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Recombinant DNA Technology in the Treatment of Diabetes.

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ABSTRACT:
After more than half a century of treating diabetics with an- imal insulins, recombinant DNA technologies and advanced protein chemistry made human insulin preparations avail- able in the early 1980s. As the next step, over the last decade, insulin analogs were constructed by changing the structure of the native protein with the goal of improving the therapeutic properties of it, because the pharmacokinetic characteristics of rapid-, intermediate-, and long-acting preparations of hu- man insulin make it almost impossible to achieve sustained normoglycemia. The first clinically available insulin analog, lispro, confirmed the hopes by showing that improved glyce- mic control can be achieved without an increase in hypogly- cemic events. Two new insulin analogs, insulin glargine and insulin aspart, have recently been approved for clinical use in the United States, and several other analogs are being inten- sively tested. Thus, it appears that a rapid acceleration of basic and clinical research in this arena will be seen, which will have direct significance to both patients and their phy- sicians. The introduction of new short-acting analogs and the development of the first truly long-acting analogs and the development of analogs with increased stability, less variabil- ity, and perhaps selective action, will help to develop more individualized treatment strategies targeted to specific pa- tient characteristics and to achieve further improvements in glycemic control. Data on the currently available and tested analogs, as well as data on those currently being developed, are reviewed.

I. INTRODUCTION

FOR THE PURPOSES of this review, we define insulin analogs as molecules brought about by modifying the structure of the human insulin molecule, which results in altered physicochemical, biological, and pharmacodynamic properties. The first such agent, insulin lispro, has been avail- able to the clinician since mid-1996. No new analogs became available for clinical use for the next 4 yr. However, at the time of the preparation of this manuscript, two new insulin analogs, insulin glargine and insulin aspart, have recently been approved for clinical use in the United States, and several other analogs are being intensively tested. Thus, it appears that a rapid acceleration of basic and clinical research will be seen in this arena, which will have direct significance to both patients and their physicians.

II. BACKGROUND

The insulin receptor is a tyrosine kinase that undergoes activation upon insulin binding, leading to the tyrosine phos- phorylation of a specific collection of intracellular proteins (1). The IGF-I receptor and the insulin receptor exhibit sub- stantial structural homology to each other, and both ligands have a easurable affinity to the other's receptor. Structure- function studies have shown that the amino acids of the insulin molecule essential for binding to the insulin receptor are A1Gly (glycine at position 1 of the A chain of insulin; Fig. 1), A2Ile, A3Val, A19Tyr, B6Leu, B12Val, B23Gly, B24Phe, and B25Phe, whereas alterations in the B10 and B26 –30 re- gions of the human insulin molecule alter its affinity to the IGF-I receptor (2, 3). Based on this knowledge, it is possible to design analogs of human insulin that preserve receptor binding but show differences in other properties.

III. WHY DO WE NEED ANALOGS?

In nondiabetic individuals, ingestion of food results in a relatively rapid rise of serum insulin concentration to a max- imum after 30 – 45 min, followed by a decline to basal levels after 2–3 h. The pharmacokinetic characteristics of the cur- rently available rapid-, intermediate-, and long-acting prep- arations of human insulin make it almost impossible to achieve sustained normoglycemia. The onset of action of sc-injected regular human insulin is too slow and the dura- tion of its action too long to mimic the insulin secretion pattern of a healthy individual during ingestion of a carbo-hydrate-containing meal (4). As a result, early postprandial hyperglycemia followed by an increased risk for hypogly-cemia before the next meal are present. Similarly, the avail- able

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intermediate/long-acting human insulin preparations are unable to provide a stable, continuous baseline insulin level. Instead, they cause peak serum insulin levels at 3– 4 h after sc injection and show considerable inter- and intra-subject variations in their bioavailability. The Diabetes Con-trol and Complications Trial confirmed the link between glycemic control and the complications of diabetes (5). There-fore, to achieve improved glucose control, the need for new insulin preparations with a faster onset and shorter duration of action and for long-acting preparations with a more flat time-action profile and less variable bioavailability became apparent in the late 1980s and early 1990s (6). However, until recently, improvements in insulin formulations were seri-ously limited; advances were only achieved in insulin purity, species, and characteristics of the retarding agent. The avail- ability of molecular genetic techniques opened new windows for creating insulin analogs by changing the structure of the native protein, improving its therapeutic properties.

In addition to its glucose-lowering effect, insulin is the most potent physiological anabolic agent known to date (7). It promotes the synthesis and storage of lipids, proteins, and carbohydrates and prevents their degradation and release back to the circulation. Despite years of intensive investiga-tion, we are still left with considerable uncertainty regarding the precise intracellular events that mediate the action of this hormone. One confounding factor has been the variety of actions of insulin, which depend on the cell type, time of exposure, and the presence or absence of other hormones (8). Another is the fact that insulin can act as a growth factor for cultured cells and shares many of the mitogenic signaling pathways elicited by other growth factors. However, the metabolic effects of insulin are unique and cannot be repro-duced by other cellular stimuli (7, 9). Taken together, these findings indicate that signaling mechanisms that respond only to insulin exist, and they allow for the specialized effects of insulin on metabolism. Designing and studying insulin analogs has helped, and without any doubt will help, our understanding of the complex processes insulin is associated with, and creating analogs selective to one or another of insulin's actions might well be of clinical significance.

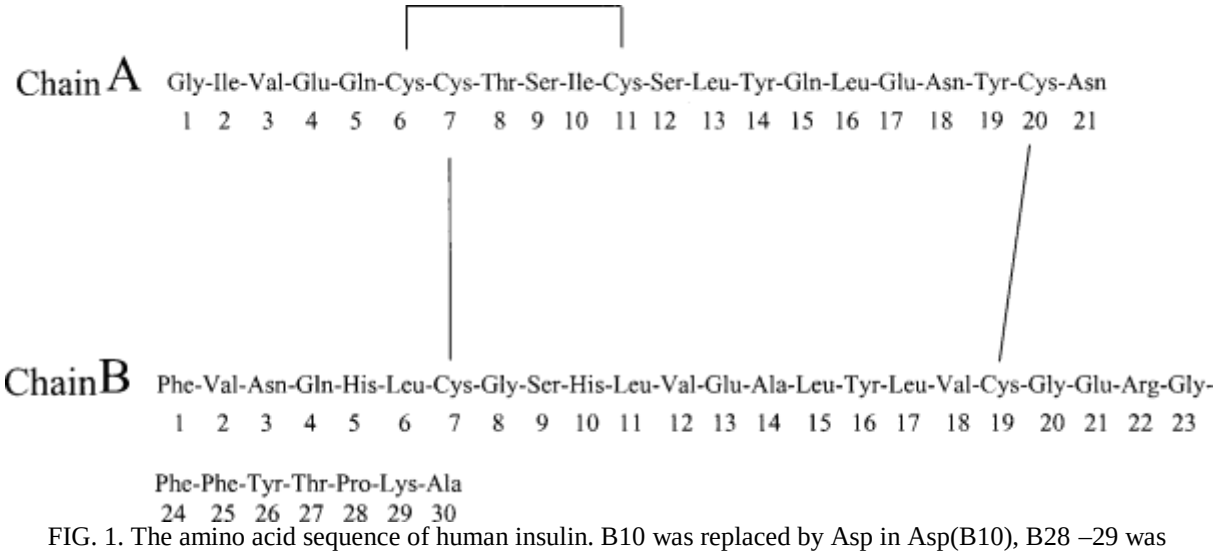

FIG. 1. The amino acid sequence of human insulin. B10 was replaced by Asp in Asp(B10), B28 –29 was reversed in lispro, B28 was replaced with Asp in aspart, A21 was replaced with Gly, and Arg was added to B31–32 in HOE 901. Alanine, Ala; arginine, Arg; asparagine, Asn; aspartic acid, Asp; cysteine, Cys; glutamic acid, Glu; glutamine, Gln; glycine, Gly; histidine; His; isoleucine, Ile; leucine, Leu; lysine, Lys; methionine, Met; phenylalanine, Phe; proline, Pro; serine, Ser; threonine, Thr; tryptophan, Trp; tyrosine, Tyr; valine, Val.

IV. SHORT-ACTINGINSULINANALOGS

In addition to the above, regular insulin has more disad- vantages. Because of its relatively slow onset of action, reg-ular insulin is optimally administered 30 – 60 min before meals. Due to the inconvenience and difficulties with pre- dicting the time of the meal, most patients do not follow this advice, even when adequately instructed (10, 11). Therefore, short-acting analogs that could be injected immediately be- fore meals would improve compliance with treatment rec- ommendations and

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the patients' overall satisfaction with the regimen. Patients consider the opportunity to inject insulin immediately before the meal an advantage, because it can increase flexibility and freedom in daily activities (12). This cannot, however, be achieved by human insulin, because above physiological concentrations, such as those present in the injectable preparations, native human insulin forms dimers and hexamers, which inhibit its rapid absorption from the injection site (13). Therefore, a possible approach to facilitate absorption and to achieve rapid action is to develop analogs with a decreased tendency to self-associate. This can be accomplished by changing the amino acid sequence of human insulin. 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 (14).

