Eur J Clin Pharmacol (1999) 55: 199–203

PHARMACODYNAMICS

P. D. Home · L. Barriocanal · A. Lindholm

Comparative pharmacokinetics and pharmacodynamics of the novel rapid-acting insulin analogue, insulin aspart, in healthy volunteers

Received: 26 October 1998 / Accepted in revised form: 23 January 1999

Abstract *Objective*: The pharmacokinetics of a new insulin analogue, insulin aspart, were compared with unmodified human insulin in a double-blind crossover study of 25 fasting healthy men following a single subcutaneous dose.
Methods: Either insulin aspart or human insulin, 0.1 $U \cdot kg\text{-body-weight}^{-1}$, was injected subcutaneously and followed by determination of 8-h profiles of serum insulin and plasma glucose concentrations.
Results: The absorption of insulin aspart was, on average, more than twice as fast and reached levels more than twice as high compared with human insulin [$t_{max(ins)}$ of 52 (23) vs 145 (93) min, $P < 0.0001$; and $C_{max(ins)}$ of 41 (11) vs 18 (4) $mU \cdot l^{-1}$, $P < 0.0001$; mean with (SD)]. However, total bioavailability did not differ between the insulins, and thus the mean residence time was significantly shorter for insulin aspart [$MRT_{(ins)}$ of 149 (26) vs 217 (30) min, $P < 0.0001$]. Plasma glucose (PG) fell more than twice as rapidly [$t_{min(PG)}$ of 94 (45) vs 226 (120) min, $P < 0.0001$], to a greater extent [$C_{min(PG)}$ 2.1 (0.6) vs 1.4 (0.4) $mmol \cdot l^{-1}$, $P < 0.0001$], and for a shorter duration with insulin aspart than with human insulin.
Conclusion: With improved subcutaneous absorption characteristics, the insulin aspart concentration–time profile resembles physiological meal-stimulated insulin release more closely than that of unmodified human insulin. This significantly alters the pharmacodynamic response in an advantageous manner in the meal-related treatment of diabetes mellitus.

P.D. Home (✉) · L. Barriocanal
Human Diabetes and Metabolism Research Centre,
The Medical School,
Framlington Place,
Newcastle upon Tyne NE2 4HH, UK
e-mail: Philip.Home@newcastle.ac.uk
Tel.: +44-191-222-7019; Fax: +44-191-222-0723

A. Lindholm
Novo Nordisk A/S,
Bagsvaerd, Denmark

Key words Insulin aspart · Insulin absorption · Rapid-acting insulin analogue

Introduction

Despite recommendations for the subcutaneous injection of unmodified human insulin some 30 min before a meal, postprandial plasma glucose (PG) levels are not well controlled in patients with diabetes. The circulating insulin level rises comparatively slowly, reaching a peak only after 1.5–2 h and declining only slowly – a situation that does not mimic the normal physiological response to the ingestion of a meal [1, 2].

Self-association of the insulin molecules to form hexamers [3] is thought to be a major reason for the delay in absorption from subcutaneous tissues into the systemic circulation; the rate of absorption is inversely correlated with the degree of self-association [4, 5]. In insulin aspart, proline at position 28 of the B chain of the insulin molecule was replaced by aspartate, discouraging hexamer formation. The resulting analogue has receptor affinity, receptor association and dissociation rates, and in vivo potency similar to human insulin [1, 6–8]. Insulin-like growth factor 1 activity is also similar to human insulin [1, 7]. In animal studies and early clinical studies, insulin aspart was absorbed faster from the subcutaneous injection site than unmodified human insulin [1, 6–9]. A pilot clinical study in healthy humans under euglycaemic glucose clamp conditions showed that insulin aspart had a faster onset of action than human insulin. Moreover, insulin aspart and human insulin appeared to be equipotent in terms of their PG lowering effect [10].

