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RESEARCH ARTICLE

Pramlintide reduces postprandial glucose excursions when added to insulin lispro in subjects with type 2 diabetes: a dose-timing study

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Abstract

Background To assess the postprandial glucose-lowering effect of the human amylin analog pramlintide when given with insulin lispro in subjects with type 2 diabetes, with an emphasis on the optimal dose timing relative to meals.

Methods In this randomized, single-blind, placebo-controlled, five-way crossover study, 19 subjects with type 2 diabetes using insulin lispro underwent five consecutive mixed-meal tests. In randomized order, subjects received subcutaneous injections of placebo at −15 min or 120-μg pramlintide at −15, 0, +15, or +30 min relative to the standardized breakfast after an overnight fast. Insulin lispro was injected at 0 min at doses that were adjusted appropriately for both the content of the standardized meal and the anticipated effects of pramlintide. Plasma glucose concentrations were measured before and during the 4-h postmeal period.

Results When injected at 0 min, pramlintide reduced the postprandial glucose excursion by 81% compared to insulin lispro + placebo (incremental $AUC_{0-4\,h}$ (mean ± SE) 2.0 ± 1.5 vs. 10.4 ± 2.2 mmol/h/L, $P < 0.05$). When pramlintide was injected at −15, +15, and +30 min, the postprandial incremental glucose $AUC_{0-4\,h}$ was also significantly reduced ($P < 0.05$), but to a lesser extent (42 to 73%). Pramlintide treatment was well tolerated and no serious adverse events were reported.

Conclusions Administration of pramlintide either at or just prior to a meal caused a greater reduction in postprandial glucose than either administration of placebo or postmeal pramlintide injections in subjects with type 2 diabetes treated with a rapid-acting insulin analog, insulin lispro. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords pramlintide; postprandial glucose; type 2 diabetes; insulin lispro

Introduction

Evidence is accumulating to implicate the contribution of postprandial hyperglycemia to poor glycemic control (HbA_{1c}) [1,2], increased microvascular and macrovascular morbidity [3,4], and increased cardiovascular and all-cause mortality [4,5] in patients with type 2 diabetes. Although the outcome of long-term intervention studies with postprandial glucose-lowering agents is awaited [6], there is general consensus that

control of postprandial glucose excursions is an important treatment goal in the pharmacological management of type 2 diabetes [7–9]. Several oral antidiabetic agents and rapid-acting insulin analogues are currently available to treat postprandial hyperglycemia in type 2 diabetes [2], but many patients are unable to achieve the postprandial glucose targets that are recommended by professional diabetes organizations [7–9].

Efforts to normalize excessive postprandial glucose excursions with rapid-acting oral insulin secretagogues or rapid-acting insulin analogues are limited because most patients with type 2 diabetes have prominent peripheral and hepatic insulin resistance. In fact, two recent carefully conducted multitracer studies were inconclusive as to whether insulin lispro, a rapid-acting insulin analogue, facilitates a more rapid rate of plasma glucose disappearance or a more rapid suppression of hepatic glucose production compared to regular insulin in people with type 2 diabetes. In both studies, only a modest reduction in postprandial glucose excursions with insulin lispro compared to regular insulin was observed [10,11].

When evaluating novel approaches to further improve postprandial glucose control in patients with type 2 diabetes, it should be noted that in healthy, nondiabetic subjects, normal postprandial glucose homeostasis is achieved by a complex interplay of several glucoregulatory hormones, including the pancreatic β-cell hormones insulin and amylin, the α-cell hormone glucagon, and the gut hormone glucagon-like peptide-1 (GLP-1) [12–15]. In insulin-treated patients with type 2 diabetes, the postprandial insulin and amylin response is markedly impaired [16–18], whereas the postprandial glucagon response is abnormally increased [19], all of which contribute to excessive postprandial glucose excursions [20].

