New Directions in Drug Development: Mixtures, Analogues, and Modeling

JOHN A. GALLOWAY, MD

Success in modern medical research is acheived when basic and clinical information about a given disorder converges, either intentionally or fortuitously, with the availability of technology or other means to design and apply interventions for the disorder in question. A prime example is the discovery of insulin and its replacement in patients with IDDM in 1923. Seven decades later, the focus of diabetes management is on improvement in metabolic control to forestall the chronic complications of the disease and improve the quality of life of patients with the disease. Metabolic control is being addressed through the development of insulin analogues using sophisticated techniques to understand the chemistry of insulin and to modify it using rDNA technology. The objective of these efforts is to simulate normal insulin secretion with subcutaneously injected agonists. Quality-of-life needs are being addressed with delivery devices, insulin mixtures, and insulin analogues. Although none of these improvements parallel the discovery of insulin, they do provide an optimistic outlook for patients with diabetes mellitus.

When the progress made in the treatment of diabetes mellitus during the past two decades is considered, the needs and challenges for the final decade of this century become evident. For instance, the introduction of patient self-monitoring of blood glucose and laboratory measurement of glycosylated hemoglobin called attention to the failure of commonly used regimens to normalize blood glucose. The shortcomings of these regimens led to and facilitated the use of conventional intensive insulin treatment programs and CSII or pumps. Acceptance of CSII has been based largely on the belief, against which no effective counter can be found, that optimizing blood glucose control is most efficiently achieved by simulating normal insulin secretion.

The use of intensified regimens has been limited by three significant factors. First, until very recently there has been a paucity of incontrovertible evidence, based on controlled, long-term clinical trials, that lowering blood glucose will forestall development of the chronic complications of diabetes. Fortunately, the results of the DCCT (1) point unmistakably to the importance of glycemic control in preventing the progression of retinopathy, nephropathy, and neuropathy in patients with IDDM. Although the DCCT results are also relevant to NIDDM, the findings of the U.K. Prospective Diabetes Study (2), which will be available in 1994, should be particularly instructive regarding the benefits and risks of glycemic control in NIDDM patients. It should be pointed out, however, that based on random, uncontrolled trials and anecdotal experience, the impressions are extant that glycemic control is very important in IDDM and useful in NIDDM. These notions will be supported by the results of the aforementioned controlled clinical trials. Second, glycemic control, particularly in individuals with IDDM, imposes enormous burdens on the patient's life-style and quality of life. Thus, those who choose to self-monitor their blood glucose are in a constant state of vigilance with respect to their well-being in general, and the possibility of hypoglycemia in particular. For this reason, interventions or treatment improvements that the person without diabetes may perceive as trivial or marginal may be highly useful to the patient with diabetes. Third, factors related to compliance and the inability or unwillingness of patients to make the necessary quality-of-life sacrifices undoubtedly constitute the major impediment to normalization of blood glucose in most patients (3). Nonetheless, it is clear that certain properties of commercially available insulin preparations preclude the simulation of normal insulin secretion and its metabolic benefits. For this reason, the principal manufacturers of insulin are engaged in vigorous competition to produce insulin agonists that can more closely replicate endogenous insulin secretion or its effects.

From the Lilly Research Laboratories, Eli Lilly, Indianapolis; and the Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana.

Address correspondence and reprint requests to John A. Galloway, MD, Lilly Research Laboratories, Mail drop, 2128, Lilly Corporate Center, Indianapolis, IN 46285.

IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; CSII, continuous subcutaneous insulin infusion; DCCT, Diabetes Control and Complications Trial; HPI, human proinsulin; IGF-I, insulin-like growth factor-I; BHCP, biosynthetic human C-peptide; HGP, hepatic glucose production; CV, coefficient of variation.

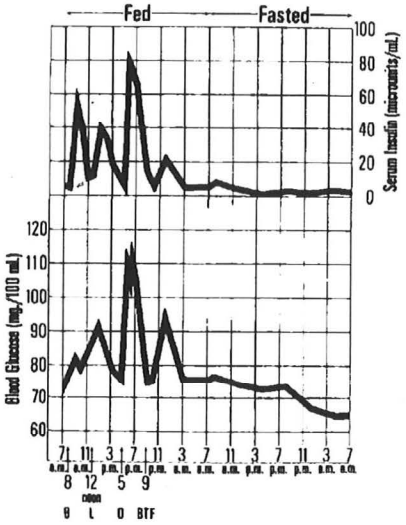

Figure 1—*Serum insulin and blood glucose responses of normal subjects to a standard diabetic diet (30 kcal/kg) given as 2/7 with each main meal and 1/7 at bedtime. From Galloway and Chance (4). © by Elsevier.*

This paper reviews the rationale for and methods used to develop specific insulin analogues, and describes how certain insulin mixtures and analogues may have a positive effect on the quality of life of patients with NIDDM as well as those with IDDM.

