Oral Modified Insulin (HIM2) in Patients With Type 1 Diabetes Mellitus: Results From a Phase I/II Clinical Trial

Stephen Clement, Paresh Dandona, J. Gordon Still, and Gordana Kosutic

An effective, orally administered insulin product would be of substantial benefit in the treatment of patients with diabetes mellitus. This phase I/II clinical trial was the first to investigate the safety and effectiveness of a single oral dose of a modified human insulin in controlling postprandial plasma glucose levels in patients with type 1 diabetes mellitus who were receiving basal continuous subcutaneous insulin infusion (CSII) therapy. Fourteen patients with type 1 diabetes mellitus were evaluated in an open-label, 2-center, dose-escalation, nonrandomized study of oral hexyl-insulin monoconjugate 2 (HIM2). After an overnight fast and prior to receiving a standardized meal (50% carbohydrates, 30% fat, 20% proteins; 650 calories), the patients received either no additional insulin (day 1), or 0.5 to 1.0 mg/kg of HIM2 (day 2). All patients received a basal insulin regimen by CSII throughout the study. Blood samples were collected for determination of glucose and insulin levels for 240 minutes post-dose. The postprandial glucose excursion versus time curves showed clear reductions in glucose values after both HIM2 doses (day 2) relative to no treatment (day 1), although the differences in the reductions were not statistically significant. When the data for both HIM2 doses were pooled, a statistically significant effect of HIM2 on glucose excursion (as measured by $AUCex_{30\text{-}240}$) was observed. Mean ± SD values for $AUCex_{30\text{-}240}$ were 501.35 ± 124.1 mg · h/dL after no treatment and 375.81 ± 215.5 mg · h/dL after HIM2 (Wilcoxon signed-rank test, $P = .042$). The results of this study suggest that oral HIM2, when added to a basal insulin regimen, was safe and may prove effective in controlling postprandial hyperglycemia in patients with type 1 diabetes mellitus. Further clinical investigation is necessary.

ELEVATED POSTPRANDIAL glucose concentrations may contribute to ineffective glycemic control in patients with type 1 and type 2 diabetes mellitus, resulting in microvascular and macrovascular complications.[1-5] The liver plays a key role in maintaining normal postprandial glucose homeostasis. At the onset of a meal, hepatic glucose production rapidly decreases while hepatic glucose uptake increases. This rapid change in glucose flux in the liver is mediated, in part, by rapid changes in portal insulin levels.[6-9] An insulin concentration gradient of approximately 2 to 3:1 exists between the portal and peripheral circulation.[10] This gradient may be necessary for normal postprandial glucose control. Also, insulin in the peripheral circulation has been shown to indirectly control hepatic glucose output, possibly by modulating gluconeogenic substrate availability.[11] However, high levels of insulin in the peripheral circulation, which frequently occur with current methods of insulin administration, can lead to hypoglycemia.[12]

We hypothesized that delivery of insulin directly into the portal circulation via intestinal absorption may result in an insulin concentration gradient that is similar to the gradient in individuals without diabetes. A portal to peripheral insulin gradient that is closer to the nondiabetic state, compared to the gradient produced by systemically delivered insulin, may provide a substantial benefit in glucose control without an associated risk of severe hypoglycemia in the treatment of patients with diabetes.

Hexyl-insulin monoconjugate 2 (HIM2) is an orally active insulin created by a site-specific oligomeric modification of recombinant human insulin. The oligomer (PEG moiety plus alkyl linker) is attached to B29 lysine. In comparison to native, nonmodified insulin, HIM2 has enhanced resistance to enzymatic degradation in the gut, greater solubility in both lipid and water-based media, and longer circulating half-life. In a recent clinical study of fasting patients with type 1 diabetes mellitus, reproducible glucose-stabilizing and glucose-lowering effects were observed after oral administration of HIM2.[13]

The primary objective of this phase I/II exploratory study was to determine the safety and tolerability of HIM2 under study conditions. The secondary goal was to determine the postprandial glucose-lowering effects of a single oral dose of HIM2 when added to a basal regimen of short-acting insulin (CSII) therapy in patients with type 1 diabetes mellitus, and to evaluate the dose-response effect over a narrow dose range of HIM2.