Pramlintide is a synthetic analogue of human amylin that is under development as an adjunct to insulin therapy in patients with type 1 and type 2 diabetes [15–17,21,22]. Short-term clinical studies in subjects with type 2 diabetes have shown that mealtime amylin replacement via preprandial subcutaneous injections of pramlintide, in addition to regular insulin injections, suppresses mealtime glucagon secretion [23] and slows the rate of gastric emptying [24]. As a result, the appearance of both endogenous (liver-derived) and exogenous (meal-derived) glucose into the circulation is controlled to better match the rate of insulin-mediated glucose disappearance, leading to a substantial reduction of postprandial glucose excursions [25,26].

The objective of the present study was to further examine the effect of pramlintide on postprandial glucose concentrations when used as an adjunct to the rapid-acting insulin analogue, insulin lispro, in people with type 2 diabetes, with an emphasis on the optimal dose timing relative to meals.

Subjects and methods

Study population

A total of 19 subjects with type 2 diabetes receiving treatment with insulin lispro underwent a standardized mixed-meal test on five consecutive days. Subjects were between 18 and 65 years of age and had the following characteristics: a history of type 2 diabetes for at least one year, a baseline HbA_{1c} value between 7 and 11% (HbA_{1c} nondiabetic range was 4.3 to 6.1%, determined by ion-exchange high-performance liquid chromatography), stable daily insulin dose (within $\pm$10% for two months), and no change in type of insulin(s) used prior to the study, if concomitantly treated with metformin, sulphonylureas, and/or thiazolidinediones then had used these agents at a stable dose for two months prior to study, free from severe hypoglycemia (see definition below) or hyperglycemia for two months, and stable weight for two months. Women who were not surgically sterile or postmenopausal were requested to practice appropriate contraception. Subjects were excluded if they had evidence of significant active cardiac disease; untreated or poorly controlled hypertension; or a clinically significant history or presence of hepatic, renal, CNS, gastrointestinal, psychiatric, pulmonary, hematological, autoimmune disease; or malignant disease requiring chemotherapy. Further exclusion criteria included treatment with drugs known to affect gastrointestinal motility (e.g. erythromycin, metoclopramide, cisapride, cholestyramine, or colestipol) or postprandial glucose (α-glucosidase inhibitors and meglitinides).

For evaluation purposes, severe hypoglycemia was defined as those events that required either the assistance of another individual, the administration of glucagon, or the administration of intravenous glucose. Moderate hypoglycemia was defined as symptoms that disrupted activities and were usually associated with a glucose concentration <3.3 mmol/L, and mild hypoglycemia was defined as symptoms consistent with hypoglycemia with or without a glucose measurement and no disruption of activities.

The Institutional Review Board of each study site approved the protocol and all subjects provided written informed consent prior to participation. This study was conducted in accordance with principles described in the Declaration of Helsinki (1964), including all amendments up to and including the South Africa revision (1996).

Study design

Within 14 days of screening, consenting subjects were admitted to the clinical research center for at least six days. Between screening and admission, subjects were asked to record their daily food intake, insulin regimen, and self-monitored blood glucose results in a diary. Upon admission, each subject underwent a mixed-meal test on five consecutive days in a randomized, single-blind,

placebo-controlled, five-way crossover design. The meal was a standardized breakfast, consisting of a bagel with margarine and jam, cheese, yogurt, milk, and orange juice. The size of the meal was calculated individually to provide 30% of a subject's total daily caloric requirements, with a macronutrient composition according to the American Diabetes Association (ADA) nutritional recommendations (55%/15%/30% of kcal from carbohydrate/protein/fat, respectively). The size of the standardized breakfast meal was the same on each study day for each individual and the meal was always consumed within 15 min.

On each day, subjects received one of five treatments (a subcutaneous injection of placebo at −15 min or 120-μg pramlintide [0.6 mg/mL, Amylin Pharmaceuticals, Inc.] at −15, 0, +15, or +30 min relative to the standardized breakfast) according to a randomized sequence, after an overnight fast. Pramlintide or placebo was injected into the subcutaneous tissue of the anterior abdominal wall on the opposite side from the insulin injection. To minimize the confounding effect of ambient glycemia on gastric emptying, and to prevent major imbalances in premeal glycemia across the five meal-test days, investigators were allowed to postpone meal tests by one day if the premeal plasma glucose concentration was <4.4 or >11.1 mmol/L.

