

CORRESPONDENCE



A Serious Adverse Event after Successful Gene Therapy for X-Linked Severe Combined Immunodeficiency

TO THE EDITOR: We recently reported (April 18 issue)¹ the sustained correction of X-linked severe combined immunodeficiency disease by *ex vivo*, retrovirally mediated transfer of the $\gamma\epsilon$ gene into CD34+ cells in four of five patients with the disease. These results have since been confirmed in four additional patients with typical X-linked severe combined immunodeficiency. Of the first four successfully treated patients, three continue to do well up to 3.6 years after gene therapy, whereas a serious adverse event occurred in the fourth patient. At a routine checkup 30 months after gene therapy, lymphocytosis consisting of a monoclonal population of V γ 9/V δ 1, γ/δ T cells of mature phenotype was detected. One proviral integration site was found, located on the short arm of chromosome 11 within the LMO-2 locus, as determined with the use of linear-amplification mediated polymerase-chain-reaction analysis.² This proviral integration within the LMO-2 locus was associated with aberrant expression of the LMO-2 transcript in the monoclonal T-cell population. Aberrant expression of LMO-2 has been reported in acute lymphoblastic leukemia arising from T cells with α/β receptors, usually with the chromosomal translocation t(11;14).³ Tests for replication-competent retrovirus were repeatedly negative in our patient's lymphocytes.

Between 30 and 34 months after gene therapy, the patient's lymphocyte count rose to 300,000 per cubic millimeter, and hepatosplenomegaly developed. Further investigations showed the presence of a t(6;13) translocation, which had not been detected 30 months after the therapy. Treatment with a chemotherapy regimen based on a high-risk protocol for acute lymphocytic leukemia (a protocol of the Dutch Childhood Leukemia Study Group) was initiated and has resulted, to date, in a dramatic reduction in the abnormal cells.

We interpret these findings as the consequence of the insertional mutagenesis event, a risk that is potentially associated with retrovirally mediated gene transfer and that has previously been considered to be very low in humans.⁴ For this reason, a thorough reassessment of the potential risk of retrovirally mediated gene therapy is warranted. It is likely that additional factors may have contributed to the adverse event in our patient, including a varicella-zoster virus infection five months before clinically detectable lymphoproliferation, which may have stimulated immune reactivity of the γ/δ T-cell clone, or a selective growth advantage conferred by $\gamma\epsilon$ expression in the transduced cells. Genetic predisposing factors for childhood cancer are also possible, since medulloblastomas have developed in the proband's sister and a first-degree relative.

We have proposed to the French regulatory authorities a halt to our trial until further evaluation of the causes of this adverse event and a careful reassessment of the risks and benefits of continuing our study of gene therapy in patients with X-linked severe combined immunodeficiency can be completed. The latter will include a comparison with the outcome of the only available alternative therapy, haploidentical stem-cell transplantation.⁵

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Editor's note: A Perspective on this report appears on pages 193–194 of this issue.

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Warfarin, Aspirin, or Both after Myocardial Infarction

TO THE EDITOR: Hurlen et al. (Sept. 26 issue)¹ showed that warfarin with or without aspirin, as compared with aspirin alone, was associated with a reduction in the risk of the composite end point of death, nonfatal myocardial infarction, or embolic stroke in patients with myocardial infarction but did not establish its clinical relevance. They did not assess the implications of the components of the end point for patients. As compared with aspirin alone, the absolute reduction in the rate of nonfatal myocardial infarction with warfarin alone was 0.6 percent per year and with warfarin plus aspirin was 1.1 percent. Patients are not likely to accept long-term warfarin therapy for such a modest reduction in a nondisabling, nonfatal condition. A similar reduction in the rate of events such as death or stroke is more likely to lead to acceptance of warfarin therapy. Hurlen et al., however, found no reduction in mortality and found a reduction in the rate of stroke of 0.3 percent per year with both warfarin and warfarin plus aspirin — too small to change clinical practice.