MATERIALS AND METHODS

Subjects

Sixteen adult patients with type 1 diabetes mellitus (fasting C-peptide levels < 0.5 nmol/L) provided written informed consent and were enrolled at 1 of 2 study centers. Prior to study enrollment, blood glucose levels for all patients were managed by using short-acting insulin injections in combination with a basal regimen of short-acting insulin using CSII therapy. Fourteen patients completed the study and were analyzed for this report.

Methods

The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practices. The study protocol and informed

From the Georgetown University Medical Center, Washington, DC; Diabetes-Endocrinology Center of Western New York, Buffalo, NY; and the Department of Clinical and Regulatory Affairs, Nobex Corp, Research Triangle Park, NC.

Submitted March 6, 2003; accepted July 11, 2003.

Supported by a grant from Nobex Corp, Research Triangle Park, NC.

Portions of this study were presented in abstract form at the 61st annual meeting of the American Diabetes Association in Philadelphia, PA, June 2001.

Address reprint requests to Stephen Clement, MD, Associate Professor of Medicine, Division of Endocrinology and Metabolism, Georgetown University Medical Center, 232 Building D, 4000 Reservoir Rd NW, Washington, DC 20007.

0026-0495/04/5301-0002$30.00/0

doi:10.1016/j.metabol.2003.07.021

consent form were approved by the Georgetown University Investigational Review Board (Washington, DC) and the Human Research Committee of Millard Fillmore Hospital (Buffalo, NY).

The treatment period consisted of 2 separate days of observation in the research unit. Each test day was separated by 2 to 7 days of rest. Throughout their participation in the study, the patients continued to receive basal insulin by CSII. Neither patients nor investigators made adjustments in the rate of basal insulin infusion on the test days during the study. No bolus insulin injections were given during the pre-dose and evaluation period on the test days. The patients did not consume food after midnight prior to each of the test days, although water was consumed as desired. On the morning of each study day, baseline assessments were performed before the treatment period began. On day 1, all patients received a meal containing an identical number of calories comprised (650 calories) of 50% carbohydrate, 30% fat, and 20% protein at approximately 8 AM. Patients completed eating the meal within 15 to 20 minutes. The percent of food consumed was recorded on the patients' case records forms. No further food was provided over the next 4 hours; however, water was consumed as desired. On day 2, the patients received a single oral dose of either 0.5 mg/kg or 1.0 mg/kg of HIM2 (prepared as a semisolid formulation in unit strengths of 3 mg and 15 mg within opaque, elongated, hard-shell, 2-piece gelatin capsules, size #3 and size #0 respectively, CAPSUGEL/Division of Warner Lambert, Greenwood, SC) with 180 mL to 240 mL of tap water at approximately 8 AM, and the meal was started 30 minutes after dosing. Doses for oral insulin HIM2 were determined based on the data of no observed adverse event levels in 2 animal species (rat and dog) when dosed for 14 days, and on the preliminary clinical data generated in healthy volunteers and type 1 diabetic patients.[13,14] Day 2 procedures were otherwise identical to day 1. This was an escalating-dose study, and neither the dose levels of HIM2 nor the sequence of HIM2 dosing days was randomized. The meal was identical in number of calories and content for all patients on both days of the study. Evaluations at screening, at baseline, during study conduct, and at completion or discontinuation included a physical examination, vital signs, electrocardiogram (ECG), and pregnancy and standard laboratory tests. Standard 12-lead ECGs were performed during screening and at the end of the study.