Each subject's short-acting insulin dose was adjusted appropriately for both the content of the standardized meal and the anticipated effects of pramlintide based upon the individual subject's history of their usual dietary intake and insulin use. Insulin lispro was injected at 0 min relative to the standardized meal, based on the package insert directions. Efforts were made to keep the short-acting insulin dose constant at the time of each of the standardized breakfast meal challenges. Deviations from the predetermined short-acting insulin dose were allowed only for safety reasons (dose reduction to avoid postprandial hypoglycemia if the premeal glucose was near normal), but not for the purpose of glycemic control (dose increase to improve postprandial hyperglycemia).

In terms of oral antihyperglycemic agents, 2 of the 19 subjects received metformin, 1 received sulfonylurea, 2 received thiazolidinediones, and 3 received a combination of metformin and either a sulfonylurea or thiazolidinedione. Dosing regimens for these agents were held constant throughout the study.

Statistical analyses

Main pharmacodynamic parameters included the incremental plasma glucose area under the concentration time curve (AUC) from 0 to 2 h ($AUC_{0-2\,h}$) incremental $AUC_{0-4\,h}$, and the incremental plasma glucose concentrations at specific sampling times. The mean ± standard error (SE) incremental plasma glucose concentration profiles were calculated and plotted by treatment and by study group. For each study group, the pharmacodynamic parameter data were summarized descriptively and were analyzed using mixed-effect models. The mixed-effect models included treatment, treatment sequence, and period as fixed effects, and subject-within-sequence as random effects.

The *P*-values for comparisons among the least square (LS) means of the incremental $AUC_{0-2\,h}$, incremental $AUC_{0-4\,h}$, and incremental glucose concentrations at various time points between dose timings were provided.

Safety evaluations were based on reports of treatment-emergent adverse events in response to nondirected questioning, clinical laboratory evaluations (hematology, serum biochemistry, urinalysis), vital signs (blood pressure and pulse rate), electrocardiograms, and physical examinations in all subjects.

Results

Subject disposition and baseline demographics

All 19 subjects who were randomized completed the study. The demographic characteristics are shown in Table 1.

Glucose pharmacodynamics

The mean premeal glucose concentrations were comparable across all five study days in the insulin lispro group (Table 2). In all four dose-timing regimens, addition of pramlintide to insulin lispro led to a significant ($P < 0.05$) reduction in postprandial glucose as measured by both $AUC_{0-2\,h}$ and $AUC_{0-4\,h}$ (Table 2 and Figure 1). Overall, pramlintide injection at 0 min reduced the postprandial glucose excursions more definitively than at other dose timings (Table 2 and Figure 1). Pramlintide injections at −15, +15 and +30 min also reduced overall postprandial glucose excursions. Each of these injection times affected the glucose profiles differently. When subjects received pramlintide injections just prior to or with the meal (0 or −15 min), plasma glucose concentrations did not rise during the first hour after the meal, increased gradually during the next 2 h, then regressed toward baseline (Table 2 and Figure 1). When subjects received pramlintide after the meal (+15 or +30 min), plasma glucose

Table 1. Demographic and baseline characteristics

Characteristic	Insulin lispro $n = 19$
Sex, male/female (*n*)	9/10
Race, Caucasian/Black/Hispanic (*n*)	8/1/10
Age (years)	50 ± 9
Weight (kg)	97.4 ± 21.7
BMI (kg/m^2)	35.2 ± 6.7
Diabetes duration (years)	15 ± 10
HbA_{1c} (%)	9.3 ± 1.6

Data, other than sex and race, are mean ± SD.

