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HUMAN INSULIN AND ITS MODIFICATIONS

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In the mid-1970s, a study by the National Diabetes Commission noted that the insulin-using diabetic population was growing faster than meat production and suggested the possibility of an animal pancreas shortage within the next 20-30 years[1]. In response, Eli Lilly and Company considered a number of alternative sources of insulin, including total chemical synthesis, tissue culture production, and production by genetic cell manipulation. After deciding upon the last[2], a project was undertaken with the University of California, San Francisco, and the Genentech Company to produce human insulin by recombinant DNA (HI rDNA) technology[2,3]. From the outset of the project, Lilly maintained a close working relationship with the Food and Drug Administration (FDA) and other regulatory agencies[2,4]. In anticipation of the New Drug Application (NDA) for HI rDNA, the Metabolic-Endocrine Division of the FDA recruited two senior scientists with the requisite expertise in biology and chemistry to deal with issues unique to recombinant technology[5]. To allay concerns about this novel manufacturing process, significant effort was made to educate both the scientific community and the public well in advance of producing human insulin for clinical trials[2].

The first dose of HI rDNA was administered to a normal volunteer in the U.K. in June 1980 and in the U.S. in August 1980, and to a diabetic patient in December 1980. An NDA was filed in the spring of 1982 with approval by the U.S. FDA the following fall. HI rDNA was the first drug for human use made by recombinant DNA technology and now accounts for 50% of insulin use in the U.S. and its use continues to grow.

Prior to the availability of human insulin prepared by recombinant DNA technology, limited quantities of material extracted from human pancreas had been given to humans[6-8]. Based on the similarities in chemical structure between human and pork insulin and on the results of these studies (Table I), it was anticipated that human insulin would be comparable to purified pork insulin (PPI) in pharmacology and multicenter clinical studies. However, as indicated below and summarized in Table II, certain differences were observed.

TABLE I
SPECIES DIFFERENCES IN AMINO ACID SEQUENCE OF MAMMALIAN INSULINS

	Positions			
	A Chain			B Chain
Source	8	9	10	30
Beef	Alanine	Serine	Valine	Alanine
Pork	Threonine	Serine	Isoleucine	Alanine
Human	Threonine	Serine	Isoleucine	Threonine

TABLE II
DIFFERENCES BETWEEN HUMAN AND ANIMAL INSULINS (PORK, MIXED BEEF-PORK) IN CLINICAL STUDIES

Clinical Pharmacology Studies:

Human insulin rDNA (neutral regular, isophane [NPH], and ultralente) slightly more rapidly absorbed and shorter acting than purified pork insulin (PPI)[2,9,10]

Multicenter Studies in Insulin-Naive ("New ") Patients:

Metabolic control comparable to that of PPI and mixed beef-pork (MBP) insulin[11-13]

Serum insulin antibody titers slightly less in patients treated with human insulin rDNA than with PPI and significantly less than with MBP[13,14]

No differences in the frequency of allergy or lipoatrophy with HI rDNA, PPI, and MBP[13]

Studies in Established ("Transfer") Patients:

Slight increase in glycemia initially following transfer from PPI or MBP to human insulin[11,15]

A marked decrease in serum insulin antibody titers following transfer from MBP and minimal changes following transfer from PPI

No differences in the frequency of allergy or lipoatrophy[15]

Of interest is the fact that the findings generated from the use of HI rDNA have generally been confirmed by studies utilizing semisynthetic human insulin[10,16].

The fact that (as indicated in Table II) HI rDNA was found to be significantly less immunogenic than mixed beef-pork (MBP) insulin and slightly less immunogenic than PPI raised a question concerning the clinical significance of antibody binding. Clearly, binding >10%, a level more frequently reached in patients receiving PPI than HI rDNA[14,17] and in virtually all patients receiving beef-containing insulins, may result in an attenuation of the effect of injected insulin[18] and delay recovery from hypoglycemia[19] (Figure 1). Finally, because antibody binding promotes placental transfer of maternal insulin[20] the fetuses of mothers with increased serum insulin antibody titers are subject to hyperinsulinemia[21]. Thus, Menon *et al*[21] have shown that in pregnant patients with insulin-dependent diabetes mellitus (IDDM) increased antibody titers (binding >19.3 ± 2.3%) was associated with a significantly increased frequency of fetal macrosomia. Patients treated with HI rDNA had low mean antibody titers (5.0 ± 1%) and produced no infants with macrosomia.