NORMAL INSULIN SECRETION AND ITS SIMULATION BY INJECTED "INSULINS"

— As suggested above, the most efficient method for achieving metabolic control in IDDM patients is to simulate normal endogenous insulin secretion. It is therefore appropriate to identify the essential components of endogenous insulin secretion in the nondiabetic individual, review the deficiencies of presently available insulin preparations, and indicate what might or should be done to simulate it or its effects with insulin agonists manufactured by rDNA technology.

As indicated in Fig. 1, normal insulin secretion consists of basal and meal-stimulated components. The function of basal insulin secretion, which is usually in the range of 5–10 U/ml (30–60 pM) (Fig. 1), is to restrain HGP in the postabsorptive state. Because fasting blood glucose has an extremely high correlation with HGP and is the base on which prandial glycemia is added during the next 24 h, simulation of this phase of insulin secretion is highly desirable, particularly in individuals with NIDDM. In Lilly clinical trials of patients with IDDM as well as NIDDM, the single blood glucose value that correlated best with glycohemoglobin was the value taken after an overnight fast and collected at the study site (4). The function of the other component of endogenous insulin secretion, the meal-stimulated phase, is to promote disposal of ingested nutrients, principally glucose, into the periphery. As indicated in Fig. 1, this phase, which is characterized by a rise in serum hormone to concentrations of 80–120 U/ml (480 to 720 pM), is exquisitely sensitive to, and therefore synchronized with, the rise in blood glucose in response to meals. In addition, once the meal glycemia has subsided, there is a prompt return to the basal or postabsorptive level. The physiological importance of proper timing of the premeal insulin dose has been clearly demonstrated in both NIDDM (5) and IDDM (6).

As suggested above, presently available insulins or insulin regimens are generally incapable of simulating the basal or meal-stimulated components of normal insulin secretion. For instance, as indicated in Fig. 2, even ultralente pork insulin, which presumably would behave like ultralente human insulin, is not truly a basal insulin, as it has peaks that occur 15–20 h after injection. With respect to the meal-stimulated component, although the serum hormone profile and glucodynamic effects of neutral regular human insulin, which is the fastest-acting commercially available insulin, would be expected to simulate that of normal insulin secretion, Fig. 3 shows that it does not. Here it is clear that the peak effects of neutral regular human insulin, 0.2 U/kg subcutaneously, do not occur until 3 to 4 h after injection and are present as long as 8 h. The administration of regular insulin by CSII would seem to be an ideal method for mimicking normal insulin secretion. However, Fig. 4 shows that patients with IDDM on CSII treated with basal-bolus programs have higher than normal serum insulin concentrations throughout the day and night, probably as a result of the long-acting nature of the premeal bolus infusions of neutral regular insulin.

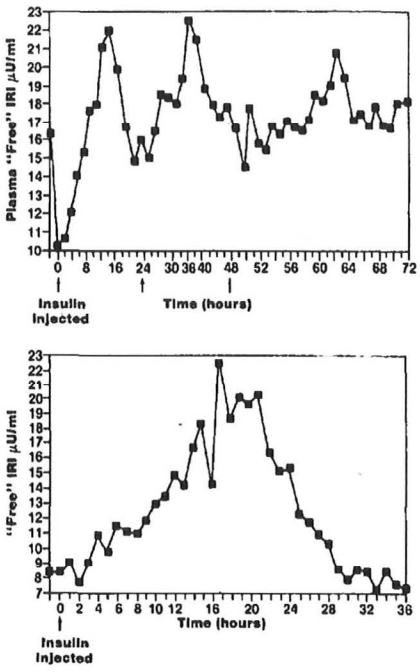

Figure 2—*Mean serum free insulin responses of 6 IDDM patients to ultralente pork insulin (0.8 U/kg subcutaneously). Patients were maintained euglycemic with a glucose clamp technique. A: Responses to dosing at 0, 24, and 48 h. B: The response over 36 h to a single dose at time 0. Courtesy of J.E. Gerich.*

The discussion above has focused on the acute benefits of simulating normal insulin secretion on glucose metabolism. However, because conventional insulin treatment usually results in average serum insulin concentrations 2–4 times normal (7), and hyperinsulinemia

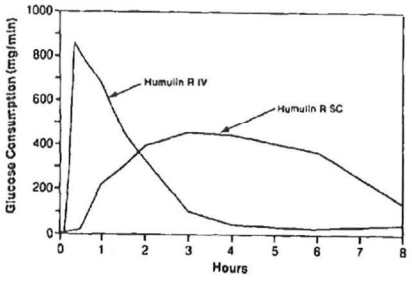

Figure 3—*Pharmacodynamic responses of 6 normal volunteer subjects to neutral regular human insulin rDNA (Humulin R) 0.2 U/kg by bolus intravenous infusion and by subcutaneous injection. Subjects were kept euglycemic with a Biostator. Note that the peak effect of subcutaneous Humulin occurs after 3 h and that glucose is still being infused at 8 h after the subcutaneous injection. From Galloway (8).* © *by the American Diabetes Association.*

may be a significant risk factor for coronary heart disease (8,9), an important long-term advantage to simulating normal insulin secretion may be reduction in chronic hyperinsulinemia.