Table 2. Glucose pharmacodynamics and preprandial insulin dose

Insulin lispro	+ Placebo	+120-μg pramlintide			
Parameter	−15 min (A)	−15 min (B)	0 min (C)	+15 min (D)	+30 min (E)
Preprandial (−5 min) plasma glucose (mmol/L)	8.3 ± 0.5	8.4 ± 0.4	9.4 ± 0.7	8.6 ± 0.5	9.2 ± 0.5
Preprandial short-acting insulin dose (U)*	17.9 ± 2.5	17.7 ± 2.5	18.1 ± 2.7	17.5 ± 2.5	17.9 ± 2.6
Incremental plasma glucose C_{30} min (mmol/L)	+2.1 ± 0.3	+0.1 ± 0.2	−0.1 ± 0.2	+1.7 ± 0.3	+2.2 ± 0.4
Incremental plasma glucose C_{90} min (mmol/L)	+4.3 ± 0.5	+1.3 ± 0.5	−0.2 ± 0.5	−0.4 ± 0.5	+0.6 ± 0.5
Postprandial incremental $AUC_{0-2\ h}$ (mmol/L·h)	+6.1 ± 0.7	+1.4 ± 0.6[a,c]	−0.2 ± 0.6[a,b,e]	+0.6 ± 0.7[a,e]	+2.8 ± 0.8[a,c,d]
Percent reduction in incremental $AUC^{\dagger}_{0-2\ h}$		77%	>100%	89%	55%
Postprandial incremental $AUC_{0-4\ h}$ (mmol/L·h)	+10.4 ± 2.2	+6.1 ± 1.9[a,c]	+2.0 ± 1.5[a,b]	+2.9 ± 1.8[a]	+4.3 ± 2.2[a]
Percent reduction in incremental $AUC^{\dagger}_{0-4\ h}$		42%	81%	73%	59%

Data are presented as mean ± SE and percent reductions were calculated using mean values. Statistically significant ($P < 0.05$) pairwise comparison of LS means denoted by [a]Treatment vs. placebo; [b]Treatment vs. −15 min; [c]Treatment vs. 0 min; [d]Treatment vs. +15 min; [e]Treatment vs. +30 min; *Day −1 insulin dose: 21.3 U; †Relative to the placebo control.

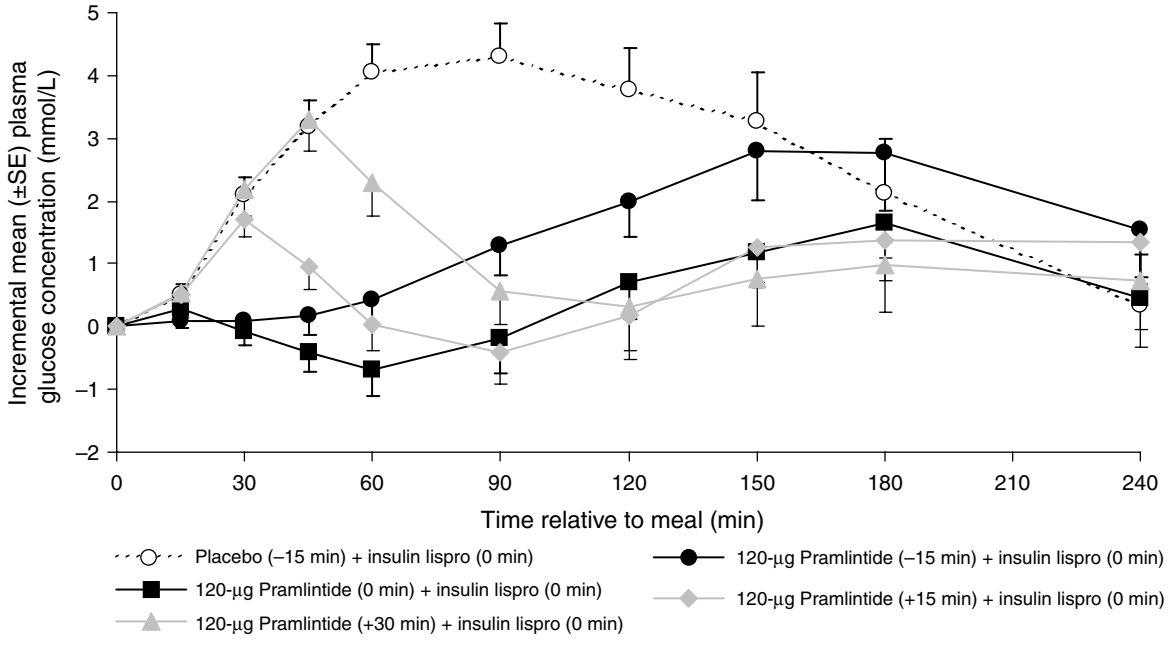

Figure 1. Postprandial glucose profiles in subjects with type 2 diabetes following injections of insulin lispro plus either placebo or 120-μg pramlintide at different time points relative to a standardized breakfast

concentrations increased for the first 30 or 45 min, then rapidly decreased after pramlintide injection, and reached a plateau in the last 2 h (Figure 1).