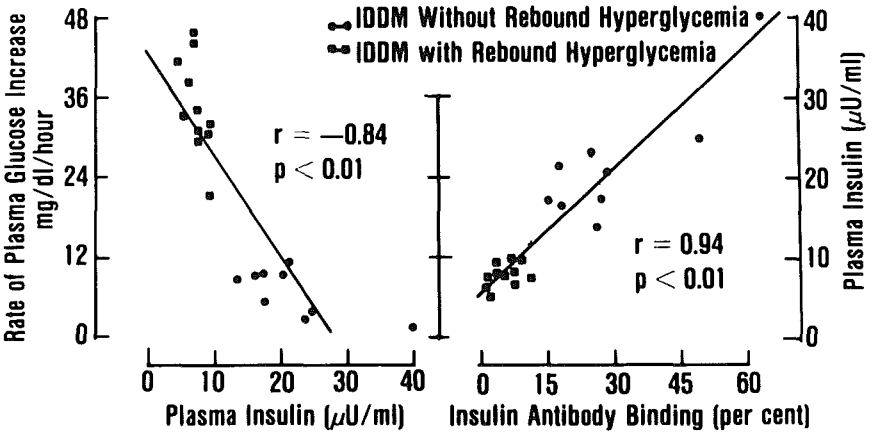

Fig.1. Effect of insulin antibodies on serum free insulin and recovery from hypoglycemia in patients with insulin-dependent diabetes mellitus. Reproduced with permission (ref. 19).

A final issue that arose from the use of HI rDNA (and semisynthetic human insulin) was the possibility that patients transferred from animal insulins (pork, beef, or a mixture) experienced fewer warning signs or symptoms of hypoglycemia[22]. Whether this phenomenon is real or apparent has been the subject of a number of studies and editorial comments[23]. The position of most authorities is that decreased awareness of hypoglycemia is more likely secondary to the diminution of cognitive function that results from "tight" glycemic control and is not related to insulin species[23,24].

Certainly, assurance of an unlimited supply of the homologous hormone which has advantages over animal-source insulin is a noteworthy scientific achievement. However, the most important therapeutic benefit for patients with diabetes mellitus to be derived from recombinant DNA technology may well prove to be the capability to design and produce analogues that are chemically and/or pharmacologically superior to native insulin. The impetus for this activity is based on the premise that failure to achieve the level of metabolic control deemed necessary to forestall the chronic complications of diabetes is in part due to the failure of conventional insulins to simulate the normal pattern of secretion of insulin in response to feeding and fasting. For example, as displayed in Figure 2, normal insulin secretion is characterized by (1) rapid and prompt increases in hormone levels to dispose of meals (peripheral glucose disposal [PGD]) followed by a correspondingly prompt return to basal levels and (2) low hormone levels in the postabsorptive, or basal state; these maintain normal glycemia between meals by reducing hepatic glucose production (HGP). The importance of the latter in the overall glycemic control of patients with diabetes is that the fasting blood glucose is highly correlated with HGP. Moreover, excessive HGP is a hallmark of noninsulin-dependent diabetes mellitus (NIDDM)[25].

Because studies with pork proinsulin had disclosed relative hepatospecificity, e.g., in comparison with insulin greater suppression of HGP versus promotion of PGD, proinsulin was viewed as a basal analogue which might be useful in suppressing HGP with a minimal risk of hypoglycemia. Moreover, pork proinsulin, which in contrast to the commercially available modified insulins that are suspensions of zinc and/or protamine, was a soluble intermediate-acting insulin agonist. Therefore, the technology that produced human insulin was applied to the production of the first biosynthetic analogue of insulin, human proinsulin (HPI), clinical testing of which began in mid-1982. The pharmacologic features of pork proinsulin were confirmed with HPI. Moreover, large intrasubject coefficient of variation of responses that are characteristic of treatment with insulin[26] were remarkably reduced by HPI[25]. Unfortunately, clinical studies of this agent were suspended due to an increased frequency of acute myocardial infarction in one multicenter study and the fact that in multicenter trials glycemic control was not

different in HPI-treated versus insulin-treated groups (i.e., the attractive pharmacologic features apparently were not translated into clinical benefit)[25,27,28].

Jorgensen *et al* have reported on an insulin analogue that is substantially more slowly absorbed than ultralente beef insulin, the slowest acting of the commercially available modified insulins[29]. However, glucodynamic data on this analogue are not available.

