Postprandial Glycemic Control With Biphasic Insulin Aspart in Patients With Type 1 Diabetes

Kjeld Hermansen, Stein Vaaler, Sten Madsbad, Marian Dalgaard, Mette Zander, Kamilla Begtrup, and Karsten Soendergaard

We sought to investigate the ability of biphasic insulin aspart 30 (BIAsp 30) to control postprandial hyperglycemia and hyperlipidemia in a meal-test comparison with biphasic human insulin 30 (BHI 30). In this randomised crossover trial, 50 patients with type 1 diabetes (mean age, 35.7 ± 9.4 years; body mass index [BMI], 24.0 ± 2.6 kg/m^2; HbA_{1c}, 8.6% ± 1.1%) were studied on 3 separate days, where the following treatments were given in random order: BIAsp 30 injected immediately before a standard breakfast, BHI 30 injected 30 minutes before breakfast (BHI $30_{t=-30}$), and BHI 30 injected immediately before breakfast (BHI $30_{t=0}$). The dose was 0.40 U/kg for all 3 treatments. BIAsp 30 reduced the area under the baseline adjusted 4-hour postprandial serum glucose curve ($AUC_{0\text{-}4h}$) by 23% compared with BHI $30_{t=0}$ ($P < .0001$) and by 9% compared with BHI $30_{t=-30}$ ($P = .013$). Maximum serum glucose concentration (C_{max}) was lower for BIAsp 30 compared with BHI $30_{t=0}$ (14.0 ± 2.4 *v* 16.5 ± 2.8 mmol/L, $P < .0001$), and time to maximal serum glucose concentration (t_{max}) was approximately 20 minutes shorter for BIAsp 30, irrespective of timing of BHI 30 injection ($P < .0001$). There were no significant differences among the 3 treatments with respect to postprandial levels of free fatty acids or triglycerides. The pharmacokinetic results were consistent with the above observations, ie, significantly larger insulin $AUC_{0\text{-}4h}$, higher C_{max} and shorter t_{max} were observed for BIAsp 30 compared with BHI 30, irrespective of timing of BHI 30 injection. We conclude that postprandial glycemic control was more effective with BIAsp 30 than with BHI 30, irrespective of timing of BHI 30 injection.

LARGE PROSPECTIVE and controlled epidemiological studies have confirmed that tight glycemic control reduces the incidence and delays the progression of late diabetic complications associated with type 1 and type 2 diabetes.[1,2] Furthermore, avoiding excessive postprandial hyperglycemia is increasingly considered important in the management of diabetes.[3]

Achieving tight glycemic control in the postprandial phase through insulin therapy is difficult. In healthy individuals, insulin is rapidly released into the systemic circulation in response to food intake. This causes blood glucose to reach peak concentrations 30 to 45 minutes after eating, followed by a decline to basal levels during the following 2 to 3 hours. By contrast, absorption of subcutaneously injected human insulin is relatively slow, in part because insulin molecules tend to self-associate at higher concentrations such as those found in injectable preparations.[4] Peak serum levels are reached after 2 to 3 hours, returning to fasting levels after 6 to 8 hours. The net effect of these nonphysiological pharmacokinetics is early postprandial hyperglycemia and a risk of hypoglycemia before the next meal or during the night.

By replacing certain amino acids, the tendency of the insulin molecule to self-associate can be reduced without affecting its interaction with insulin receptors.[5] One such insulin analog is insulin aspart (IAsp), which is homologous to human insulin except for the substitution of proline with aspartic acid at position B28. Also, insulin aspart is equipotent with human insulin.[6] Faster absorption as well as improved postprandial and long-term glycemic control have been shown for IAsp compared with regular human insulin.[7-11]

Biphasic insulin aspart 30 (BIAsp 30) is a premixed formulation of 30% soluble and 70% protamine-crystallized IAsp. The main advantage of premixed insulin is that fewer daily injections are necessary to obtain reasonably adequate glycemic control. Thus, premixed insulins are widely used by patients with type 1 or type 2 diabetes when more intensive injection regimens are unwanted or impractical.