METHODS TO DEVELOP INSULIN ANALOGUES— With the deficiencies of conventional insulins described, the discussion now turns to methods used in the development of improved

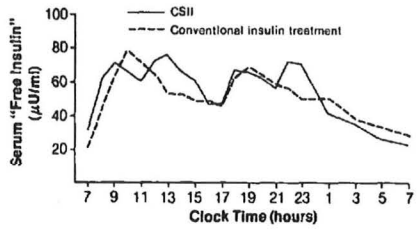

Figure 4—*Mean serum free insulin concentrations in 5 patients with IDDM who received pork insulin either by CSII or with conventional twice-daily regimens of NPH and neutral insulin at 0730 and 0430. With CSII, meal boluses were given at 0700 and 1100 and at 1630 and 2130. Basal insulin was 50–60% of the total daily dose. From Galloway and Chance (4).* © *by Elsevier.*

"insulins," or insulin analogues. (It should be noted that the first analogue of human insulin was beef insulin, which has been in use for 70 years.) Basically, two approaches can be used in developing insulin analogues. The first is to identify an "insulin" that has been found to have attractive pharmacological properties and adapt it for human use. An example of this process is the selection and development of HPI (10). Studies in animals and humans demonstrated that pork proinsulin was a soluble, intermediate-acting insulin agonist that appeared to be relatively hepatospecific. Thus, in proinsulin there seemed to be the possibility of developing an intermediate-acting insulin agonist that was free of protamine or excess zinc, and with effects on the liver that might be uniquely efficacious in NIDDM, a disorder characterized by excessive HGP among other defects (8,11). Accordingly, HPI was produced by rDNA technology and clinical studies were undertaken.

Pharmacology studies in dogs, normal volunteer subjects, and patients with NIDDM demonstrated that, compared with insulin for equivalent suppression of HGP, the effect of HPI on peripheral glucose disposal was less than that of insulin; that is, consistent with the finding with pork proinsulin, HPI was relatively hepatospecific (10). Although the differences between HPI and insulin were clear and distinct in all studies, they were quantitatively unimpressive. However, an unexpected pharmacological finding was that the intrapatient CV in serum hormone concentrations and glycemic response was ~50% of that observed with insulin. Despite these apparent pharmacological benefits, controlled clinical trials failed to demonstrate better glycemic control in the HPI-treated group (10).

Of note is that as the multicenter clinical trials were nearing completion, a report occurred indicating that HPI was more active when injected into the abdomen than into the arm or thigh, suggesting the possibility of partial uptake by the portal vein with direct access to the liver. The potential importance of this finding is evidenced by another preliminary report that showed that HPI was equipotent with human insulin when administered intraperitoneally (12). Although injection sites were random in the multicenter trials, at one center where the investigator used just the abdominal site, HPI was found to be superior to insulin (13). However, before an appropriate study to evaluate the importance of injection site on the bioactivity of HPI could be undertaken, a potentially significant toxicity issue arose. Specifically, in one study of insulin-naive patients, in which ~70 were randomized to insulin and 70 to HPI, there were 6 myocardial infarctions, including 2 deaths, in the HPI-treated group after 1 year of treatment, but none in the insulin-treated group (10). This finding, combined with the failure to demonstrate improved metabolic control with HPI versus insulin across the multicenter clinical trials, led to the suspension of development of HPI. Although a causal relationship between the use of HPI and the cardiovascular events was not established, it should be noted that because the hypoglycemic potency of HPI was only 4.0 to 5.7 U/mg, serum hormone concentrations in HPI-treated patients were exceedingly high—up to 1000 times normal. Nonetheless, experience with HPI combined with an expanding literature linking the possible mitogenic effects of hyperinsulinemia to atherosclerosis (9,10,14) has clearly added the issues of mitogenicity, including both atherogenicity and, by implication, carcinogenicity, to the developmental agenda for insulin analogues. Indeed, in vitro studies have suggested that certain insulin analogues have greater effects (binding to vascular smooth muscle and/or cell proliferation) than human insulin or HPI (15).