Both Novo-Nordisk[30] and Lilly have utilized recombinant DNA technology to prepare insulin analogues which promote PGD more efficiently than insulin. The need for such analogues can be seen by examining Figures 2-4.

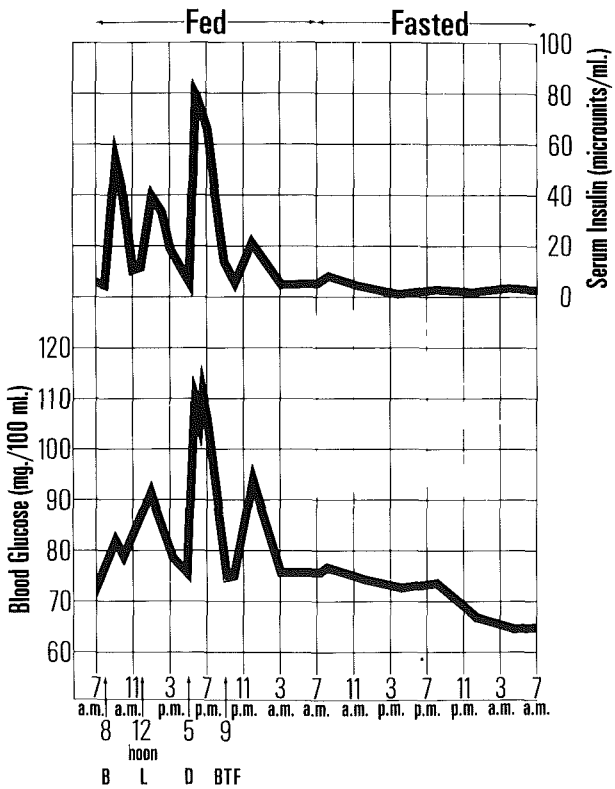

Fig. 2. The blood glucose and serum insulin response of six normal volunteers to fasting and feeding. Diet consisted of 30 calories per kg of body weight, 2/7 at each main meal and 1/7 at bedtime.

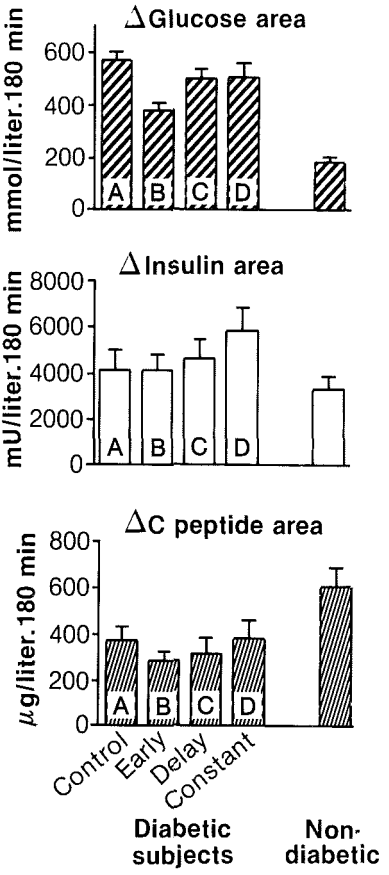

Fig. 3. The integrated glycemic, serum insulin, and C-peptide response of slightly overweight persons with NIDDM to a mixed meal containing 68 grams of carbohydrate to (A) no exogenous insulin; (B) a ramped intravenous infusion of 1.8 U of insulin administered between 0 and 30 min before the meal to simulate normal endogenous insulin secretion; (C) the same as (B) but the insulin infusion was begun with the meal; and (D) 1.8 U of insulin infused over 180 min. This figure demonstrates that the most efficient treatment (low blood glucose and serum insulin) was treatment (B). Reproduced with permission from Diabetes (1988) 37: 736-44.