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1. Hurlen M, Abdelnoor M, Smith P, Erikssen J, Arnesen H. War-

farin, aspirin, or both after myocardial infarction. *N Engl J Med* 2002;347:969-74.

TO THE EDITOR: Hurlen et al. found warfarin, alone or in combination with aspirin, to be superior to aspirin alone after acute myocardial infarction. Becker, in the accompanying editorial,¹ concluded on the basis of this and another study (the Antithrombotics in the Prevention of Reocclusion in Coronary Thrombolysis 2 trial²) that anticoagulation therapy should be “strongly considered” after acute myocardial infarction.

We believe that the general applicability of the findings of these two trials is severely limited by the restricted use of coronary intervention. American College of Cardiology–American Heart Association guidelines³ recommend coronary angiography for patients with acute myocardial infarction associated with ST-segment elevation who have spontaneous or provokable ischemia and for most patients with acute myocardial infarction not associated with ST-segment elevation, who clearly benefit from an early, aggressive approach. The value of warfarin has been demonstrated in patients who, for the most part, do not undergo early coronary intervention. Conceivably, anticoagulation may be beneficial in patients who leave the hospital with severe narrowing of the infarct-related artery, but it may not be as beneficial in patients who are discharged af-

ter successful coronary intervention. We believe that the value of warfarin in patients who are treated according to current guidelines remains to be determined.

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TO THE EDITOR: Hurlen et al. do not report on the prevalence of atrial fibrillation in their patients with myocardial infarction after random assignment to treatment with aspirin, warfarin, or aspirin plus warfarin. These data need to be reported. In their patients with sinus rhythm, what were the incidence rates of death, reinfarction, and thromboembolic stroke in the three treatment groups?

Patients 75 years of age or older were excluded from the study. The mean age of the patients was 60 years. Because the efficacy and safety of cardiovascular drugs may differ between elderly patients and younger patients, it is important to enroll elderly patients in randomized, controlled trials so that physicians can provide evidence-based medical care to this high-risk population.

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THE AUTHORS REPLY: The Warfarin, Aspirin, Reinfarction Study (WARIS II) showed that warfarin, alone or in combination with aspirin, was superior to aspirin alone in reducing the incidence of the composite end point of death, nonfatal reinfarction,

or nonfatal thromboembolic stroke after acute myocardial infarction. Pullicino and Thompson question the clinical relevance of the findings for patients with nonfatal myocardial infarction. Nonfatal infarction may have serious implications clinically, socially, psychologically, and economically. Thus, we believe that a reduction in the incidence of nonfatal reinfarction is important. We agree, however, that there is a need to identify subgroups in which anticoagulant therapy may be either particularly beneficial or harmful.

Amit et al. argue that the applicability of the WARIS II trial results may be limited by the restricted use of acute percutaneous coronary interventions. The rationale for long-term antithrombotic therapy after acute myocardial infarction does not vary according to whether or not patients have had initial percutaneous coronary interventions. A recent study involving the use of intracoronary ultrasonography demonstrated multiple ruptured plaques in addition to the culprit lesion in patients with acute coronary syndromes.¹ Thus, atherosclerotic disease associated with the risk of thrombotic complications extends beyond the culprit lesion responsible for a single acute clinical episode in most patients.

Aronow raises the issue of patients with atrial fibrillation. Patients with paroxysmal, persistent, or permanent atrial fibrillation at the time of randomization were considered to have an indication for warfarin therapy. Hence, they were ineligible for the study. Exceptions were made for patients 60 years of age or younger who did not have conventional risk factors and who had structurally normal hearts. In general, it is important to enroll elderly patients in studies. However, we believe that the increased risk of bleeding associated with older age may limit the number of elderly patients who will eventually take warfarin in this context.

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Botulinum Toxin for Spasticity after Stroke

TO THE EDITOR: The design of the study by Brashear et al. of botulinum toxin for stroke (Aug. 8 issue)¹ fails to meet the claim of a “double-blind, placebo-controlled trial.” The investigators were open partners with the manufacturer in conducting a study in which the outcome measures were patients’ reports of qualitative improvement after a dramatic injection that produces weakness. Whether the injection is paralytic or placebo, patients are likely to be biased toward reports of better performance and physicians toward observations of decreased reaction to flexor-muscle stretch. Thus, the 27 percent response to placebo is no surprise. Observation of true muscle weakness by both patients and physicians after the injection of toxin must inevitably reinforce this subjective sense of improvement.