Analog	Structure	Characteristics	Status
NovoSol Basal	Arg(B27)Gly(A21) Thr(B30)	Long action, low bioavailability	Trials discontinued
Asp(B10)	Asp(B10)	Short acting, rapidly absorbed, increased metabolic potency	Trials discontinued
Lispro	Lys(B28)Pro(B29)	Short acting, rapidly absorbed	Available since 1996
Aspart	Asp(B28)	Short acting, rapidly absorbed	Approved, but not marketed yet
HOE 901	Gly(A21)Arg(B31)Arg(B32)	Long acting, peakless action, low rates of hypoglycemia	Available for clinical use Preclinical
WW99-S32	N(s)-palmitoyl Lys(B29)	Long action, less variation, highly reproducible pharmacokinetic profile	and early clinical trialsPreclinical
NN304 (detemir)	LysB29-tetradecanoyl, des(B30)	Long-acting, peakless action, less variation	and early clinical trials

TABLE1. Structure, characteristics, and status of insulin analogs

A. InsulinAsp(B10)

Elucidating the genetic basis for a case of familial hyper-proinsulinemia (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 proposed for clinical use (15) (Fig. 1 and Table 1). Insulin Asp(B10) is absorbed twice as rapidly as regular insulin and offers potential therapeutic benefits (16). However, studies with Asp(B10) pointed out that a potential problem with altering the amino acid se-quence 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 IGF-I receptor. This analog has been demonstrated to have an increased affinity both for the insulin and for the IGF-I re- ceptor, a decreased rate of dissociation from the insulin re-ceptor, as well as prolonged cellular processing (17–19). This results in a much greater metabolic effect compared with human insulin, which would be a potential therapeutic ad-vantage. In addition to carbohydrate metabolism, insulin Asp(B10) been shown to have an increased effect on lipo-genesis as well (20). Unfortunately, the above characteristics also lead to increased mitogenic activity in several cell lines, and as a result, suprapharmacological doses of Asp(B10) cause a dose-dependent increase in the incidence of adeno- carcinomas in laboratory animals (20 –22). Further clinical studies with this analog were therefore halted. However, realizing the enormous potential implications brought about by modifying the human insulin molecule encouraged re-searchers to continue developing new insulin analogs. At the same time, the significantly different clinical properties of Asp(B10) also boosted a new area of insulin research inves-tigating the biochemical rocesses other than carbohydrate metabolism in which insulin participates and the processes through which the insulin molecule goes after receptor bind- ing. Research on the analog Asp(B10) has provided useful information in the context of insulin action.

In contrast to human insulin, Asp(B10) induces a pro- longed phosphorylation state of the 95-kDa receptor β-subunit and of the insulin receptor substrates 1/2 and Shc (23). In addition, an increased and prolonged tyrosine phos-phorylation of a yet unidentified 60-kDa protein has been observed with Asp(B10). Asp(B10) also shows increased [3 H]thymidine incorporation into DNA compared with reg-ular insulin (23).

It appears that the increased mitogenic

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activity of Asp(B10) could result from at least two mechanisms, increased IGF-I receptor affinity and decreased dissociation from the insulin receptor. This could be of clinical significance, because when a new analog is developed, it is possible that one or both of these characteristics will be altered. The lesson learned from insulin Asp(B10) was that assessing the molecular pharma- cological properties, such as insulin and IGF-I receptor bind- ing and metabolic and mitogenic potency, is of clinical im- portance in the evaluation of newly developed insulin analogs.

B. Insulin lispro (EliLilly&Co.,Indianapolis,IN)

The B26 –30 region of the insulin molecule is not critical in binding to the insulin receptor. However, it is clearly important in mediating the formation of insulin dimers (24). Therefore, structural modifications of the molecule at these positions would be expected to generate insulin analogs with minimal tendency for self-association but unaltered affinity to the insulin receptor compared with regular human insulin (3).

The first genetically engineered rapid-acting insulin ana- log to become available for the clinician was insulin lispro, which was approved for clinical use in Europe in April of 1996 and in the United States in June of 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) (Fig. 1 and Table 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 with regular insulin (25). Importantly, as discussed above, the amino acid se- quence 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. Although lispro's affinity for the IGF-I receptor is slightly higher, it is not enough to cause a difference in its cell growth-stimulat- ing activity compared with regular insulin (26, 27). Also, in the case of lispro, growth-promoting activity in human mam-mary epithelial cells has been found to be correlated more with dissociation kinetics from the insulin receptor, which were shown to be identical with those of human insulin (3). Insulin lispro was also found to have a low mitogenic po-tency when studied using a human osteosarcoma cell line (20), and in contrast to Asp(B10), the cellular processing of lispro is essentially identical with that of human insulin (19). Therefore, unlike Asp(B10), lispro was found to be safe for clinical use.

In terms of activity on lipogenesis, insulin lispro was foundto be essentially the same as human insulin (20). Phar- macokinetic studies indicate that insulin lispro acts within 15 min, peaks in approximately 1 h, and disappears within 2– 4 h after sc injection (25, 28). In clinical studies, as expected from a short-acting analog, insulin lispro achieved signifi-cant improvements in postprandial glucose levels with a lower rate of hypoglycemic events compared with regular insulin (29 –31). This can be observed even if insulin lispro is administered immediately before meals and regular insulin is injected 30 – 45 min before meals. Unfortunately,
in most cases, these beneficial effects were not accompanied by im- provements in glycosylated hemoglobin values (29, 30). In addition to 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. There- fore, 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 daily injections, instead of one, of neutral protamine Hagendorn (NPH) insulin (32, 33). Therefore, for the intensive therapy of diabetes by multiple daily injections, the addition of a few units of NPH to lispro at each meal, combined with bedtime NPH, can be recommended (33–35). This regimen may even improve unawareness of, and im- paired counterregulation to, hypoglycemia (35).

Similarly, because continuous sc insulin infusion (CSII) systems are able to provide a reasonable basal insulin sub- stitution, 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 (36), 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 as compared with patients receiving pump treatment with regular insulin (37, 38). Importantly, the im- proved glycemic control is achieved without an increase (or even with a decrease) 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

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might be at more risk for developing ketoacidosis in the case of catheter occlusion or pump malfunction (39). This was, however, not confirmed by a recent study, in which no difference with respect to the rate of rise in plasma glucose or serum ketone levels after dis-rupting sc infusion was found between patients receiving CSII treatment with lispro or those receiving treatment with regular insulin (40). The frequency of catheter occlusion or other site-related problems is similar with lispro and buff-ered regular insulin (37, 38). When comparing regimens us-ing lispro, it was found that using lispro in CSII provides better glycemic control with lower doses of insulin than multiple daily injections of lispro and NPH (41). This, in addition to supporting the suitability of lispro in pump sys-tems, also highlights the fact that the real advantages of a short-acting analog can be better translated into clinical ben-efits when they are used in a regimen with optimal basal insulin coverage (i.e., insulin pumps or a truly long-acting insulin, but not NPH).

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 prota-mine lispro (25/75 and 50/50) (42, 43). Compared with hu-man 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 (44).

Managing diabetes in patients with end-stage renal dis-ease 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, mak-ing the dose-effect profile of insulin difficult to control and making hypoglycemia more likely. There is evidence that using insulin lispro might make the calculation of insulin requirements easier and might help to avoid large fluctua-tions in blood glucose levels of these patients (45).

Insulin lispro has also been tested for use in pregnancy and gestational diabetes (46, 47). Compared with regular
human insulin, during a meal test, areas under the curve for glucose, insulin, and C-peptide were found to be significantly lower with insulin lispro. Mean fasting and postprandial glucose concentrations and end-point HbA 1c levels were similar to those with regular insulin, but patients on lispro demon- strated fewer hypoglycemic episodes. No fetal or neonatal abnormalities were noted in either treatment group. Anti- insulin antibody levels were similar in the two groups, and insulin lispro was not detectable in the cord blood (46). A recent study found that, whereas no patients on insulin lispro showed any change in their retinopathy status, 14% of pa- tients on regular insulin had worsening of retinopathy (48). Based on the limited available data on its long-term ef-fectiveness, it appears that insulin lispro remains effective in treating diabetic patients up to 5.4 yr of treatment (49). No differences have been reported between insulin lispro and regular insulin in the likelihood of developing allergic reac- tions, adverse events, or abnormal laboratory values (50). The immunogenicity of insulin lispro is similar to that of regular insulin (51). Antibodies specific against insulin lispro hardly ever develop and do not affect dose requirements (49, 52). Interestingly, there have been reports of patients in whom severe resistance to human insulin due to antibody formation was successfully overcome by switching them to insulin lispro (53, 54).