Preprandial insulin dose

The mean preprandial insulin lispro dose administered with the standardized test meal were comparable on each of the five meal-test days (Table 2). The insulin lispro dose administered at each of the five meal-challenge tests was ∼17% lower than the dose administered on Day-1 (Table 2).

Safety

Pramlintide was generally well tolerated. There were no severe hypoglycemic episodes, no serious adverse events, and no clinically relevant changes in laboratory tests, vital signs, electrocardiograms, or abnormal findings upon physical examinations.

Compared to placebo administration, there was an increased incidence of mild nausea in all pramlintide dose timings and an increased incidence of mild to moderate hypoglycemia (see definitions in Subjects and methods) in the 0, +15, and +30 min pramlintide dose timings (Table 3). During the 4-h postprandial period, the majority of hypoglycemic events associated with pramlintide treatment (6 of 7 hypoglycemic episodes) occurred when the fasting plasma glucose concentration was <7.0 mmol/L.

Discussion

It has previously been shown that pramlintide reduces postprandial glucose excursions when used in conjunction

Table 3. Incidence of hypoglycemia and nausea

	+ Placebo		+120-μg pramlintide							
	−15 min ($n = 19$)		−15 min ($n = 19$)		0 min ($n = 19$)		+15 min ($n = 19$)		+30 min ($n = 19$)	
Insulin lispro	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Hypoglycemia*	2	10.5	0	0.0	3	15.8	3	15.8	5	26.3
Occurred during 4-h postprandial period	0	0.0	0	0.0	3	15.8	2	10.5	1	5.3
Did not occur during 4-h postprandial period	2	10.5	0	0.0	1	5.3	1	5.3	4	21.1
Nausea	1	5.3	3	15.8	4	21.1	2	10.5	3	15.8

Subjects may appear in more than one crossover period. Subjects experiencing multiple episodes of an adverse event within a treatment period were counted once within that treatment period. A subject may appear in more than one crossover dosing period or hypoglycemic timing event.
*Hypoglycemic events were all of mild to moderate intensity and occurred mostly when the fasting plasma glucose concentration was <7.0 mmol/L.

with regular insulin in patients with type 2 diabetes [25]. Reduction in postprandial hyperglucagonemia [23] and slowing of gastric emptying [24] have been identified as key mechanisms underlying this postprandial glucose-lowering effect. The present study revealed several additional important findings.

The results from this study indicate that pramlintide reduced postprandial glucose excursions when used in conjunction with the rapid-acting insulin analogue, insulin lispro. This reduction has importance, given that rapid-acting insulin analogues have themselves been shown to improve postprandial glucose excursions compared to soluble insulin in people with type 2 diabetes [10,11]. The advantage of rapid-acting insulin analogues over regular insulin in these studies was modest and primarily evident in the later part of the postprandial period [10,11]. In fact, the initial surge in plasma glucose (during the first 60 to 90 min) after the meal was not reduced compared to soluble insulin [10,11]. This observation may in part be attributable to the inability of subcutaneously administered insulin (even of a rapid-acting insulin analogue) to mimic the immediate and robust release of endogenous insulin into the portal vein that occurs in nondiabetic subjects after meals [16,17]. Moreover, most patients with type 2 diabetes have marked hepatic and peripheral insulin resistance, further hindering efforts to normalize postprandial glucose excursions with injection of exogenous insulin alone. Unlike insulin and its analogues, which reduce postprandial glucose excursions primarily via a stimulation of glucose disappearance from the circulation, pramlintide reduces postprandial glucose excursions by redressing both endogenous (liver-derived) and exogenous (meal-derived) glucose appearance into the circulation [15–17,21,22]. As a result, the early surge in plasma glucose after a meal is prevented and the overall postprandial glucose excursion reduced. Because the mechanism of action of pramlintide is complementary to that of insulin and its analogues, the postprandial glucose-lowering effect is present regardless of which type of insulin is used.

