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# Genetically Engineered Insulin Analogs: Diabetes in the New Millenium

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***Abstract*——Tight glucose control is essential to minimize complications in diabetic patients. However, the pharmacokinetic characteristics of the currently available rapid-, intermediate-, and long-acting preparations of human insulin make it almost impossible to achieve sustained normoglycemia. Until recently, improvements in insulin formulations were seriously limited as advances were only achieved in insulin purity, species, and characteristics of the retarding agent. The availability of molecular genetic techniques opened new windows to create insulin analogs by changing the structure of the native protein and to improve the therapeutic properties. The first clinically available insulin analog, Lispro, confirmed the hopes by showing that improved glycemic control can be achieved without an increase in hypoglycemic events. This requires, however, optimal basal insulin replacement, either by multiple daily injections of neutral protein Hagedorn (NPH) insulin or by insulin pump. Evidence suggests that short-acting insulin analogs would be better matched by a true basal insulin than by the erratically absorbed and rather short-acting NPH insulin. Therefore, future availability of long-acting analogs raises the hope to realize the true potential benefits of the currently available short-acting analog, Lispro, and of those still awaiting approval. The introduction of new short-acting and the first truly long-acting analogs, the development of analogs with increased stability, less variability and perhaps selective action will help to develop more individualized treatment strategies targeted to specific patient characteristics and to achieve further improvements in glycemic control.**

## I. Introduction

In nondiabetic individuals, ingestion of food results in a rise of serum insulin concentration to a maximum after 30 to 45 min, followed by a decline to basal levels after 2 to 3 h. The pharmacokinetic characteristics of the currently available rapid-, intermediate-, and long-acting preparations of human insulin make it almost impossible to achieve sustained normoglycemia. The onset of action of s.c. injected regular insulin is too slow, and the duration of its action is too long to mimic the insulin secretion pattern of a healthy

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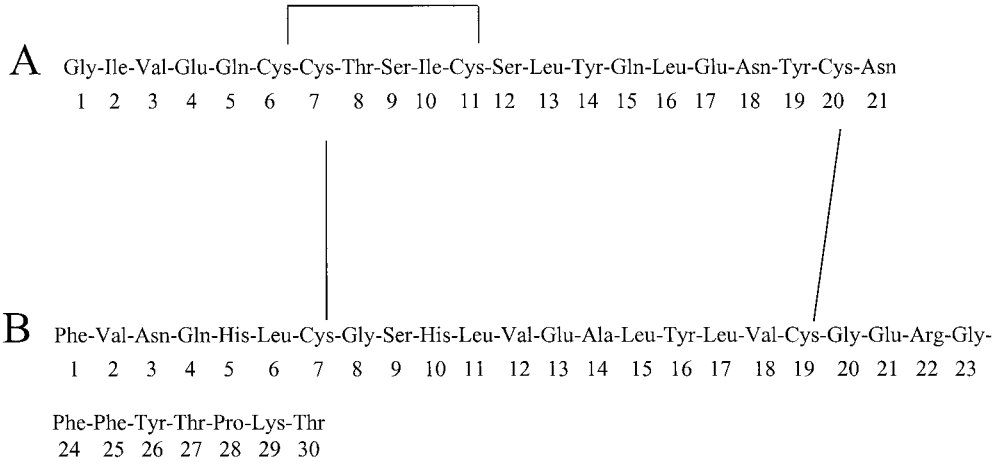


FIG. 1. The amino acid sequence of human insulin. B28–29 reversed in Lispro, B28 replaced with Asp in Aspart, A21 replaced with Gly and Arg added to B31–32 in HOE 901.