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 (29, 38, 55). This can improve patient motivation and compliance, which are very important components of treatment success in diabetic patients.

C. Insulin aspart [NovoLog (Novo Nordisk, Princeton, NJ)

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 (Fig. 1 and Table 1) (56). This analog was approved for clinical use in the United States in June of 2000. Preclinical studies of insulin aspart have dem- onstrated that receptor interaction kinetics with the insulin receptor and with the IGF-I receptor are essentially equiv- alent to those seen with human insulin (22), and an equiv- alent metabolic effect of insulin aspart and human insulin has been shown with iv administration (57). The potency on lipogenesis of insulin aspart is similar to that on human insulin, whereas its affinity to the IGF-I receptor is slightly

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lower, and thus, it does not result in greater mitogenic po- tency (20). When administered iv, insulin aspart shows a similar safety profile with that of human insulin (58). When further assessing its safety, it was found that insulin aspart and soluble human insulin elicit the same counterregulatory and symptomatic responses to acute hypoglycemia in pa- tients with type 1 diabetes (59). 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 (60 – 62). As expected, the post- prandial glucose control achieved with this analog is supe- rior to regular human insulin, whereas their bioavailability is comparable (61). Mean postprandial glucose levels after any meal are lower, even when aspart is injected immedi- ately before the meal and regular human insulin is admin- istered 30 min before meals (63). These results are consistent with those reported with the other short-acting analog, lis- pro, but there is evidence that the improvement in postpran- dial control can be achieved without deterioration of late postprandial plasma glucose concentrations (64). The expec- tation 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, which showed more than a 50% reduction in major hypoglycemic events compared with human insulin (64). In a very interesting study with type 1 diabetics, it was found that, because of its rapid absorption, insulin aspart provided reasonable glucose control even when injected 15 min after the start of meals (65). In the same study, it was also found that after abdominal injections, aspart had a shorter duration of glucose lowering effect than after administration in the thigh or deltoid area (65). The beneficial effects of insulin aspart have also been confirmed in type 2 diabetics (66) and in a pediatric population with type 1 diabetes (67). Importantly, this analog retains its ben- eficial pharmacodynamic properties in a stable 30/70 pre-mixed formulation, as it shows a significantly greater
met- abolic effect in the first 4 h with more rapid absorption and higher peak serum concentration than the 30/70
mixture of human insulin (68, 69). Because of its promising character- istics, studies are presently underway to evaluate long-term metabolic control with insulin aspart.

V. 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 isoelec- tric point of human insulin from pH 5.4 toward neutral by developing analogs with more positively charged amino ac- ids (70). This will make the analog less soluble at the neutral pH of the injection site, and the injection of the analog into the sc tissue will result in crystallization of the molecules, causing delayed absorption into the circulation.

A. NovoSol Basal (Novo Nordisk)

One of the first analogs developed by recombinant DNA technology based on the above therapeutic goals was No-voSol Basal (B27Arg, A21Gly, B30Thr-NH 2). As evidenced by longer half-life than that of Ultratard (Novo Nordisk) HM insulin, one of the longest acting preparations of human insulin, the task of prolonged absorption was successfully completed with this NovoSol Basal, but nearly 2 times higher doses of this 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 also because of its reduced bioavailability, NovoSol Basal was withdrawn from further studies (71, 72).

B. Insulin glargine [HOE 901, LANTUS (Aventis Pharmaceuticals, Parsippany, NJ)]

HOE 901 (insulin glargine, LANTUS) is a new long-acting biosynthetic human insulin analog developed by Aventis Pharmaceuticals, which was approved for use in patients with type 1 and type 2 diabetes mellitus by the United States Food and Drug Administration in April of 2000 and by the European Agency for the Evaluation of Medicinal Products in June of 2000. This analog results from elongation of the C-terminal end of the insulin B chain by two arginine resi-dues, as well as substitution of the A21 asparagine residue with glycine (A21Gly, B31Arg, B32Arg human insulin) (Fig. 1 and Table 1). These modifications led to a shift of the isoelectric point from pH 5.4 of human insulin to 6.7, making insulin glargine less soluble at physiological pH levels. After sc injection, insulin glargine precipitates in the sc tissues,which delays its absorption and prolongs its duration of action (73). The substitution at position A21

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largely increased the bioavailability of this analog, so unlike NovoSol Basal, it is suitable for clinical use (74).

With respect to insulin receptor binding, receptor auto-phosphorylation, phosphorylation of signaling elements, and promotion of mitogenesis in muscle cells, insulin glargine behaves like regular human insulin (23). Moreover,the 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, whereas its lipogenic activity is slightly lower (20, 75). However, insulin glargine's therapeutic properties and potentials are remark-able 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 and induced a smoother metabolic effect than NPH insulin (73, 76). Thus, HOE 901 is expected to better substitute basal insulin re-quirements. Moreover, although it is well known from clin- ical practice that the effect of NPH insulin can vary with the site of injection, it has been found that changes in the injection site do not alter the time-action profile of HOE 901 (77, 78). In one of the first small, short-term clinical studies investi-gating this analog in 1996, once-daily injections of HOE 901 resulted in similar glycemic control as compared with four daily injections of the same total units of NPH in type 1 diabetics (79). 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 (80) and hemoglobin A 1c values compared with patients on NPH and regular insulin (81). Remarkably, the better glucose control was associated with similar or even lower incidences of hypoglycemia. Studies of type 2 diabetic subjects showed similar fasting plasma glu- cose values with one injection of HOE 901 compared with those found with one or two injections of NPH insulin. Again, the incidence of hypoglycemia was similar or lower among patients on HOE 901 (82–84). More recently, the findings of less frequent hypoglycemic episodes and lower fasting plasma glucose levels compared with NPH were con-firmed in large, multicenter clinical trials with type 1 and type 2 diabetics in Europe and the United States (85– 88). Considering that less hypoglycemia was consistently ob- served, these data suggest that the target fasting plasma glucose level can be lower for insulin glargine than for NPH (88). The technical difficulties with blinding the studies com- paring NPH and HOE 901 should be noted, as the two prep- arations can be easily identified because HOE 901 is a clear solution as opposed to the cloudy solution of NPH. It might make designing blinded research studies more difficult, but in daily clinical life, it could actually be an advantage that insulin glargine is a clear solution. It has been shown that patients do not sufficiently shake suspensions like NPH in- sulin before administration (89). Because it is not necessary to shake HOE 901 before usage, it may have a lower intra- individual variability of its metabolic effect. In recent clinical trials, patients treated with insulin glargine had less vari-ability of their fasting plasma glucose values than those re-ceiving NPH (84, 90).

Insulin glargine has a greater affinity to the IGF-I receptor than human insulin (20). The observation of a progression of retinopathy in some patients with type 2 diabetes treated ith insulin glargine raised concerns, partly because IGF-I has been implicated in the development of retinopathy (91).

A review of the retinopathy data and the lack of optic disc swelling, which is the most common ocular side effect of treatment with IGF-I, led to the conclusion that this finding was probably not related to insulin glargine (92).

A potential problem with altering the structure of the insulin molecule is increasing the risk of antibody develop-
ment and adverse reactions at the site of injection. Impor-tantly, 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 by modifying the hormone's structure to achieve binding to a serum pro-tein. It is well known that a number of hormones bind to a specific serum-binding protein, which extends their half-life. The same can be done with insulin by coupling 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 sc tissue fluid with a slow disap-pearance rate. Binding insulin to albumin can therefore re-tard the absorption of the molecule and prolong its action.The binding to albumin apparently involves both nonpolar and ionic

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interactions with the protein (93). 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 (Indianapolis, IN) (WW99- S32) and Novo Nordisk (Copenhagen, Denmark) (NN304).

1. NN304 (insulin detemir). In animal studies, the time for 50% disappearance from the sc space of NN304 (LysB29- tetrade- canoyl, des(B30)-insulin) was 14.3 h, significantly longer than that of NPH insulin (10.5 h) and with significantly less in-teranimal variation (94). In healthy volunteers, the metabolic response induced by sc 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 maximal life compared with NPH in- sulin (95). This analog has been found to be less effective than human insulin when given in equimolar doses to healthy volunteers (95). Insulin detemir was also found to have a lower affinity to the insulin receptor, but a prolonged re- ceptor dissociation time compared with human insulin (20). Insulin detemir is less potent than human insulin in binding to the IGF-I receptor and stimulating lipogenesis, and unlike Asp(B10), it is less mitogenic than human insulin. Thus, the in vitro profile of insulin detemir did not cause any safety concerns (20). 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 al- bumin binding level (96).