A faster onset of action has been shown in healthy subjects for BIAsp 30 when compared with a similar biphasic preparation of human insulin (BHI 30).[12] The present trial was the first to directly compare BIAsp 30 and BHI 30 with respect to pharmacokinetics and pharmacodynamics in patients with type 1 diabetes. Postprandial levels of glucose and insulin after a single dose of BIAsp 30 were compared with those after a single dose of BHI 30 injected either immediately before or 30 minutes before a test meal. Furthermore, the effect on postprandial concentrations of free fatty acids and triglycerides was evaluated.

MATERIALS AND METHODS

Subjects

Fifty patients diagnosed with type 1 diabetes for at least 2 years and treated with human insulin for at least 12 months were recruited in Denmark and Norway. Key inclusion criteria were age ≥ 18 years, body mass index (BMI) less than 30.0 kg/m^2, and glycosylated hemoglobin A_{1c} (HbA_{1c}) less than 11.0%. The exclusion criteria included impaired renal or hepatic function, cardiac disease, profilerative retinopathy and/or advanced neuropathy, recurrent severe hypoglycemia, and total daily insulin dose ≥ 1.40 IU/kg. Of the 50 enrolled patients, 46 completed the trial and 4 were withdrawn: 3 because they wished to discontinue. Key subject characteristics are listed in Table 1.

From the University Department of Endocrinology and Metabolism, Århus Amtssygehus, Århus, Denmark; Section of Endocrinology, Department of Medicine, Rikshospitalet, Oslo, Norway; Department of Endocrinology, Hvidovre Hospital, Hvidovre, Denmark; and Novo Nordisk A/S, Bagsværd, Denmark.

Submitted October 25, 2001; accepted January 31, 2002.

Address reprint requests to Kjeld Hermansen, MD, Department of Endocrinology and Metabolism, Aachus Amtssygenus, Aarhus University Hospital, Tage Hansens gade 2, 8000 Århus, Denmark.

0026-0495/02/5107-0022$35.00/0

doi:10.1053/meta.2002.33358

Table 1. Subject Characteristics at Baseline

Age (yr)	35.7 ± 9.4
BMI (kg/m^2)	24.0 ± 2.6
Duration of diabetes (yr)	13.3 ± 8.8
HbA_{1c} (%)*	8.6 ± 1.1

NOTE. Data are presented as means ± SD. n = 50.
*The upper normal limit of HbA_{1c} is 6.2%

Trial Design

The trial was conducted according to a randomized 3-way crossover open-label design. There were three trial days, separated by 5 to 21 days. During these 3 trial days, the following 3 treatments were given in random order: BIAsp 30 (Biphasic insulin aspart 30, Novo Nordisk A/S, Bagsvaerd, Denmark) injected immediately before a standard breakfast, BHI 30 (Mixtard 30, Novo Nordisk A/S) injected 30 minutes before breakfast (BHI $30_{t=-30}$), and BHI 30 injected immediately before breakfast (BHI $30_{t=0}$). The insulin dose was 0.40 U/kg body weight for all 3 treatments. Blood samples were drawn for obtaining 0- to 4-hour postprandial glucose and lipid profiles and 0- to 4-hour postinjection insulin profiles.

Trial Day Procedures

Subjects were on their usual insulin regimen between visits but were instructed not to inject intermediate- or long-acting insulin after breakfast on the day preceding trial days. During the night preceding trial days and until 15 minutes before meals, they were given human insulin (Actrapid, Novo Nordisk A/S) by intravenous infusion to stabilize the preinjection blood glucose concentration between 5 and 8 mmol/L. The rate of insulin infusion was adjusted using a predefined algorithm based on blood glucose concentrations measured every 30 to 60 minutes throughout the night.