The study lacked an essential blind crossover component. If, for example, patients were videotaped in the process of dressing on several occasions after the injection of drug and after the injection of placebo, unprejudiced observers who did not know the patients could have served to validate the presence of a therapeutic effect. Reports of conventional clinical tests of agility and coordination might have provided information about the physiologic mechanisms behind subjective improvement. Other interventions, such as physical therapy, splinting, and medication, which may affect outcomes, were not mentioned.

This study does not justify extended injections for most patients with stroke. As Rowland observes in the accompanying Perspective,² treatment of spasticity is a popular and profitable practice. But no well-controlled outcome studies have yet shown that the suppression of reflexes by drugs (baclofen or tizanidine), surgery, or physical therapy “can significantly improve coordinated movement in hemiplegic limbs.”³⁻⁶

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TO THE EDITOR: Patients with chronic hemiplegic effects of stroke and physicians may interpret the study of Brashear et al. as showing that functional disability decreased, so functional ability to use the hand must have increased. The authors use the term “disability” in a fashion that requires close reading. The clinical effectiveness of the intervention can be summed up as a means to loosen the wrist and finger flexors, so that the hand is easier to maintain open passively. The study was powered to detect a change of 0.5 point on the Ashworth Scale of tone—a moot change in terms of clinical effectiveness. The small absolute but statistically significant changes in scores on the Disability Assessment Scale are a subjective measure of a narrow band of disability: patients’ perceptions about odd arm postures, difficulty in cleaning a clenched hand, pain possibly related to stiffness, and dressing skills not necessarily requiring the use of the affected hand. The authors offer no information about sensorimotor impairment or functionality of the hand for grasping and reaching before and after treatment. Did the subjects use the affected hand in performing daily activities before or after receiving injections?

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TO THE EDITOR: Brashear et al. report that patients assigned to injections of botulinum toxin type A “had greater improvement in the principal target of treatment than did subjects who received placebo” but do not report the actual degree of benefit seen in each of the four measures of disability. Can they provide these data?

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THE AUTHORS REPLY: In response to Dr. Landau’s comments regarding our study, consideration was

given to the issue of blinding. The source of funding for the study and the relationships between the investigators and the sponsor were clearly defined in our article in a manner that is consistent with the policy of the *Journal*. We do not believe that the results were biased.

Landau and Dobkin suggest videotaping, tests of agility, or sensorimotor or functional testing before and after injection. No data on sensorimotor impairment or specific functionality of the hand before treatment were obtained in this study. Videotaping was not used because it does not have the sensitivity to detect a change. Functional testing may not be appropriate, since negative symptoms of the upper motoneuron syndrome (e.g., weakness) prevent voluntary, goal-directed movement, irrespective of the presence or absence of spasticity.¹ The Disability Assessment Scale measures a range of variables and is an appropriate first step in understanding the effects of the toxin treatment.

Clinically meaningful end points were explored in the development of this assessment scale. Input from patients and physicians indicates that for many, a primary goal is to reduce muscle tone, not necessarily to improve the active function of the affected limb.

A difference between groups in the mean change on the Ashworth Scale of tone that is smaller than that which we observed with botulinum toxin A has been considered to demonstrate the effectiveness of oral antispasticity treatment.² With regard to concomitant medication and physical therapy, both treatment groups were asked to maintain the regimens they had been receiving before the study.

We reported the results of a single treatment with botulinum toxin A and mentioned the results of an open-label extension of the study. Readers should wait for those results to be published before concluding that our “study does not justify extended injections for most patients with stroke.”

The data requested by Drs. Buitrago and Koolwijk are listed in Table 1. Pain was not a primary symptom for the majority of patients; thus, it would be inappropriate to draw population-based conclusions.