individual during a carbohydrate-containing meal (Heinemann et al., 1992). As a result, early postprandial hyperglycemia followed by an increased risk for hypoglycemia before the next meal are present. Similarly, the available intermediate/long-acting insulin preparations are unable to provide a stable, continuous baseline insulin level. Instead, they cause peak serum insulin levels at 3 to 4 h after s.c. injection and show considerable inter- and intrasubject variations in their bioavailability. The Diabetes Control and Complications Trial confirmed the link between glycemic control and the complications of diabetes (DCCT Research Group, 1993). Therefore, to achieve improved glucose control, the need for new insulin preparations with a faster onset and shorter duration of action, and long-acting preparations with a more flat time-action profile and less variable bioavailability became apparent in the 1990s (Berger, 1989). Until recently, however, improvements in insulin formulations were seriously limited, because advances were only achieved in insulin purity, species, and characteristics of the retarding agent. The availability of molecular genetic techniques opened new windows to create insulin analogs by changing the structure of the native protein and to improve the therapeutic properties of it.

## II. Short-Acting Insulin Analogs

Regular insulin has additional disadvantages. Because of its relatively slow onset of action, regular insulin is optimally administered 30 to 60 min before meals. Due to the inconvenience and difficulties with predicting the time of the meal, most patients do not follow this advice, even when adequately instructed (Jorgensen and Nielsen, 1990; Heinemann, 1995). Therefore, short-acting analogs that can be injected immediately before meals could improve compliance with treatment recommendations and the patients' overall satisfaction with the regimen. Patients consider the opportunity to inject insulin immediately before the meal an advantage, as it can increase flexibility and freedom in daily activities (Desmet et al., 1994). However, this cannot be achieved by human insulin, since above physiologic concentrations such as those present in the injectable preparations native human insulin forms dimers and hexamers, which inhibits its rapid absorption from the injection site (Mosekilde et al., 1989). Therefore, a possible approach to facilitate absorption and achieve rapid action is to develop analogs with a decreased tendency to self-association. This can be accomplished by changing the amino acid sequence of human insulin (Fig. 1). Because of faster absorption, a substantial reduction in the postprandial glucose excursion is expected with such analogs, and the more rapid decline in the serum concentration of the analog should result in a reduced risk of late hypoglycemia compared with regular human insulin (Brange, 1997).

### *A. Insulin Asp(B10)*

Elucidating the genetic basis for a case of familial hyperproinsulinemia (it involves a single point mutation in the proinsulin gene resulting in the substitution of aspartic acid for the naturally occurring histidine for residue 10 of the B chain of insulin) led to the development of insulin Asp(B10), one of the first insulin analogs (Schwartz et al., 1987). Insulin Asp(B10) is absorbed twice as rapidly as regular insulin and offers potential therapeutic benefits (Nielsen et al., 1995). However, studies with Asp(B10) pointed out that a potential problem with altering the amino acid sequence of human insulin is that it can change the three-dimensional structure of the molecule in a way that results in altered interaction with the insulin receptor and the insulin growth factor (IGF[2])-I receptor. In relative terms, the IGF-I receptor mediates cell growth to a greater extent than the insulin receptor. Insulin analogs, which have a

[2] Abbreviations: IGF, insulin growth factor; NPH, neutral protein Hagedorn; CSII, continuous s.c. insulin infusion; NN304, LysB29-tetradecanoyl, des(B30); WW99–532, *N*(ε)-palmitoyl Lys(B29); HOE 901, insulin glargine.

