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A 75% insulin lispro/25% NPL mixture provides a longer duration of insulin activity compared with insulin lispro alone in patients with Type 1 diabetes

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Abstract

Aims To compare a new insulin formulation, high mix (HM) [75% lispro (LP) and 25% neutral protamine lispro (NPL)], to regular human insulin (HR) and LP with respect to glucose response and pharmacokinetics following a test meal in patients with Type 1 diabetes.

Methods After fasting overnight, patients received an intravenous insulin infusion to standardize blood glucose (BG) to 7.5 mmol/l (135 mg/dl). In a randomised, three-way crossover study, HR was injected 30 min before, and LP or HM was injected immediately before the test meal on three separate occasions. For each patient, LP and HR were administered at identical doses; the HM dose was one and one third times that of HR and LP to maintain the same dose of short or rapid-acting insulin. The insulin infusion was stopped 15 min after the insulin injection. Free insulin and BG concentrations were measured frequently for 7 h following the test meal.

Results HM and LP resulted in better glycaemic control than HR during the observation period. BG concentrations during the first 4–5 h did not differ between HM and LP. However, HM exhibited prolonged insulin activity relative to LP beyond 5 h, extending the duration of action by approximately 1 h, and resulting in lower overall BG concentrations when the 0–6- and 0–7-h intervals were considered.

Conclusions Compared with LP, HM provided similar glycaemic control for up to 5 h and superior glycaemic control from 5 to 7 h following a standard test meal

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Introduction

Insulin lispro (LP) provides a more physiologic insulin activity profile after subcutaneous (s.c.) injection than regular human insulin (HR) [1]. However, due to the relatively short duration of action of LP, the basal insulin regimen may require optimization, especially in patients with minimal or no residual pancreatic beta cell function [2]. The use of twice daily or multiple daily injections of basal insulin (NPH or ultralente) may be necessary to provide optimal basal insulin replacement in such patients [3–5].

High mixture (HM) is a novel insulin mixture containing 75% LP and 25% neutral protamine lispro (NPL), a protamine-based intermediate-acting LP formulation. Glucose clamp studies comparing HM with LP in non-diabetic subjects have demonstrated that the rapid action of LP is retained within HM and that treatment with HM is associated with a prolongation

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of insulin activity [6]. These characteristics suggest that the use of HM may provide a mechanism for optimising basal insulin therapy in patients using insulin lispro, especially when long time intervals exist between meals. In that setting, unless basal insulin is adequately replaced, blood glucose (BG) concentrations will rise during the late post-prandial period due to the relatively short duration of action of LP. Treatment with a self-prepared mixture consisting of two-thirds LP and one-third NPH has been shown to result in better glycaemic control than either LP or HR alone following a standard test meal in patients with Type 1 diabetes [2]. The LP-to-NPL ratio present in HM may provide a similar improvement in glycaemic control following a similar test meal, while obviating the need for the use of two separate insulins. Thus, the goals of this study were to compare HM with LP and HR with respect to glucose response and pharmacokinetics in patients with Type 1 diabetes mellitus in the setting of a standard test meal.

Patients and methods

Patient population

Thirty-three patients with Type 1 diabetes mellitus enrolled in this study; 31 patients (16 men, 15 women) mean age (± SD) 37.2 ± 7.1 years, mean body mass index (BMI) 24.4 ± 2.5 kg/m^2, and mean duration of diabetes 21.4 ± 8.4 years completed the study. All patients gave a medical history and underwent a physical examination and clinical laboratory tests at a screening visit. In order to enter the study, patients must have been maintained on intensive insulin therapy, defined as pre-meal short-acting HR or LP plus NPH given once or more daily via injection *or* a continuous subcutaneous insulin infusion pump for at least 60 days. They also must have had an HbA_{1c} level of ≤ 150% of the upper limit of the normal (= 9.0%) measured within 60 days prior to the start of the study and a fasting C-peptide concentration of < 0.9 ng/ml. Patients who used ultralente insulin or systemic glucocortocoids within 1 month of the study, had a history of renal transplant, or were undergoing renal dialysis were excluded from the study. All participants provided written informed consent in accordance with the Declaration of Helsinki and local regulations.

Study design

The study was a randomised, open-label, three-way crossover study. All patients were randomised to receive a single subcutaneous (s.c.) dose of each of the three study insulins (LP, HR and HM) across three separate visits. LP (Humalog®), regular human insulin (Humulin R®), and HM were provided by Eli Lilly and Company (Indianapolis, IN, USA) as 100 units/ml preparations.