2. WW99-S32. In a diabetic animal model, the duration of action of the other fatty acid-acylated insulin nalog, WW99- S32 [Nϵ-palmitoyl Lys(B29)] human insulin, administered iv was nearly twice that of unmodified human insulin, and the plasma half-life was nearly 7-fold that of the unmodified protein. Administered sc, [Nϵ-palmitoyl Lys(B29)] 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 (Eli Lilly & Co.) (97). The combination of these attributes resulted in prolonged stabilization of fasting glu-cose levels in insulin-dependent animals. The binding of this analog to albumin was confirmed. In human studies with healthy volunteers, this analog showed a highly reproduc-ible, linear pharmacokinetic profile, but showed less potency when compared with NPH (98). The latter finding was sub-sequently confirmed in C-peptide-negative patients (99). Based on the results with insulin acylation, derivatization with albumin-binding ligands could be generally applicable to prolong the action profile of peptide drugs (93).

VI. VI. REMAINING TASKS

A. Selective action

Insulin has a number of effects in addition to carbohydrate metabolism. Some of these effects depend on the cell or tissue type studied. Therefore, selectivity of an analog can be de- fined as selectivity to a certain tissue, or to a certain effect. Insulin influences glucose metabolism by inhibiting he- patic 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. Another consideration is that the pancreas delivers insulin to the portal vein, and the liver is therefore subject to relatively high insulin concentrations compared with peripheral tissues. With sc insulin therapy, this portal/peripheral insulin gradient is lost, resulting in nonphysiological insulin distribution. The result, even in patients able to achieve near-normal HbA 1c levels, is multiple and profound metabolic abnormalities, including excessive glycemic fluctuations, dyslipidemia, and alterations in IGF-I and GH levels. These abnormalities have been implicated in the complications of diabetes (100, 101). Currently, insulin can only be delivered into the portal circulation by surgically implanted ip pumps, certain types of pancreatic transplan-tation, or islet cell transplantation (102, 103). The importance of the issue is underlined by the findings of decreased re- quirement for antihypertensive therapy and decreased total and free insulin and insulin antibodies in patients with sur- gically implanted pumps (104). Unfortunately, despite the recent promising preliminary results with islet cell trans- plantation using a glucocorticoid-free immunosuppressive regimen, all of the above methods have significant difficul-ties, which preclude their use in the majority of patients (105, 106). An alternative could be the development of insulin analogs
with a greater effect on the liver than on the periphery.

1. Proinsulin. Proinsulin, the single-chain precursor of insu-lin, is more effective in the liver than in the periphery (107,108). Reasons for this selectivity are not fully understood, but the increased

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molecular size of proinsulin compared with insulin has been proposed as a potential mechanism. Endo-thelial cells in peripheral tissues limit the transfer of sub- stances from the circulation into the tissues with a rate in-versely related to the molecular size of the transferred substance. However, hepatocytes are freely in contact with all blood constituents in the hepatic sinusoids. Dose require-ments for proinsulin are approximately 4-fold higher than for human insulin, and there is a possible association between its use and myocardial infarction (109, 110). Proinsulin was therefore withdrawn from clinical trials, but the recognition of its selective action has stimulated the search for analogs with greater hepatic effects relative to peripheral tissues.

2. Thyroxyl-insulin complex. Two insulin analogs with in-creased molecular size due to covalent dimerization have been shown to have a greater effect on hepatic glucose production than peripheral glucose disposal after iv admin- istration (111). These dimeric analogs (NαB1, NαB1,-suberoyl-insulin dimer, and NϵB29, NϵB'29,-suberoyl-insu-lin dimer) are probably not suitable for clinical use because of their relatively low potency, but they confirm the possi-bility that analogs with selective action due to increased molecular size might be developed. Another interesting find-ing is that two insulin analogs covalently linked to T 4 (Nαβ1-thyroxyl-insulin and Nαβ1-thyroxyl-aminohexanoyl insu- lin) also show greater selectivity for hepatic glucose production in dogs (112). These insulin analogs bind thyroid hormone binding proteins to form high molecular weight complexes. Nαβ1 L-thyroxyl-insulin was recently found to be well tolerated and well absorbed in humans after sc injection and to show hepatoselectivity compared with NPH insulin (113). 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 recep- tor sites, which results from high molecular weight.

3. Further possibilities for selective action. 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 resi- dence of the material and alter activity profile. The analog Asp(B10) is an example of this (19). The metabolic effects of insulin analogs, such as increased glucose uptake and me- tabolism, and the mitogenic effects generally correlate well with binding to the insulin receptor (20). However, some metabolic actions, such as inhibition of protein degradation, do not. We have recently shown that the inhibition of protein degradation in cultured cells by insulin and the analogs lispro, Asp(B10), and B4Glu,B16Gln,B17Phe insulin does not correlate to insulin receptor binding and is dependent on cell type (114). For example, Asp(B10), which shows markedly increased receptor binding compared with insulin, has a similar effect to insulin on the inhibition of protein degra- dation in the human hepatoma cell line HepG2. This means that relative to its receptor binding, Asp(B10) is less effective in inhibiting protein degradation than human insulin in HepG2 cells. The effects in two other cell lines are dependent on the class of proteins being investigated. Effects similar to insulin were seen on short-lived proteins, but intermediate- lived protein degradation was inhibited to a greater degree with Asp(B10) than with insulin. Further work has suggested that the action to inhibit protein degradation is more closely correlated to cellular insulin/analog processing. In fact, it has been shown that insulin inhibition of protein degradation in isolated rat hepatocytes requires cellular insulin degradation (115, 116). For future development of specific analogs, more information is needed on properties of the insulin molecule important for different biological activities, e.g., carbohy- drate vs. fat vs. protein metabolic effects (117). An example for this might be insulin detemir, which was found to have less lipogenic activity than human insulin relative to its in- sulin receptor affinity (20).

B. Increased stability

Insulin is not a stable chemical entity. A variety of chemical changes of the primary structure affect insulin during han-dling, storage, and even use. Insulin decomposition is mainlydue 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 (118). 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

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covalent dimers (14). In a very interesting recent study, it was shown that attachment of short-chain (750- and 2000-Da) methoxypoly (ethylene gly-col) to the amino groups of either residue PheB1 or LysB29 of insulin's B-chain improves the conjugates' physical sta- bility without appreciable perturbations to its tertiary struc- ture, self-association behavior, or in vivo biological activity (119). However, designing and testing more analogs with increased stability still remains an important task for the future.

C. Less variability

The high intra- and interindividual variability of the re-sponse 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 (60). 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 sc injection of the same doses (120, 121). Pharmacodynamic variability, on the other hand, can be caused by differences in insulin action, causing different metabolic effects by sim- ilar plasma insulin concentrations (122). In short-acting prep-arations, a decreased variability in serum insulin concentra-tions compared with regular human insulin has been shown after sc injections of the analog insulin lispro (123). Also, interindividual variability in pharmacodynamic and phar-macokinetic parameters with insulin aspart was found to be generally less than that with human insulin, whereas the intraindividual variability in these parameters was similar for the two (124). Generally, variability is even more prob-lematic with long-acting insulin products; this is due to their insoluble nature. The long-acting preparations of human insulin are mostly suspensions, which require shaking before use, adding another factor to variability as adequate mixing usually does not occur (89). It is therefore expected that soluble long-acting analogs will have less variability in their pharmacokinetics. 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 (71). Nevertheless, developing insulin analogs with lower inter- and intraindi-vidual pharmacokinetic and pharmacodynamic variability remains an important task.

D. Ultrarapid 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 actually be 15–30 min before meals (125). When administered at least 15 min before meals, lispro achieves a greater improvement in postprandial val- ues as opposed to being injected immediately before meals. This suggests that developing even more rapidly absorbed short-acting analogs could offer potential benefits.

E. Ultralong 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. These patients could potentially use ultralong-acting analogs that could be injected once weekly or even less frequently. This type of preparation obviously would not provide good con-trol but could offer basal coverage sufficient to prevent ke-toacidosis or other acute complications. The concept may seem utopian at first, but a recent study reported that a single sc injection of a new analog, in which two 9-fluorenyl-methoxycarbonyl moieties are covalently linked to the phe-nylalanine at position B1 and to the lysine at B29 of human insulin, normalized blood sugar levels for 2–3 d of rats with streptozotocin-induced diabetes (126). The analog itself has only 1–2% of the biological potency of insulin, but undergoes a time-dependent spontaneous conversion to fully active insulin. The conversion takes place slowly under physiolog-ical conditions, with a t 1/2 of 12 d.