Blood glucose and insulin concentrations were measured serially over the 240 minutes post-dose observation period. All specimens were centralized and processed in a single laboratory (MDS Pharma, Toronto, Canada). To assure safety blood glucose levels were determined on site using a Yellow Springs Instrument glucose analyzer (YSI Inc, Yellow Springs, OH). Plasma glucose concentrations were determined using a glucose oxidase-based enzymatic assay (Roche Diagnostics/Boehringer Mannheim Hitachi 911 analyzer, Laval, Canada), with a lower limit of detection of 2 mg/dL. Plasma insulin concentrations were determined using a radioimmunoassay kit (Linco Research, St Charles, MO); the cross-reactivity of HIM2 in the insulin assay was 100% and the lower limit of detection was 2 μU/mL (0.07 ng/mL). Insulin concentration values less than the lower limit of detection were assigned a value of 2 μU/mL (0.07 ng/mL).

Data Analysis

This was an early exploratory (phase I/II) study, and the sample size was established accordingly. Efficacy hypotheses were not prespecified, so results are presented descriptively, reported *P* values are uncorrected for multiple comparisons, and all conclusions are to be considered tentative. Data are expressed as means ± SD or means ± SEM. Calculations were performed with SAS version 6.12 (SAS Institute, Cary, NC). Two-hour postprandial glucose, glucose excursion concentrations, and areas under the concentration-time curves (AUCs) for plasma glucose, glucose excursion, and insulin during the post-dose evaluation period were calculated for each patient. Plasma glucose excursions were calculated by subtracting the 30-minute plasma glucose concentration (start of the meal) from each plasma glucose concentration. The linear trapezoidal method was used for calculating the areas under the plasma concentration-time curves. When an additional bolus of insulin ("insulin rescue") was given to the patients during the post-dose/meal observation period the plasma insulin concentration value associated with the collection time immediately prior to rescue was carried forward to all scheduled times after rescue through 240 minutes. Comparisons of glucodynamic parameters between placebo treatments and HIM2 treatments for individual dose groups and for the pooled groups were performed using the Wilcoxon signed-rank test.

RESULTS

Demographic and Baseline Characteristics

A total of 16 patients (all Caucasians, even distribution between genders, means ± SD data: age, 36.9 ± 8.6 years; body mass index [BMI], 26.26 ± 3.05; daily insulin intake, 40.0 ± 11.1 U; and hemoglobin A_{1c} [HbA_{1c}], 7.47% ± 0.67%) with type 1 diabetes mellitus were enrolled in the study. None of the subjects had clinically significant laboratory abnormalities other than those associated with type 1 diabetes. Their average daily insulin requirements ranged from 0.26 to 0.65 U/kg, suggesting a lack of significant resistance to insulin. Two of the 16 patients did not complete the study. Of the 2 patients who did not complete, 1 patient was lost to follow-up and 1 did not complete due to poor venous access.

Safety

A total of 15 patients experienced 58 adverse events. There were no discontinuations due to HIM2-related adverse events. The most frequently reported events were asthenia (4 patients) and headache (4 patients). The following adverse events were reported in 1 or 2 patients: thirst, nausea, anemia, dry mouth, somnolence, ataxia, dizziness, nervousness, paresthesia, amblyopia, urinary urgency, and back pain. There was no dose-related trend in the incidence of adverse events. All adverse events experienced by patients were classified as mild or moderate in intensity; none of the moderate adverse events were associated with study drug. One adverse event of mild intensity (urinary urgency), which was experienced on day 2, was considered to be of unknown relationship to study drug. Review of clinical laboratory test results (hematology, clinical chemistry, and urinalysis) revealed no clinically important changes related to HIM2 administration. Individual clinical laboratory values were comparable before and after administration of the study drug, with some hematology and clinical chemistry parameters falling outside of normal reference ranges. The majority of the out of range values was expected for patients with diabetes and was not considered clinically important. With the exception of pulse rate, mean vital sign values were comparable between dose groups at each assessment. Mean pulse rates were higher for the HIM2 1.0-mg/kg dose group than for the HIM2 0.5-mg/kg group at several post-baseline assessments; however, this difference was not considered clinically important.