Trial day activities were started only if blood glucose measurements were within the 5- to 8-mmol/L range at 15 and 30 minutes before injection of trial product. Insulin was injected subcutaneously into the anterior abdominal wall, midway between the umbilicus and the anterior superior iliac spine, into a raised skin fold and at a 45-degree angle to the skin, alternating between the right and left side. Either of 2 standard breakfasts containing approximately 1,750 kJ and with nearly identical composition was allowed, but each patient had the same breakfast type on each trial day. The calorie distribution of the meal was approximately 15% from protein, 30% from fat, and 55% from carbohydrate. Meals were to be ingested within 15 minutes. During the period from –30 to 240 minutes relative to the time of starting breakfast, 16 blood samples were drawn at regular intervals for measuring glucose and insulin, and 10 blood samples were drawn for measuring triglycerides and free fatty acids. Blood glucose was checked on-site using a Beckman Yellow Spring analyzer for (Palo Alto, CA) control and safety purposes.

Bioanalysis

Serum insulin aspart was measured using an IAsp-specific enzyme-linked immunosorbent assay (ELISA) developed by Novo Nordisk A/S.[13] Serum human insulin was measured using an ELISA assay (DAKO insulin kit, Cambridgeshire, UK; reference code no. K6219). No measurement bias due to the presence of insulin antibodies in the blood samples was expected as both ELISA assays react similarly to insulin antibodies.[13,14] Free fatty acids were measured by a coupled enzymatic method involving acyl-conenzyme A (CoA) synthetase and acyl-CoA oxidase (Wako Chemicals GmbH, Neuss, Germany). Glucose and triglycerides were measured by standard enzymatic methods. Nova Medical Medi-Lab A/S was responsible for carrying out the analyses.

Efficacy and Safety End Points

Glucose: Baseline corrected area under the 4-hour postprandial serum glucose concentration-time curve ($AUC_{0\text{-}4h(SG)}$) was analyzed. Postprandial glucose dynamics were further characterized by the maximum serum concentration of glucose ($C_{max(SG)}$) and the time to reach maximum serum concentration of glucose ($t_{max(SG)}$). Lipids: Areas under the 4-hour postprandial serum concentration-time curve were analyzed for free fatty acids ($AUC_{0\text{-}4h(FFA)}$) and triglycerides ($AUC_{0\text{-}4h(triglycerides)}$). Insulin: Area under the 4-hour post-injection serum insulin concentration-time curve ($AUC_{0\text{-}4h(ins)}$), maximum serum concentration of insulin ($C_{max(ins)}$), and time to reach maximum serum concentration of insulin ($t_{max(ins)}$) were analyzed. Safety: Occurrence of adverse events and hypoglycemic episodes, as well as standard blood biochemistry and hematology parameters were evaluated.

Statistical Analysis

AUC values were calculated using the trapezoidal method. Pharmacokinetic and pharmacodynamic endpoints were analyzed by 2-way analysis of variance (ANOVA) with subject and treatment as factors. All tests were 2-tailed with a significance level of 5%. Analyses of serum glucose AUC were adjusted for baseline glucose level. Except for t_{max} end points, all data were log-transformed when analyzed. Statistical analyses were done using SAS for UNIX version 6.12 (SAS Institute, Cary, NC). Safety data were summarized.

RESULTS

Serum Glucose and Lipids

BIAsp 30 reduced the postprandial serum glucose $AUC_{0\text{-}4h}$ by 23% compared with BHI $30_{t=0}$ ($P < .0001$) and by 9% compared with BHI $30_{t=-30}$ ($P = .013$). Thus, injection of BIAsp 30 immediately before eating resulted in significantly improved postprandial glycemic control compared with BHI 30, irrespective of whether BHI 30 was injected 30 minutes before or immediately before the test breakfast. Serum glucose C_{max} was lower for BIAsp 30 compared with BHI $30_{t=0}$ (14.0 ± 2.4 *v* 16.5 ± 2.8 mmol/L, $P < .0001$), and t_{max} was approximately 20 minutes shorter for BIAsp 30, irrespective of timing of BHI 30 injection ($P < .0001$) (Table 2). Mean pretest fasting serum glucose levels were similar for the 3 treatments (6.7 to 6.9 mmol/L). The mean serum glucose profiles are depicted in Fig 1.