F. Benefit without metabolic activity?

The insulin analog Asp(B25) practically does not bind to the insulin receptor or IGF-I receptor and has no hypogly-cemic effect (17). 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 (127). 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. Pre- liminary trials have suggested that treatment of high-risk prediabetic patients with human insulin can prevent the onset of diabetes, but of course, this

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carries the risk of hy- poglycemia, even more so than in patients with fully devel- oped diabetes. Several large-scale controlled trials have been organized (e.g., the Diabetes Prevention Trial 1 and the Eu- ropean Pediatric Prediabetes Subcutaneous Insulin Trial) to evaluate the effect of prophylactic insulin therapy in the prevention or delay of diabetes in high-risk pediatric indi- viduals (128, 129). Although it is still unclear whether the analog Asp(B25) can be used for preventing diabetes in pre- diabetic children and young adults, the theory of using an- alogs without the potentially harmful hypoglycemic effects for diabetes prevention is certainly an interesting one.

VII. CONCLUSIONS

After more than half a century of treating diabetics with animal insulins, recombinant DNA technologies and ad-
vanced protein chemistry made human insulin preparations available in the early 1980s. As the next step, over the last decade, a number of insulin analogs were constructed and tested to further improve the therapy of diabetes (20, 130). The need for nearly optimal glucose control in diabetics to minimize complications clearly exists. Without insulin ana-logs, however, this can only be accomplished at the expense of an increase in hypoglycemic reactions. The Diabetes Con- trol and Complications Trial demonstrated that a 10% im- provement of glycosylated hemoglobin levels results in a 43% improvement of retinopathy, but is accompanied by an 18% increase of severe hypoglycemic episodes (5). 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 mul- tiple 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 (64). Therefore, the avail- ability of long-acting analogs raises the hope to take advan- tage of the true potential benefits of the currently available short-acting analog lispro, and of those still awaiting ap- proval. The introduction of new short- and long-acting an- alogs and the development of analogs with increased sta- bility, less variability, and perhaps selective action will help to develop more individualized treatment strategies targeted to specific patient characteristics and to achieve further im-provements in glycemic control. Combining different insulin analogs may even help to treat the multiple metabolic ab- normalities diabetics have beyond their carbohydrate metabolism.

Insulin analogs also represent a unique tool to unravel structure-function relationships in insulin biochemistry and insulin action (20). Recombinant insulin analogs have been and will be important in mapping the putative receptor bind- ing domain(s) of the insulin molecule and elucidating the specificity of the pathways leading to the metabolic and mitogenic effects of the hormone.

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REFERENCES

1. **WhiteMF,KahnCR**1994Theinsulinsignalingsystem.JBiolChem269:1–4
2. **PullenRA,LindsayDG,WoodSP,TickleIJ,BlundellTL,WollmerA,KrailG,BrandenburgD,ZahnH,GliemannJ,Gammeltoft S**1976Receptor-bindingregionofinsulin.Nature259:369–373
3. **Slieker LJ, Brooke GS, DiMarchi RD, Flora DB, Green LK, Hoff-mann JA, Long HB, Fan L, Shields JE, Sundell KL, Surface PL,ChanceRE**1997ModificationsintheB10andB26–30regionsoftheBchainofhumaninsulinalteraffinityforthehumanIGF-Ireceptormorethanfortheinsulinreceptor.Diabetologia40(Suppl2):S54–S61
4. **Heinemann L, Starke AAR, Hohmann A, Berger M** 1992 Timingbetweenthesubcutaneousadministrationofinsulinandconsump-tionofacarbohydraterichmeal.HormMetabResSuppl26:137–139
5. **Diabetes Control and Complications Trial Research Group** 1993The effect of intensive treatment of diabetes on the developmentand the progression of long-term complications in insulin-dependentdiabetesmellitus.NEnglJMed329:977–978
6. **Berger M** 1989 Towards more physiological insulin therapy in the1990s:acomment.DiabetesResClinPract6:S25–S31
7. **Mastick CC, Brady MJ, Printen JA,**

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1210-1227 www.ijprajournal.com ISSN: 2456-4494

Ribon V, Saltiel AR 1998Spatial determinants of specificity in insulin action. Mol Cell Bio-chem182:65–71

8. **Saltiel AR** 1996 Diverse signaling pathways in the cellular actionsofinsulin.AmJPhysiol270:E375–E85
9. **AzpiazuI,SaltielAR,DePaoli-RoachAA,LawrenceJC** 1996Regulationofbothglycogensynthaseand PHAS-Ibyinsulininratskeletal muscle involves mitogen-activated protein kinase-inde-pendentandrapamycin-sensitivepathways.JBiolChem271:5033–5039
10. **Jorgensen LN, Nielsen FS** 1990 Timing of premeal insulins indiabetic patients on a multiple daily injection regimen: a question-nairestudy.Diabetologia33(Suppl1):A116(Abstract)
11. **Heinemann L** 1995 Do insulin-treated diabetic patients use aninjection-meal-intervalindailylife?DiabetMed12:449–450
12. **Desmet M, Rutters A, Schmitt H, Satter E** 1994 [Lys (B28), Pro(B29)]humaninsulin(LysPro):patientstreatedwithLysProversushumanregularinsulin–qualityoflifeassessment.Diabetes43(Suppl1):167.A(Abstract)
13. **Mosekilde E, Jensen KS, Binder C, Pramming S, Thorsteinson B**1989Modelingabsorptionkineticsofsubcutaneousinjectedsolubleinsulin.JPharmacokinetBiopharm17:67–87
14. **Brange J** 1997 The new era of biotech insulin analogues. Diabeto-logia40:S48–S53
15. **Schwartz GP, Thompson Burke G, Katsoyannis PG** 1987 A su-peractive insulin: [B10-Aspartic acid]insulin (human). Proc NatlAcadSciUSA84:6408–6411
16. **Nielsen FS, Jorgensen LN, Ipsen M, Voldsgaard AI, Parving HH**1995 Long-term comparison of human insulin analogue B10Aspand soluble human insulin in IDDM patients on a basal/bolusinsulinregimen.Diabetologia38:592–598
17. **DrejerK,KruseV,LarsenUD,HougaardP,BjornS,GammeltoftS** 1991 Receptor binding and tyrosine kinase activation by insulinanalogueswithextremeaffinitiesstudiedinhumanhepatomaHepG2cells.Diabetes40:1488–1495
18. **Bornfeldt KE, Gidlof RA, Wasteson A, Lake M, Skottner A, Arn-qvist HJ** 1991 Binding and biological effect of insulin, insulin an-aloguesandinsulin-likegrowthfactorsinrataorticsmoothmusclecells: comparison of maximal growth promoting activities. Diabe-tologia34:307–313
19. **Hamel FG, Siford GL, Fawcett J, Chance RE, Frank BH, Duck-worth WC** 1999 Differences in the cellular processing of AspB10human insulin compared with human insulin and LysB28ProB29humaninsulin.Metabolism48:611–617
20. **Kurtzhals P, Schaffer L, Sorensen A, Kristensen C, Jonassen I,SchmidC,TrubT**2000Correlationsofreceptorbindingandmi-togenic potencies of insulin analogs designed for clinical use. Di-abetes49:999–1005
21. **Jorgensen LN, Dideriksen LH, Drejer K** 1992 Carcinogenic effectof the human insulin analogue B-10 Asp in female rats. Diabeto-logia35(Suppl1):A3(Abstract)
22. **Drejer K** 1992 The bioactivity of insulin analogues from in vitroreceptor binding to in vivo glucose uptake. Diabetes Metab Rev8:259–286
23. **Berti L, Kellerer M, Bossenmaier B, Seffer E, Seipke G, HaringHU** 1998 The long-acting human insulin analog HOE 901: characteristicsofinsulinsignallingincomparisontoAsp(B10)andregularinsulin.HormMetabRes30:123–129
24. **Baker EN, Blundell TL, Cutfield JF, Cutfield SM, Dodson EJ,Dodson GG, Hodgkin DM, Hubbard RE, Isaacs NW, ReynoldsCD** 1988 The structure of 2Zn pig insulin crystals at 1.5 A resolution.PhilosTransRSocLondBBiolSci319:369–456
25. **HoweyDC,BowsherRR,BrunelleRL,WoodworthJR**1994[Lys(B28),Pro(B29)]-humaninsulin:arapidlyabsorbedanalogueofhumaninsulin.Diabetes43:396–402
26. **Slieker LJ, Sundell K** 1991 Modifications in the 28 –29 position oftheinsulinB-chainalterbindingtotheIGF-Ireceptorwithminimaleffectoninsulinreceptorbinding.Diabetes40(Suppl1):168.A(Abstract)
27. **SliekerLJ,BrookeGS,ChanceRE**1994InsulinandIGF-Ianalogs:novel approaches to

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1210-1227 www.ijprajournal.com ISSN: 2456-4494

improved insulin pharmacokinetics. In: Le-RoithD,RaizadaMK,eds.Currentdirectionsinisinsulin-likegrowthfactorresearch.Vol343ofAdvancesinexperimentalmedicineandbiology.NewYork:PlenumPress;25–32

