Since its discovery and first clinical use in the 1920s, insulin therapy has revolutionized the treatment and natural history of both type 1 and type 2 diabetes mellitus. This article highlights selected milestones in insulin discovery and its continued development as a pivotal therapy for diabetes (Fig. 1).

The history of insulin begins at the turn of the twentieth century, with the culmination of early landmark research on endocrine pancreas (patho)physiology, which provided the intellectual and methodological framework for insulin discovery. In 1890, von Mering and Minkowski identified the crucial link between the pancreas and diabetes as evidenced by the diabetic phenotype they induced in dogs after pancreatectomy. Subsequent histologic observations during 1900-1901 of hyaline changes in the islets of Langerhans from patients with diabetes by Opie, coupled with the pioneering animal pancreatic duct ligation experiments of Ssobolew from 1900 to 1902, resulting in atrophied acinar cells accompanied by normal, intact islets and a nondiabetic phenotype, collectively implicated the pancreatic islets in the etiology of diabetes. Physiologist Sharpey-Schäfer hypothesized that pancreatic islets might produce an “internal secretion” or hormone involved in glucose homeostasis. Consequently, the first 2 decades of the twentieth century witnessed numerous attempts to isolate this internal secretion. Although several investigators prepared and administered pancreatic extracts with transient hypoglycemic effects in animals and even humans, adverse side effects ultimately precluded their clinical utility.

In 1920, the pathologist Barron recognized that pancreatic duct obstruction, whether secondary to pancreatolithiasis in a rare post-mortem case or pancreatic duct ligation in prior animal experiments, led to atrophy of the exocrine but not...
endocrine pancreas. Primarily inspired by Barron’s paper, Frederick G. Banting conceived of a novel method of isolating the internal secretion by deliberately inducing atrophy of the acinar cells of the exocrine pancreas via duct ligation in dogs, to diminish the potentially destructive effect of digestive enzymes on the islet hormone. In the summer of 1921, assisted by Charles H. Best in the laboratory of John J.R. MacLeod at the University of Toronto, Banting became the first to demonstrate that pancreatic islet extracts consistently reduced hyperglycemia and glycosuria in depancreatized, diabetic dogs. Later that year, with the expertise of biochemist James B. Collip, a novel protocol was developed to purify what they later named “insulin” (Latin: insula, island), from pancreatic islets of whole bovine pancreata without the need for pancreatic duct ligation experiments. The first successful therapeutic use of pancreas extracts of bovine insulin occurred at the Toronto General Hospital on January 11, 1922 on a 14-year-old patient, Leonard Thompson, who was admitted with type 1 diabetes. Even this very unphysiologic rather crude form of insulin therapy rapidly transformed type 1 diabetes from an inevitably fatal condition to a chronic metabolic disease characterized by significant risk of hypoglycemia, diabetic ketoacidosis, and ultimately the long-term complications associated with diabetes. In 1923, the Nobel Prize in Medicine and Physiology was jointly awarded to Banting and MacLeod for what is considered one of the greatest advancements in modern medicine.

Insulin therapy has significantly evolved since 1922, with major improvements in insulin purification, production, formulation, regimens, and delivery systems. Until the 1980s animal insulins, extracted from either bovine or porcine pancreata, comprised all commercially available insulin formulations. Such soluble, “regular,” animal insulin products were initially very impure, leading to immunologic reactions (e.g., insulin allergy, immune-mediated lipoatrophy at the injection site, and antibody-mediated insulin
resistance) and significant variability in pharmacokinetics and pharmacodynamics. Early advances in purification techniques led to better-quality products with more consistent biological action. To prolong the glucose-lowering effects of insulin and reduce the number of daily injections required with soluble regular insulin, longer-acting preparations were designed by combining insulin with zinc and/or basic proteins (protamines) to delay subcutaneous absorption. These formulations included protamine insulin and protamine zinc insulin (PZI) developed in the 1930s,\textsuperscript{14–16} isophane neutral protamine Hagedorn (NPH) launched in the 1940s\textsuperscript{17} and the trilogy of “lente” insulins introduced during the 1950s.\textsuperscript{18} Among the latter, NPH insulin has retained its clinical utility to this day, as a twice-daily insulin, used alone or in conjunction with soluble insulin as a premixed product.\textsuperscript{19} During the 1950s, Sanger\textsuperscript{20} elucidated the primary structure of bovine insulin. Through advances in protein chromatography techniques, the 1970s witnessed the production of highly purified animal insulin, denoted monocomponent or single-peak insulin.\textsuperscript{21} Although chemically synthesized human insulin was first produced in the 1960s\textsuperscript{22–26} and studied in preliminary clinical trials,\textsuperscript{27} this breakthrough was overshadowed by the advent of recombinant DNA technology in the late 1970s, which gave rise to recombinant human insulin in 1978, a much more commercially viable product.\textsuperscript{28} This milestone marked a new era in the evolution of insulin therapy and peptide drug development in general. Since the 1980s, insulin replacement strategies have been incrementally optimized, with the development of structurally modified, so-called designer insulin analogues,\textsuperscript{29,30} the evaluation of alternative delivery routes (eg, nasal, pulmonary inhaled, oral, peritoneal insulin formulations),\textsuperscript{31} continuous subcutaneous insulin infusion therapy (ie, insulin pumps),\textsuperscript{32,33} and most recently the potential to use continuous glucose monitoring with glucose sensor technology (ie, closed-loop insulin delivery, also referred to as the artificial pancreas).\textsuperscript{34,35} The remainder of this article focuses on current and future injectable insulin preparations.