There were no significant differences between treatments with respect to postprandial levels of free fatty acids and triglycerides (Table 2).

Insulin

The pharmacokinetics of BIAsp 30 differed from those of BHI 30: The insulin $AUC_{0\text{-}4h}$ observed for BIAsp 30 was larger by 24% when compared with BHI $30_{t=0}$ ($P < .0001$) and by 16% compared with BHI $30_{t=-30}$ ($P = .0024$) (Fig 2). The faster absorption of BIAsp 30 compared with BHI 30 is reflected in the significantly higher $C_{max\ (ins)}$ and shorter $t_{max\ (ins)}$ values observed for BIAsp 30 (Table 2).

Safety

There were no major but 34 minor hypoglycemic events on trial days: 16 events occurred with BIAsp 30, 9 events with BHI 30 injected at t = 0, and 9 events with BHI 30 injected at t = −30. There were no clinically significant abnormalities in

Table 2. Derived Pharmacodynamic and Pharmacokinetic Parameters

	BIAsp 30	BHI 30	
	$t = 0$	$t = 0$	$t = -30$
Pharmacodynamics			
$AUC_{0-4h(SG)}$			
mmol/L × min	2,280.7 ± 569.9	2,894.1 ± 658.5	2,528.5 ± 731.8
Ratio		0.77 (0.72-0.83)	0.91 (0.84-0.98)
P value		<.0001	.0125
$C_{max(SG)}$			
mmol/L	14.0 ± 2.42	16.5 ± 2.81	14.3 ± 3.41
Ratio		0.85 (0.79-0.90)	1.00 (0.94-1.06)
P value		<.0001	.9380
$t_{max(SG)}$			
Minutes	71.1 ± 24.68	90.8 ± 29.48	91.4 ± 32.89
Difference (min)		−23.2 (−32.6 to −13.8)	−21.8 (−31.2 to −12.3)
P value		<.0001	<.0001
$AUC_{0-4h(FFA)}$			
mmol/L × min	46.8 ± 20.92	54.7 ± 30.93	42.7 ± 27.71
Ratio		0.89 (0.72-1.09)	1.19 (0.96-1.47)
P value		.2592	.1048
$AUC_{0-4h(triglycerides)}$			
μmol/L × min	228.4 ± 91.08	221.7 ± 79.93	218.7 ± 87.13
Ratio		1.01 (0.92-1.10)	1.03 (0.94-1.13)
P value		.8863	.4705
Pharmacokinetics			
$AUC_{0-4h(ins)}$			
mU/L × min	11,141 ± 6,935	9,167.7 ± 6,448.2	9,607.6 ± 6,064.9*
Ratio		1.24 (1.13-1.36)	1.16 (1.05-1.27)
P value		<.0001	.0024
$C_{max(ins)}$			
mU/L	68.2 ± 39.20	49.8 ± 33.02	55.6 ± 29.37
Ratio		1.36 (1.20-1.54)	1.20 (1.07-1.36)
P value		<.0001	.0033
$t_{max(ins)}$			
Minutes	98.1 ± 36.31	139.8 ± 52.94	127.6 ± 71.62*
Difference (min)		−41.1 (−65.9-−16.2)	−29.3 (−54.3-−4.41)
P value		.0015	.0218

NOTE. Results and ANOVA comparisons of BIAsp 30 (injected at mealtime) with BHI 30 injected at mealtime and 30 minutes beforehand, respectively. Data are listed as means ± SD. The ratios and differences (with 95% confidence intervals) refer to ANOVA analyses comparing BIAsp 30 and BHI 30 treatments.

*Data obtained from $t = -30$ to $t = 210$.

vital signs or the investigated biochemical and hematological parameters.

DISCUSSION

This was the first trial to directly compare postprandial pharmacodynamics and pharmacokinetics of the biphasic insulin preparations BIAsp 30 and BHI 30, when administered to subjects with type 1 diabetes in a rigorously controlled meal test setting.