We also wish to make the following corrections.

Table 1. Mean Scores at Base Line and Mean Change at Week 6 for All Variables According to the Disability Assessment Scale.

Variable	Botulinum Toxin		P Value
	Type A (N=64)	Placebo (N=62)	
Hygiene			
Base-line score	1.89	1.69	0.33
Week 6 change	-0.72	-0.14	0.004
Dressing			
Base-line score	2.25	1.94	0.04
Week 6 change	-0.49	-0.15	0.03
Pain			
Base-line score	0.78	0.94	0.27
Week 6 change	-0.37	-0.25	0.72
Limb position			
Base-line score	2.03	1.92	0.73
Week 6 change	-0.62	-0.24	0.02

The primary end point of this study, according to the protocol, was the change in the tone of the wrist flexor muscle from base line to week 6. Assessment of functional disability was an additional prespecified end point; week 6 was considered the primary end point of interest. In addition, on page 397, the fifth line of the section on “Efficacy” should read “62 in the placebo group” rather than “64 in the placebo group.”

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Editor's note: The study was supported by Allergan. Drs. Brashear and Gordon have received research grants and honorariums for speeches from Allergan.

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Peginterferon Alfa-2a plus Ribavirin for Chronic Hepatitis C

TO THE EDITOR: Fried et al. (Sept. 26 issue)¹ compared three treatment regimens in terms of sus-

tained virologic response in patients with chronic hepatitis C virus (HCV) infection; the regimens were

peginterferon alfa-2a plus ribavirin, interferon alfa-2b plus ribavirin, and peginterferon alfa-2a alone. Naturally, the patients receiving peginterferon alfa-2a plus ribavirin had the highest rate of sustained virologic response. In the community, the current treatment of choice is peginterferon alfa-2b with ribavirin. Thus, this study did not compare the newer regimen, peginterferon alfa-2a with ribavirin, with the best therapy currently available. The rate of sustained virologic response of 56 percent with peginterferon alfa-2a plus ribavirin is quite similar to the rates for peginterferon alfa-2b plus ribavirin in published reports. The media blitz by Hoffmann-LaRoche regarding the publication of this article in the *Journal* is much ado about nothing.

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1. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-82.

DR. FRIED REPLIES: Dr. Rakov correctly notes that our study compared peginterferon alfa-2a and ribavirin with standard interferon alfa-2b and ribavirin. Thrice-weekly treatment with interferon alfa-2b and ribavirin, which was considered the standard of care when our study was initiated in 1999,¹ represented the appropriate treatment for the control group. Peginterferon alfa-2a in combination with ribavirin recently received a unanimous recommendation for

approval by an advisory panel to the Food and Drug Administration.

Our study demonstrated the superiority of peginterferon alfa-2a and ribavirin to standard therapy in terms of the overall sustained virologic response as well as for patients with characteristics associated with resistance to treatment, such as HCV genotype 1 and high levels of HCV RNA before treatment. These higher response rates were attained with a lower incidence of self-reported side effects, particularly influenza-like symptoms and depression. Furthermore, we demonstrated the predictability of a response to antiviral therapy by week 12 on the basis of changes in HCV RNA levels. These data provide extremely important information for the clinician who is treating patients with chronic hepatitis C. The study was not designed to compare peginterferon alfa-2a with combination regimens containing peginterferon alfa-2b.² Physicians and other health care providers should be encouraged to review carefully all the available data from studies such as ours and determine which agent is best suited for their individual patients.

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Increase in Nocturnal Blood Pressure and Progression to Microalbuminuria in Diabetes

TO THE EDITOR: Lurbe et al. (Sept. 12 issue)¹ report that the normal decrease in nocturnal blood pressure may be blunted before the development of microalbuminuria in patients with type 1 diabetes. The authors' suggestion that subtly increased blood pressure is the mechanism behind the nephropathy may be an oversimplification. Although average blood pressure was slightly higher in the patients in whom microalbuminuria subsequently developed, hyperglycemia and higher heart rates were also more common in these patients. Altered autonomic tone may be associated with all these observations, including the increased rate of microalbuminuria.

