

Insulin Analogues: Reviewing the Pros and Cons in Managing Diabetes Mellitus

Jennifer H. Martin^{1,2*}, Anthony Russell^{1,3}, Trisha O'Moore-Sullivan^{1,3} and Johannes B. Prins^{1,3,4}

¹The University of Queensland, Brisbane, Australia

²Department of Internal Medicine, Princess Alexandra Hospital, Woolloongabba, Brisbane, Australia

³Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Australia

⁴CEO, Mater Medical Research Institute, Brisbane, Australia

Abstract

The issue of planning the timing and dosing of insulin in relation to food is one of the most difficult issues confronting people with diabetes. Recent focus on improving quality of life in this area has focused on developing different modes of administration of insulin thereby avoiding subcutaneous injections and developing new analogues of insulin. Both inhalational and buccal administration technologies have been developed, and have essentially overcome some of the difficult pharmacokinetic issues regarding large peptide molecules, however there remain some clinical problems. Advances in the practicalities of treating insulin have occurred, such as more accurate and less expensive glucometers, new administration alternatives such as implantable pumps, with further developments in the pipeline including islet and gene replacement for Type I disease. However all of these newer options have limitations and currently subcutaneous administration is the only real option for most people. Insulin analogues have so far been relatively disappointing in terms of improvement in mortality and morbidity although for some patients the ability to alter the dosage of insulin depending on the planned meal size or reduction of between meal snacks has been helpful. Furthermore there is a yet unknown question around long term safety. This review will discuss the major clinical issues surrounding the new insulin analogues as they relate to efficacy and side effects.

Keywords: History; Insulin; Recombinant; Methods of delivery

Discovery and Development of Insulin

Insulin is an anabolic polypeptide hormone secreted by the beta cells of the pancreatic islets of Langerhans. Before the discovery of insulin, scientists used various extracts from the pancreas to lower blood glucose in laboratory animals. However it was re-infusion of a pancreatic extract into a pancreatectomised dog that improved hyperglycemia. This discovery led to the development of a procedure for beef pancreas extract and in 1923, Lilly[®] patented *Iletin* (mixed porcine/bovine isophane). This was followed in 1926 with the isolation of crystalline insulin and the unravelling of the sequence and structure of insulin in 1956 (Figure 1).

Zinc and a low molecular weight protein called protamine were subsequently developed and isophane neutral Hagedorn (NPH) insulin, bound to protamine, became available. Long acting insulins such as semilente, lente and ultra- lente were developed. However antibody allergies and lipoatrophy started to be noticed due to the antigenicity from the porcine and bovine proteins.

In 1975 human insulin was synthesized which was timely as by then Lilly[®] was using 1 ton of pancreas per hour to make enough insulin. Within 3 years a genetically manipulated plasmid of *E. coli* bacteria was used to individually express the A and B insulin chain and thus successfully produce recombinant human insulin. This breakthrough provided the first opportunity to mass-produce 'human' insulin using gene technology resulting in recombinant Humulin R (rapid), Humulin N (NPH) and the semi-synthetic insulins Actrapid and Monotard. It also offered the opportunity to manipulate the insulin sequence enabling development of genetically modified insulins to improve the pharmacokinetic profile. Currently a large number of additional analogue insulin formulae are being tested.

Specific Analogues

Absorption and Distribution

Prandial insulin: A) Regular Human Insulin (RHI): When zinc atoms are added to human insulin in solution, insulin monomers self associate to form hexameric insulin. These larger hexamers are slowly absorbed and then slowly dissociate into dimers and monomers that then diffuse into the circulation more rapidly, but with similar total absorption. After subcutaneous injection, RHI concentrations peak at 1-2 hours and return to baseline after 6-8 hours. However there is great variability, with up to 15-25% variability in intra- and inter-individual concentrations. This is due to a number of factors including dose, re-suspension technique, site, amount of subcutaneous adipose tissue (with less fat increasing absorption) and blood flow [1,2]. The desire to both mimic endogenous meal related insulin secretion more closely and to improve the consistency of absorption and distribution kinetics, encouraged the development and subsequent identification of molecular modifications to reduce hexamer formation and improve absorption. These modifications have underpinned the development of the "new" synthetic insulins.

