with IGF-I. Finally, IGF-I has been associated previously with several forms of cancer, including carcinoma of the colon (2-4), carcinoma of the prostate (5,6), carcinoma of the breast (7), as well as hematologic malignancies (8).

We believe that failure to mention this serious adverse event by Thrailkill et al. in

their report of the IGF-I study (1) is a most regrettable omission that may be substantially misleading to readers of this article regarding the safety of the IGF-I regimen.

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## Cootherapy With Recombinant Human IGF-I and Insulin Improves Glycemic Control in Type 1 Diabetes

In their article "Cootherapy With Recombinant Human Insulin-Like Growth Factor I and Insulin Improves Glycemic Control in Type 1 Diabetes," Thraikill et al. (1) demonstrate that 12 weeks of recombinant human IGF-I (rhIGF-I) and insulin cotherapy improves glycemic control in patients with type 1 diabetes better than optimized insulin management alone. In contrast with the Diabetes Control and Complications Trial (DCCT) (2), the improvement in glycemic control was not associated with an increased frequency of hypoglycemic events. Although a previous study evidenced a loss of therapeutic effect after 3 months of therapy (3), the authors are to be commended for exploring new strategies for a better treatment of patients with type 1 diabetes. However, we are very much concerned about the ophthalmological changes of the patients treated with rhIGF-I as an adjunct to insulin therapy.

In the study, 16 of 199 patients showed a three-step or greater increase in the Early Treatment of Diabetic Retinopathy Study score after 12 weeks of treatment. Of these 16 patients, 12 had concomitant optic disk swelling (ODS), and 3 of these 12 patients received laser photocoagulation. In 4 of the 16 patients who experienced an early worsening of retinopathy, retinal changes were extensive, including neovascularization of the optic disk with associated ODS. In summary, a total of 24 patients (12%) had ODS (17 with definite ODS and 7 with questionable ODS) (1).

It is remarkable that despite the relatively short period of cotherapy (12 weeks) and the absence of hypoglycemic events, 16 patients had a significant worsening of retinopathy including neovascularization at the optic disk, which represents a severe form of proliferative retinopathy. The authors state that the progression of the retinal disease is probably dose-related, since most of these patients were receiving one of the two highest rhIGF-I doses. The hypothesis that a worsening of diabetic retinopathy might also be associated with long-term therapy at the lowest rhIGF-I

doses should be carefully investigated and hopefully excluded.

The high prevalence of ODS among the treated patients deserves a more accurate evaluation of the causative effects. It is unfortunate that patients showing ODS did not undergo further and more extensive examination. There appears to be a causal relationship between the initiation of rhIGF-I with the development of ODS. The pathogenesis of rhIGF-I-induced papilledema is probably related to the occurrence of pseudotumor cerebri (PTC). In 1993, Malozowski et al. (6) described 22 children and 1 adult identified by a Food and Drug Administration database who were treated with recombinant growth hormone or IGF-I and developed PTC. The pathogenesis of rhIGF-I-induced PTC remains unknown and warrants further studies. It may involve alterations in cerebrospinal fluid (CSF) drainage across the arachnoid villi (7). Increased levels of IGF-I in the CSF may act on IGF-I receptors in the choroid plexus to increase CSF production (8). Alterations in sodium and water retention may also play a role.

In conclusion, the safety of cotherapy with rhIGF-I in regard to ODS should be more carefully investigated before rhIGF-I in adjunct with insulin therapy is proposed and initiated as a new treatment of patients with type 1 diabetes.

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## Response to Frank and Grunberger

The authors greatly appreciate the comments of Drs. Frank and Grunberger (1) in reference to our article titled "Cootherapy With Recombinant Human Insulin-Like Growth Factor I and Insulin Improves Glycemic Control in Type 1 Diabetes."

A review of the chronology of events related to this particular patient is provided for the reader. The patient described was a 57-year-old man who was enrolled in the study and began treatment with active IGF-I therapy (40 µg/kg twice daily) on 5 April 1996. On 15 May 1996, the subject reported increased hip pain bilaterally and began physical therapy. Hip X-rays, labo-

ratory tests, and a sedimentation rate were all normal at that time. A work-up for rheumatoid arthritis was also negative. However, the hip pain continued and in January 1997 (after study completion, and 6 months after drug exposure), a magnetic resonance imaging scan showed neoplastic marrow and the patient was diagnosed with non-Hodgkin's lymphoma (NHL). In March 1997, an investigational new drug (IND) safety report describing this adverse event was filed with the Food and Drug Administration.

The intent of this work was to report on events as they relate to the described pretreatment, 12-week treatment, and reasonable post-treatment follow-up period for the study. The one patient death reported in this article occurred while that subject was participating in this study. A summary of adverse events during this trial was included in Table 2 in that article. The patient described by Drs. Frank and Grunberger is included among 10 subjects reported with arthralgias in the 40/40-dose group; in this subject, symptoms of arthralgias were present during the period of his participation in the study.

As noted by Drs. Frank and Grunberger, the relationship of this event to previous recombinant human IGF-I exposure remains uncertain. Among men of this patient's age, the incidence of NHL is reported as ~35/100,000 person-years. It is possible that the diagnosis of NHL in a male patient of this age bears no relationship to previous recombinant human IGF-I exposure. Alternatively, because the subject's hip pain was described by investigators as an increase in preexisting hip pain, the presence of microscopic malignant disease might also have predated this subject's participation in the study.

Among all Phase II/III recombinant human IGF-I studies conducted by Genentech, three additional IND safety

reports describing neoplastic disease have been submitted. In two patients, post-study excision of irregular nevi present before study drug administration revealed a diagnosis of malignant melanoma. One patient had received recombinant human IGF-I for 12 weeks. The other patient had received placebo. Another subject with inactive Kaposi's lesions received recombinant human growth hormone and recombinant human IGF-I for 12 weeks. After discontinuation of therapy, new lesions developed. The investigator considered the new lesions possibly related to the study drug and possibly related to underlying disease. At this time, review of safety data from all Genentech recombinant human IGF-I clinical trials does not demonstrate an increased risk of malignancy after recombinant human IGF-I exposure.

The authors acknowledge the concern of Drs. Frank and Grunberger and agree that the potential risk of malignancy after recombinant human IGF-I treatment should be carefully monitored in future clinical trials. To date, however, no causal relationships between recombinant human IGF-I treatment and human cancers have been documented either in the literature cited by Drs. Frank and Grunberger or in the study that we have presented.

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