The primary objective of the present study was to compare the serum insulin profile of insulin aspart with that of human insulin in non-diabetic humans to ascertain whether the analogue is absorbed faster after subcutaneous injection and how its duration of action compares with that of unmodified human insulin. The

secondary objective was to compare the efficacies of the two insulins as indicated by the PG profiles.

Subjects and methods

Subjects and study design

Twenty-five subjects were enrolled and 19 completed the study. All 25 were healthy, non-smoking male Caucasians, aged 19–50 years, with a body mass index below 30.0 $kg \cdot m^{-2}$, an HbA_{1c} below 6.1% and a fasting PG below 6.0 $mmol \cdot l^{-1}$. All subjects gave written informed consent. The trial protocol and the consent form were reviewed by the local ethics committee and the trial was carried out in accordance with Good Clinical Practice (GCP) [11].

The trial had a randomized double-blind, crossover design in which each subject acted as his own control. On the first study day, after an overnight fast, each subject received a single dose of 0.1 $U \cdot kg\text{-body-weight}^{-1}$ of either insulin aspart or unmodified human insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark). On the second study day, 1–3 weeks later, subjects received the alternative insulin at the same dose.

Blood samples were collected 10 min before insulin injection, every 5 min until +20 min, then every 10 min until +90 min, every 15 min to +150 min, every 30 min to +240 min and every 60 min to +480 min. The subjects were fasting during the 8-hour sampling period after which they were given a carbohydrate meal, and were subsequently discharged. The subjects returned 1–3 weeks after the second study day for a post-trial examination. The insulin aspart and human insulin cartridges (Penfill, Novo Nordisk A/S) and pen injectors (Novopen II, Novo Nordisk) were identical so that the trial could be performed double-blind.

Pharmacokinetic and pharmacodynamic assessments

Venous blood samples were analysed for serum insulin and C-peptide concentrations, and the C-peptide values were used to correct for endogenous insulin using the basal-insulin:C-peptide ratio to obtain a corrected exogenous insulin profile:

$$I_{ex(t)} = I_{tot(t)} - (C_{(t)} I_{(t \le 0)} / C_{(t \le 0)})$$

where $I_{ex(t)}$ is the exogenous insulin concentration at time t post-injection, $I_{tot(t)}$ is the total insulin concentration at time t post-injection, $C_{(t)}$ is the C-peptide concentration at time t; $I_{(t \le 0)}$ is the initial endogenous insulin concentration; and $C_{(t \le 0)}$ is initial C-peptide concentration [12]. This method assumes equivalent plasma clearance of insulin and C-peptide, while in practice that of the latter is 4–6 times longer. As a result the contribution of endogenous insulin will be overestimated when secretion is falling, and underestimated when rising, the degree of error varying with its rate of change. Total area under curve between two steady states will not, however, be affected.

Serum insulin was determined using a standard radioimmunoassay kit from Pharmacia (Uppsala, Sweden). This assay does not completely cross-react with insulin aspart. Therefore, after extensive validation (data unpublished), the following correction formula was applied to calculate the corrected insulin aspart concentration:

$$\text{Insulin aspart}_{\text{corrected}} = F \times (1503 \times \text{insulin aspart}_{\text{fraction}}) / (1398 - \text{insulin aspart}_{\text{fraction}})$$

where F denotes the dilution factor and insulin $\text{aspart}_{\text{fraction}}$ is in $pmol \cdot l^{-1}$ as is the diluted assay result.

Endpoints derived from the serum insulin profiles were the mean residence time ($MRT_{(ins)}$), maximum serum insulin concentration ($C_{max(ins)}$), the time of maximum serum insulin concentration ($t_{max(ins)}$) and the area under the insulin concentration-time curve ($AUC_{(ins)}$). All endpoints were calculated from the time of insulin administration (t = 0 min) to the last measured time point (t = 480 min). The terminal elimination rate constant ($\lambda_{z(ins)}$) and the apparent absorption half-life ($t_{1/2(ins)}$) for each insulin preparation after subcutaneous administration were estimated by non-compartmental pharmacokinetic analysis using WinNonlin software version 1.1 (Scientific Consulting, Cary, N.C., USA).