B) Rapid acting analogues (Aspart [AspB28 human insulin]),

***Corresponding author:** Jennifer H. Martin, Department of Medicine, The University of Queensland, Princess Alexandra Hospital, Woolloongabba 4102, Queensland, Australia, Tel: +617 3176 3072; E-mail: j.martin4@uq.edu.au

Received November 20, 2010; **Accepted** December 21, 2011; **Published** December 23, 2011

Citation: Martin JH, Russell A, O'Moore-Sullivan T, Prins JB (2011) Insulin Analogues: Reviewing the Pros and Cons in Managing Diabetes Mellitus. J Pharmacogenom Pharmacoproteomics 2:106. doi:10.4172/2153-0645.1000106

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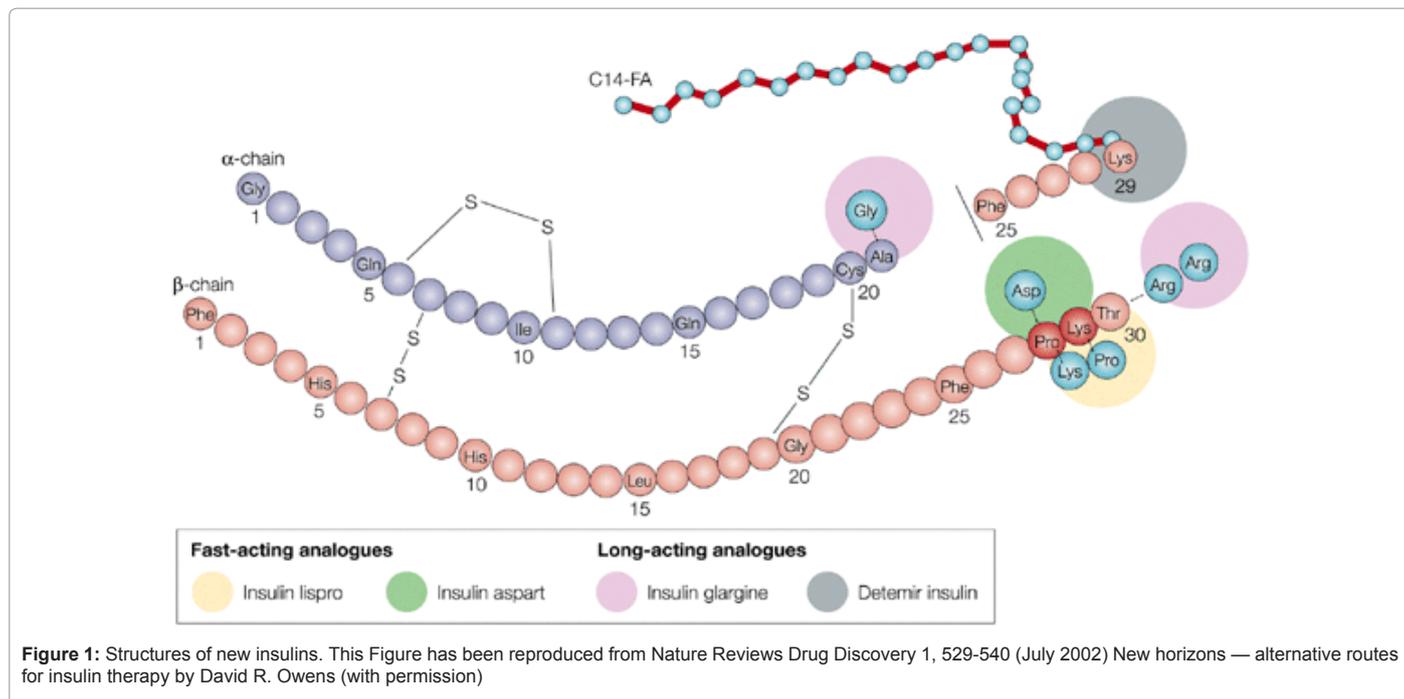


Figure 1: Structures of new insulins. This Figure has been reproduced from Nature Reviews Drug Discovery 1, 529-540 (July 2002) New horizons — alternative routes for insulin therapy by David R. Owens (with permission)

Lispro [28(B)-L-lysine-29(B)-L-proline-human insulin], Glulisine [3(B)-Lys, 29(B)-Glu-human insulin]: These synthetic insulin analogues have molecular modifications that delay self association of insulin monomers, enabling more rapid absorption of injected drug and more rapid attainment of maximum concentrations. This leads to closer replication of the normal prandial insulin response when compared to RHI [3]. Insulin lispro, aspart, and glulisine have nearly identical pharmacology profiles [4] and may have less intra- and inter-individual variation in absorption than human insulin [3]. The peak activity of rapid acting analogues is at about 1 hour and duration of action is approximately 3-4 hours [5]. Glulisine formulation, unlike the other analogues, does not have added hexamer-producing zinc. This may have benefits in the absorption, although this has so far not translated into any measurable benefit in diabetes control or side effects profile.