Prior to each of the three test meal visits, patients fasted for a minimum of 14 h after receiving their evening dose of NPH. Patients using insulin pumps discontinued their basal infusion 2 h prior to the beginning of the experimental period. At each visit, regular human insulin was administered as an intravenous infusion over a period of 3–5 h prior to the test meal. Once the BG had stabilised at 7.5 ± 0.8 mmol/l (135 ± 15 mg/dl) for at least 1 h, patients received a single s.c. dose of the test insulin, according to their sequence group assignment, in either lower abdominal quadrant. The insulin infusion was discontinued 15 min following the injection of the study insulin. LP and HM were administered immediately before and HR 30 min before the test meal. Identical doses of HR and LP were administered, while the doses of HM tested were increased by one-third relative to the HR and LP doses. This adjustment in the HM dose provided identical doses of rapid- or short-acting insulin across all treatments. For example, if a patient were to receive 12 units of LP or HR in conjunction with the test meal, they would be given 16 units (12 multiplied by one-and-one-third) of HM. The dose of insulin to be administered before the test meal was individually selected for each patient based upon the individual experience of the patient and physician.

The standard test meal was a 770 kcal lunch consisting of 57% carbohydrate (110 g), 14% protein, and 29% fat. The test meal was designed such that an insulin dose (LP or HR) of at least 10 units could be administered. Patients were instructed to consume the test meal within 30 min. After the patient finished the test meal, blood samples were collected every 30 min over a 7-h period for measurement of BG and serum insulin concentrations. Patient monitoring was discontinued early if the BG concentration rose to levels ≥ 13.9 mmol/l (250 mg/dl) and if the investigator felt that this represented a significant clinical risk to the patient. In addition, if a patient's blood glucose were to fall below 3.3 mmol/l (60 mg/dl) and remain below that level for a period of time that posed risk to the patient, the patient was to be given carbohydrate, and only serum insulin concentrations would be measured and analysed from that point forward.

Analytical methods

BG concentrations were measured using the YSI Stat Glucose Analyser 2300 (YSI Incorporated, Yellow Springs, OH, USA). Serum obtained during the study was analysed for free immunoreactive insulin (FIRI) concentrations after HR administration and for free LP concentrations after HM and LP administrations. Free insulin concentrations were determined after stripping serum of antibody-bound insulin by the addition of 20% polyethylene glycol and precipitation prior to measurement of insulin concentration. FIRI levels were measured using a double-antibody radioimmunoassay technique (Diagnostic Products Corp., Los Angeles, CA, USA). A separate double-antibody radioimmunoassay method specific for LP was used to measure free LP [7]. A lower limit of quantification of 43.0 pmol/l was established for this assay.

Pharmacokinetic parameters were calculated using non-compartmental methods for adjusted FIRI and free LP concentrations. Adjustments were made for the contribution of the insulin infusion to serum FIRI concentrations. This adjustment was performed to permit comparisons to the assay-specific free LP measurements made during the HM and LP treatments, in which cases there was no concern regarding an effect of the insulin infusion. To make this adjustment, mean pharmacokinetic data from a 0.1-U/kg insulin bolus [9] were fitted to a two-compartment model, and the model was applied to the insulin infused for each patient. The modelled concentrations were then subtracted from the measured concentrations, and

the residual concentrations represented those from the administered regular human insulin. Maximum serum concentrations (C_{max}), time to C_{max} (t_{max}), and area under the curve (AUC_0^∞) were reported, as were the terminal elimination rate constant (λ_z) and half-life ($t_{1/2}$).

Methods similar to non-compartmental pharmacokinetic calculations were employed to assess glucose response parameters. The maximum blood glucose occurring during the first 4 h after the standard meal (0–4 h BG_{max}) was reported. The average blood glucose values during the first 4, 5, 6 and 7 h after dosing (BG_{avg}) were calculated by first calculating the area under the blood glucose-vs.-time curve and then dividing by the time interval over which it was calculated. The BG area under the curve (AUC) was calculated using the trapezoidal rule. When monitoring was suspended because of hyperglycaemia, glucose response parameters were calculated utilising the last observed value carried forward for the remainder of the 7-h observation period (i.e. the last value measured was assigned to all subsequent scheduled measurement times).

