

Acknowledgments— This work was supported in part by a grant from the Ministry of Health and Welfare, Government of Japan.

We thank T. Yamamoto for her excellent technical assistance.

References

1. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Turner RC, Velho G, Chevre J-C, Froguel P, Bell GI: Mutations in the hepatocyte nuclear factor-1 α gene in maturity-onset diabetes of the young (MODY3). *Nature* 384:455–458, 1996
2. Kaisaki PJ, Menzel S, Lindner T, Oda N, Rjasanowski I, Sahm J, Meincke G, Schulze J, Schmechel H, Petzold C, Ledermann HM, Sachse G, Boriraj VV, Menzel R, Kerner W, Turner RC, Yamagata K, Bell GI: Mutations in the hepatocyte nuclear factor-1 α gene in MODY and early-onset NIDDM: evidence for a mutational hotspot in exon 4. *Diabetes* 46:528–535, 1997
3. Iwasaki N, Oda N, Ogata M, Hara M, Hinokio Y, Oda Y, Yamagata K, Kanematsu S, Ohgawara H, Omori Y, Bell GI: Mutations in the hepatocyte nuclear factor-1 α /MODY3 gene in Japanese subjects with early- and late-onset NIDDM. *Diabetes* 46:1504–1508, 1997
4. Yamada S, Nishigori H, Onda H, Utsugi T, Yanagawa T, Maruyama T, Onigata K, Nagashima K, Nagai R, Morikawa A, Takeuchi T, Takeda J: Identification of mutations in the hepatocyte nuclear factor (HNF)-1 α gene in Japanese subjects with IDDM. *Diabetes* 46:1643–1647, 1997
5. Gehring W, Affolter M, Burglin T: Homeo-domain proteins. *Ann Rev Biochem* 63:487–526, 1994
6. Lehto M, Tuomi T, Mahtani M, Widen E, Forsblom C, Sarelin L, Gullstrom M, Iso-maa B, Lehtovirta M, Hyrkko A, Kanninen T, Orho M, Manley S, Turner R, Brettn T, Kirby A, Thomas J, Duyk G, Lander E, Taskinen M-R, Groop L: Characterization of the MODY3 phenotype: early-onset diabetes caused by an insulin secretion defect. *J Clin Invest* 99:582–591, 1997
7. Awata T, Matsumoto T, Iwamoto Y, Matsuda A, Kuzuya T, Saito T: Japanese case of diabetes mellitus and deafness with mutation in mitochondrial tRNA^{Leu(UUR)} gene. *Lancet* 341:1291–1292, 1993
8. Gerbitz KD, Van den Ouweland JMW, Maassen JA, Jaksch M: Mitochondrial diabetes mellitus: a review. *Biochim Biophys Acta* 1271:253–260, 1995
9. Bell GI, Pilkis SJ, Weber IT, Polonsky KS: Glucokinase mutations, insulin secretion, and diabetes mellitus. *Ann Rev Physiol* 58:171–186, 1996

An Epitaph for Sulfated Insulin

Immunologic profile of the last patients as they are switched from sulfated beef to human insulin

Immunologic reaction to exogenous insulin can lead to several clinical manifestations, ranging from local cutaneous reaction at the injection site to severe anaphylaxis (1). High levels of anti-insulin immunoglobulin (Ig) G antibodies can also bind to exogenous insulin and cause immune insulin resistance (IIR), which could lead to frequent ketoacidosis (2,3). Sulfated insulin (SI) was developed in 1964 by adding two to eight sulfate groups to beef insulin. It was commercialized mainly for the treatment of patients suffering from IIR and also from insulin allergy. The use of SI has been shown to decrease insulin requirement in IIR, with improvement of insulin peak action kinetics in these SI-treated patients (4). It may also induce a specific T-cell suppressor response to insulin (3).

Novo Nordisk has been the only pharmaceutical company to produce this form of insulin in recent years. According to the company's most recent survey, performed in 1993, SI was used by only 26 patients worldwide, all of whom resided in Canada. Of the 26 patients originally identified, 11 were no longer using SI for various reasons at study commencement in 1996. The present study was undertaken to monitor the clinical condition and immunologic status of these patients while switching from sulfated beef insulin to recombinant human insulin (HI). Every attempt was made to contact the patients directly and through their physicians, and a standardized questionnaire was sent to each patient's primary care physician to complete in conjunction with the patient. The attending physicians of patients willing to participate in the study were advised to initially decrease the daily insulin dose by approximately 25% when switching from SI to HI (Novolin ge insulin, Human Biosynthetic; Novo Nordisk) and, subsequently, to titrate the insulin dose upward to achieve good glycemic control. Diabetes management was left to their discretion. All patients participating in this study gave their informed written consent, and the study protocol was approved by the

human ethics committee of the Toronto Hospital, University of Toronto.