TABLE 1
*Structure, characteristics, and status of insulin analogs*

| Analog | Structure | Characteristics | Status | References |
|---|---|---|---|---|
| NovoSol Basal | Arg(B27)Gly(A21)Thr(B30) | Long action, low bioavailability | Trials discontinued | Jorgensen et al., 1989; Jorgensen and Drejer, 1990 |
| Lispro | Lys(B28)Pro(B29) | Short-acting, rapidly absorbed | Available | Howey et al., 1994; Torlone et al., 1994; Pfutzner et al., 1996; Anderson et al., 1997; Brunelle et al., 1998; Karsidag et al., 1996; Zinman et al., 1997; Ciofetta et al., 1999; Lalli et al., 1999; Renner et al., 1999; Roach et al., 1999; Garg et al., 1999[a] |
| Aspart | Asp(B28) | Short-acting, rapidly absorbed | Advanced clinical trials, awaiting approval | Home et al., 1998; Heinemann et al., 1998; Dall, 1999; Lindholm et al., 1999 |
| HOE 901 | Gly(A21)Arg(B31)Arg(B32) | Long-acting, peakless action, low rates of hypoglycemia | Advanced clinical trials, awaiting approval | Talaicular et al., 1996; Pieber et al., 1998; Rosenstock et al., 1998; Raskin et al., 1998; Matthews et al., 1998 |
| WW99-S32 | N(epsilon)-palmitoyl Lys(B29) | Long action, less variation, highly reproducible pharmacokinetic profile | Pre-clinical and early clinical trials | Myers et al., 1997; Howey et al., 1997; Radziuk et al., 1998 |
| NN304 | LysB29-tetradecanoyl,des(B30) | Long-acting, peakless action, less variation | Pre-clinical and early clinical trials | Markussen et al., 1996; Kurtzhals et al., 1997; Heinemann et al., 1999 |

[a] See text for more references on insulin Lispro.

greater affinity for the IGF-I receptor than human insulin, would therefore have increased growth and mitogenic effects compared with the native insulin molecule. The analog Asp(B10) has been demonstrated to have an increased affinity for the IGF-I receptor and a decreased rate of dissociation from the insulin receptor, as well as prolonged cellular processing (Bornfeldt et al., 1991; Hamel et al., 1999). Asp(B10) has been shown to have an increased cellular residence time, which results from an increased binding and internalization without a concomitant increase in intracellular degradation by insulin-degrading enzyme (Drejer, 1992; Hansen et al., 1996; Hamel et al., 1999). The consequence is cellular retention of biologically active insulin. These properties of Asp(B10) result in a greater metabolic effect compared with human insulin, which would be a potential advantage. However, the above characteristics also lead to increased mitogenic activity, and as a result, supraphar-macological doses of Asp(B10) cause a dose-dependent increase in the incidence of adenocarcinomas in laboratory animals (Jorgensen et al., 1992; Drejer, 1992). Further clinical studies with this analog were therefore halted.

### *B. Insulin Lispro*

The first genetically engineered rapid-acting analog to become available for the clinician was insulin Lispro, which was approved for clinical use in Europe in April 1996 and in the United States in June 1996. In insulin Lispro the normal sequence of proline at position 28 of the B chain and lysine at position 29 is reversed (LysB28,ProB29; Lispro) (Fig. 1). This reversal causes a decreased tendency for self-association, and as a result, faster absorption, higher peak serum levels, and shorter duration of action can be observed with insulin Lispro compared to regular insulin (Howey et al., 1994). Importantly, the amino acid sequence changes in Lispro do not affect its receptor-binding domain. Therefore, the affinity to the insulin receptor of insulin Lispro is similar to that of regular insulin, whereas Lispro's affinity for the IGF-I receptor is slightly higher but not enough to cause a difference in its cell growth-stimulating activity compared with regular insulin (Slieker et al., 1991, 1994). Pharmacokinetic studies indicate that insulin Lispro acts within 15 min, peaks in approximately 1 h, and disappears within 2 to 4 h after s.c. injection (Howey et al., 1994; Torlone et al., 1994). In clinical studies, as expected from a short-acting analog, insulin Lispro achieved significant improvements in postprandial glucose levels with a lower rate of hypoglycemic events compared with regular insulin (Pfutzner et al., 1996; Anderson et al., 1997; Brunelle et al., 1998). This can be observed even if insulin Lispro is administered immediately before meals, and regular insulin is injected 30 to 45 min before meals. Unfortunately, in most cases these beneficial effects were not accompanied by improvements in glycosylated hemoglobin values (Pfutzner et al., 1996; Anderson et al., 1997). Besides the decrease in hypoglycemic events, the most likely explanation for this is the inability of the currently used long-acting insulins to provide true basal coverage. Therefore, increased preprandial plasma glucose concentrations are present in patients on insulin Lispro. Supporting this theory, a clinically and statistically significant decrease of hemoglobin $A_1c$ levels was seen when insulin Lispro was used with two or more instead of one daily injections of NPH insulin (Karsidag et al., 1996; Ciofetta et al., 1999). Therefore, adding a few units of NPH to Lispro at each meal, combined with bedtime NPH (Del Sindaco et al., 1998; Ciofetta et al., 1999; Lalli et al., 1999) can be recommended for the intensive therapy of diabetes by multiple daily injections. This regimen may even improve unawareness of, and impaired counter-regulation to hypoglycemia (Lalli et al., 1999).