$MRT_{(ins)}$ was calculated from:

$$MRT_{(ins)} = AUMC_{(ins)} / AUC_{(ins)}$$

where AUMC is the area under the statistical moment curve and AUC is the area under the concentration-time curve, calculated by the trapezoidal rule [13]. The apparent terminal $t_{1/2}$ and terminal elimination rate constant were calculated as the slope of the terminal linear part of the ln(conc) versus time curve. The area under the serum insulin concentration-time curve from time 0 min to infinity ($AUC_{0-\infty(ins)}$) was estimated using the equation:

$$AUC_{0-\infty} = AUC_{0-t_n} + \hat{C}_n \hat{\lambda}_z$$

where C_n is the estimated concentration at time t_n; and λ_z is the estimated terminal rate constant.

The relative bioavailability of insulin aspart (IAsp) versus human insulin (HI) [F(AUC)] was derived as the ratio:

$$F(AUC) = \frac{AUC_{0-\infty(IAsp)}}{AUC_{0-\infty(HI)}}$$

where $AUC_{0-\infty}$ is the area under the serum concentration-time curve from time 0 min to infinity.

PG levels were measured at the Bioanalytical Research Corporation (BARC), Gent, Belgium, using the hexokinase method on fluoride plasma. Endpoints derived from the PG profiles were the negative excursion of glucose as assessed as the area below the baseline plasma glucose level and above the glucose concentration-time curve ($EXC_{(PG)}$), the maximum change in plasma glucose concentration ($\Delta C_{min(PG)}$, defined as $PG_0 - C_{min(PG)}$), and the time ($t_{min(PG)}$) when $\Delta C_{min(PG)}$ first occurs.

Statistical methods

With a significance level of 5%, a sample size of 20 subjects ensured that the trial had an 80% chance of detecting a true relative difference between the insulin preparations. $MRT_{(ins)}$, $C_{max(ins)}$, $AUC_{(ins)}$ and $EXC_{(PG)}$ were logarithmically transformed and then analysed by ANOVA with subject as a random effect and treatment condition as a fixed effect. Median differences in $t_{max(ins)}$ and $t_{min(PG)}$ were compared by the Wilcoxon Signed Rank test using the Hodges-Lehmann approach. All tests were made as within-subject comparisons at the 5% significance level. Statistical analyses were made using SAS for UNIX, version 6.0 (Statistical Analysis Systems, SAS Institute, Raleigh, N.C., USA).

Results

One subject was withdrawn from the trial due to a protocol violation and a further five subjects were excluded as complete profiles were not recorded while taking insulin aspart due to hypoglycaemia. These subjects could therefore not act as their own controls. The maximum serum concentration of insulin aspart ($C_{max(ins)}$) was significantly higher than for human insulin [41 (11) vs 18 (4) $mU \cdot l^{-1}$, $P < 0.001$], while the time taken to reach this concentration ($t_{max(ins)}$) was significantly shorter [52 (23) vs 145 (93) min, $P < 0.001$; Fig. 1]. $MRT_{(ins)}$ was significantly shorter for insulin aspart than for human insulin, indicating a shorter residence time for insulin aspart in subcutaneous tissue (Table 1). The harmonic apparent terminal half-lives, $t_{1/2(ins)}$, for insulin aspart and human insulin were