BIAsp 30 injected immediately before the test meal improved postprandial glycemic control compared with BHI 30, irrespective of whether BHI 30 was injected immediately before or 30 minutes before the test meal. Absorption of BIAsp 30 at 0 to 4 hours after injection was significantly faster compared with BHI 30, which accounts for the observed improvement in ability of BIAsp 30 to control postprandial blood glucose levels.

No safety concerns regarding BIAsp 30 have been raised in this or other trials.

The improvement in postprandial glycemic control with BIAsp 30 was greatest when compared with BHI 30 injected at mealtime, which supports the recommendation of injecting human insulins 30 minutes before meals to compensate for the slower absorption. However, it is well known that many patients with diabetes inject human insulin immediately before eating, partly because this often is perceived as more convenient or practical, partly because of the fear of hypoglycemia before meals.[15,16] Due to the rapid absorption and effect of the soluble fraction of BIAsp 30, superior postprandial glycemic control can be gained by injecting BIAsp 30 at mealtimes, which should ensure optimum flexibility as well as the possibility of adjusting insulin doses according to meal size and composition.

ANOVA analyses of 0- to 4-hour postprandial AUC for free

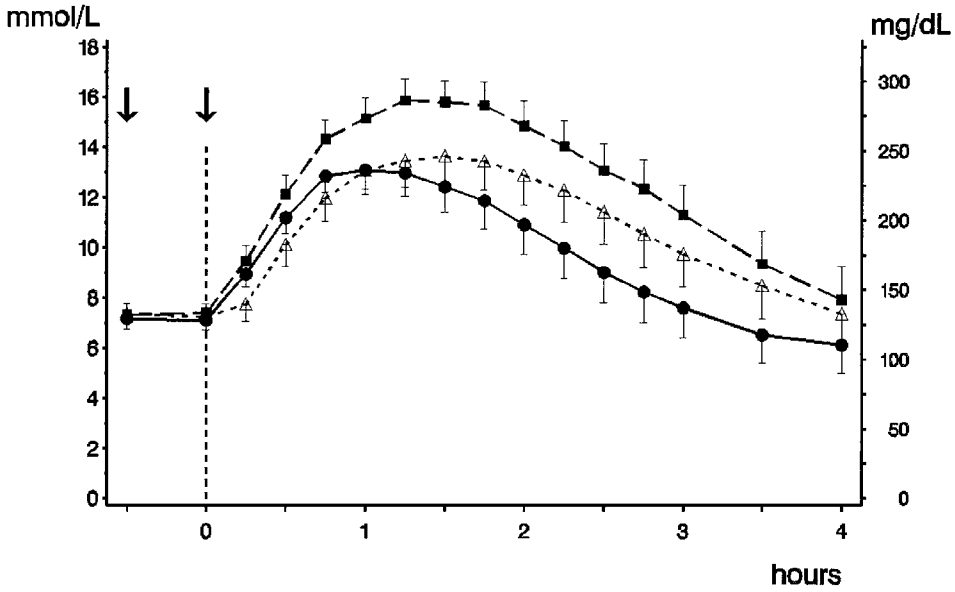

Fig 1. Mean postprandial serum glucose profiles for 46 subjects with type 1 diabetes after injection of BIAsp 30 immediately before a test meal (●); BHI 30 injected immediately before a test meal (■); or BHI 30 injected 30 minutes before a test meal (△). Arrows indicate the injection times, and the vertical dotted line marks the time of meal intake. Error bars represent 2 × SEM.

fatty acids and triglycerides did not show any significant differences between BIAsp 30 and BHI 30. This is not surprising when considering the single-dose trial design. In another single-dose trial in subjects with type 1 diabetes,[17] widely different levels of insulin replacement at meals (effected by varying postmeal insulin infusion rates incrementally) did not significantly influence postprandial triglyceride concentrations. Postprandial levels of free fatty acids were significantly affected, but this was in response to several-fold differences in postprandial insulin concentrations, which was not seen in the present trial. Extension of the observation period to 8 hours, meals with a higher fat content, and multiple-dose trials are probably better suited to assess the effect of insulin therapies on postprandial lipidemia.