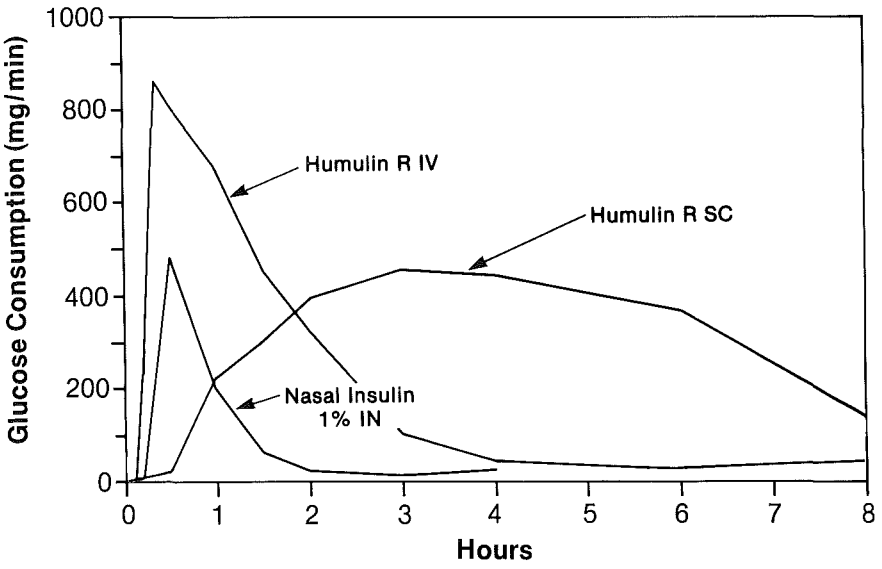

Fig. 4. The results of a glucose clamp (Biostator) study demonstrating the pharmacodynamic response of normal volunteers to human insulin rDNA 0.2 U/kg administered intravenously or subcutaneously or 1.4 U/kg applied to the nasal mucosa. Courtesy of D. C. Howey and S. A. Hooper.

Figure 2 shows that the prompt rise of serum hormone levels is useful in optimizing control of meal-induced hyperglycemia. Figure 3 demonstrates the increased effectiveness of insulin when given slightly before a meal. However, Figure 4 shows that although intravenous and nasally applied insulin simulate a serum insulin profile which is comparable to a normal response to a meal, because of a marked delay in peak after subcutaneous injection, regular or soluble insulin clearly does not. Moreover, the latter has an extended duration of action, features that can contribute to hypoglycemia between meals. Another disadvantage of subcutaneous regular insulin is that prolonged hyperinsulinemia has been implicated in the development of atherosclerosis and coronary heart disease[31]. The long-acting nature of soluble insulin has been shown to be due to its tendency to self-associate into dimers, tetramers, and hexamers. Preservation of the peptide in the monomeric form significantly speeds its absorption and disposal[30]. While it is beyond the scope of this paper to describe the chemistry of self-association of insulin, it is appropriate to point out that recombinant DNA technology provides a relatively convenient technique for the substitution of amino acids in the insulin molecule to minimize monomer-monomer interactions. Fortunately, the chemical goal of monomer preservation usually can be achieved with changing fewer than four, usually one or two,