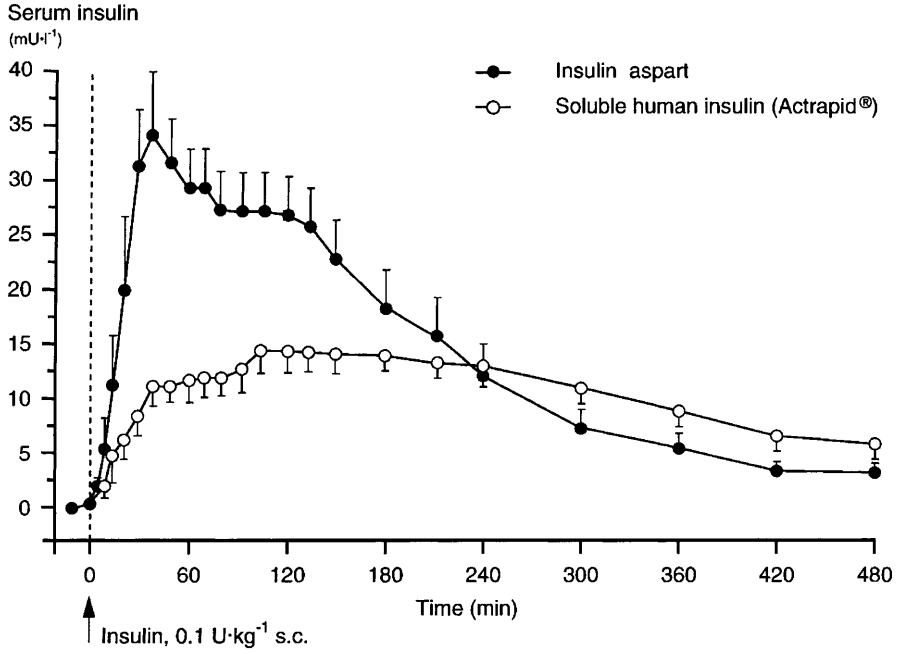

Fig. 1 Serum insulin profiles (corrected for endogenous insulin) in healthy male volunteers ($n = 19$) following subcutaneous injection of the insulin aspart analogue (●) or of unmodified human insulin (○)

76 min and 122 min, respectively, and the terminal elimination constants, $\lambda_{z(ins)}$, were 0.34 (0.18) h^{-1} and 0.54 (0.29) h^{-1} ($P < 0.05$). $AUC_{(ins)}$ was significantly greater for insulin aspart than for human insulin (Table 1). However, the relative bioavailability of insulin aspart to human insulin did not differ significantly and the median value was 1.12 (IQ range 0.86–1.40).

The change in plasma glucose concentration ($\Delta C_{min(PG)}$) was greater with insulin aspart than with unmodified human insulin [2.1 (0.6) vs 1.4 (0.4) $mmol \cdot l^{-1}$; $P < 0.001$; Fig. 2] and the time to reach the minimum concentration ($t_{min(PG)}$) was shorter [94 (45) vs 226 (120) min; $P < 0.001$]. However, the overall dynamic responses were similar, as indicated by the absence of a significant difference between $EXC_{(PG)}$ for insulin aspart and human insulin (Table 2).

Discussion

The serum insulin profiles of insulin aspart, and unmodified human insulin were markedly different. While the effects of calculation of endogenous insulin secretion (see Methods) would distort the rising and falling parts of both profiles to some extent, such effects would be relatively small, as they can only operate on the effective endogenous insulin concentration, which was much smaller than the contribution from exogenous insulin. After subcutaneous injection of insulin aspart, the circulating insulin concentrations rose much faster, reached a greater peak ($C_{max(ins)}$) much earlier ($t_{max(ins)}$) and returned to baseline more rapidly than after injection of human insulin. Assuming clearance is similar (which previous studies have indicated [14, data on file, Novo Nordisk], then the rapid and higher peaks are presumed to be due to faster absorption from the subcutaneous tissues for the insulin analogue. The faster rate of absorption is due to the rapid dissociation into monomers and dimers after injection, these then being readily absorbed [1].

The plasma insulin profile of insulin aspart has several important clinical implications for the use of this insulin analogue in a multiple injection regimen by patients with diabetes. It approaches the ideal mealtime administration profile, which should mimic the normal physiological response to food intake [10]. The rapid rise in serum insulin following subcutaneous injection of the insulin aspart allows injection at meal-time to be effective in controlling postprandial PG concentrations, while human insulin needs to be injected some 30 min or more before a meal for an optimal effect [15, 16].