Because of the attractiveness of an intermediate-insulin agonist that would be soluble and contain no excess zinc, researchers proceeded with the development of a normal metabolite of HPI, des

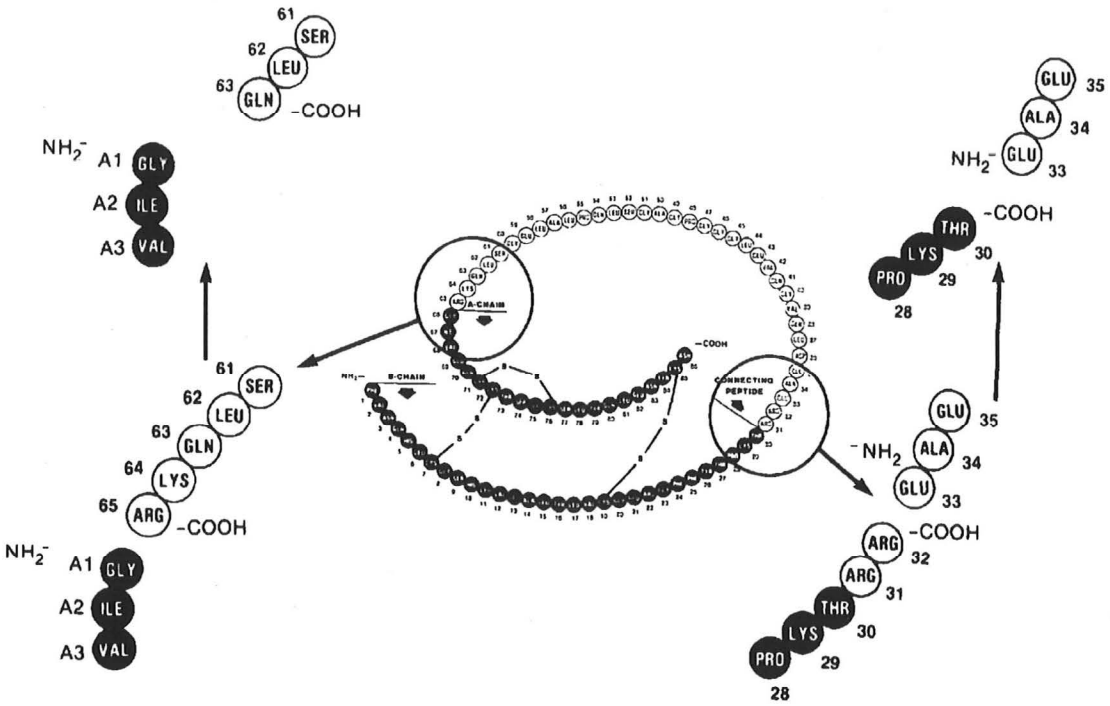

Figure 5—*Metabolites or intermediates formed by the conversion of HPI to insulin. The des 64,65 HPI (dPRO) form (shown upper left) is the result of a split between 65 and 66 (or A1 and C65), followed by removal of the Arg and Lys residues at positions 64 and 65. From Galloway et al. (10).* © *by the American Diabetes Association.*

64,65 HPI (des 64,65 HPI, or "dPRO") (Fig. 5). Data generated in humans with this analogue are very preliminary, whereas studies in dogs (16) suggest that des 64,65 HPI is intermediate acting and has a hypoglycemic potency comparable to that of insulin. Therefore, serum hormone concentrations after administration of des 64,65 HPI should be significantly lower than those seen after HPI. The fact that animal studies comparing des 64,65 HPI with insulin and HPI (17) have disclosed that the hepatic extraction coefficient and hepatic half-life of the des 64,65 analogue is closer to that of insulin than HPI reduces the likelihood that the des 64,65 analogue will be hepatospecific. On the other hand, the consistent absorption kinetics seen with HPI would be expected with des 64,65 HPI.

The second method for developing improved insulins is that of computer modeling, which is usually performed by a computational chemist. This is a multicomponent technique that uses a computer programmed with physicochemical and biological information developed on insulin over several decades. Computer modeling allows the chemist to generate virtually infinite iterations of the possibilities of intramolecular relationships to explain or extrapolate the behavior of insulin or its analogues under various conditions without actually undertaking arduous and expensive preclinical or clinical tests (18–20). This use of a computer can generate vital information concerning stability, self-association, and pharmacological activity, including receptor binding, mitogenicity, and immunogenicity.

Modeling (computational chemistry) is usually used in conjunction with experimental chemistry. An example is the development of the Lys,Pro analogue of human insulin, a fast-acting insulin analogue that has been synthesized in the Lilly Research Laboratories (5,21). For instance, the long-acting nature of neutral regular insulin (described above) has been understood to be attributed to its tendency to self-associate into dimers, tetramers, hexamers, and polymers, with absorption of subcutaneously injected insulin occurring only after it has dissociated into a less aggregated form (6). It was noted that IGF-I has many structural similarities to insulin in the COOH-terminus of the B-chain but does not self-associate. Therefore, attention was focused on an important difference between the two polypeptides B28 and B29 on the B-chain, where the sequence is Pro,Lys for insulin and Lys,Pro for IGF-I (22). Using molecular modeling, with help from a Cray 2 supercomputer, it was possible to visualize and comprehend the dynamics of the intramolecular behavior that results in self-association.