28. **Torlone E, Fanelli C, Rambotti AM, Kassi G, Modarelli F, DiVincenzoA,EpifanoL,CiofettaM,PampanelliS,BrunettiP**1994Pharmacokinetics, pharmacodynamics and glucose counterregu-lation following subcutaneous injection of the monomeric insulinanalogue[Lys(B28),Pro(B29)]inIDDM.Diabetologia37:713–720
29. **PfutznerA,KustnerE,ForstT,Schulze-SchleppinghoffB,Traut-mann ME, Haslbeck M, Schatz H, Beyer J** 1996 Intensive insulintherapywithinsulinlisproinpatientswithtype1diabetesreducesthe frequency of hypoglycemic episodes. Exp Clin Endocrinol Di-abetes104:25–30
30. **Anderson Jr JH, Brunelle RL, Koivisto VA, Pfutzner A, Traut-mann ME, Vignati L, DiMarchi R** 1997 Reduction of postprandialhyperglycemia and frequency of hypoglycemia in IDDM patientsoninsulin-analogtreatment.MulticenterInsulinLisproStudyGroup.Diabetes46:265–270
31. **Brunelle BL, Llewelyn J, Anderson Jr JH, Gale EA, Koivisto VA**1998 Meta-analysis of the effect of insulin lispro on severe hypoglycemia in patients with type 1 diabetes. Diabetes Care 21:1726 –1731
32. **KarsidagK,SatmanI,DinccagN,AltunasY, KaradenizS,YilmazMT** 1996 Comparison of metabolic control in IDDM with twodifferentintensiveregimensof[Lys(B28),Pro(B29)]humaninsulin(lispro)plusNPHinsulin.Diabetologia39(Suppl1):A222(Ab-stract)
33. **Ciofetta M, Lalli C, Del Sindaco P, Torlone E, Pampanelli S,Mauro L, Chiara DL, Brunetti P, Bolli GB** 1999 Contribution ofpostprandial versus interprandial blood glucose to HbA_1c in type1 diabetes on physiologic intensive therapy with lispro insulin atmealtime.DiabetesCare22:795–800
34. **Del Sindaco P, Ciofetta M, Lalli C, Perriello G, Pampanelli S,TorloneE,BrunettiP,BolliGB**1998Useoftheshort-actinginsulinanalogue lispro in intensive treatment of type 1 diabetes mellitus:importance of appropriate replacement of basal insulin and time-intervalinjection-meal.DiabetMed15:592–600
35. **Lalli C, Ciofetta M, Del Sindaco P, Torlone E, Pampanelli S,Compagnucci P, Cartechini MG, Bartocci L, Brunetti P, Bolli GB**1999 Long-term intensive treatment of type 1 diabetes with theshort-acting insulin analog lispro in variable combination withNPHinsulinatmealtime.DiabetesCare22:468–477
36. **Lougheed WD, Zinman B, Strack TR, Janis LJ, Weymouth AB,Bernstein EA, Korbas AM, Frank BH** 1997 Stability of insulinlisproininsulininfusionsystems.DiabetesCare20:1061–1065
37. **Zinman B, Tildesley H, Chiasson JL, Tsui E, Strack TR** 1997Insulin lispro in CSII: results of a double-blind cross-over study.Diabetes46:440–443
38. **Renner R, Pfutzner A, Trautmann M, Harzer O, Sauter K, Land-graf R** 1999 Use of insulin lispro in continuous subcutaneous in-sulin infusion treatment: results of a multicenter trial. GermanHumalog-CSIIStudyGroup.DiabetesCare22:784–788
39. **Pen P, Hinselmann C, Pfutzner A, Dreyer M** 1996 Catheter dis-connection in type 1 diabetes treated with CSII: comparison ofinsulin lispro and human regular insulin. Diabetologia 39(Suppl1):A847(Abstract)
40. **Attia N, Jones TW, Holcombe J, Tamborlane WV** 1998 Compar-ison of human regular and lispro insulins after interruption ofcontinuous subcutaneous insulin infusion and in the treatment ofacutelydecompensatedIDDM.DiabetesCare21:817–821
41. **Hanaire-Broutin H, Melki V, Bessieres-Lacombe S, Tauber JP**2000Comparisonofcontinuoussubcutaneousinsulininfusionandmultiple daily injection regimens using insulin lispro in type 1diabeticpatientsonintensifiedtreatment:arandomizedstudy.TheStudy Group for the Development of Pump Therapy in Diabetes.DiabetesCare23:1232–1235
42. **JanssenMM,CasteleijnS,DevilleW,Popp-SnijdersC,RoachP,Heine RJ** 1997 Nighttime insulin kinetics and glycemic control intype 1 diabetes patients following

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1210-1227 www.ijprajournal.com ISSN: 2456-4494

administration of an interme-diate-actinglispropreparation.DiabetesCare20:1870–1873

43. **Roach P, Trautmann M, Arora V, Sun B, Anderson Jr JH** 1999Improved postprandial blood glucose control and reduced noc-turnal hypoglycemia during treatment with two novel insulin lis-pro-protamineformulations,insulinlispromix25andinsulinlispromix50.Mix50StudyGroup.ClinTher21:523–534
44. **Malone JK, Woodworth JR, Arora V, Yang H, Campaigne BN,Halle JP, Yale JF, Grossman LD** 2000 Improved postprandial gly-cemic control with Humalog Mix 75/25 after a standard test mealinpatientswithtype2diabetesmellitus.ClinTher22:222–230
45. **Jehle PM, Aisenpreis U, Bundschu D, Keller F** 1999 AdvantagesofinsulinLispro(short-acting)interminalkidneyfailure.FortschrMed117:41–42
46. **JovanovicL,IlicS,PettittDJ,HugoK,GutierrezM,BowsherRR,BastyrEJ**1999Metabolicandimmunologiceffectsofinsulinlisproingestationaldiabetes.DiabetesCare22:1422–1427
47. **Calle-Pascual AL, Bagazgoitia J, Calle JR, Charro A, Maranes JP**2000Useofinsulinlisproinpregnancy.DiabetesNutrMetab13:173–177
48. **Buchbinder A, Miodovnik M, McElvy S, Rosenn B, Kranias G,Khoury J, Siddiqi TA** 2000 Is insulin lispro associated with thedevelopment or progression of diabetic retinopathy during pregnancy?AmJObstetGynecol183:1162–1165
49. **GargSK,AndersonJH,PerrySV,Mackenzie T,KeithP,JenningsMK, Hansen MM, Chase HP** 1999 Long-term efficacy of humaloginsubjectswithType1diabetesmellitus.DiabetMed16:384–387
50. **AndersonJ,SymanowskiS,BrunelleR**1994Safetyof[Lys(B28),Pro(B29)humaninsulin]analoginlong-termclinicaltrials.Diabetes43(Suppl1):61A(Abstract)
51. **FinebergNS,FinebergSE,AndersonJH,BirkettMA,GibsonRG,HufferdS**1996Immunologiceffectsofinsulinlispro[Lys(B28),Pro(B29) human insulin] in IDDM and NIDDM patientspreviouslytreatedwithinsulin.Diabetes45:1750–1754
52. **Roach P, Varshavsky JA, Gantner K, Anderson JH** 1996 Insulinantibody formation during treatment with human insulin or in-sulin lispro does not affect insulin dose requirements. Diabetes45(Suppl2):261A(Abstract)
53. **Henrichs HR, Unger H, Trautmann ME, Pfutzner A** 1996 Severeinsulinresistancetreatedwithinsulinlispro.Lancet348:1248(Letter)
54. **LahtelaJT,KnipM,PaulR,AntonenJ,Salmi J**1997Severeantibody-mediatedhumaninsulinresistance:successfultreatmentwiththeinsulinanaloglispro:acasereport.DiabetesCare20:71–73
55. **Kotsanos JG, Vignati L, Huster W, Andrejasich C, Boggs MB,JacobsonAM,MarreroD,MathiasSD,PatrickD,ZalaniS,Anderson J** 1997 Health-related quality-of-life results from multi-national clinical trials of insulin lispro: assessing benefits of a newdiabetestherapy.DiabetesCare20:948–958
56. **Brange J, Ribel U, Hansen JF, Dodson G, Hansen MT, HavelundS, Melberg SG, Norris F, Norris K, Snel L** 1988 Monomeric in-sulins obtained by protein engineering and their medical implications.Nature333:679–682
57. **Kang S, Creagh FM, Ara J, Owens DR Peters JR** 1991 Insulinanalogues and human insulin: near-equivalent in vivo biologicalactivity in healthy males in spite of widely different in vitro potencies.Diabetes40(Suppl1):243A(Abstract)
58. **Dall V** 1999 Preclinical safety pharmacology studies on the rapid-acting insulin analogue insulin aspart. Arzneimittelforschung 49:463–470
59. **Frier BM, Ewing FM, Lindholm A, Hylleberg B, Kanc K** 2000Symptomatic and counterregulatory hormonal responses to acutehypoglycaemia induced by insulin aspart and soluble human insulininType1diabetes.DiabetesMetabResRev16:262–268
60. **Heinemann L, Weyer C, Rauhaus M, Heinrichs S, Heise T** 1998Variability of the metabolic effect of soluble insulin and the rapid-actinginsulinanaloginsulinaspart.DiabetesCare21:1910–1914
61. **Lindholm A, McEwen J, Riis AP** 1999 Improved postprandialglycemic control with insulin aspart: a randomized double-