Prevention and delay of late diabetic complications has been shown to be dependent on glycemic control as measured by level of HbA_{1c}.[1,2] HbA_{1c} reflects overall glycemic control, but is not dependent on blood glucose fluctuations. The significance of such fluctuations with respect to the development of diabetic complications is not definitely established.[18] However, in the Diabetes Control and Complications Trial (DCCT)[1] it was observed that, at equivalent levels of HbA_{1c}, patients on intensive basal-bolus therapy had a reduced risk of complications compared with patients on conventional insulin therapy. As was speculated by the investigators of the DCCT, this may imply that features of glycemic control not reflected by HbA_{1c} may add to or modify the risk of complications.

Recent studies suggest that postprandial glucose peaks is one such specific risk factor, especially with respect to cardiovascular disease. The large Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study

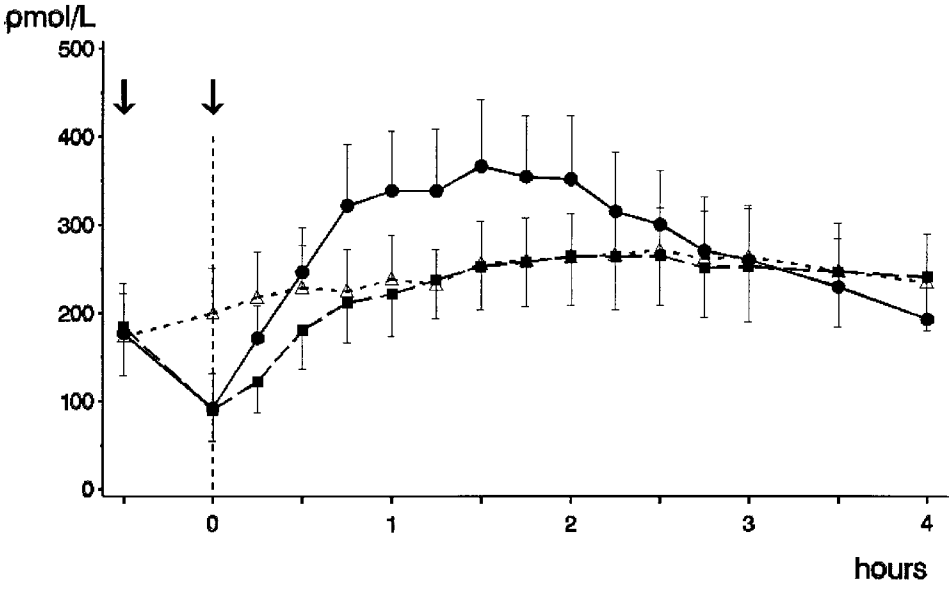

Fig 2. Mean postprandial serum insulin profiles (specific IAsp + human insulin = total insulin) for 46 subjects with type 1 diabetes after injection of BIAsp 30 immediately before a test meal (●); BHI 30 injected immediately before a test meal (■); or BHI 30 injected 30 minutes before a test meal (△). Arrows indicate the injection times, and the vertical dotted line marks the time of meal ingestion. Error bars represent 2 × SEM.

compared 2 standard diagnostic criteria for diabetes—fasting blood glucose concentration and 2-hour postprandial blood glucose concentration following a standard glucose challenge—with regard to prediction of mortality.[19] It was found that 2-hour postchallenge blood glucose was a better predictor of cardiovascular disease and deaths from all causes than was fasting blood glucose. Other studies support these observations.[20-22] This hypothesis of an added benefit of good postprandial glycemic control needs to be tested in a controlled long-term outcome trial.

In conclusion, this trial demonstrated improved postprandial blood glucose control with BIAsp 30 compared with BHI 30, which was caused by a relatively faster absorption of BIAsp 30. Although the relative importance of acute versus chronic hyperglycemia in the development of diabetic complications is not well defined, clinically it seems reasonable to aim at optimizing postprandial as well as overall glycemic control. To this end, biphasic preparations of insulin analogs seem well suited for those who wish to limit the number of daily injections.