Similarly, because continuous s.c. insulin infusion (CSII) systems are able to provide a reasonable basal

insulin substitution, improved glycosylated hemoglobin values would be expected with pump treatment using insulin Lispro. After the stability of Lispro in insulin pump systems had been confirmed (Lougheed et al., 1997), clinical trials began to assess its effectiveness in CSII treatment. As assumed, results with insulin Lispro in patients receiving CSII are promising as evidenced by lower glycosylated hemoglobin values and improved postprandial glucose levels when compared with patients receiving pump treatment with regular insulin (Zinman et al., 1997; Renner et al., 1999). Importantly, the improved glycemic control is achieved without an increase in the number of hypoglycemic events. A potential disadvantage of using insulin Lispro in pump systems as opposed to regular insulin is that because of its more rapid disappearance, patients might be at more risk for developing ketoacidosis in case of catheter occlusion or pump malfunction (Pen et al., 1996). However, this was not confirmed by a recent study (Attia et al., 1998) in which no difference was found between patients receiving CSII treatment with Lispro or regular insulin with respect to the rate of rise in plasma glucose or serum ketone levels after disrupting s.c. infusion. The frequency of catheter occlusion or other site-related problems is similar with Lispro and buffered regular insulin (Zinman et al., 1997; Renner et al., 1999).

A protamine formulation of insulin Lispro with prolonged action (neutral protamine Lispro) has been developed and shown to be suitable as an intermediate-acting agent or as part of premixed preparations of Lispro and neutral protamine Lispro (25/75 and 50/50) (Janssen et al., 1997; Roach et al., 1999). Compared with human insulin mixtures, twice-daily administration of insulin Lispro mixtures resulted in improved postprandial glycemic control, similar overall glycemic control, and less nocturnal hypoglycemia, as well as offering the convenience of dosing closer to meals.

Managing diabetes in patients with end-stage renal disease is often problematic, because renal failure interferes with the metabolism of glucose and insulin. Many of these diabetics have wide fluctuations in their daily blood glucose profile. The action of regular insulin may be prolonged as a consequence of the failure of renal insulin degradation, making the dose-effect profile of insulin difficult to control, and hypoglycemia more likely. There is evidence that using insulin Lispro may make the calculation of insulin requirements easier and help to avoid large fluctuations in blood glucose levels of these patients (Jehle et al., 1999).

Based on the limited available data on its long-term effectiveness, it appears that insulin Lispro remains effective in treating diabetic patients up to 5.4 years of treatment (Garg et al., 1999). No differences have been reported between insulin Lispro and regular insulin in the likelihood of developing allergic reactions, adverse events, or abnormal laboratory values (Anderson et al., 1994). The immunogenicity of insulin Lispro is similar to that of regular insulin (Fineberg et al., 1996). Antibodies specific against insulin Lispro rarely develop and do not affect dose requirements (Roach et al., 1996; Garg et al., 1999). Interestingly, patients have been reported in whom severe resistance to human insulin because of antibody formation was successfully overcome by switching them to insulin Lispro (Henrichs et al., 1996; Lahtela et al., 1997).

Despite the difficulties with standardizing quality of life assessments, the available data are surprisingly consistent and show a greatly increased treatment satisfaction among patients receiving Lispro by CSII or as multiple injections (Pfutzner et al., 1996; Kotsanos et al., 1997; Renner et al., 1999). This can improve patient motivation and compliance, which are very important components of treatment success in diabetic patients.