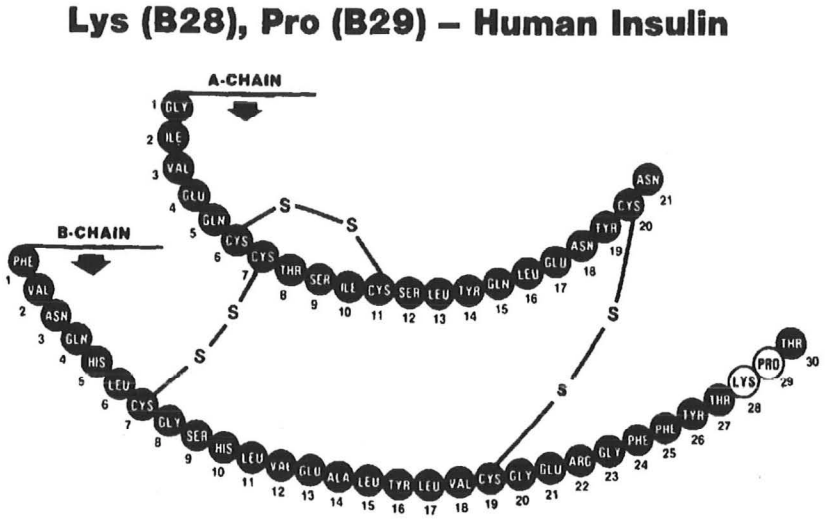

Figure 6—*Lys(B28), Pro(B29)-Human insulin. This analogue is identical to human insulin except at positions B28 and B29 where the sequence of the two residues has been reversed and are in the same order as in IGF-I, a polypeptide which does not self-associate. Courtesy of R.E. Chance.*

It was ascertained that the reversal of the Pro,Lys sequence in the B28–29 region of insulin (Fig. 6) increased the free-energy barrier to self-association. Modeling also predicted that the Lys,Lys analogue would be close to native insulin in self-association and showed that the position of the Pro in the sequence was critical to controlling self-association. As indicated first in studies in dogs (23) (Fig. 7), in normal humans (24) the absorption and glucodynamic effects of the Lys,Pro analogue of human insulin, Lys,Pro human insulin, are significantly more rapid in onset that those of human insulin rDNA (Fig. 6). Of interest is the fact that the slight change in the structure of human insulin had an insignificant effect on affinity for IGF-I receptors or insulin receptors on placental membranes (21,25). Thus, it would appear that the Lys-Pro analogue and other fast-acting analogues (6,26) will have the pharmacological properties necessary for producing a rapid, timely, and adequate increase in serum hormone concentration to promote disposal of ingested nutrients efficiently. Table 1 shows the profile of an ideal fast-acting insulin analogue.

An issue not addressed above is that of immunogenicity. The importance of lack of immunogenicity is based on studies in patients with and without antibodies to animal insulins, which have demonstrated that antibodies may delay the effect of injected insulin (27). This being the case, if a fast-acting analogue is immunogenic, antibodies could be formed that would delay and therefore neutralize the pharmacodynamic effect it was designed to deliver. One can only speculate on the potential importance of the proimmunogenic effects of episodic exposure, a sine qua non of fast-acting analogue use, and the anti-immunogenic effects of reducing the size of the peptide that might stimulate antibody formation.

As indicated above, based on the pharmacokinetic shortcomings of ultralente insulin and the suspension of trials with HPI, a significant clinical need exists for a basal insulin agonist. Table 2 lists the features identified as being desirable in the ideal long-acting insulin analogue. Novo-Nordisk has reported an analogue (NovoSol basal) (28) with absorption and duration of action apparently substantially slower and more consistent than that of ultralente beef insulin and without peaks. However, the absence of additional information on this analogue precludes further discussion. Because of the extraordinary challenge of

Table 1—***Features of an ideal fast-acting insulin analogue***

Primary metabolic function
Promotion of peripheral glucose disposal
Specifications
Time-action profile:
Onset <1 h after subcutaneous injection
Duration of <4 h
Metabolic >>> mitogenic effects
Nonimmunogenic
Chemically stable
Mixable with long-acting insulin and insulin analogues

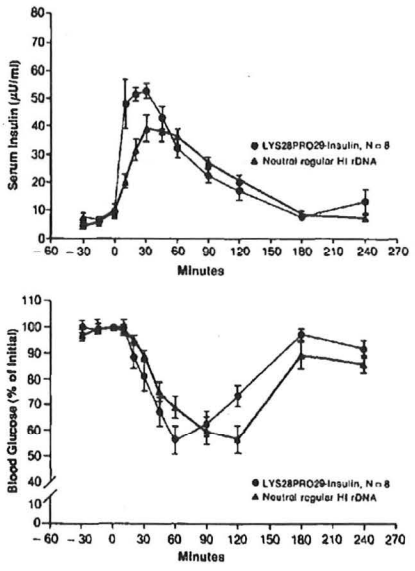

Figure 7—*Serum insulin and blood glucose responses of normal fasted anesthetized male beagle dogs after subcutaneous administration of 0.1 U/kg of neutral regular human insulin rDNA or the Lys,Pro analogue of human insulin. From Galloway et al. (23). © by Elsevier.*