To ensure the safety of all study participants, supplemental intravenous glucose (glucose rescue) was to be administered to patients with blood glucose levels less than 60 mg/dL (YSI data) and clinical symptoms of hypoglycemia. Also, any patient whose blood glucose levels were greater than 400 mg/dL

Table 1. Effect of HIM2 on Postprandial Glucodynamic Parameters

Parameter	0.5 mg/kg HIM2 (n = 8)			1.0 mg/kg HIM2 (n = 6)			Pooled (0.5- and 1.0-mg/kg groups) (N = 14)		
	Basal Insulin Only*	Basal Insulin and HIM2*	*P* Value†	Basal Insulin Only*	Basal Insulin and HIM2*	*P* Value†	Basal Insulin Only*	Basal Insulin and HIM2*	*P* Value†
Baseline plasma glucose (mg/dL)	132.9 ± 33.5	154.6 ± 20.9		147.7 ± 13.4	153.8 ± 19.7		139.2 ± 27.1	154.3 ± 19.6	
2 pp (mg/dL)	319.4 ± 53.0	286.9 ± 91.1	.250	342.5 ± 59.2	285.0 ± 112.8	.563	329.3 ± 54.8	286.1 ± 96.8	.177
$AUC_{0\text{-}240}$ ($mg \cdot h \cdot dL^{-1}$)	1,039.49 ± 180.58	961.87 ± 272.71	.641	1,151.09 ± 188.36	950.49 ± 345.69	.563	1,087.32 ± 185.71	956.99 ± 293.33	.391
2 pp_{ex} (mg/dL)	183.6 ± 53.7	142.4 ± 77.7	.211	179.7 ± 35.1	144.2 ± 81.4	.563	181.9 ± 45.1	143.1 ± 76.2	.135
$AUCex_{30\text{-}240}$ ($mg \cdot h \cdot dL^{-1}$)	497.10 ± 142.83	373.09 ± 221.62	.109	507.02 ± 106.85	379.44 ± 227.88	.313	501.35 ± 124.10	375.81 ± 215.48	.042

Abbreviations: 2 pp, 2-hour postprandial plasma glucose concentration; $AUC_{0\text{-}240}$, plasma glucose concentration-time curve from 0 to 240 minutes after HIM2 plus basal insulin or after basal insulin or after basal insulin alone (calculated using the linear method. 2 pp_{ex}, glucose excursion at 2 hours after the standard meal, calculated by subtracting the baseline (immediately prior to meal) glucose concentration from each postprandial glucose concentration; $AUCex_{30\text{-}240}$, area under the glucose excursion concentration-time curve from 30 to 240 minutes after HIM2 plus basal insulin or after basal insulin alone (calculated using the linear trapezoidal method).

*Data are expressed as mean ± SD.

†P values for differences between Day 1 (basal insulin only) and Day 2 (basal insulin and HIM2) were determined using the Wilcoxon signed rank test and were based on within patient comparisons. P-values were not determined for differences in baseline plasma glucose levels.

received supplemental intravenous insulin (insulin rescue) to lower blood glucose concentrations to safe levels. A total of 12 hyperglycemic events were reported throughout the study; of these 12 events only 3 were reported during the HIM2 administration post-dose/post-meal observation period. These events occurred at 165 minutes, 105 minutes and 240 minutes post-meal with reported glucose values of 407 mg/dL, 378 mg/dL, and 399 mg/dL, respectively. All other hyperglycemic events occurred on study day 1, when no additional pre-meal insulin was administered. No patient required supplemental glucose for hypoglycemia on days 1 and 2. One patient experienced hypoglycemia (glucose < 60 mg/dL) after HIM2 dosing on day 2 at 150 minutes after the start of the postdose evaluation period, but the patient did not receive any supplemental glucose.

Effect of oral HIM2 on Pharmacokinetic and Glucodynamic Parameters

All patients but one consumed 100% of standardized meal on both study days. Female patient #224 consumed on both study days 75% of the offered meal.