### *C. Insulin Aspart*

The next example of changing the amino acid sequence of the insulin molecule to achieve short-acting insulin analogs is insulin aspart (AspB28), in which substitution of proline with the charged aspartic acid is carried out to reduce self-association of the molecule (Brange et al., 1988) (Fig. 1). Preclinical studies of insulin aspart have demonstrated that receptor interaction kinetics with the insulin receptor and with the IGF-I receptor are equivalent to those seen with human insulin (Drejer, 1992), and an equivalent metabolic effect of insulin aspart and human insulin has been shown with i.v. administration (Kang et al., 1991). When administered i.v., insulin aspart shows a similar safety profile as human insulin (Dall, 1999). Insulin aspart has been shown to be absorbed twice as fast as human insulin and to reach maximum concentrations twice as high, whereas its duration of action is shorter (Heinemann et al., 1998; Lindholm et al., 1999). As expected, the postprandial glucose control achieved with this analog is superior to regular human insulin, although their bioavailability is comparable (Lindholm et al., 1999). These results are consistent with those reported with the other short-acting analog, Lispro, but there is evidence that the improvement in postprandial control can be achieved without deterioration of late postprandial plasma glucose concentrations (Home et al., 1998). The expectation of lower rates of hypoglycemia also seems to have been met with insulin aspart as evidenced by a recent multicenter trial of type 1 diabetic patients, who showed more than a 50% reduction in major hypoglycemic events compared with human insulin (Home et al., 1998). Importantly, this analog retains its beneficial pharmacodynamic properties in a stable 30/70 premixed formulation as it shows a significantly greater metabolic effect in the first 4 h than the 30/70 mixture of human insulin (Weyer et al., 1997). Because of its promising characteristics, studies are presently underway to evaluate long-term metabolic control with insulin aspart.

## III. Long-Acting Insulin Analogs

A number of alterations of the insulin molecule by genetic engineering are currently being tested to retard and stabilize absorption kinetics of long-acting insulin preparations. One possibility to prolong insulin action is to elevate the isoelectric point of human insulin from pH 5.4 toward neutral by developing analogs with more positively charged amino acids (Rosskamp and Park, 1999). This will make the analog less soluble at the neutral pH of the injection site, and the injection of the analog into the s.c. tissue will result in crystallization of the molecules, causing delayed absorption into the circulation.

### *A. NovoSol Basal*

One of the first analogs developed by protein-engineering technology based on the above therapeutic goals was NovoSol Basal (B27Arg, A21Gly, B30Thr-NH2). Whereas the task of prolonged absorption was successfully completed with that analog as evidenced by longer $T_{50}$ than that of Ultratard HM insulin, nearly two times higher doses of the analog were required to achieve compatible glucose control. Also, whereas NovoSol Basal showed less intraindividual variability in its action, the interindividual variation remained high. Therefore, and because of its reduced bioavailability NovoSol Basal was withdrawn from further studies (Jorgensen et al., 1989; Jorgensen and Drejer, 1990).