Table 1 Pharmacokinetic serum insulin endpoints for insulin aspart and human insulin. Data are given as mean with (SD); $n = 19$. $MRT_{(ins)}$ mean residence time of injected insulin; $C_{max(ins)}$ maximum serum insulin concentration, corrected for endogenous insulin; $t_{max(ins)}$ time to reach maximum serum insulin concentration; $AUC_{(ins)}$ area under the serum insulin concentration-time curve from time 0 min to the last measured time point at 8 h; $AUC_{0-\infty(ins)}$ area under the serum insulin concentration-time curve from time 0 min to infinity; *NS* not significant, *SD* standard deviation

	Insulin aspart	Human insulin	*P*
$t_{max(ins)}$ (min)	52 (23)	145 (93)	< 0.0001
$C_{max(ins)}$ ($mU \cdot l^{-1}$)	41 (11)	18 (4)	< 0.0001
$MRT_{(ins)}$ (min)	149 (26)	217 (30)	< 0.0001
$AUC_{(ins)}$ ($mU \cdot l^{-1} \cdot min$)	6461 (1207)	4669 (836)	< 0.0001
$AUC_{0-\infty(ins)}$ ($mU \cdot l^{-1} \cdot min$)	6740 (1294)	6961 (4192)	NS

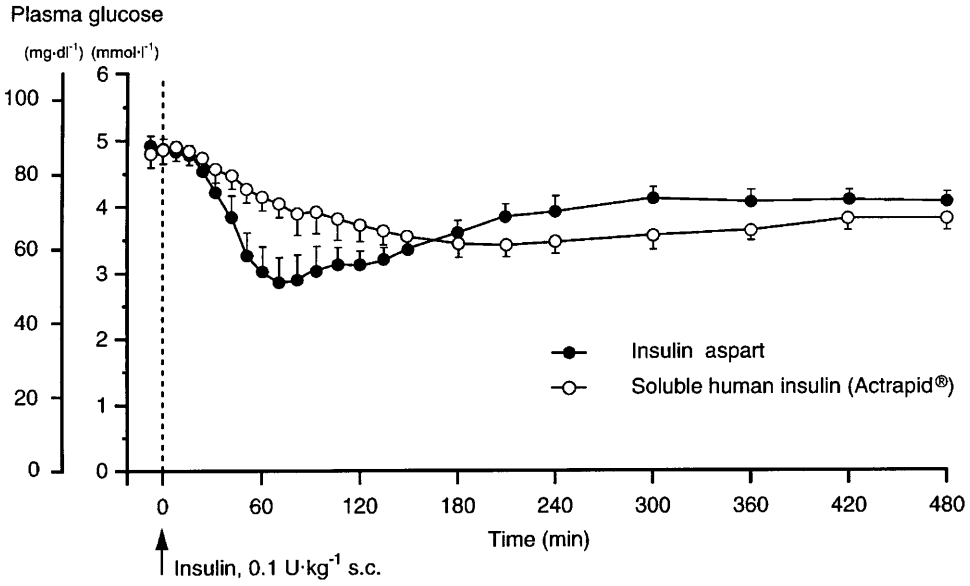

Fig. 2 Plasma glucose profiles in healthy male volunteers ($n = 19$) following subcutaneous injection of the insulin aspart analogue (●) or of unmodified human insulin (○)

Mealtime injections are significantly more convenient for the patient [17]. The rapid rise to a higher peak insulin level corresponds to the period when postprandial glucose absorption is at its maximum [18–20] and therefore should reduce the postprandial plasma glucose peak.