All patients completing the study were included in the final analysis. A mixed model for a three-period crossover design was used in the analyses of the pharmacokinetic and glucose response parameters using a two-sided test with a nominal significance level of 0.05. This model included sequence and treatment as fixed factors and patient nested within sequence as a random effect. The distribution of all parameters was tested for normality using a chi-squared test. The data were log-transformed if not normal, with a mixed model applied to the log-transformed data. Additional pairwise comparisons were performed for HM vs. LP, HM vs. HR, and LP vs. HR. Data were analysed using SAS® PROC MIXED statistical software (Cary, NC, USA) [8].

Results

Thirty-one of the 33 patients enrolled in the study received all three study treatments (HR, LP, and HM). One patient was discontinued from the study during the first test meal period due to experiencing hypoglycaemia (blood glucose 59 mg/dl) 75 min after injection of HM. One patient failed to consume the test meal and became hypoglycaemic 20 min after injection of LP. Both patients were treated appropriately without sequelae, and results from these patients were not included in the analyses.

Glucose response

Fig. 1 shows the mean blood glucose profiles from all three treatments. All patients achieved comparable baseline BG concentrations during the intravenous insulin infusion. The mean (± SD) doses of study drugs used in conjunction with the test meals were 14.8 ± 4.1 units for LP and HR (0.20 ± 0.05 units/kg) and 20.0 ± 5.44 units for HM (0.27 ± 0.07 units/kg).

Following the test meal, the mean BG rose to 10–11 mmol/l (180–198 mg/dl) and remained at this level after the HR treatment. The mean peak BG following both LP and HM administration was approximately 9.5 mmol/l (171 mg/dl), but quickly fell to baseline or slightly below, and gradually rose thereafter. Following the decline, the rise in BG concentrations was delayed following treatment with HM when compared with that observed following treatment with LP.

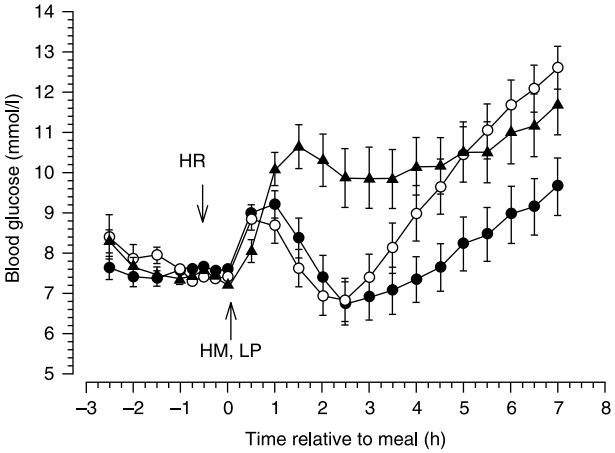

Figure 1 Mean BG concentration vs. time profiles: concentrations with last value carried forward. Closed circles (●), open circles (○), and closed triangles (▲) represent the glucose concentrations after administration of HM, LP, and HR, respectively. Later times (beyond 2.5 h) reflect mean concentrations with the last measured value carried forward. Bars represent standard error of the mean. The arrows indicate the times of administration for the three formulations. All points represent data from at least two-thirds of patients studied.

As stated in Patients and methods, monitoring was discontinued early and supplemental insulin was administered if BG concentrations rose above 13.9 mmol/l (250 mg/dl) anytime between 3.0 and 6.5 h after the test meal, and if this was judged to represent a clinical risk to the patient, i.e. if BG concentrations remained above the stated threshold for a prolonged period, or if the BG concentration was observed to be rising rapidly. Monitoring was discontinued early for three patients after treatment with HM, for 12 patients after LP, and for seven patients after HR. Times of discontinuation (hours) for HM were 5 and 6 (n = 2); for LP were 3.5, 4, 4.5, 5 (n = 3), 5.5, 6 (n = 3) and 6.5 (n = 2); and for HR were 3, 3.5, 5 (n = 2), 5.5 (n = 2) and 6.5. The inclusion of data from patients who discontinued due to hyperglycaemia probably resulted in an underestimate of the mean BG concentration later in the observation period because the BG would almost certainly have continued to rise to concentrations above 13.9 mmol/l (250 mg/dl) in these patients had monitoring been continued. While a minority of the 31 patients completing the study developed blood glucose concentrations of less than 3.3 mmol/l (60 mg/dl) during the observation period (eight after HM, five after LP, and one after HR), none experienced signs or symptoms of or required treatment for hypoglycaemia, and therefore were able to complete the study. All patients experiencing BG below 3.3 mmol/l (60 mg/dl) did so within 2–3.5 h after the administration of study insulin, with the exception of one patient who fell below this threshold at 5.5 h after HM.