Table 2—*Features of an ideal long-acting insulin analogue*

Primary metabolic function
Suppression of HGP
Specifications
Time-action profile:
Onset >4 h after subcutaneous injection
Duration of >24 h
Once-daily injection
Low intrasubject CV of response
Metabolic >>> mitogenic effects
Hypoglycemic potency = or > human insulin
Nonimmunogenic
Chemically stable
Mixable with short-acting insulin and insulin analogues

developing an analogue to meet the specifications listed in Table 2, it seems unlikely that such a compound will be available in the immediate future. In addition, the possibility of developing a long-acting analogue that could simulate the pulsatility of normal-basal insulin secretion (29) seems exceedingly remote.

HUMAN C-PEPTIDE — A substance prepared by rDNA (30) that has attractive physiological properties, as opposed to HPI, which has desirable features but only in pharmacological doses, is BHCP. Although C-peptide is cosecreted from normal β in quantities that are equimolar with insulin and is present in the circulation, until recently, no physiological function had been assigned to this peptide. For this reason, the C-peptide deficiency that accompanies insulin deficiency in IDDM has been regarded as having no consequence. Recently, investigators in Stockholm reported that acute infusions of BHCP to mimic normal plasma concentration of C-peptide in C-peptide-negative patients with IDDM reduced glomerular hyperfiltration without affecting renal plasma flow (31). In a preliminary report, this group indicated that the addition of BHCP, administered for 30 days by CSII in amounts equimolar with insulin in patients with IDDM, reduced glomerular hyperfiltration and capillary leakage (measured by vitreous fluorophotometry) and possibly improved metabolic control (32). In vitro studies have shown that BHCP promoted the transport of 3-O-methylglucose in a dose-dependent manner in muscle specimens taken from healthy volunteer subjects (33) but not in muscle from NIDDM patients (34). These findings, if confirmed, could have important implications for treatment of patients with IDDM. However, because C-peptide concentrations usually are normal or elevated in patients with NIDDM (35), C-peptide replacement therapy is unlikely to be of benefit. Nonetheless, if the findings in IDDM are confirmed, serious consideration will be given to adding BHCP to insulin for patients with IDDM.

QUALITY-OF-LIFE ISSUES — The above discussion has focused on how improvements in the pharmacokinetics and/or pharmacodynamics of insulin preparations might result in improved metabolic control, which, in turn, may reduce the complications of diabetes. No less important to the patient with diabetes are interventions and techniques that will improve his or her quality of life. Undoubtedly, the most important tool in recent years for improving the quality of life of patients with diabetes mellitus has been self-monitoring of blood glucose. Two other developments, however, also deserve mention: pen injectors and premixtures of NPH and regular insulin. The availability of these penlike devices, which accurately deliver insulin (regular alone or in combination with NPH) from a prefilled cartridge, give patients a simple, convenient method for delivering insulin without having to carry insulin vials (36). These devices undoubtedly have improved quality of life for patients by facilitating multiple-dose insulin therapy (37,38). Although occasional reports indicate that use of pen injectors improves metabolic control (39), in general, metabolic control with this device has not been shown to be superior to that achieved with intensive conventional treatment. Nonetheless, the convenience afforded to patients by pen injectors is undeniable.

The availability of insulin mixtures, especially 70% NPH and 30% regular insulin, has provided patients with a convenient method for taking two insulins and has obviated errors inherent in the multiple-step procedure of self-mixing. The use of premixtures of insulin may or may not improve metabolic control. Bell et al. (40) demonstrated better control in patients with NIDDM using prefilled insulin mixtures. However, in a study of patients with IDDM, Corcoran and Yudkin (41) failed to demonstrate an advantage in metabolic control. The increasing acceptance in the marketplace of premixed insulins suggests that the convenience they offer is clearly satisfying a patient need and supports the introduction of mixtures with other proportions of NPH and regular insulin (e.g., 50/50, 80/20).

Finally, insulin analogues have the potential to improve the quality of life of patients with diabetes (6). For instance, it has been proposed that the fast-acting insulin analogues will have two important benefits. First, because of their rapid absorption, analogues can be given substantially closer to meals than conventional neutral regular insulin, which typically must be administered 30 to 45 minute before a meal to match the pharmacodynamic effect with the prandial glucose rise. Second, the rapid decline in serum hormone concentrations and effect of the fast-acting insulin analogues may decrease the frequency of between-meal hypoglycemia. Indeed, this may also result in a major reduction of the nearly threefold increase in the frequency of serious hypoglycemia, as compared with patients with only moderately to poorly controlled diabetes (42).