In patients with diabetes, nocturnal heart-rate variability is blunted, reflecting an altered sympathetic-parasympathetic balance²; the higher nocturnal heart rates in the study by Lurbe et al. can be attributed to this physiologic process. Glycemia may be correlated with increased sympathetic tone — in the presence and in the absence of diabetes.^{3,4} Finally, there is now evidence that sympathetic overactivity, independently of blood pressure, can accelerate nephropathy.⁵

If autonomic tone is what we are measuring with 24-hour blood-pressure monitoring, then perhaps we should consider other options for measuring it. For example, at our institution, ambulatory blood-

pressure monitoring for 24 hours costs \$279, whereas measurement of heart-rate variability with respiratory maneuvers can be performed in 20 minutes at a cost of \$170. Before we advocate ambulatory blood-pressure monitoring to predict which patients with diabetes will have subsequent target-organ disease, we should determine whether the alterations in autonomic balance that are probably responsible for the findings reported by Lurbe et al. can be identified by other (perhaps less expensive) means.

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Editor's note: Dr. Brotman reports having received research support from Boston Medical Technologies, the company that makes the machine that measures heart-rate variability, mentioned above.

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TO THE EDITOR: Lurbe et al. define abnormal blood pressure as a ratio of nighttime to daytime systolic pressure of more than 0.90, which was found in 43 percent of the patients with normoalbuminuria. We have proposed the 90th percentile (ratio of nighttime to daytime systolic pressure, 0.95) as a more appropriate cutoff point.¹

Urinary albumin excretion was essentially unchanged during the 28 months from the first evaluation (11.6 mg per 24 hours) to the final evaluation (16.8 mg per 24 hours) in patients in whom microalbuminuria subsequently developed. This very unexpected finding conflicts with the natural history, which is characterized by a gradual increase in uri-

nary albumin excretion in patients with progression to microalbuminuria.

At the first evaluation, the two groups had the same ratio of nighttime to daytime systolic pressure, and the frequency of microalbuminuria was similar in patients with a normal pattern of blood pressure and those with an abnormal pattern. Thus, ambulatory blood-pressure monitoring two years before progression to microalbuminuria cannot predict this event. The finding of high nighttime blood pressure at the final measurement, just before the patients met the criteria for microalbuminuria, is of limited clinical value. At this late stage of progression to microalbuminuria, the demonstration of elevated nighttime blood pressure suggests a parallel progression of the kidney disease and increase in nighttime blood pressure.

There is a large overlap in the ratio of nighttime to daytime blood pressure both between normoalbuminuric patients with progression to microalbuminuria and those without progression² and between patients with normoalbuminuria and those with microalbuminuria.³ Ambulatory blood pressure cannot predict the development of microalbuminuria years ahead. An early increase in the indicator itself (urinary albumin excretion) within the normoalbuminuric range is still the most useful risk factor.

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TO THE EDITOR: Lurbe et al. found that progression to microalbuminuria was preceded by increased nocturnal blood pressure in patients with type 1 diabetes. However, it is questionable whether this conclusion can be generalized to all patients with type 1 diabetes, since the poor metabolic control (glycosylated hemoglobin level, approximately 10.0 percent) in their patients may have influenced the

results. Moreover, fixed intervals, instead of the patient's records, were used to define daytime and nighttime periods, which may have led to misclassification. Nocturnal blood pressure is associated with the glycosylated hemoglobin level,¹ and inclusion of both variables in the same regression model may therefore be inappropriate.² Finally, microalbuminuria may be transient, and reversal to normoalbuminuria occurs in about 30 percent of patients with type 1 diabetes.³