Both LP and HM resulted in lower BG concentrations than did HR during the 7-h observation period (Table 1). The early

Table 1 Glucose response measurements for LP, HM, and HR following s.c. administration

Glucodynamic parameter*	LP	HM	HR	P†
0–4 h BG_{max} (mmol/l)	10.7 ± 2.34	10.3 ± 1.89	11.9 ± 3.33	0.003
		$P = 0.376$	$P < 0.001$	
		$P = 0.012$		
0–4 h BG_{avg} (mmol/l)	7.84 ± 2.17	7.76 ± 2.16	9.67 ± 2.93	< 0.001
		$P = 0.834$	$P < 0.001$	
		$P < 0.001$		
0–5 h BG_{avg} (mmol/l)	8.21 ± 2.36	7.75 ± 2.29	9.79 ± 3.02	< 0.001
		$P = 0.238$	$P < 0.001$	
		$P < 0.001$		
0–6 h BG_{avg} (mmol/l)	8.68 ± 2.51	7.88 ± 2.42	9.93 ± 3.08	< 0.001
		$P = 0.045$	$P < 0.001$	
		$P = 0.002$		
0–7 h BG_{avg} (mmol/l)	9.16 ± 2.55	8.08 ± 2.53	10.12 ± 3.13	< 0.001
		$P = 0.007$	$P < 0.001$	
		$P = 0.017$		

*Parameter definitions: 0–4 h BG_{max}, maximum post-prandial glucose concentration during the first 4 h after the meal; 0–4 h BG_{avg}, 0–5 h BG_{avg}, 0–6 h BG_{avg}, 0–7 h BG_{avg}, average glucose concentration during the first 4, 5, 6 and 7 h post-prandial (respectively), with the last value carried forward.
†*P*-value across all comparisons analysed by SAS® PROC MIXED.

peak in BG (0–4 h BG_{max}) was significantly lower for HM and LP than for HR. All measures related to overall BG control showed that HM and LP provided better glycaemic control than HR. In addition, BG measurements over several shorter intervals —0–4, 0–5 and 0–6 h (0–4, 0–5 and 0–6 BG_{avg})—also showed that LP and HM provided better BG control than HR.

Comparisons between LP and HM show similar BG control during the first 4 h following the test meal. BG concentrations began to rise above the pre-meal BG level beyond the 0- to 3-h interval following the test meal after administration of LP and beyond the 0- to 4-h interval for HM (Fig. 1); the increase in BG occurred more rapidly after LP. Thus, HM resulted in significantly lower BG levels at 6 and 7 h after the test meal, although BG_{avg} values began to separate between the two treatments as early as 5 h after dosing (see 0–5 h BG_{avg}, 0–6 h BG_{avg} and 0–7 h BG_{avg} values, Table 1). These results support the conclusion that HM extends the duration of action of LP by approximately 1 hour at the doses employed.

Pharmacokinetics

Serum FIRI concentrations were corrected to account for early contributions to the circulating insulin concentrations by the intravenous insulin infusion. Figure 2 shows the mean serum concentration-vs.-time plots for FIRI (after this adjustment) and for free LP after HM and LP dosing. Mean free LP concentrations after LP and HM injection were similar and revealed rapid absorption and elimination profiles. Free LP concentrations were measurable for a longer period of time after administration