A systematic evaluation of the different dose-timing regimens showed that when pramlintide was given at 0 min, the postprandial glucose-lowering effect was most pronounced (81% reduction in $AUC_{0-4\,h}$). This potent postprandial glucose-lowering effect occurred, despite a concomitant lowering of the mean preprandial short-acting insulin dose by ~17%. This is also a clinically relevant finding, indicating that optimal postprandial glucose control can be achieved by administration of pramlintide and insulin lispro at the same time, immediately prior to meals. Administration of pramlintide either before (−15 min) or after (+15 min or +30 min) the meal was also capable of reducing postprandial glucose excursions compared to injection of insulin lispro alone, albeit to a lesser extent (42, 73, and 59% reductions in $AUC_{0-4\,h}$, respectively, compared to insulin lispro alone). Specifically, when pramlintide was administered at −15 min, plasma glucose tended to rise in the later half of the postprandial period, whereas when pramlintide was administered at +15 or +30 min, the early postprandial surge in plasma glucose was not prevented. This is consistent with the known mechanism of action of pramlintide; as the rate of glucose appearance into the circulation is reduced to better match the rate of insulin-mediated glucose disappearance, even after the ingestion of the meal, the glycemic surge is curbed and plasma glucose concentrations revert toward lower levels.

In this study, the occurrence of hypoglycemia was examined thoroughly in order to understand the interactions of pramlintide dosing, insulin dosing, glucose pharmacodynamics, and subsequent hypoglycemic events. The finding that most subjects who experienced hypoglycemia during the 4-h postprandial period had premeal glucose concentrations <7 mmol/L indicates that a reduction in dose of preprandial short-acting insulin should be considered when initiating pramlintide treatment, especially in subjects with preprandial blood glucose concentrations close to the normal range.

The present pharmacodynamic study assessed the acute postprandial glucose-lowering effect of pramlintide in a carefully controlled, domiciled setting. However, two 1-year, randomized, double-blind, placebo-controlled trials in insulin-treated patients with type 2 diabetes, have shown that the postprandial glucose-lowering effect of pramlintide resulted in significant and sustained reductions in HbA_{1c} that were accompanied by a relative

reduction in insulin use and no increase in hypoglycemic event rates [27,28].

In conclusion, administration of pramlintide either at, or just prior to, a meal caused a greater reduction in postprandial glucose than either administration of placebo or postmeal pramlintide injections in subjects with type 2 diabetes treated with a rapid-acting insulin analogue, insulin lispro.