We previously reported⁴ that a blunted fall in nocturnal blood pressure in normotensive patients with type 1 diabetes and normoalbuminuria was associated with increased levels of urinary albumin excretion (within the normal range) and with a predominance of sympathetic activity. Furthermore, after a similar follow-up period (mean [\pm SD], 66.0 \pm 38.9 months) in 51 patients with type 1 dia-

betes (mean age, 34.0 \pm 8.2 years; duration of diabetes, 10.5 \pm 7.6 years), we found that only 1 patient had progression to microalbuminuria, and another had progression to proteinuria. The better metabolic control (glycosylated hemoglobin level, 8.1 \pm 1.8 percent; reference range, 4.6 to 6.0 percent) in our patients may explain the differences between our results and those of Lurbe et al. We hypothesize that glycemic control, rather than nocturnal blood pressure, is the main determinant of progression to microalbuminuria in patients with type 1 diabetes.

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TO THE EDITOR: Lurbe and colleagues report that the nondipping status precedes the development of microalbuminuria in normotensive patients with type 1 diabetes. Moreover, when microalbuminuria and a reduced fall in nocturnal blood pressure are detected in persons with type 1 diabetes, hypertension is usually absent, whereas persons with type 2 diabetes usually have overt hypertension when microalbuminuria and a nondipping status are first detected.¹

We investigated the relation between urinary albumin excretion and morning blood-pressure variations in 31 normotensive patients with newly diagnosed type 2 diabetes. In a comparison of patients who did not have microalbuminuria with those who did, the latter had significantly higher morning blood pressure ($P<0.003$), whereas daytime and nighttime blood pressures were similar in the two groups (Table 1). The association between morning blood pressure and microalbuminuria persisted after adjustment for potential confounders such as sex, age, and duration of diabetes.

Table 1. Characteristics of Patients with Type 2 Diabetes According to the Presence or Absence of Microalbuminuria.*

Characteristic	Micro-albuminuria (50.7 \pm 10.9 mg/ 24 hr)	Normo-albuminuria (10.2 \pm 9.2 mg/ 24 hr)	P Value
No. of patients	14	17	
Age (yr)	50 \pm 8	49 \pm 7	0.32
Male sex (no.)	7	9	0.41
Current smoker (no.)	3	5	0.25
Body-mass index†	26 \pm 3	27 \pm 2	0.11
Duration of diabetes (yr)	7.6 \pm 1.2	7.2 \pm 1.1	0.16
Glucose (mmol/liter)	8.0 \pm 1.0	7.9 \pm 0.6	0.19
Glycosylated hemoglobin (%)	8.8 \pm 0.8	7.8 \pm 0.8	0.01
Clinic blood pressure (mm Hg)			
Systolic	126 \pm 11	124 \pm 9	0.15
Diastolic	79 \pm 7	80 \pm 8	0.11
Clinic heart rate (beats/min)	74 \pm 9	75 \pm 11	0.55
Ambulatory blood pressure (mm Hg)			
Morning (mean, 6 a.m. to 10 a.m.)			
Systolic	146 \pm 12	119 \pm 11	<0.003
Diastolic	91 \pm 8	70 \pm 9	<0.005
Daytime (mean, 10 a.m. to 10 p.m.)			
Systolic	120 \pm 10	118 \pm 11	0.12
Diastolic	74 \pm 6	74 \pm 5	0.21
Nighttime (mean, 10 p.m. to 6 a.m.)			
Systolic	110 \pm 7	109 \pm 8	0.16
Diastolic	63 \pm 4	62 \pm 5	0.12

* Plus-minus values are means \pm SD.

† The body-mass index is the weight in kilograms divided by the square of the height in meters.

Blood pressure falls markedly in the nighttime because of the reduction in sympathetic activity that is brought about by sleep and then increases steeply in the morning when the person awakens and resumes his or her daily activities.² In patients with type 2 diabetes, this increase occurs together with a peak incidence in the onset of acute cardiovascular disease in the morning hours,³ which is why an enhanced rise in morning blood pressure is widely regarded as an adverse phenomenon that needs to be counteracted by the blood-pressure-lowering effect of treatment.⁴ The increase in morning blood pressure and microalbuminuria coexist in normotensive patients with newly diagnosed type 2 diabetes and may contribute to the increased cardiovascular risk among these patients.