No statistically significant differences were observed in pharmacokinetic and glucodynamic parameters between the 0.5-mg/kg and the 1.0-mg/kg HIM2 groups, suggesting that the effects of HIM2 were not dose-dependent under the conditions of this study (Table 1). Therefore, the 2 treatment groups were combined for analysis. Figure 1 illustrates mean ± SE plasma glucose and insulin concentrations during the post-dose /post-meal evaluation period after combining data from the 0.5-mg/kg group and the 1.0-mg/kg treatment groups on day 1 (after no treatment) and on day 2 (after receiving HIM2). Mean postprandial plasma glucose concentrations were consistently higher throughout the evaluation period with CSII therapy alone when compared with HIM2 in combination with CSII therapy.

The following glucodynamic parameters were calculated by combining data from the 0.5-mg/kg and 1.0-mg/kg treatment groups: 2-hour postprandial plasma glucose concentration (2 pp); area under the plasma glucose concentration-time curve from 0 to 240 minutes ($AUC_{0\text{-}240}$); 2-hour postprandial glucose

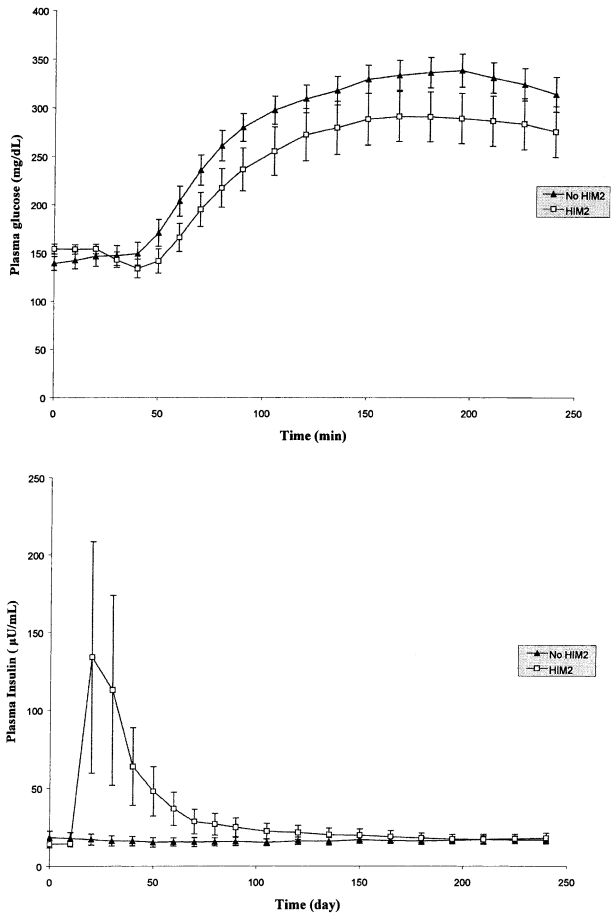

Fig 1. Mean postprandial plasma insulin and glucose concentrations. The mean ± SEM plasma glucose (A) and insulin (B) concentrations during the 4-hour post-dose observation period are shown. (A) Time 0 is defined as the start of the meal. (B) Time 0 is defined as the time of the dose (HIM2 or no HIM2). For 2 patients who required insulin rescue due to hyperglycemia (see Results), the insulin concentration immediately prior to rescue was carried forward for subsequent time points during the post-dose observation period.

excursion concentration ($2pp_{ex}$); and area under the glucose excursion versus time curves from 30 minutes (start of meal) to 240 minutes ($AUCex_{30\text{-}240}$) (Table 1). Glucodynamic variables after receiving HIM2 (ie, CSII therapy plus HIM2) or not receiving HIM2 (ie, CSII therapy alone) 30 minutes before the meal were compared. Baseline plasma glucose levels were not significantly different between no treatment and HIM2 treatment. The most distinct effect of HIM2 was the decrease in postprandial glucose $AUCex_{30\text{-}240}$ observed after HIM2 treatment when compared to no treatment ($P = .042$ by Wilcoxon signed rank test), although in light of the multiple analyses performed this must be considered only suggestive of a real effect.