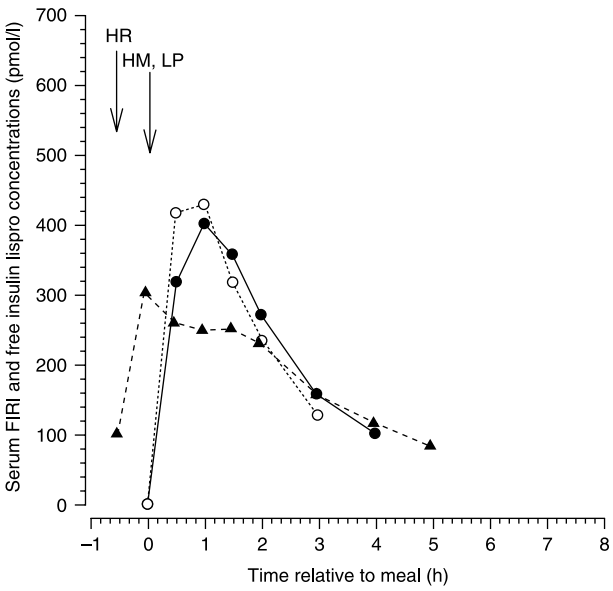

Figure 2 Mean serum insulin concentration vs. time profiles: LP, HM, and HR. Free serum LP concentrations after the administration of LP (○) and HM (●) are compared with the FIRI concentrations after HR administration (▲). The serum FIRI concentrations for HR reflect correction of concentrations for the i.v. insulin infusion. Arrows refer to administration times. All points represent data from at least two-thirds of patients studied. Data do not appear beyond 5 h as the majority of insulin concentrations beyond this time point were below the detection limits of the assay.

Table 2 Non-compartmental pharmacokinetic measurements for LP, HM, and HR following s.c. administration

Pharmacokinetic parameter*	LP	HM	HR	*P*†
C_{max} (pmol/l)	511 ± 296	428 ± 181	382 ± 212	0.031
		P = 0.086	*P* = 0.362	
		P = 0.010		
t_{max} (min)	47 ± 19	62 ± 22	89 ± 56	< 0.001
		P = 0.116	*P* = 0.005	
		P < 0.001		
AUC_0^∞ (pmol h/l)	963 ± 348	1096 ± 448	1038 ± 407	0.057
		P = 0.017	*P* = 0.262	
		P = 0.197		
λ_z (h^{-1})	0.0140 ± 0.0044	0.0107 ± 0.0040	0.0085 ± 0.0039	< 0.001
		P < 0.001	*P* = 0.027	
		P < 0.001		
$t_{1/2}$ (min)	54.2 ± 17.4	72.9 ± 24.1	94.6 ± 35.9	< 0.001
		P = 0.004	*P* = 0.001	
		P < 0.001		

*Parameter definition: C_{max}, maximum plasma concentration; t_{max}, time to achieve C_{max}; λ_z, terminal elimination rate constant; $t_{1/2}$, terminal half-life; AUC_0^∞, area under the plasma concentration vs. time curve from time of administration until infinity.
†*P*-value across all comparisons.

of HM than after LP, reflecting the contribution of the NPL component of the mixture to the pharmacokinetic profile. As expected, HR exhibited a longer half-life than both LP and HM. Figure 2 suggests that the maximum FIRI concentration achieved after administration of HR is reached after 30 min. This artificial 'peak' reflects a bias introduced by a minority of the study patients who had an incomplete correction of the FIRI concentrations contributed by the intravenous insulin infusion.

A summary of the pharmacokinetic measurements is provided in Table 2. Statistically significant differences were observed across the three treatments for all parameters, with the exception of the AUC_0^∞, but a trend toward a significant difference between the treatments was observed for this parameter ($P = 0.057$). Pairwise comparisons between treatments showed statistically significant differences between LP and HM for AUC_0^∞, λ_z, and $t_{1/2}$, presumably reflecting the higher dose of HM given, as well as the contribution of the NPL component of the mixture. As expected based upon the HM dose adjustment, C_{max} and t_{max} did not differ between LP and HM.

Statistically significant differences were also observed between HR and LP, with LP exhibiting a higher C_{max} and earlier t_{max} value than HR. HM also displayed an earlier t_{max} than HR, but no other differences were observed between these two treatments.

Discussion

HM exhibited an extended duration of activity when compared with LP as evidenced by lower BG levels during the 4- to 7-h time interval following the test meal. In addition, both LP and HM provided better glucose control than HR during the 7-h post-test meal observation period. Both of these observations are consistent with the findings of a study that compared LP alone at a dose of 0.15 units/kg with the same dose of HR and to the same dose of LP combined with 0.07 units/kg of NPH in patients with Type 1 diabetes [2]. As in the present study, treatments were compared in the setting of a standard test meal and in the absence of basal insulin therapy. In that study, BG was better controlled by LP than by HR during the early post-prandial period, but began to rise earlier after administration of LP (3–4 h) than after HR (5–6 h). The addition of 0.07 units/kg of NPH to the original LP dose of 0.15 units/kg resulted in a prolongation of insulin activity similar to that provided by the HM formulation in the present study. Taken together, these findings indicate that the NPL component of the HM formulation was responsible for the increased duration of insulin action observed in the present study.