After the insulin was switched, the body weight, daily insulin dose required, type of insulin, frequency of injections, fasting glucose level, and frequency of mild and severe hypoglycemia were recorded at each visit. Blood was drawn at baseline, at 2–4 weeks, at 8–12 weeks, and at or after 25 weeks to monitor HbA_{1c} (normal range, 4.3–6.1%) and to measure specific anti-insulin antibodies and insulin-specific T-cell proliferation. Insulin-specific antibodies were quantitated by enzyme-linked immunosorbent assay using horseradish peroxidase (HRP)-conjugated polyclonal goat anti-human IgM, IgG, IgA, and IgE (Caltag, San Francisco, CA), or HRP-conjugated anti-human IgG1, IgG2, IgG3, and IgG4 monoclonal antibody (PharMingen, San Diego, CA) as developing antibodies. Assays were performed in triplicate, and the results expressed in optical density (OD) units. Results from individual assays were standardized using sera from individuals with high-titer insulin-specific antibodies. For measurement of insulin-specific proliferation to various concentrations of HI, pork insulin, beef insulin, or SI (0, 50, 100, and 500 μ g/ml), peripheral blood lymphocytes were isolated by Ficoll-Hypaque density centrifugation, and proliferation was measured by addition of tritiated thymidine (1 μ Ci per well) for the final 18 h of incubation. All assays were performed in triplicate, and results were expressed as counts per minute above background proliferation in the absence of antigen.

Of the 15 patients identified as using SI in 1996, 13 agreed to participate in the study. SI was used by eight patients for IIR, by four for insulin allergy, and by one for severe lipoatrophy. The mean duration of SI use was 16.2 ± 9.1 years (mean \pm SEM). One patient withdrew from the study because of a suicide attempt. Another patient died during follow-up from causes unrelated to diabetes. After the switch from SI to HI, there was no significant change in HbA_{1c} ($9.2 \pm 1.7\%$ at baseline vs. $9.5 \pm 1.7\%$ 12 weeks after the switch), although there was a statistically significant increase in daily insulin dose required after 12 weeks (73 ± 41 U/day at baseline [$n = 9$] vs. 86 ± 53 U/day at >12 weeks [$n = 6$], $P < 0.05$). This small but significant increase in daily insulin requirement at 12 weeks, which was not associated with a reduction in HbA_{1c}, could be interpreted as suggesting that SI may have some beneficial effect

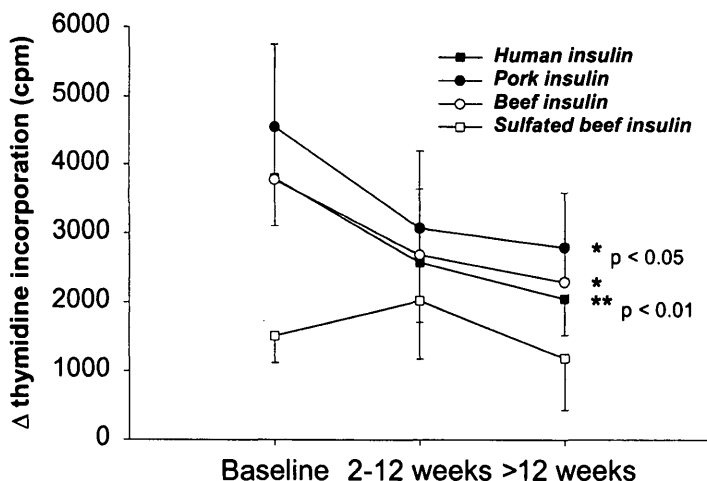


Figure 1—T-cell proliferative response to different insulins (500 µg/ml) at baseline and after switch to HI.

with respect to reducing IIR. However, we caution the reader against overinterpretation of these data, based on the small and clinically insignificant increase in insulin dose and the small number of cases in which data were available for analysis. No patient had any local or systemic allergic reaction to or untoward effect from the introduction of HI. The patients' weight did not significantly change during the study (85.3 ± 25.2 kg at baseline vs. 87.4 ± 25.2 kg at >12 weeks, *P* = NS).