Basal insulin:

A) Neutral Protamine Hagedorn (NPH): NPH has peak activity at 4-6 hours post injection and total duration of action of 12-16 hours.

B) Glargine [Gly A21, Arg B31, Arg B32] insulin: The molecular modifications made to insulin glargine ensure that it is soluble at acidic pH (resulting in a clear insulin in the vial) with less solubility at neutral pH resulting in a micro-precipitate at the injection site. This effect delays absorption from the subcutaneous site resulting in a delayed onset of action (1.5 vs. 0.8 hours for NPH), flatter (but not “flat”) absorption profile and a prolonged duration of action (10-26 vs. 14 hours) [6]. Recently it has been noted that up to 33% of adult patients with T1DM appear to require twice rather than once daily dosing of glargine.

C) Detemir [LysB29(Nε-tetradecanoyl) des(B30) human insulin]: The addition of the fatty acid side chain in detemir allows the molecule to bind reversibly to albumin with high affinity in the subcutaneous, intravascular and extracellular compartments. This

slows both absorption and excretion however twice daily dosing is still often required. The fatty acid side-chain enhances self-association of monomers in the subcutaneous depot, which as well as contributing to prolongation of action reduces variability of the time effect profile [7].

Clearance

Prandial insulin: Hepatic clearance is the major site of metabolism for circulating insulin although approximately 30% of systemically circulating insulin is renally cleared with a small amount of degradation by circulation proteases. The renal clearance is theoretically an issue for people with impaired renal function, with reduced insulin requirements sometimes but not always required as renal function decreases, perhaps due to other insulin degradation pathways including greater endocytosis-lysosomal and enzymatic degradation in proximal tubular cells [8].

Insulin aspart pharmacokinetics are not affected in a clinically

<ul style="list-style-type: none"> · The newer insulins have greater similarity to each other than to the older insulins
<ul style="list-style-type: none"> · The newer short acting insulins may enable more flexibility with dosing regimens, meal composition and requirement for between meal snacks than older insulins
<ul style="list-style-type: none"> · Both detemir and glargine may need to be used in a twice daily regimen to enable control of HbA1c
<ul style="list-style-type: none"> · Detemir may have weight advantages over older insulins in obese people with type 2 diabetes
<ul style="list-style-type: none"> · Older insulins have a wealth of safety data and as yet the mitogenic potential of the new insulins, especially over a lifetime is not fully known
<ul style="list-style-type: none"> · Newer insulins do not provide any efficacy advantage (in terms of HbA1c) but do offer convenience and may provide a reduction (but not elimination) of nocturnal hypoglycaemia

Table 1: Summary Points.

significant manner by liver disease, mild-moderate renal impairment or BMI [9]. Glulisine does not appear to accumulate in patients with renal impairment [10].

Basal insulin:

A) Glargine: Glargine is a pro-drug which undergoes partial rapid clearance at the injection site by metabolism to two active metabolites M1 and M2, the remainder of which is metabolized by plasma peptidases in the circulation [11].

B) Detemir: The molecular modifications present in detemir (removal of threonine at position B30 and the acylation of a 14-carbon myristoyl fatty acid to lysine at position B29) enable tight but reversible binding to albumin, reducing hepatic clearance. Neither hepatic nor renal impairment exert a clinically relevant effect on the pharmacokinetic profile of detemir [12].

Dose Response

Prandial insulin: Insulin glulisine is the newest human insulin analogue product for the control of prandial blood glucose. As with aspart and lispro, glulisine displays faster absorption and onset of action, with a shorter duration of action than that of RHI [13].

The duration of action of lispro, aspart and glulisine only modestly increase with higher doses [2,10] however with increasing doses of RHI both the duration of action and the maximal metabolic response increase.

Basal insulin:

A) Glargine: A recent meta-analysis of insulin analogues showed that the duration of action for both insulin detemir and insulin glargine displayed dose dependency, with an average 24 hour duration of action in a clinically relevant dosing range of 0.35 U/kg to 0.8 U/kg in patients with type 1 diabetes [14]. Similar findings have been seen in type 2 patients [15], however clinically BD dosing is used for both.

B) Detemir: Data in healthy subjects show that insulin detemir has a flatter time-action profile than NPH, reaching its peak concentration nearly 90 min later [16].