The relationship between postprandial plasma glucose excursion concentrations and plasma insulin concentrations was investigated by plotting plasma glucose $AUCex_{30\text{-}240}$ values and plasma insulin $AUC_{0\text{-}240}$ values for all patients (Fig 2). Regression analysis revealed an inverse relationship, in that patients with lower plasma glucose $AUCex_{30\text{-}240}$ values also had higher plasma insulin $AUC_{0\text{-}240}$ values (Pearson $r = -0.85$, $P < .001$).

DISCUSSION

An effective, orally administered insulin product would be of substantial benefit in the treatment of patients with diabetes. The present study was the first to investigate the effectiveness of a single oral dose of a modified human insulin in controlling postprandial plasma glucose levels in patients with type 1 diabetes mellitus who were receiving basal CSII therapy. Under the conditions of the study, oral HIM2 was safe and well tolerated. A decrease of 13% to 26% in all glucodynamic parameters that reflect postprandial glucose control was observed during the HIM2 dosing period. The decrease in glucose excursion (as measured by $AUCex_{30\text{-}240}$) was statistically significant after combining data from patients who received 0.5 and 1.0 mg/mL of HIM2. The inverse correlation between peripheral insulin concentrations and blood glucose levels is of interest since it is possible that the primary action of HIM2 is at the hepatic level after its absorption and transport through the portal channels. Since the peripheral systemic concentrations of insulin represents the insulin that is not removed by the liver it is likely that the peripheral insulin concentration observed are in proportion to the amount of insulin delivered and utilized by the liver. An inverse correlation between peripheral insulin levels and blood glucose levels was also observed in a study of HIM2 effects in fasting patients with type 1 diabetes mellitus.[13] The observation that the extent of glycemic control was related to the level of peripheral insulin concentrations in patients who received HIM2 suggests that systemic insulin was likely the major contributor to glucose uptake by skeletal muscle and adipose tissue. Results reported in a recently published study of postprandial control with HIM2 in type 2 diabetic patients suggest a different perspective.[15] In that study oral HIM2 at doses of 0.5 mg and 1.0 mg/kg, provided postprandial glucose levels comparable to 8 U of subcutaneous regular insulin, but at lower peripheral insulin concentrations. This observation is consistent with the hypothesis that oral delivery of insulin may lead to portal-to-peripheral insulin gradients similar to that observed in healthy individuals with normal endogenous insulin secretion.

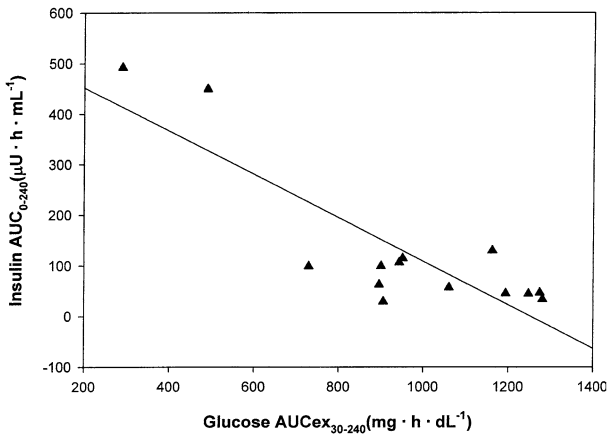

Fig 2. Postprandial plasma glucose $AUCex_{0\text{-}240}$ *v* plasma insulin $AUC_{0\text{-}240}$ values for all patients. The values for postprandial plasma glucose $AUCex_{30\text{-}240}$ and plasma insulin $AUC_{0\text{-}240}$ after treatment with HIM2 on day 2 of the study were plotted for each patient. Pearson $r = -0.85$, $P < .001$

To further investigate the relationship between peripheral insulin levels and glycemic control in patients after oral HIM2 treatment, studies of a multiple-dose HIM2 treatment regimen are necessary.

The lack of a dose-dependent effect could be the result of the narrow dose range studied or variability in absorption of this early prototype formulation. Future work will include studies of potentially improved formulations that are expected to enhance absorption of HIM2.