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1210-1227 www.ijprajournal.com ISSN: 2456-4494

blindcross-overtrialintype1diabetes.DiabetesCare22:801–805

62. **Mudaliar SR, Lindberg FA, Joyce M, Beerdsen P, Strange P, LinA, Henry RR** 1999 Insulin aspart (B28 asp-insulin): a fast-actinganalog of human insulin: absorption kinetics and action profilecompared with regular human insulin in healthy nondiabetic sub-jects.DiabetesCare22:1501–1506
63. **Raskin P, Guthrie RA, Leiter L, Riis A, Jovanovic L** 2000 Use ofinsulin aspart, a fast-acting insulin analog, as the mealtime insulinin the management of patients with type 1 diabetes. Diabetes Care23:583–588
64. **Home PD, Lindholm A, Hylleberg B, Round P** 1998 Improvedglycemic control with insulin aspart: a multicenter randomizeddouble-blind crossover trial in type 1 diabetic patients. UK InsulinAspartStudyGroup.DiabetesCare21:1904–1909
65. **BrunnerGA,HirschbergerS,SendlhoferG, WutteA,EllmererM,Balent B, Schaupp L, Krejs GJ, Pieber TR** 2000 Post-prandialadministration of the insulin analogue insulin aspart in patientswithType1diabetesmellitus.DiabetMed17:371–375
66. **Rosenfalck AM, Thorsby P, Kjems L, Birkeland K, Dejgaard A,Hanssen KF, Madsbad S** 2000 Improved postprandial glycaemiccontrol with insulin Aspart in type 2 diabetic patients treated withinsulin.ActaDiabetol37:41–46
67. **Mortensen HB, Lindholm A, Olsen BS, Hylleberg B** 2000 Rapidappearance and onset of action of insulin aspart in paediatric sub-jectswithtype1diabetes.EurJPediatr159:483–488
68. **Weyer C, Heise T, Heinemann L** 1997 Insulin aspart in a 30/70premixed formulation: pharmacodynamic properties of a rapid-actinginsulinanaloginstablemixture.Diabetes Care20:1612–1614
69. **Jacobsen LV, Sogaard B, Riis A** 2000 Pharmacokinetics and phar-macodynamics of a premixed formulation of soluble and prota-mine-retardedinsulinaspart.EurJClinPharmacol56:399–403
70. **RosskampRH,ParkG**1999Long-actinginsulinanalogs.DiabetesCare22(Suppl2):B109–B113
71. **JorgensenS,VaagA,LangkjaerL,HougaardP,MarkussenJ**1989NovoSol Basal: pharmacokinetics of a novel soluble long actinginsulinanalog.BrMedJ299:415–459
72. **Jorgensen S, Drejer K** 1990 Insulin analogs and nasal insulin de-livery. In: Bailey CJ, Flatt PR, eds. New antidiabetic drugs. 1st ed.London:Smith-Gordon;83–92
73. **Heinemann L, Linkeschova R, Rave K, Hompesch B, Sedlak M,Heise T** 2000 Time-action profile of the long-acting insulin analoginsulin glargine (HOE901) in comparison with those of NPH in-sulinandplacebo.DiabetesCare23:644–649
74. **Seipke GK, Geisen HP, Neubauer C, Pittius R, Rosskamp R,Schwabe D** 1992 New insulin preparations with prolonged actionprofiles: A21 modified arginine insulins. Diabetologia 35(Suppl1):A4(Abstract)
75. **Bahr M, Kolter T, Seipke G, Eckel J** 1997 Growth promoting andmetabolicactivityofthehumaninsulinanalogue[GlyA21,ArgB31,ArgB32]insulin(HOE901)inmusclecells.EurJPharmacol320:259–265
76. **DreyerM,PeinM,SchmidtB,HeidtmannB,SchlunzenM,Rosskamp R** 1994 Comparison of the pharmacokinetics/dynamicsofGLY(A21)-ARG(B31,B32)-human-insulin(HOE71GT)withNPH-insulin following subcutaneous injection by using euglyce-micclamptechnique.Diabetologia37(Suppl1):A78(Abstract)
77. **OwensD,LuzioS,BeckP,CoatesP,TinbergenJ,KurzhalsR**1997TheabsorptionoftheinsulinanalogueHOE901fromdifferentsitesinhealthysubjects.Diabetes46(Suppl1):329A(Abstract)
78. **OwensDR,CoatesPA,LuzioSD,TinbergenJP,KurzhalsR**2000Pharmacokineticsof ^{125}I-labeledinsulinglargine(HOE901)inhealthy men: comparison with NPH insulin and the influence ofdifferentsubcutaneousinjectionsites.DiabetesCare23:813–819
79. **Talaulicar M, Willms B, Rosskamp R** 1996 HOE 901, ein neuesInsulinanalogzurSubstitutiondesbasalenInsulin-

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1210-1227 www.ijprajournal.com ISSN: 2456-4494

bedarfsbeiTypeIDiabetes.DiabetesundStoffwechsel5:3–6

80. **Rosenstock J, Park G, Zimmermann J** 1998 Efficacy and safety ofHOE 901 in patients with type 1 DM: a four-week randomized,NPHinsulin-controlledtrial.Diabetes47(Suppl1):A92(Abstract)
81. **Pieber T, Eugene-Jolchine I, DeRobert E** 1998 Efficacy and safetyof HOE 901 in patients with type 1 diabetes: a four-week random-ized, NPH insulin-controlled trial. Diabetes 47(Suppl 1):A62 (Ab-stract)
82. **Raskin P, Park G, Zimmerman J** 1998 The effect of HOE 901 onglycemic control in type 2 diabetes. Diabetes 47(Suppl 1):A103(Abstract)
83. **Matthews DR, Pfeiffer C** 1998 A new long-acting insulin (HOE901) demonstrates less nocturnal hypoglycemia when comparedwith protamine insulin in a clinical trial. Diabetologia 41(Suppl1):A245(Abstract)
84. **Rosenstock J, Schwartz S, Clark C, Edwards M, Donley D** 1999Efficacy and safety of HOE 901 (Insulin Glargine) in subjects withtype 2 DM: a 28-week randomized, NPH insulin-controlled trial.Diabetes48(Suppl1):A100(Abstract)
85. **RatnerRE,HirschIB,NeifingJL,GargSK, MeccaTE,WilsonCA**2000 Less hypoglycemia with insulin glargine in intensive insulintherapy for type 1 diabetes. U.S. Study Group of Insulin GlargineinType1Diabetes.DiabetesCare23:639–643
86. **PieberTR,Eugene-JolchineI,DerobertE**2000Efficacyandsafety of HOE 901 versus NPH insulin in patients with type 1 diabetes.The European Study Group of HOE 901 in type 1 diabetes. DiabetesCare23:157–162
87. **Rosenstock J, Park G, Zimmerman J** 2000 Basal insulin glargine(HOE 901) versus NPH insulin in patients with type 1 diabetes onmultiple daily insulin regimens. U.S. Insulin Glargine (HOE 901)Type1DiabetesInvestigatorGroup.DiabetesCare23:1137–1142
88. **Yki-Jarvinen H, Dressler A, Ziemen M** 2000 Less nocturnal hy-poglycemia and better post-dinner glucose control with bedtimeinsulin glargine compared with bedtime NPH insulin during in-sulincombinationtherapyintype2diabetes.HOE901/3002StudyGroup.DiabetesCare23:1130–1136
89. **JehlePM,MichelerC,JehleDR,BreitigD,BoehmBO**1999Inadequate suspension of neutral protamine Hagendorn (NPH)insulininpens.Lancet6:1604–1607
90. **Ratner RE, Hirsch IB, Mecca TE, Wilson CA** 1999 Efficacy andsafetyofinsulinglargineinsubjectswithtype1diabetes:a28-weekrandomized, NPH insulin-controlled trial. Diabetes 48(Suppl 1):A120
91. **SmithLE,ShenW,PerruzziC,SokerS,KinoseF,XuX,RobinsonG, Driver S, Bischoff J, Zhang B, Schaeffer JM, Senger DR** 1999Regulationofvascularendothelialgrowthfactor-dependentretinalneovascularization by insulin-like growth factor-1 receptor. NatMed5:1390–1395
92. **BolliGB,OwensDR**2000Insulinglargine.Lancet356:443–445
93. **Kurtzhals P, Havelund S, Jonassen I, Kiehr B, Larsen UD, RibelU, Markussen J** 1995 Albumin binding of insulins acylated withfatty acids: characterization of the ligand-protein interaction andcorrelationbetweenbindingaffinityandtimingoftheinsulineffectinvivo.BiochemJ312:725–731
94. **Markussen J, Havelund S, Kurtzhals P, Andersen AS, HalstromJ,HasselagerE,LarsenUD,RibelU,SchafferL,VadK,JonassenI** 1996 Soluble, fatty acid acylated insulins bind to albumin andshowprotractedactioninpigs.Diabetologia 39:281–288
95. **Heinemann L, Sinha K, Weyer C, Loftager M, Hirschberger S,HeiseT**1999Time-actionprofileofthesoluble,fattyacidacylated,long-actinginsulinanalogueNN304.DiabetMed16:332–338
96. **Kurtzhals P, Havelund S, Jonassen I, Markussen J** 1997 Effect offatty acids and selected drugs on the albumin binding of a long-acting,acylatedinsulinanalogue.JPharmSci86:1365–1368
97. **MyersSR,Yakubu-MadusFE,JohnsonWT,BakerJE,CusickTS,WilliamsVK,TinsleyFC,KriauciunasA,**