### *B. HOE 901*

HOE 901 (insulin glargine) is a new long-acting biosynthetic human insulin analog currently being investigated by Hoechst, which results from elongation of the C-terminal end of the insulin B chain by two glycine residues, as well as substitution of the A21 asparagine residue with glycine (Fig. 1). This analog behaves with respect to insulin receptor binding, receptor autophosphorylation, phosphorylation of signaling elements, and promotion of mitogenesis like regular human insulin (Berti et al., 1998). Moreover, the IGF-I receptor-mediated growth promoting activity of HOE 901 in muscle cells and the maximal metabolic activity of this analog are not different from those of native human insulin (Bahr et al., 1997). However, its therapeutic properties and potentials are remarkable and different from human insulin. HOE 901 was shown to exert a glucose-lowering effect for 24 h after a single daily injection without a pronounced plasma peak (Dreyer et al., 1994). In one of the first small short-term clinical studies investigating this analog in 1996, once-daily injections of HOE 901 resulted in similar glycemic control as four daily injections of the same total units of NPH in type 1 diabetics (Talaulicar et al., 1996). Later on, the characteristics of HOE 901 have been investigated in both type 1 and type 2 diabetic patients. In Phase II trials conducted in Europe and the United States with type 1 diabetics, once-daily injections of HOE 901 along with premeal regular insulin achieved significantly lower fasting plasma glucose levels (Rosenstock et al., 1998) and hemoglobin $A_1c$ values compared with patients on NPH and regular insulin (Pieber et al., 1998). Remarkably, the better glucose control was associated with similar or even lower incidence of hypoglycemia. Studies of type 2 diabetic subjects showed similar fasting plasma glucose values with one injection of HOE 901 as those found with one or two injections of NPH insulin. Again, the incidence of hypoglycemia was similar or lower among patients on HOE 901 (Matthews and Pfeiffer, 1998; Raskin et al., 1998; Rosenstock et al., 1999). The technical difficulties with blinding the studies comparing NPH and HOE 901 should be noted, as the two preparations can be easily identified, because HOE 901 is a clear solution as opposed to the cloudy solution of NPH. A potential problem with altering the structure of the insulin molecule is increasing the risk of antibody development and adverse reactions at the site of injection. Importantly, adverse events and injection-site reactions associated with HOE 901 were not different from those found with NPH insulin, and antibody formation was also similar with the two preparations.

### *C. Fatty Acid Acylated Insulins*

Another way of prolonging insulin action is to couple the insulin molecule to nonesterified fatty acids, which bind to albumin. Albumin serves as a multifunctional transport protein that binds a wide variety of endogenous substances and drugs. Albumin is present in the s.c. tissue fluid with a slow disappearance rate. Binding insulin to albumin can therefore retard the absorption of the molecule and prolong its action. The binding to albumin apparently involves both nonpolar and ionic interactions with the protein (Kurtzhals et al., 1995). Acylation of the insulin molecule is usually performed in the side chain of lysine at position 29 of the B chain. Such insulin analogs are currently being studied by Lilly [*N*($\epsilon$)-palmitoyl Lys(B29); WW99-S32] and Novo Nordisk [LysB29-tetradecanoyl, des(B30); NN304] (Table 1).

In animal studies, the time for 50% disappearance from the s.c. space of NN304 was 14.3 h, significantly longer than that of NPH insulin (10.5 h) and with significantly less interanimal variation (Markussen et al., 1996). In healthy volunteers the metabolic response induced by s.c. injection of NN304 does not show the pronounced peak seen with NPH insulin in an identical dose. NN304 also shows a slower onset of action, as indicated by a significantly higher $t_{max}$ compared with NPH insulin (Heinemann et al., 1999). Importantly, the binding of NN304 has been shown to be independent of the binding of drugs in the two major binding pockets that are located in domains IIA and IIIA of the albumin molecule. Thus, NN304 is unlikely to be involved in clinically significant drug interactions at the albumin binding level (Kurtzhals et al., 1997).

In a diabetic animal model, the duration of action of the other analog WW99-S32 human insulin administered i.v. was nearly twice that of unmodified human insulin, and the plasma half-life was nearly 7-fold that of the unmodified protein. Administered s.c., WW99-S32 human insulin had a longer duration of action, a flatter, more basal, plasma insulin profile, and a lower intersubject variability of response than the intermediate-acting insulin suspension Humulin L (Myers et al., 1997). In healthy volunteers, this analog showed a highly reproducible linear pharmacokinetic profile but less potency when compared with NPH (Howey et al., 1997). The latter finding was subsequently confirmed in C-peptide negative patients (Radziuk et al., 1998). Based on the results with insulin acylation, derivatization with albumin-binding ligands could be applicable generally to prolong the action profile of peptide drugs (Kurtzhals et al., 1995).