CONCLUSIONS — The perpetual interaction of new basic and clinical infor-

mation on diabetes, modern technology, and patients' quality-of-life needs is producing a slowly rising spiral of improvement in the treatment of and outlook for patients with diabetes mellitus. It is certain that these positive trends will continue. Their absolute impact at a given future date, however, remains uncertain.

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References

1. The Diabetes Control and Complications Trial Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl J Med* 329:977–86, 1993
2. UK Prospective Diabetes Study (UKPDS): VIII. Study design, progress and performance. *Diabetologia* 34:877–90, 1991
3. Galloway JA: Chemistry and clinical use of insulin. In *Diabetes Mellitus*, 9th ed. Galloway JA, Potvin JH, Shuman CR, Eds. Indianapolis, Eli Lilly, 1988, p. 106–36
4. Galloway JA, Chance RE: Insulin agonist therapy: a challenge for the 1990s. *Clin Ther* 12:460–72, 1990
5. Bruce DG, Chisholm DJ, Storlien LH, Kraegen EW: Physiological importance of deficiency in early prandial insulin secretion in non-insulin-dependent diabetes. *Diabetes* 37:736–44, 1988
6. Brange J, Owens DR, Kang S, Volund A: Monomeric insulins and their experimental and clinical implications. *Diabetes Care* 13:923–54, 1990
7. Hayford JT, Thompson RG: Free and total insulin integrated concentrations in insulin-dependent diabetes. *Metabolism* 31:387–97, 1982
8. Galloway JA: Treatment of NIDDM with insulin agonists or substitutes. *Diabetes Care* 13:1209–39, 1990
9. Stout RW: Insulin and atheroma: 20-yr perspective. *Diabetes Care* 13:631–54, 1990
10. Galloway JA, Hooper SA, Spradlin CT, Howey DC, Frank BH, Bowsher RR, Anderson JH: Biosynthetic human proinsulin: review of chemistry, in vitro and in vivo receptor binding, animal and human pharmacology studies, and clinical trial experience. *Diabetes Care* 14:666–92, 1992
11. DeFronzo RA: Lilly Lecture 1987. The triumvirate: β-cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes* 37:667–87, 1988
12. Nippe A, Walter H, Bachmann W, Mehnert H: Intraperitoneal (IP) versus intravenous (IV) application of human proinsulin and insulin in type I diabetics (Abstract). *Diabetes* 34 (Suppl. 1):59A, 1985
13. Davidson JK, Satterfield LD, Rolfes RM: rDNA human proinsulin produces excellent metabolic control of type I (insulin-dependent) diabetes (Abstract). *Diabetologia* 30:511A, 1987
14. Stout RW: Diabetes and atherosclerosis—the role of insulin. *Diabetologia* 16: 141–50, 1979
15. Bornfeldt KE, Gidlöf RA, Wasteson A, Lake M, Skottner A, Arnqvist HJ: Binding and biological effects of insulin, insulin analogueues and insulin-like growth factors in rat aortic smooth muscle cells. Comparison of maximal growth promoting activities. *Diabetologia* 34:307–13, 1991
16. Tillil H, Frank BH, Pekar AH, Broelsch C, Rubenstein AH, Polonsky KS: Hypoglycemic potency and metabolic clearance rate of intravenously administered human proinsulin and metabolites. *Endocrinology* 127:2418–22, 1990
17. Sodoyez-Goffaux F, Sodoyez JC, Koch M, DeVos CJ, Frank BH: Scintigraphic distribution of ^{123} I labelled proinsulin, split conversion intermediates and insulin in rats. *Diabetologia* 31:848–54, 1988
18. Caves LSD, Nguyen DT, Hubbard RE: Conformational variability of insulin: a molecular dynamics analysis. In *Molecular Dynamics: Applications in Molecular Biology*. Goodfellow JM, Ed. Boca Raton, FL, CRC Press Inc., 1990, p. 27–68
19. Hua QX, Shoelson SE, Kochoyan M, Weiss MA: Receptor binding redefined by a structural switch in a mutant human insulin. *Nature* 354:238–41, 1991
20. Mark AE, Berendsen HJC, van Gunsteren WF: Conformational flexibility of aqueous monomeric and dimeric insulin: a molecular dynamics study. *Biochemistry* 30:10866–72, 1991
21. Long HB, Baker JC, Belagaje RM, DiMarchi RD, Frank BH, Green LK, Hoffmann JA, Muth WL, Pekar AH, Reams SG, Shaw WN, Shields JE, Slieker LJ, Su KSE, Sundell K, Chance RE: Human insulin analogues with rapid onset and short duration of action. In *Peptides XII* (Proceedings of the 12th American Peptide Symposium). Smith J, Rivier J, Eds., Leiden, Netherlands, ESCOM Science Publishers, B. V., 1992 p. 88–90
22. DiMarchi RD, Mayer JP, Fan L, Brems DN, Frank BH, Green LK, Hoffmann JA, Howey DC, Long HB, Shaw WN, Shields JE, Slieker LJ, Su KS, Sundell K, Chance RE: Synthesis of a fast-acting insulin based on structural homology with insulin-like growth factor I. In *Peptides XII* (Proceedings of the 12th American Peptide Symposium). Smith J, Rivier J, Eds., Leiden, Netherlands, ESCOM Science Publishers, B. V., 1992, p. 26–28
23. Galloway JA, Chance RE, Su KSE: Human insulin and its modifications. In *The Clinical Pharmacology of Biotechnology Products*. Reidenberg MM, Ed. Amsterdam, Elsevier Science Publishers BV, 1991, p. 23–34
24. Howey DC, Hooper SA, Bowsher RR: [Lys(B28), Pro(B29)]-human insulin: an equipotent analogue of human insulin with rapid onset and short duration of action (Abstract 1688). *Diabetes* 40 (Suppl. 1):423A, 1991
25. Slieker LJ, Sundell K: Modifications in the 28–29 position of the insulin B-chain alter binding to the IGF-I receptor with minimal effect on insulin receptor binding (Abstract 670). *Diabetes* 40 (Suppl. 1):168A, 1991
26. Vølund Aa, Brange J, Drejer K, Jensen I, Markussen J, Ribel U, Sørensen AR, Schlichtkrull J: In vitro and in vivo potency of insulin analogueues designed for