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THE AUTHORS REPLY: We agree with Brotman and colleagues that nocturnal hypertension and an elevated heart rate in patients with diabetes may reflect an altered sympathetic-parasympathetic balance. Blood pressure and its circadian variation depend on multiple factors, not only autonomic tone. The idea that hyperglycemia increases sympathetic tone and, independently of blood pressure, accelerates nephropathy is nevertheless intriguing. Consideration of the cost of testing was beyond the scope of our article.

Poulsen and associates suggest a nighttime-to-daytime ratio of 0.95 as a criterion for nocturnal hypertension. We chose the widely used but equally arbitrary ratio of 0.90 as a cutoff point.¹ They note the absence of a significant increase in urinary albumin excretion, within the normoalbuminuric range, in the patients in our study in whom microalbuminuria ultimately developed. The increase in nocturnal

blood pressure occurred during a mean interval of 28 months, during which urinary albumin excretion had not changed significantly (from 11.6 to 16.8 mg per 24 hours).² Thus, our key finding, an increase in nocturnal blood pressure that antedated the development of microalbuminuria, is not affected by our not having documented a gradual increase in urinary albumin excretion. Regardless of whether an increase in urinary albumin excretion within the normoalbuminuric range is or is not a good marker of progression, a normal ratio of nighttime to daytime systolic blood pressure has a strong negative predictive value for the development of microalbuminuria.²

Caramori et al. comment on glycemic control. We stated that a higher level of glycosylated hemoglobin is a predictor of the risk of microalbuminuria.² The patients in their study were older and possibly more compliant with regard to insulin and diet, which may explain, in part, the low rate of progression to microalbuminuria. In the absence of an elevation in blood pressure, however, poor glycemic control alone may not have a decisive role in the progression to microalbuminuria, as suggested by our subgroup analysis based on the glycosylated hemoglobin level.² Moreover, progression to overt nephropathy is influenced by the blood pressure level perhaps more than by the degree of glycemic control.³ Regarding fixed intervals as compared with the patient's records, the nighttime-to-daytime ratio taken from a fixed interval avoids transitional effects and, in our experience, yields results similar to those with self-reporting.

Marfella et al. provide their data from patients with type 2 diabetes. We appreciate the importance of examining blood pressure in the early morning hours because of the high incidence of cardiovascular events at this time of the day.⁴ Their data, however, show normal blood pressure in the clinic, which is contrary to the view that patients with type 2 diabetes are clearly hypertensive when microalbuminuria develops.⁵

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Nephrolithiasis, Osteoporosis, and Mutations in the Type 2a Sodium–Phosphate Cotransporter

TO THE EDITOR: In the report by Prié et al. (Sept. 26 issue)¹ indicating that inactivating mutations in the sodium–phosphate cotransporter gene, *NPT2a*, are associated with renal phosphate wasting and bone demineralization, the data on expression do not support a dominant negative effect of the V147M mutation. As the authors themselves acknowledge, coinjection of 10 ng of wild-type RNA and 10 ng of mutant (V147M) RNA yielded a phosphate-induced current “similar to that in oocytes expressing 10 ng of wild-type *NPT2a* RNA alone.” The comparison of this current with that produced by injection of 20 ng of wild-type RNA is largely irrelevant. Furthermore, the V147M mutation predicts an amino acid substitution in a transmembrane domain, a less likely site for interaction with other proteins.

In contrast, the data on the A48F mutation do support a dominant negative mechanism. This mutation is in the NH₂ terminal portion of the protein, thought to reside inside the cell,² and could plausibly alter protein–protein interactions. The serum phosphorus concentration in the patient with this mutation was distinctly lower than those in the other 19 patients in the study. Although heterozygous for the A48F mutation, this patient has many of the phenotypic features of mice homozygous for the disrupted *NPT2a* gene,³ including renal calcification.⁴ However, it is important to note that heterozygous mutant mice (with one disrupted *NPT2a* allele) have neither hypercalciuria³ nor nephrocalcinosis.