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References

1. Riddle MC. Evening insulin strategy. *Diabetes Care* 1990; **13**: 676–686.
2. Del Prato S. In search of normoglycaemia in diabetes: controlling postprandial glucose. *Int J Obes Relat Metab Disord* 2002; **26**(Suppl. 3): S9–S17.
3. DECODE Study Group. Glucose tolerance and cardiovascular mortality. Comparison of fasting and 2-hour diagnostic criteria. *Arch Intern Med* 2001; **161**: 397–404.
4. Donahue RP, Abbott RD, Reed DM, Yano K. Postchallenge glucose concentration and coronary heart disease in men of Japanese ancestry. Honolulu heart program. *Diabetes* 1987; **36**: 689–692.
5. 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* 1999; **42**: 926–931.
6. American Diabetes Association. Postprandial blood glucose (consensus statement). *Diabetes Care* 2001; **24**: 775–778.
7. American Diabetes Association. Standards of medical care for patients with diabetes mellitus (position statement). *Diabetes Care* 2003; **26**(Suppl. 1): 33–50.
8. American Association of Clinical Endocrinologists. The American association of clinical endocrinologists medical guidelines for the management of diabetes mellitus: The AACE system of intensive diabetes self-management – 2002 update. *Endocr Pract* 2002; **8**(Suppl. 1): 40–65.
9. European Diabetes Policy Group. A desktop guide to type 2 diabetes mellitus. *Diabet Med* 1999; **16**: 716–730.
10. Bruttomesso D, Pianta A, Mari A, *et al*. Restoration of early rise in plasma insulin levels improves the glucose tolerance of type 2 diabetic patients. *Diabetes* 1999; **48**: 99–105.
11. Paquot N, Roulin D, Schneiter P, *et al*. Effects of regular insulin or insulin LISPRO on glucose metabolism after an oral glucose load in patients with type 2 diabetes mellitus. *Diabetes Metab* 1998; **24**: 523–528.
12. Scherbaum WA. The role of amylin in the physiology of glycemic control. *Exp Clin Endocrinol Diabetes* 1998; **106**: 97–102.
13. Unger RH. Glucagon physiology and pathophysiology. *N Engl J Med* 1971; **285**: 443–449.
14. Drucker DJ. Glucagon-like peptides. *Diabetes* 1998; **47**: 159–169.
15. Baron AD, Kim D, Weyer C. Novel peptides under development for the treatment of type 1 and type 2 diabetes mellitus. *Curr Drug Targets* 2002; **2**: 62–83.
16. Edelman SV, Weyer C. Unresolved challenges with insulin therapy in type 1 and type 2 diabetes: potential benefit of replacing amylin, a second β-cell hormone. *Diabetes Technol Ther* 2002; **4**: 175–189.
17. Buse JB, Weyer C, Maggs DG. Amylin replacement with pramlintide in type 1 and type 2 diabetes: a physiological approach to overcome barriers with insulin therapy. *Clin Diabetes* 2002; **20**: 137–144.
18. Fineman MS, Giotta MP, Thompson RG, Kolterman OG, Koda JE. Amylin response following Sustacal® ingestion is diminished in type II diabetic patients treated with insulin. *Diabetologia* 1996; **39**: A149 (abstract 566).
19. Dinneen S, Gerich J, Rizza R. Carbohydrate metabolism in non-insulin-dependent diabetes mellitus. *N Engl J Med* 1992; **327**: 707–713.
20. Shah P, Vella A, Basu A, Basu R, Schwenk WF, Rizza RA. Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2000; **85**: 4053–4059.
21. Weyer C, Maggs DG, Young AA, Kolterman OG. Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: a physiological approach toward improved metabolic control. *Curr Pharm Des* 2001; **7**: 1353–1373.
22. Nyholm B, Brock B, Orskov L, Schmitz O. Amylin receptor agonists: a novel pharmacological approach in the management of insulin-treated diabetes mellitus. *Expert Opin Investig Drugs* 2001; **10**: 1641–1652.
23. Fineman M, Weyer C, Maggs DG, Strobel S, Kolterman O. The human amylin analog, pramlintide, reduces postprandial hyperglucagonemia in patients with type 2 diabetes mellitus. *Horm Metab Res* 2002; **34**: 504–508.
24. Vella A, Lee JS, Camilleri M, *et al*. Effects of pramlintide, an amylin analogue, on gastric emptying in type 1 and 2 diabetes mellitus. *Neurogastroenterol Motil* 2002; **14**: 123–131.
25. Thompson RG, Gottlieb A, Organ K, Koda J, Kisicki J, Kolterman OG. Pramlintide: a human amylin analogue reduced postprandial plasma glucose, insulin and c-peptide concentrations in patients with type 2 diabetes. *Diabet Med* 1997; **14**: 547–555.
26. Kolterman OG, Schwartz S, Corder C, *et al*. Effect of 14 days' subcutaneous administration of the human amylin analogue, pramlintide (AC137), on an intravenous insulin challenge and response to a standard liquid meal in patients with IDDM. *Diabetologia* 1996; **39**: 492–499.
27. Ratner RE, Want LL, Fineman MS, *et al*. Adjunctive therapy with the amylin analogue pramlintide leads to a combined improvement in glycemic and weight control in insulin-treated subjects with type 2 diabetes. *Diabetes Technol Ther* 2002; **4**: 51–61.
28. Hollander PA, Levy P, Fineman M, *et al*. Pramlintide as an adjunct to insulin therapy improves long-term glycemic and weight control in patients with type 2 diabetes mellitus: a one year randomized controlled trial. *Diabetes Care* 2003; **26**: 784–790.