This study has certain limitations. It was exploratory in nature and was not designed to test any specific efficacy hypothesis. Sample size was determined on the basis of the phase I/II safety and dose-finding requirements (the primary objectives of the study), not on the basis of power to demonstrate efficacy (a secondary objective); the limited number of patients studied did not allow for complete assessment of the glucose-lowering effects of HIM2, or for assessment of the dose-dependent effects. Because doses were always administered in ascending order, consistent with standard Phase I study design, it is not possible to separate dose effects from sequence effects. This could result in false-positive or false-negative dose-response findings, although the length of the washout period renders sequence effects unlikely.

It is anticipated that oral HIM2 may provide a useful alternative to insulin injections. Because orally administered HIM2 is absorbed into the portal circulation, this method of delivery has the potential to provide more complete liver insulinization and to reduce the risk of hypoglycemia that is associated with subcutaneous insulin administration.

ACKNOWLEDGMENT

The authors thank David Sorscher, PhD, and Caren Carver, MTS, for drafting the manuscript; Diana Gibson, MS, for logistical study support and drafting the manuscript; and Susan Howard, MSN, and Lisa Martin, RN, for managing patients.

REFERENCES

1. American Diabetes Association: Postprandial blood glucose. Diabetes Care 24:775-778, 2001

2. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 20:1183-1197, 1997

3. Mohan V, Vijayaprabha R, Rema M: Vascular complications in long-term South Indian NIDDM over 25 years duration. Diabetes Res Clin Pract 31:133-140, 1996

4. Beghi E, Monticelli ML: Diabetic polyneuropathy in the elderly. Prevalence and risk factors in two geographic areas of Italy. Italian General Practitioner Study Group. Acta Neurol Scand 96:223-228, 1997

5. Bavenholm P, de Faire U, Landou C, et al: Progression of coronary artery disease in young male post-infarction patients is linked to disturbances of carbohydrate and lipoprotein metabolism and to impaired fibrinolytic function. Eur Heart J 19:402-410, 1998

6. Cherrington A: Banting Lecture 1997: Control of glucose uptake and release by the liver in vivo. Diabetes 48:1198-1214, 1999

7. Nilsson LH, Hultman E: Liver and muscle glycogen in man after glucose and fructose infusion. Scand J Clin Lab Invest 33:5-10, 1974

8. Taylor R, Magnusson I, Rothman DL, et al: Direct assessment of liver glycogen storage by ^{13}C-nuclear magnetic resonance spectroscopy and regulation of glucose homeostasis after a mixed meal in normal subjects. J Clin Invest 97:126-132, 1996

9. Radziuk J: Hepatic glycogen formation by direct uptake of glucose following oral glucose loading in man. Can J Physiol Pharmacol 57:1196-1199, 1979

10. DeFeo P, Perriello G, de Cosmo S, et al: Comparison of glucose counterregulation during short-term and prolonged hypoglycemia in normal humans. Diabetes 35:563-569, 1986

11. Ader M, Bergman N: Peripheral effects of insulin dominate suppression of fasting hepatic glucose production. Am J Physiol 258: E1020-E1032, 1990

12. Davidson JK: Hypoglycemia, in Conn RB (ed): Current Diagnosis (ed 7). Philadelphia, PA, Saunders, 1985, pp 747-752

13. Clement S, Still G, Kosutic G, et al: Oral insulin product hexyl-insulin monoconjugate 2 (HIM2) in type 1 diabetes mellitus: The glucose stabilization effects of HIM2. Diabetes Tech Therapeut 4:459-466, 2002

14. Still JGL: Development of oral insulin: Progress and current status. Diabet Metab Res Rev 18:S29-37, 2002 (suppl)

15. Kipnes M, Dandona P, Tripathy D, et al: Control of postprandial plasma glucose by an oral insulin product (HIM2) in patients with type 2 diabetes. Diabetes Care 26:421-426, 2003