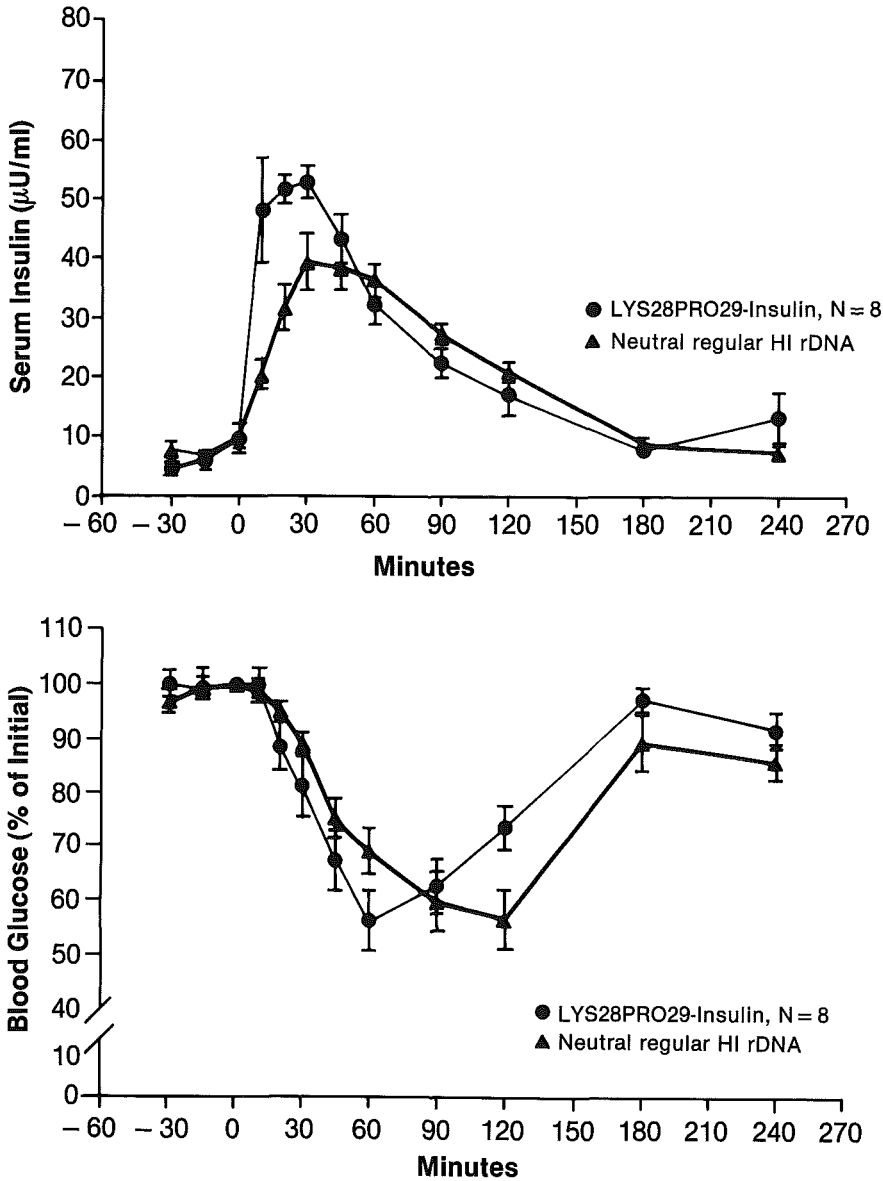

Fig. 5. The serum insulin and blood glucose response of anesthetized normal fasted male beagle dogs to the administration of neutral regular HI rDNA or the "Lys-Pro" analogue 0.1 U/kg subcutaneously. (The antibody used in the radioimmunoassay binds the two insulin agonists equally.)

of the 51 amino acids in the human insulin molecule. However, the task is complicated by the fact that the resulting new entity must (1) have the capacity of native insulin to interact with the insulin receptor and/or a slower metabolic clearance rate so that its net hypoglycemic potency is comparable to that of insulin, (2) be chemically stable, and (3) not be immunogenic. If the last condition is not met, antibodies might be formed which would bind the analogue, thereby defeating the pharmacodynamic purpose for which it was created[30]. An example of a quick-acting "monomeric" insulin is Lilly compound 275585 in which the proline and lysine at positions B28 and B29 of human insulin have been reversed to produce "Lys-Pro" insulin[32]. As shown in Figure 5, the Tmax is significantly higher and occurs sooner with the analogue than with HI rDNA. Moreover, serum concentrations of the "Lys-Pro" analogue dissipate more quickly than with HI rDNA. Studies are in progress to confirm the clinical utility of this and other monomeric analogues.

Recombinant DNA technology has also produced naturally occurring peptides that can be used as research probes. For instance, the availability of biosynthetic human C-peptide has made possible the detailed investigation of endogenous insulin secretion in health and disease by a noninvasive technique[33-37]. In addition, insulin-like growth factors (IGF-I and II) have been cloned and their interactions with other hormone receptors studied[38]. Metabolic studies in normal volunteers[39] have demonstrated that IGF-I has a hypoglycemic potency comparable to that of HPI and suggested an insulin-sparing effect. A recent report has suggested that IGF-I reduces insulin resistance in the muscle of normal-weight but not obese patients with NIDDM[40].

Overall, recombinant DNA technology has precluded a worldwide insulin shortage and provided means for producing a number of insulin agonists all of which will be useful research tools and some of which may well be found to be superior to existing insulin preparations in the treatment of diabetes mellitus. Clearly, the acceptance and success of the recombinant human insulin project has had a major positive impact on all drug development.

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Discussion - HUMAN INSULIN AND ITS MODIFICATIONS

D. Maruhn

I was somewhat surprised that you showed only results on glucose and no results on long term diabetic control, for example using HBA 1c or something like that.

J.A. Galloway

In the transfer studies, where the blood sugars were the highest, there was no statistically significant difference between the glycohemoglobin levels. The insulin dose was the same for both groups.

R.G. Werner

The insulin derivatives that you mentioned may have a great advantage because of their mode of action, but they might be immunogenic. Do you see any possibility to clear out the risk of immunogenicity prior to clinical trials?

J.A. Galloway

No, we are aware of no way to assess immunogenicity pre-clinically.