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THE AUTHORS REPLY: Drs. Scheinman and Tenenhouse question the possibility that the V147M mutation has a dominant negative effect on phosphate transport. As shown in Figure 3 of our article, phosphate-induced current increases with increasing doses of *NPT2a* RNA and reaches a plateau at approximately 30 ng of RNA. Thus, in the absence of a dominant negative effect, the current measured in oocytes coexpressing 10 ng of wild-type RNA and 10 ng of mutant RNA should approximately equal the sum of the currents measured in oocytes expressing either wild-type or mutant *NPT2a* RNA alone. We found that phosphate-induced current in oocytes coinjected with wild-type RNA and either V147M or A48F mutant RNA (10 ng of each) was significantly lower than the sum of these two values ($P < 0.005$ for both comparisons). These results demonstrate a dominant negative effect. The current produced by 20 ng of wild-type RNA was measured (Fig. 4 of the article) to demonstrate that the reduction in phosphate-induced current in coinjected oocytes could not be explained by a saturation of the cellular pathways required to increase the current.

Mutations in transmembrane domains leading to a dominant negative effect have been reported for various proteins, including ion channels and seven transmembrane receptors.¹⁻³ These effects may be attributable to modifications in the secondary structure of the protein resulting from amino acid substitution. In addition, involvement of transmembrane domains in protein dimerization has been report-

ed.⁴ Therefore, changes in the ability of NPT2a to form multimers or participate in other macromolecular complexes could explain the dominant negative effect that we observed for the V147M mutation.

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Insulin-Injection-Site Reactions Associated with Type I Latex Allergy

TO THE EDITOR: A 35-year-old woman with diabetes presented with pruritic, erythematous, urticated plaques at insulin-injection sites, persisting for up to 48 hours (Fig. 1). Reactions were associated with the use of Humulin I and Humulin S (Lilly) injected with a prefilled cartridge pen and Human Monotard (Novo Nordisk) aspirated from a vial and injected with a latex-free Microfine syringe.

The patient had positive skin-prick-test reactions to an aqueous powdered-latex-glove solution and commercial solutions ALK (960) Latex 100 and 10 HEP (Soluprick SQ). Skin-prick testing directly through an insulin-vial bung was also positive. We then compared injection of 10 U of Human Monotard subcutaneously with a natural-rubber-latex-free syringe, aspirated through the stopper and directly from the glass vial. A pruritic erythematous

response was noted 20 minutes after injection from the “punctured” solution but not after aspiration directly from the vial. Our patient also had a reaction to all constituents of the Novo Nordisk intradermal kit, presumably because of needle penetration of the latex-containing bung in each vial.

Total serum IgE was normal but specific IgE tests with latex and protamine were negative. Patch testing with parabens, zinc sulfate, thiuram mix, and Human Monotard solution “as is” was negative. The manufacturers of the insulin preparations subsequently informed us that the cartridge bungs contain butyl rubber with added dry natural rubber latex, whereas the vial bungs contain butyl rubber with a natural-rubber-latex diaphragm on the outside. Synthetic butyl rubber should pose no hazard to latex-sensitized persons, but the natural rubber latex added to the bungs to provide optimal durability must have been responsible for our patient’s reactions. With a switch to latex-free vials of Hypurin Bovine Lente (CP Pharmaceuticals), our patient had no further injection problems.

Despite documentation of localized and systemic type I reactions to latex in patients with diabetes,^{1,2} the possibility of such a reaction has not been ruled out in the majority of reports of injection-site reactions in the literature. Although evidence suggests that the risk among latex-sensitive patients with diabetes is very low,³ Primeau et al. demonstrated that natural-rubber vial stoppers release sufficient latex protein into solution during storage to elicit positive intradermal skin reactions in latex-allergic persons.⁴

Since the introduction of highly purified human recombinant insulin, injection-site reactions have



Figure 1. Urticated Plaques at Insulin-Injection Sites in a Patient with Diabetes.

been relatively rare, but this case illustrates that type I latex allergy should be considered and that latex-specific IgE measurements may give false negative results.

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