The mean BG-vs.-time profile for LP in the present study is consistent with previously published results [2], but the BG-vs.-time profile for HR is not. In the present study, BG peaked between 1 and 2 h following treatment with HR, and remained at or near this level for the remainder of the observation period, whereas in the previous study [2] the BG concentration fell briefly to near-pre-meal levels before rising again during the late post-prandial period. In another test meal study [10], BG levels had returned to the pre-meal baseline by the end of a 7-h observation period. The reason for the somewhat uncharacteristic response to HR observed in the present study is unclear. Pharmacokinetic comparisons between LP and HM and between LP and HR were consistent with previous observations [1,6].

The greater duration of control provided by HM relative to LP suggests that HM may be preferable to LP as a pre-meal insulin within certain types of insulin regimens. For example, HM before meals plus NPH at bedtime may be preferable to LP before meals plus NPH at bedtime due to the extended insulin activity observed with HM, at least at the doses employed in the present study. This extended activity may result in better between-meal glycaemic control than would be provided by LP alone as the activity of the bedtime NPH wanes during the daytime hours. Several studies in which LP combined with relatively small doses of NPH was given before meals and NPH was given at bedtime have shown that the LP : NPL ratio present within HM may be appropriate before one or more meals within this type of regimen [3–5,11]. Further study will be required to determine whether HM has a role in regimens employing the new long-acting insulin analogue insulin glargine.

While post-prandial BG levels did indeed rise after both LP and HM in this study, BG concentrations after HM were minimally higher than pre-meal levels at 5 h and were substantially lower than BG concentrations after LP from this time point forward (Fig. 1). Nonetheless, it could be argued that the level of glycaemic control beyond 5 h may have been better if another regimen had been utilised such as free mixed LP and NPH prior to the meal with individual optimization of the two insulins. In cases where pre-meal HM does maintain BG concentrations at or near pre-meal levels during the patient's between-meal interval, the use of HM provides convenience, in that it obviates the need for two separate injections for the patient using insulin injection pens, and the mixing of insulins for the patient using vials and syringes. On a related point, due to the relatively large test meal utilised in this study, the LP doses (and therefore the HM doses) utilised in this study were relatively high compared with typical pre-meal LP doses in many patients with Type 1 diabetes, and the relative responses to these insulins may be different at lower doses due to smaller absolute doses of NPL. Further patient-based studies comparing LP with HM at lower doses will help to clarify this point. Finally, a minority of patients experienced relatively low blood glucose concentrations without signs or symptoms of hypoglycaemia following the test meal, suggesting that the dose of study insulin chosen may have been relatively high for these patients. The fact that all but one of these relatively low blood glucose concentrations were observed within 2 to 3 h after the dose of HM suggests that this was due to the action of the lispro component as opposed that of the NPL component of the mixture.

An appreciable degree of dose flexibility for varying carbohydrate intake could be maintained during the use of HM, as the fractional NPL dose would change only slightly in relation to larger changes in the fractional LP dose as the total dose of the mixture is adjusted. For example, increasing the pre-meal HM dose from 12 to 16 units would increase the LP dose from 9 to 12 units, but would only increase the NPL dose from 3 to 4 units, a dose increase that would not likely be clinically relevant in most cases. In practice, the dose of LP would theoretically be chosen based upon the examination of the 2-h post-prandial BG level, whereas the dose of HM would be chosen based upon both the 2-h post-prandial BG and the BG just prior to the next meal. If more NPL is required than allowed by the HM ratio, it is conceivable that a 50 : 50 mix of LP and NPL could be utilised.

In summary, when HM was dosed at one-and-one-third times the comparator dose of LP, HM exhibited prolonged insulin activity relative to LP, extending the effective duration of LP action by approximately 1 h following a standard test meal in C-peptide negative Type 1 diabetes. These findings indicate that HM is capable of providing both rapid-acting insulin action in the early post-prandial period and prolonged insulin action during the late post-prandial period. This mixture has a unique time-action profile which, along with fixed LP : NPL mixtures in other ratios, may be useful in bridging long-between meal intervals within a multiple daily injection regimen.

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