Receptor Affinity, Signaling and Mitogenicity

Upon binding the insulin receptor (IR) or insulin-like growth factor 1 receptor (IGF-1R) autophosphorylation and downstream signalling cascades ensue. In very simple terms, insulin can signal via the IR to "metabolic" pathways (via IRS-1/PI3K/AKT) or "mitogenic" pathways (via MAP kinase). "Metabolic" responses include glucose uptake, suppression of gluconeogenesis and anabolism. "Mitogenic" effects include cell proliferation and anti-apoptosis. IGF-1R signalling is predominately "mitogenic".

The net "mitogenic" vs "metabolic" effect of insulin and its analogues is determined by cellular expression of IR, IGF-1R and hybrid receptors, insulin concentration (higher concentrations favour mitogenic signalling) and insulin sequence. This is of therapeutic relevance because numerous insulin analogues, including inhaled insulins such as Exubera® (which require high dosages) have not proceeded to clinical trial because of a number of factors including cost and acceptability but also due to concerns regarding mitogenic effects [17]. *In vitro* studies for example have shown that particular modifications to the insulin molecule can alter affinity for the insulin and IGF-1

receptor and receptor occupancy time, and that these alterations can affect mitogenic potential [18]. Analogues with reduced dissociation time (i.e. longer receptor occupancy) have a disproportionate increase in mitogenic activity compared to metabolic activity. Specifically, insulin glargine was found to have a six to eight fold increase in IGF-1 receptor affinity and mitogenic potency compared with human insulin. Glargine metabolites show mitogenicity but less than the parent [11]. Rodent *in vivo* studies have not shown any increase in carcinogenicity with glargine however [19].

Insulin aspart and lispro appear to be similar to human insulin in metabolic potency and insulin receptor affinity although lispro has been noted to have a 1.5 fold higher IGF-1 receptor affinity compared to human insulin [20]. However mitogenic potential was not altered compared to aspart or regular human insulin. In a mammary cell line only glargine of the insulin analogues showed a significantly higher proliferative effect on MCF 7 breast cancer cells [21]. More recently studies of glulisine suggest that this agent has similar insulin receptor association, dissociation, affinity kinetics and similar mitogenic and metabolic potency to RHI [22].

Detemir, as a consequence of its fatty acyl chain, has a lower affinity for the insulin receptor [7]. However it dissociates from the insulin receptor 2-fold faster and is associated with reduced mitogenic potency compared to human insulin [7].

The clinical and long-term implications of these *in vitro* studies are not fully known. However since 2008 when an observational study on inhaled insulin analogues was associated with six new cases of lung cancer in smokers/ex-smokers as opposed to one in the comparator arm [23] there have been concerns regarding the use of insulin analogues and cancer. A prospective observational study in Sweden from 2006-7 has shown an adjusted increased risk of breast cancers in users of monotherapy glargine as compared to other types of insulin (RR 1.97, 95% CI 1.29-3) [24]. Further dose-adjusted observational human studies from Germany [25] and Scotland [26] have supported these findings, but not a database review from the United Kingdom [27] nor other manuscripts [19,28,29]. As these studies were observational, care needs to be exercised with interpretation. Thus although there is mechanistic data supporting mitogenicity of insulin glargine and observational human data suggesting an association with carcinogenicity, human clinical trial data to date have not shown clear evidence of an increased cancer risk.

Maternofetal Transport

Most data on risk of congenital malformations with the new insulins in pregnancy has come from observational data. So far the largest amount of data regarding pregnancy outcomes for any insulin analogue is for insulin lispro. Reassuringly several retrospective studies have shown no clear association between the use of this analogue as compared to regular insulin [30].

Similar data on outcomes are not available for insulin glulisine (which is FDA pregnancy category C) or insulin glargine. A prospective study of Aspart in 322 subjects with Type 1 diabetes during pregnancy has not raised any safety concerns [31]. The results of a prospective study of detemir in pregnancy is awaited. However as the increased incidence or malformations above baseline diabetic risk is small, much larger exposures will be necessary to study the question. Due to the strong affinity for IGF-1-receptor binding with glargine and the mitogenic potential discussed above, there are theoretical toxicological concerns with using glargine in pregnancy [32].

Hypoglycaemia

There has been increased concern recently about hypoglycaemia, with evidence that this event could increase morbidity, mortality [33] and cognitive impairment [34]. There are also concerns with hypoglycaemic risk in pregnancy. As the insulin analogues glargine and detemir display a flatter pharmacokinetic profile suggesting a clinically significant reduction in hypoglycaemia incidence compared with NPH insulin, most studies have only shown a small reduction in nocturnal hypoglycaemic incidence leaving the "fear of hypoglycaemia to remain. A recent study in patients with type 1 diabetes mellitus with severe hypoglycaemia on NPH who were changed to glargine or detemir still experienced severe hypoglycaemia and few patients reached internationally accepted glycaemic treatment goals [35]. In fact a Cochrane review article showed no glycaemic benefit of long-acting insulin analogues compared with intermediate-acting insulin [36].