The levels of IgG anti-insulin antibodies observed at baseline in patients treated with SI were similar to those of diabetic patients treated with HI (data not shown). There was a trend toward a decline of anti-insulin IgG levels after the switch from SI to HI (0.200 ± 0.034 OD at baseline vs. 0.166 ± 0.039 OD at 2–12 weeks vs. 0.114 ± 0.031 OD at >12 weeks, *P* = 0.07). The profile of anti-insulin antibodies detected was not altered after switching to HI. There was a statistically significant decline (*P* < 0.05) in the specific T-cell proliferation response to pork, beef, and HI over time (Fig. 1).

Our results are consistent with the study by Davidson et al. (5) showing a decrease of ¹²⁵I-labeled insulin binding after the switch to HI in patients taking sulfated beef insulin for IIR. In that study, the only insulin associated with a recurrence of IIR was beef insulin, and the use of SI for >1 year before the switch provided protection against recurrence. Of note, none of our patients used SI for <3 years before changing to HI.

Several of the patients in our study had never used HI before. In accordance with

the view that HI is less immunogenic than animal insulin, particularly beef insulin, it is possible that the majority of the patients would never have had clinically significant IIR if HI had been available for use earlier (5). However, even the highly purified HI preparations available today can still lead to immune complications (6). Recently, the insulin analog lispro (Lys [B28], Pro [B29]) has been used successfully to treat severe IIR, although the precise mechanism of its decreased immunogenicity remains uncertain (2). It is our conviction that the universal use of HI as well as the advent of synthetic insulin analogs renders treatment with SI outdated and that patients using SI can be safely switched to these newer forms of insulin treatment.

Since the completion of our study, Novo Nordisk has discontinued the production of SI, the last lot having expired in September 1997. With the discontinuation of SI production, another page in the history of diabetes treatment has been turned.

ANDRÉ CARPENTIER, MD, FRCPC

JOAN WITHER, MD, PHD, FRCPC

BRIAN VUKUSIC, BSC

KATHLEEN LAWDAY, BPHARM, MIINFSC

ANDERS H. BOSS, MD

GARY F. LEWIS, MD, FRCPC

From the Divisions of Endocrinology (A.C., G.F.L.) and Rheumatology (J.W., B.V.), Department of Medicine, University of Toronto, Toronto; and Novo Nordisk Canada (K.L., A.B.), Mississauga, Ontario, Canada.

Address correspondence to Dr. Gary Lewis, The Toronto Hospital, General Division, Room EN 11–229, 200 Elizabeth St., Toronto, On, M5G 2C4. E-mail: glewis@torhosp.toronto.on.ca.

G.F.L. has received consulting fees from Novo Nordisk Canada.

Acknowledgments— This work was supported by a research grant from Novo Nordisk Canada. G.F.L. is the recipient of a Career Investigator Award from the Heart and Stroke Foundation of Canada. A.C. is supported by a research fellowship grant from the Juvenile Diabetes Foundation International. J.W. is supported by a Research Scholarship from the Arthritis Society of Canada.

References

1. Patterson R, Roberts M, Grammer LC: Insulin allergy: re-evaluation after two decades. *Ann Allergy* 64:459–462, 1990
2. Lahtela JT, Knip M, Paul R, Antonen J, Salmi J: Severe antibody-mediated human insulin resistance: successful treatment with the insulin analog lispro: a case report. *Diabetes Care* 20:71–73, 1997
3. Naquet P, Ellis J, Kenshole A, Semple JW, Delovitch TL: Sulfated beef insulin treatment elicits CD8⁺ T cells that may abrogate immunologic insulin resistance in type 1 diabetes. *J Clin Invest* 84:1479–1487, 1989
4. Davidson MB, Kumar D, Smith W: Successful treatment of unusual case of brittle diabetes with sulfated beef insulin. *Diabetes Care* 14:1109–1110, 1991
5. Davidson JK, Fineberg SE, De Meyts P, Fineberg NS, Galloway JA: Immunological and metabolic responses of patients with history of antibody-induced beef insulin resistance to treatment with beef, pork, human, and sulfated beef insulin. *Diabetes Care* 15:702–704, 1992
6. Yamagishi S, Toshio A, Taisei S: A case of newly diagnosed non-insulin-dependent diabetes associated with immediate-type allergy against human insulin. *Horm Res* 43:300–302, 1995

Is Diabetic Ketoacidosis a Cause of Meningeal Syndrome?

Case report

Infectious agents have to be ruled out when a patient presents with meningeal syndrome. However, several entities can cause aseptic meningitis (1), and fever per se can produce meningeal irritation (2). We report a patient presenting with diabetic ketoacidosis (DKA) and meningeal syndrome, which resolved with metabolic abnormalities.