Changing the structure of a protein hormone like insulin raises the question of whether it will still bind to the insulin receptors and whether, therefore, it will be effective in lowering the PG level. Receptor binding studies have shown insulin aspart to be identical to unmodified insulin in this respect [1]. In the present study, the PG profiles show that the insulin aspart analogue was more effective at rapidly lowering the PG level than unmodified human insulin, as the $C_{min(PG)}$ was reached faster than with human insulin and the fall in PG ($\Delta C_{min(PG)}$) was greater at the same dose. The findings confirm data of euglycaemic glucose clamp studies on healthy volunteers [9, 10], showing that the half-maximal glucose infusion rate was attained significantly earlier after subcutaneous injection of insulin aspart than after unmodified human insulin. Another recent euglycaemic clamp study continued for 10-h post-injection and showed that insulin aspart has a short duration of action [21]. The shorter duration of action may also have the advantage of a lower risk of hypoglycaemia before the next meal.

Pilot studies on diabetic patients have shown smaller meal-related plasma glucose excursions with insulin analogues (including insulin aspart) than with human insulin [22]. A study on Type 1 (insulin-dependent) diabetic patients who were maintained on their normal basal insulin delivered by a pump, confirmed the results of the present study, and showed mean peak serum insulin concentration 45 min earlier with insulin aspart than with human insulin; the postprandial increase in PG concentration was correspondingly less pronounced than with human insulin [23].

The doses of insulin aspart and human insulin were determined to produce reliably high serum insulin concentrations after subcutaneous administration during fasting conditions. In five cases this resulted in hypoglycaemia. This represents a potential bias and the possibility cannot be excluded that the pharmacokinetic and pharmacodynamic responses in the subjects that withdrew from the trial differed from those of the subjects that completed the trial. However, the crossover design partly compensates for this potential bias. The early hypoglycaemia occurs at a time when people with diabetes are currently markedly hyperglycaemic, and should therefore be seen as potentially beneficial rather than a problem. This matter is being further studied in clinical trials.

To conclude, the results demonstrate that the pharmacokinetics of insulin aspart mimic the physiological response to a meal better than human insulin, with a correspondingly more rapid effect on plasma glucose. Whether this improvement is followed by better overall glycaemic control needs to be determined in diabetic subjects in true treatment situations. Given the very appropriate efficacy profile obtained in the present study, such treatment trials are now well under way.

Table 2 Pharmacodynamic plasma glucose endpoints for insulin aspart and human insulin. Data are given as mean with (SD); $n = 19$. $EXC_{(PG)}$ area under the baseline plasma glucose level and above the plasma glucose concentration–time curve from time 0 min to the last measured time point at 8 h; $\Delta C_{min(PG)}$ maximum change in plasma glucose concentration; $t_{min(PG)}$ time to reach $\Delta C_{min(PG)}$; *NS* not significant, *SD* standard deviation

	Insulin aspart	Human insulin	*P*
$EXC_{(PG)}$ (mmol · l^{-1} · min)	445 (110)	436 (131)	NS
$DC_{min(PG)}$ (mmol · l^{-1})	2.1 (0.6)	1.4 (0.4)	< 0.0001
$t_{min(PG)}$ (min)	94 (45)	226 (120)	< 0.0001

Acknowledgements and duality of interest This study was part of the commercial development programme of insulin aspart by Novo Nordisk A/S. Dr. Barriocanal and Professor Home are grateful for the research support required to carry out the study. Dr. Lindholm is an employee of Novo Nordisk A/S. Professor Home, on behalf of the University of Newcastle, provides consultancy services to all major insulin manufacturers, including Novo Nordisk.