## IV. Future Directions

### *A. Increased Stability*

Insulin is not a stable chemical entity. A variety of chemical changes of the primary structure affect insulin during handling, storage, and even use. Insulin decomposition is mainly due to two categories of chemical reactions: hydrolysis and intermolecular transformation leading to covalent insulin dimers. Identification of the residues undergoing chemical changes during storage allows designing insulin analogs with improved stability. The advantage of such analogs would be prolonged shelf-life and more convenient storage conditions. Improved stability is also essential for pump usage. The above discussed Asp(B10) analog has increased stability but is unfortunately not suitable for clinical use (Brems et al., 1992). Substitution of AsnB3 by Gln, and AsnA21 by Ala or Gly results in analogs with 30 times less deamination and 10 times reduced formation of covalent dimers (Brange 1997). Designing and testing more analogs with increased stability remains an important task for the future.

### *B. Less Variability*

The high intra-and interindividual variability of the response to identical insulin doses is a serious problem for patients and their clinicians as well, and can hamper the achievement of reasonable glycemic control without the risk for hypoglycemic events (Heinemann et al., 1998). There are two explanations for the variability of insulin responsiveness. Pharmacokinetic variability can result from variations in insulin absorption, leading to different plasma concentrations of insulin after s.c. injection of the same doses (Lauritzen et al., 1979; Binder et al., 1984). Pharmacodynamic variability, on the other hand, can be caused by differences in insulin action, causing different metabolic effects by similar plasma insulin concentrations (Ziel et al., 1988). The above discussed long-acting analog NovoSol Basal shows less intraindividual variation in its pharmacokinetics than the longest acting currently available human insulin preparation Ultratard HM (Jorgensen et al., 1989). Similarly, a decreased variability in serum insulin concentrations compared with regular human insulin has been shown after s.c. injections of the analog insulin Lispro (Antsiferov et al., 1995). Nevertheless, developing insulin analogs with lower pharmacokinetic and pharmacodynamic variability remains to be an important task.

### *C. Selective Action*

Insulin influences glucose metabolism by inhibiting hepatic glucose production and stimulating peripheral glucose disposal. Insulin analogs with relatively greater effect on hepatic glucose production could offer potential therapeutic benefits for selected patients. Proinsulin, the single chain precursor of insulin is more effective in the liver than in the periphery (Revers et al., 1984; Lavelle-Jones et al., 1987). Reasons for this selectivity are not fully understood, but the increased molecular size of proinsulin compared with insulin has been proposed as a potential mechanism. Endothelial cells in peripheral tissues limit the transfer of substances from the circulation into the tissues with a rate inversely related to the molecular size of the transferred substance. However, hepatocytes are freely in contact with all blood constituents in the hepatic sinusoids. Dose requirements for proinsulin are approximately 4-fold higher than for human insulin, and there is a possible association between its use and myocardial infarction (Spradlin et al., 1990; Galloway et al., 1992). Proinsulin was therefore withdrawn from clinical trials, but the recognition of its selective action has stimulated the search for analogs with greater hepatic effects. Two insulin analogs with increased molecular size due to covalent dimerization have been shown to have a greater effect on hepatic glucose production than peripheral glucose disposal after i.v. administration (Shojaee-Moradie et al., 1995). These dimeric analogs [$N(\alpha)$B1, $N(\alpha)$B′1,-suberoyl insulin dimer, and $N(\epsilon)$B29, $N(\epsilon)$B′29,-suberoyl insulin dimer] are probably not suitable for clinical use because of their relatively low potency, but they confirm the possibility that analogs with selective action due to increased molecular size might be developed. Another interesting finding is that $N\alpha\beta$1-thyroxyl insulin and $N\alpha\beta$1-thyroxyl-aminohexanoyl insulin also show greater selectivity for hepatic glucose production (Shojaee-Moradie et al., 1998). These insulin analogs bind thyroid hormone-binding proteins to form high molecular weight complexes. These findings provide further support to the theory that the reduced peripheral insulin-like effect could be due to reduced transcapillary access to peripheral insulin receptor sites, which results from high molecular weight.