clinical use. *Diabetic Med* 8:839–47, 1991
27. Francis AJ, Hanning I, Alberti KGMM: The influence of insulin antibody levels on plasma profiles and action of subcutaneously injected human and bovine short-acting insulins. *Diabetologia* 28: 330–34, 1985
28. Jorgensen S, Vaag A, Langkjaer L, Hougaard P, Markussen J: NovoSol basal: pharmacokinetics of a novel soluble long-acting insulin analogueue. *Br Med J* 299:415–19, 1989
29. Paolisso G, Scheen AJ, Giugliano D, Sgambato S, Albert A, Varricchio M, D'Onofrio F, Lefèbvre PJ: Pulsatile insulin delivery has greater metabolic effects than continuous hormone administration in man: importance of pulse frequency. *J Clin Endocrinol Metab* 72:607–15, 1991
30. Frank BH, Pettee JM, Zimmerman RE, Burck PJ: The production of human proinsulin and its transformation to human insulin and C-peptide. In *Peptides: Synthesis-Structure-Function*. Rich DH, Gross E, Eds. Rockford, IL, Pierce Chemical Company, 1981, p. 729–38
31. Johansson B-L, Sjöberg S, Wahren J. The influence of human C-peptide on renal function and glucose utilization in Type 1 (insulin-dependent) diabetic patients. *Diabetologia* 35:121–28, 1992
32. Johansson B-L, Kernell A, Sjoberg S, Wahren J: Effects of C-peptide on renal function, blood-retinal barrier leakage and metabolic control in type I diabetes. *Diabetologia* 34 (Suppl. 2):A184, 1991
33. Zierath JR, Galuska D, Johansson B-L, Wallberg-Henriksson H: Effect of human C-peptide on glucose transport in in vitro incubated human skeletal muscle. *Diabetologia* 34:899–900, 1991
34. Wallberg-Henriksson H, Andréasson K, Galuska D, Johansson B-L, Thörne A, Henriksson J, Sonnenfeld T: C-peptide stimulates glucose transport in incubated normal muscle but not muscle from type 2 (non-insulin-dependent) diabetic patients (Abstract 540). *Diabetologia* 32: 555A, 1989
35. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, Van Cauter E: Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 318:1231–39, 1988
36. Houtzagers CMGJ: Subcutaneous insulin delivery: present status. *Diabetic Med* 6:754–61, 1989
37. Wikby A, Hörnquist JO, Andersson P-O: Background, quality of life and metabolic control in patients with insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* 13:53–62, 1991
38. Sangeleer M, Czarka M: An evaluation of the use of a pen-injecting device in a diabetic population. *Acta Therapeutica* 18:41–51, 1992
39. McCaughey ES, Betts PR, Rowe DJ: Improved diabetic control in adolescents using the penject syringe for multiple insulin injections. *Diabetic Med* 3:234–36, 1986
40. Bell DSH, Cutter GR, Lauritano AA: Efficacy of a premixed semisynthetic human insulin regimen. *Clin Ther* 11:795–801, 1989
41. Corcoran JS, Yudkin JS: A comparison of pre-mixed with patient-mixed insulins. *Diabetic Med* 3:246–49, 1986
42. The DCCT Research Group: Epidemiology of severe hypoglycemia in the diabetes and control complications trial (DCCT). *Am J Med* 90:450–59, 1991