References

1. Brange J, Owens DR, Kang S, Vølund A (1990) Monomeric insulins and their experimental and clinical applications. Diabetes Care 13: 923–954
2. Zinman B (1989) The physiological replacement of insulin: an elusive goal. New Engl J Med 321: 367–370
3. Blundell T, Dodson G, Hodgkin D, Mercola D (1972) Insulin: the structure in the crystal and its reflection in chemistry and biology. Adv Protein Chem 26: 279–402
4. Brange J, Ribel U, Hansen JF, Dodson G, Hansen MT, Havelund S, Melberg SG, Norris F, Norris K, Snel L, Sørensen AR, Voigt HO (1988) Monomeric insulins obtained by protein engineering and their medical implications. Nature 333: 679–682
5. Ribel U (1993) Subcutaneous absorption of insulin analogues. Front Insulin Pharmacol: 70–78
6. Vølund A, Brange J, Drejer K, Jensen I, Markussen J, Ribel U, Sørensen AR, Schlichtkrull J (1991) In vitro and in vivo potency of insulin analogues designed for clinical use. Diabet Med 8: 839–847
7. Drejer K, Kruse V, Larsen UD, Hougaard P, Soren B, Gammeltoft S (1991) Receptor binding and tyrosine kinase activation by insulin analogues with extreme affinities studied in human hepatoma HepG2 cells. Diabetes 40: 1488–1495
8. Jensen I, Kruse V, Larsen UD (1991) Scintigraphic studies in rats. Kinetics of insulin analogues covering wide range of receptor affinities. Diabetes 40: 628–632
9. Kang S, Brange J, Burch A, Vølund A, Owens DR (1991) Subcutaneous insulin absorption explained by insulin's physicochemical properties: evidence from absorption studies of soluble human insulin and insulin analogues in humans. Diabetes Care 14: 942–948
10. Heinemann L, Heise T, Jorgensen LN, Starke AAR (1993) Action profile of the rapid acting insulin analogue: human insulin B28Asp. Diabet Med 10: 535–539
11. Commission of the European Communities CPMP working party on efficacy of medical products (1990) Good clinical practice for trials on medicinal products in the European Community. Pharmacol Toxicol 67: 361–372
12. Owens DR (1986) Human insulin: clinical pharmacological studies in man. MTP Press, Lancaster, pp 137–139
13. Veng-Pedersen V (1989) Mean time parameters in pharmacokinetics: definition, computation and clinical implications (parts 1 and 2). Clin Pharmacokinet 17: 345–366 and 424–440
14. Kang S, Creagh FM, Ara J, Owens DR, Peters JR (1991) Insulin analogues and human insulin: near-equivalent in vivo biological activity in healthy males in spite of widely different in vitro potencies. Diabetes 40 [Suppl 1]: A969
15. Dimitriadis GD, Gerich JE (1983) Importance of timing of preprandial subcutaneous insulin administration in the management of diabetes mellitus. Diabetes Care 6: 374–377
16. Lean MEJ, Ng LL, Tennison BR (1985) Interval between insulin injection and eating in relation to blood glucose control in adult diabetics. Br Med J 290: 105–108
17. Jorgensen LN, Nielsen FS (1990) Timing of pre-meal insulins in diabetic patients on a multiple daily injection regimen: a questionnaire study. Diabetologia 33 [Suppl 1]: A116
18. Eaton PR, Allen RC, Schade DS, Standefer JC (1980) Normal insulin secretion: the goal of artificial insulin delivery systems. Diabetes Care 3: 270–273
19. Olsson PO, Arnqvist HJ, von Schenck HV (1988) Free insulin profiles during intensive treatment with biosynthetic human insulin. Diabete Metab 14: 253–258
20. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, Van Cauter E (1988) Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. New Engl J Med 318: 1231–1239
21. Heinemann L, Kapitza C, Starke AAR, Heise T (1996) Time-action profile of the insulin analogue B28Asp. Diabet Med 13: 683–687
22. Kang S, Creagh FM, Peters JR, Brange J, Vølund A, Owens DR (1991) Comparison of subcutaneous soluble human insulin and insulin analogues (AspB9, GluB27; AspB10; AspB28) on meal-related plasma glucose excursions in type I diabetic subjects. Diabetes Care 14: 571–577
23. Wiefels K, Hübinger A, Dannehl K, Gries FA (1995) Insulin-kinetic and -dynamic in diabetic patients under insulin pump therapy after injections of human insulin or the insulin analogue (B28Asp). Horm Metab Res 27: 421–424