REFERENCES

1. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977-986, 1993
2. UK Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352:837-853, 1998
3. Slama G: Clinical significance of post-prandial blood glucose excursions in type 1 and type 2 diabetes mellitus. Int J Clin Pract 112:9-12, 2000 (suppl)
4. Brange J, Owens DR, Kang S, et al: Monomeric insulins and their experimental and clinical implications. Diabetes Care 13:923-954, 1990
5. Brange J, Ribel U, Hansen JF, et al: Monomeric insulins obtained by protein engineering and their medical implications. Nature 333:679-682, 1988
6. Volund A, Brange J, Drejer K, et al: In vitro and in vivo potency of insulin analogues designed for clinical use. Diabet Med 8:839-847, 1991
7. Raskin P, Guthrie RA, Leiter L, et al: Use of insulin aspart, a fast-acting insulin analog, as the mealtime insulin in the management of patients with type 1 diabetes. Diabetes Care 23:583-588, 2000
8. Lindholm A, McEwen J, Riis AP: Improved postprandial glycemic control with insulin aspart. A randomized double-blind cross-over trial in type 1 diabetes. Diabetes Care 22:801-805, 1999
9. Home PD, Barriocanal L, Lindholm A: Comparative pharmacokinetics and pharmacodynamics of the novel rapid-acting insulin analogue, insulin aspart, in healthy volunteers. Eur J Clin Pharmacol 55:199-203, 1999
10. Home PD, Lindholm A, Riis A: Insulin aspart vs. human insulin in the management of long-term blood glucose control in type 1 diabetes mellitus: A randomized controlled trial. Diabet Med 17:762-770, 2000
11. Rosenfalck AM, Thorsby P, Kjems L, et al: Improved postprandial glycemic control with insulin aspart in type 2 diabetic patients treated with insulin. Acta Diabetol 37:41-46, 2000
12. Jacobsen LV, Sogaard B, Riis A: Pharmacokinetics and pharmacodynamics of a premixed formulation of soluble and protamine-retarded insulin aspart. Eur J Clin Pharmacol 56:399-403, 2000
13. Andersen L, Jorgensen PN, Jensen LB, et al: A new insulin immunoassay specific for the rapid-acting insulin analog, insulin aspart, suitable for bioavailability, bioequivalence, and pharmacokinetic studies. Clin Biochem 33:627-633, 2000
14. Andersen L, Dinesen B, Jorgensen PN, et al: Enzyme immunoassay for intact human insulin in serum or plasma. Clin Chem 39:578-582, 1993
15. Overmann H, Heinemann L: Injection-meal interval: Recommendations of diabetologists and how patients handle it. Diabetes Res Clin Pract 43:137-142, 1999
16. Heinemann L: Do insulin-treated diabetic patients use an injection-meal-interval in daily life? Diabet Med 12:449-450, 1995
17. Lewis GF, O'Meara NM, Cabana VG, et al: Postprandial triglyceride response in type 1 (insulin-dependent) diabetes mellitus is not altered by short-term deterioration in glycemic control or level of postprandial insulin replacement. Diabetologia 34:253-259, 1991
18. American Diabetes Association: Postprandial blood glucose. Diabetes Care 24:775-779, 2001
19. The DECODE Study Group–On behalf of the European Diabetes Epidemiology Group: Glucose tolerance and cardiovascular mortality. Comparison of fasting and 2-hour diagnostic criteria. Arch Intern Med 161:397-405, 2001
20. Barrett-Connor E, Ferrara A: Isolated postchallenge hyperglycemia and the risk of fatal cardiovascular disease in older women and men. The Rancho Bernardo Study. Diabetes Care 21:1236-1239, 1998
21. de Vegt F, Dekker JM, Ruhe HG, et al: Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: The Hoorn study. Diabetologia 42:926-931, 1999
22. Hanefeld M, Koehler C, Schaper F, et al: Postprandial plasma glucose is an independent risk factor for increased carotid intima-media thickness in non-diabetic individuals. Atherosclerosis 144:229-235, 1999