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1210-1227 www.ijprajournal.com ISSN: 2456-4494

ManettaJ,ChenVJ1997Acylation of human insulin with palmitic acid extends the timeactionofhumaninsulinindiabeticdogs.Diabetes46:637–642

98. **Howey DC, Woodworth JR, Bowsher RR, Reviergo J** 1997 Pharmacokineticandglucodynamicassessmentsof palmitoyl,LYS(B29) human insulin in healthy volunteers. Diabetologia(Suppl 1):A354(Abstract)

99. **Radziuk J, Pye S, Bradley B, Braaten J, Vignati L, Roach P, BowsherR,DiMarchiR,ChanceR**1998BasalactivityprofilesofNPHand[N^{ϵ}-palmitoylLys(B29)]humaninsulinsinsubjects withIDDM.Diabetologia41:116–120

100. **Sonksen PH, Russell-Jones D, Jones RH** 1993 Growth hormoneand diabetes mellitus: a review of sixty-three years of medicalresearchandaglimpseintothefuture?HormRes40:68–79

101. **Ruotolo G, Micossi P, Galimberti G, Librenti MC, Petrella G,MarcovinaS,PozzaG,HowardBV**1990Effectsofintraperitonealversus subcutaneous insulin administration on lipoprotein metabolismintypeIdiabetes.Metabolism39:598–604

102. **Rosenberg L** 2000 Pancreatic and islet transplantation. Curr GastroenterolRep2:165–172

103. **Inoue K, Miyamoto M** 2000 Islet transplantation. J HepatobiliaryPancreatSurg7:163–177

104. **DuckworthWC,SaudekCD,Giobbie-HurderA,HendersonWG,HenryRR,KelleyDE,EdelmanSV,ZieveFJ,AdlerRA,AndersonJW,AndersonRJ,HamiltonBP,DonnerTW,KirkmanMS,MorganNA**1998TheVeteransAffairsimplantableinsulinpumpstudy:effectoncardiovascularriskfactors.DiabetesCare21:1596–1602

105. **Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, WarnockGL,KnetemanNM,RajotteRV**2000Islettransplantationinsevenpatients with type 1 diabetes mellitus using a glucocorticoid-freeimmunosuppressiveregimen.NEnglJMed343:230–238

106. **Robertson RP** 2000 Successful islet transplantation for patientswithdiabetes–factorfantasy?NEnglJMed343:289–290

107. **Revers RR, Henry R, Schmeiser L, Kolterman O, Cohen R, BergenstalR,PolonskyK,JaspanJ,Rubenstein A,FrankB**1984Theeffects of biosynthetic human proinsulin on carbohydrate metabolism.Diabetes33:762–770

108. **Lavelle-Jones M, Scott MH, Kolterman O, Rubenstein AH, Olef-sky JM, Moossa AR** 1987 Selective suppression of hepatic glucoseoutput by human proinsulin in the dog. Am J Physiol 252:E230 –E236

109. **Spradlin CT, Galloway JA, Anderson JH** 1990 Apparent increasein coronary heart disease with human proinsulin (HPI)–randomizationfailureorHPIeffect?Diabetologia33(Suppl):A60(Abstract)

110. **Galloway JA, Hooper SA, Spradlin CT, Howey DC, Frank BH,Bowsher RR, Anderson JH** 1992 Biosynthetic human proinsulin:review of chemistry, in vitro and in vivo receptor binding, animaland human pharmacology studies, and clinical trial experience.DiabetesCare15:666–692

111. **Shojaee-Moradie F, Jackson NC, Boroujerdi M, Brandenburg D,Sonksen PH, Jones RH** 1995 Demonstration of a relatively hepatoselectiveeffectofcovalentinsulindimersonglucosemetabolismindogs.Diabetologia38:1007–1013

112. **Shojaee-Moradie F, Eckey H, Jackson NC, Schuttler A, BrandenburgD,SonksenPH,JonesRH**1998Novelhepatoselectiveinsulinanalogues: studies with covalently linked thyroxyl-insulin complexes.DiabetMed15:928–936

113. **Shojaee-MoradieF,PowrieJK,SundermannE,SpringMW,Schuttler A, Sonksen PH, Brandenburg D, Jones RH** 2000 Novelhepatoselective insulin analog: studies with a covalently linkedthyroxyl-insulincomplexinhumans.DiabetesCare23:1124–1129

114. **Fawcett J, Hamel FG, Bennett RG, Vajo Z, Duckworth WC** 2001Insulin and analogue effects on protein degradation in different celltypes: dissociation between binding and activity. J Biol Chem 276:11552–11558

115. **Peavy DE, Edmondson JW, Duckworth WC** 1984 Selective effectsof inhibitors of hormone processing on insulin action in isolatedhepatocytes.Endocrinology114:753–

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1210-1227 www.ijprajournal.com ISSN: 2456-4494

760

116. **Draznin B, Trowbridge M** 1982 Inhibition of intracellular prote-olysis by insulin in isolated rat hepatocytes: possible role of inter-nalizedhormone.JBiolChem257:11988–11993
117. **DuckworthWC**1997Tumornecrosisfactoran dinsulinresistance:specificityofsequenceacco untsofinhibitionofinsulinaction.JLabClinMe d130:114–115
118. **BremsDN,BrownPL,BryantC,ChanceRE, GreenLK,LongHB,Miller AA, Millican R, Shields JE, Frank BH** 1992 Improvedinsulinstabilitythroughaminoacidsu bstitution.ProteinEng5:519–525
119. **HindsK,KohJJ,JossL,LiuF,BaudysM,Kim SW**2000Synthesisand characterization of poly(ethylene glycol)-insulin conjugates.BioconjugChem11:195–201
120. **Lauritzen T, Faber OK, Binder C** 1979 Variation in ^{125}I-insulinabsorptionandbloodglucoseconcentrat ion.Diabetologia17:291–295
121. **Binder C, Lauritzen T, Faber O, Pramming S** 1984 Insulin phar-macokinetics.DiabetesCare7:188–199
122. **Ziel FH, Davidson MB, Harris MD, Rosenberg CS** 1988 The vari-ability in the action of unmodified insulin is more dependent onchanges in tissue insulin sensitivity than on insulin absorption.DiabetMed5:662–666
123. **AntsiferovM,WoodworthJR,MayorovA,R isticS,DedovI**1995Within patient variability in postprandial glucose excursion withlispro insulin analog compared with regular insulin. Diabetologia38(Suppl1):A190(Abstract)
124. **SimpsonKL,SpencerCM**1999Insulinaspart. Drugs57:759–765
125. **RassamAG,ZeiseTM,BurgeMR,SchadeDS** 1999Optimaladministration of lispro insulin in hyperglycemic type 1 diabetes.DiabetesCare22:133–136
126. **GershonovE,ShechterY,FridkinM**1999Spo ntaneousconversionofaninactivemodifiedins ulintotheactivehormoneincirculation:9-fluorenylmethoxycarbonilderivativeofinsulin .Diabetes48:1437–1442
127. **Karounos DG, Bryson JS, Cohen DA** 1997 Metabolically inactiveinsulinanalogpreventstypeIdiabetesin prediabeticNODmice.JClinInvest100:1344–1348
128. **Carel JC, Bougneres PF** 1996 Treatment of prediabetic patientswith insulin: experience and future. European Prediabetes StudyGroup.HormRes45(Suppl1):44–47
129. **JuliusMC,SchatzDA,SilversteinJH**1999Th epreventionoftypeIdiabetesmellitus.PediatrA nn28:585–588
130. **Berger M, Heinemann L** 1997 Are presently available insulin an-alogues clinically beneficial? Diabetologia40: S91–S96