Another possibility for selectivity of an analog, either to a specific tissue or for a specific action (e.g., increased mitogenicity compared with metabolic effects) would be altered cellular metabolism of the analog. Reduced degradation would prolong cellular residence of the material and alter activity profile. The analog Asp(B10) is an example of this (Hamel et al., 1999). For future development of specific analogs more information is needed on properties of the insulin molecule important for different biological activities, e.g., carbohydrate versus fat versus protein metabolic effects (Duckworth, 1997).

### *D. Ultra Rapid Onset*

Although significant improvements in postprandial plasma glucose levels can be achieved with the presently available short-acting analog, insulin Lispro, even when it is injected immediately before meals, there is evidence that its optimal administration would be actually 15 to 30 min before meals (Rassam et al., 1999). When administered at least 15 min before meals, Lispro achieves a greater improvement in postprandial values as opposed to being injected immediately before meals. This suggests that developing even more rapidly absorbed short-acting analogs could offer potential benefits.

### *E. Ultra Long Activity*

Some insulin-requiring patients simply do not have the background or resources needed for insulin treatment. They may not have access to a refrigerator or are unable to use insulin without getting help because of disabilities. Potentially these patients could use ultra long-acting analogs that could be injected once weekly or for even longer periods of time. This type of preparation obviously would not provide good control but could offer basal coverage sufficient to prevent ketoacidosis or other acute complications. The concept may seem utopistic at first sight, but a recent study reported that a single s.c. injection of a new analog, in which two 9-fluorenylmethoxycarbonyl moieties are covalently linked to the phenylalanine at position B1 and to the lysine at B29 of human insulin, normalized blood sugar levels of rats for 2 to 3 days with streptozotocin-induced diabetes (Gershonov et al., 1999). The analog itself has only 1 to 2% of the biological potency of insulin, but undergoes a time-dependent spontaneous conversion to fully active insulin. The conversion takes place slowly under physiological conditions, with a $t_{1/2}$ of 12 (!) days.

### *F. Benefit without Metabolic Activity?*

The insulin analog Asp(B25) does not bind to the insulin receptor or IGF-I receptor and has no hypoglycemic effect (Drejer et al., 1991). However, this analog has been shown to prevent diabetes in an animal model of spontaneous diabetes that shares many features of human type 1 diabetes (Karounos et al., 1997). The analog prevented diabetes in the animals even when it was initiated after the onset of extensive lymphocytic infiltration of the pancreatic islets. The mechanism—because it did not involve metabolic effects—appears to be immunological. Although it is yet unclear, whether Asp(B25) can be used for preventing diabetes in prediabetic children and young adults, the theory of using analogs without the potentially harmful hypoglycemic effects for diabetes prevention is certainly an interesting one.

## V. Closing Thoughts

The need for nearly optimal glucose control in diabetics to minimize complications clearly exists. Without insulin analogs, however, this can only be accomplished at the expense of hypoglycemic reactions. The DCCT trial demonstrated that a 10% improvement of glycosylated hemoglobin levels results in a 43% improvement of retinopathy but is accompanied by an 18% increase of severe hypoglycemic episodes (DCCT Research Group, 1993). The first clinically available insulin analog, Lispro, opened new hopes by showing that improved glycemic control can be achieved without an increase in hypoglycemic events. This requires, however, optimal basal insulin replacement, either by multiple daily injections of NPH or by CSII. Evidence suggests that short-acting insulin analogs would be better matched by a true basal insulin than by the erratically absorbed and rather short-acting NPH insulin (Home et al., 1998). Therefore, future availability of long-acting analogs raises the hope of realizing the true potential benefits of the currently available short-acting analog, Lispro, and of those still awaiting approval. The introduction of new short-acting and the first truly long-acting analogs, the development of analogs with increased stability, less variability, and perhaps selective action will help to develop more individualized treatment strategies targeted to specific patient characteristics and to achieve further improvements in glycemic control.

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