Clinical Effectiveness

In T1D, there is some evidence that glargine is more effective than NPH in reducing FBG, and other evidence that shows they are the same. Both have the same effect on HbA_{1c}. To date there has not been reported any well conducted studies that show a difference in clinical outcome between NPH and the newer insulins. The number of episodes of hypoglycaemia may be slightly less with glargine but the clinical relevance of this is unknown. Further this effect was seen when glargine was compared with once daily and not twice daily NPH. An 'older' review of the literature is documented in the Health Technology Assessment systematic reviews, summarized in [37]. In a more recent systematic review of randomised controlled trials, there was a clinically non significant difference between rapid-acting insulin analogues and RHI in adults with both T1D and T2D. Similarly, differences between long-acting insulin analogues and NPH insulin in terms of HbA_{1c} were marginal among adults with either T1D or T2D. Benefits in terms of reduced hypoglycemia were inconsistent. Similarly to the earlier systematic reviews there was insufficient data to have any certainty as to whether insulin analogues are better than conventional insulins in reducing long-term diabetes-related complications or death [38]. To date, the lack of long-term and good quality studies means we can only suggest that rapid-and long-acting insulin analogues appear to offer little comparative benefit relative to conventional insulins in terms of glycemic control or reduced hypoglycemia; and that comparative efficacy on reductions in long-term complications of diabetes is unknown.

Premixed Insulins

Similarly, evidence from clinical trials on clinical outcome with premixed insulins is inconclusive. A systematic review of surrogate endpoints has found that premixed insulin analogues provide glycemic control similar to that of premixed human insulin. Premixed analogues may provide slightly tighter glycemic control than long-acting insulin analogues. Specifically premixed insulin analogues were similar to premixed human insulin in decreasing FBG, HbA_{1c} and the incidence of hypoglycemia. They were slightly more effective in decreasing postprandial glucose levels (mean difference -1.1 mmol/L; 95% CI, -1.4 to -0.7 mmol/L). Compared with long-acting insulin analogues, premixed insulin analogues were superior in decreasing postprandial glucose levels (mean difference, -1.5 mmol/L; CI, -1.9 to -1.2 mmol/L) and HbA_{1c} (mean difference, -0.39% [CI, -0.50% to -0.28%])

but were inferior in decreasing fasting glucose levels (mean difference, 0.7 mmol/L; CI, 0.3 to 1.0 mmol/L) and were associated with a higher incidence of hypoglycemia [39].

Other Issues

Continuous subcutaneous insulin infusions (CSII) have been used to treat diabetes since the 1970s with efficacy data comparable to that of multiple daily insulin regimes, particularly in adults with Type 1 diabetes. CSII therapy is designed to provide both rapid insulin action to cover meal-time requirements and variable basal insulin delivery. The development of rapid acting insulin analogues with uniform absorption characteristics has further assisted the effectiveness of CSII therapy, reducing the size of the subcutaneous insulin depot and shortening the time interval between insulin administration and action. Recently the option of linked subcutaneous sensor capacity has emerged in the new generation pumps, clinical trials are underway. Clinical studies have validated comparable HbA_{1c} lowering effects of insulin aspart and lispro compared with regular insulin when the boluses of soluble insulin were administered 30 minutes prior to the meal, and better than regular insulin when aspart and lispro was administered immediately prior to the meal [40], however there is a risk of rapid development of ketoacidosis if there is an interruption in insulin delivery such as may occur in pump failure or catheter occlusion.

The Future

The experience of Exubera[®] led most companies who were developing inhaled insulin to cease their programmes. An exception has been MannKind who have developed a small device utilising technosphere technology which allows delivery and absorption of insulin very rapidly to the circulation and with small but reversible changes in pulmonary function [41]. Oral insulin continues to be pursued as does further research into the development of ultra-long-acting insulins such as pegylated insulins that only need administration every few days. Other novel concepts include "SmartInsulin" which releases insulin in a glucose dependent manner [42].

Summary

There have been major advances in insulin therapy over the last 50 years. It is noteworthy that although both inhalational and buccal technologies have been developed, clinical and delivery problems with the use of these technologies remain. Further developments including islet and gene replacement are exciting, but in the meantime we must work with subcutaneous administration of insulins for the majority of people, and work to streamline the approach of using appropriate insulins for the individual patient.

Acknowledgements

Breeann Silvester, School of Medicine, UQ for administrative support.

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