**INSULIN PHYSIOLOGY**

Endogenous insulin, a 51-amino-acid anabolic hormone comprising 2 peptide chains (A and B; Fig. 2A), is a key regulator of glucose, protein, and fat homeostasis. Insulin is synthesized as proinsulin, then processed and secreted by pancreatic β cells of the islets of Langerhans into the portal circulation via the hepatic vein, whereby the liver extracts a substantial fraction before entering the systemic circulation.\textsuperscript{36} To maintain euglycemia (ie, plasma glucose between 3.5 and 7.0 mmol/L), insulin release occurs both (1) at a constitutive, basal rate and (2) in short-lived large bursts, secondary to physiologic stimuli related to nutrient intake and mediated in great part by the gastrointestinal system incretin hormones GLP1 and GIP. Basal insulin secretion occurs during fasting/resting states, to inhibit hepatic glycogenolysis, ketogenesis, and gluconeogenesis, and accounts for approximately 40% of total insulin output in a 24-hour period. Stimulated insulin secretion occurs when plasma glucose levels rise to above 4.4 to 5.6 mmol/L (80–100 mg/dL), particularly after meals (postprandial) to restore euglycemia via promotion of peripheral glucose uptake and fuel storage. In addition, insulin secretion in response to a meal occurs in 2 phases: an initial transient surge (first phase) followed by a prolonged steady increase (second phase). Although glucose is the most potent insulin secretagogue, additional dietary nutrients (eg, amino acids), enteric hormones (eg, incretins), and neural signals are also implicated in insulin regulation. In healthy individuals, plasma glucose and insulin excursions occur in parallel and are tightly linked throughout the day, thereby ensuring adequate glucoregulation (Fig. 3).
GOALS OF INSULIN THERAPY

In using insulin it would of course be ideal if it could be supplied so as to imitate the natural process.

—J.J.R. Macleod and W.R. Campbell, 1925

Fig. 2. The primary structure of human insulin and insulin analogues. (A) Native human insulin; (B) rapid-acting insulin analogues (lispro, aspart, glulisine); (C) long-acting insulin analogues (glargine, detemir, degludec). Modifications of each insulin analogue are shown.

Fig. 3. Glucose and insulin excursions throughout a 24-hour period in healthy individuals. Mean levels with 95% confidence intervals are shown. (Adapted from Owens DR, Zinman B, Bolli GB. Insulins today and beyond. Lancet 2001;358:739–46.)
Insulin administration is the sole pharmacologic treatment currently available for patients with type 1 diabetes, and represents an important therapy for many patients with type 2 diabetes. Unfortunately, despite many important advances in the 90 years since its discovery, physiologic insulin replacement remains an elusive goal. Indeed, despite the advent of novel insulin formulations, treatment regimens, delivery systems, continuous glucose monitoring, and multidisciplinary educational programs, diabetes remains a major cause of morbidity and mortality worldwide. The latter is attributed to the significant microvascular and macrovascular complications associated with this disease. However, as demonstrated by several epidemiologic studies and clinical trials, including the landmark Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS), the risk of diabetic complications can be prevented and substantially reduced with intensive glycemic control (Table 1).

Given this unequivocal evidence, the fundamental treatment goals in diabetes care are to strive for optimal glycemic control and minimize the risk of hypoglycemia. To achieve these goals, the aim of insulin therapy is to approximate the physiologic insulin profile (see Fig. 3) via insulin replacement. However, this is often hindered by patient-related (eg, noncompliance) and treatment-related factors. Compared with its endogenous portal secretion from the pancreas, exogenous insulin is administered peripherally via subcutaneous injection, leading to peripheral hyperinsulinemia and portal hypoinsulinemia. Moreover, the variability of subcutaneous insulin absorption and the risk of hypoglycemia further complicate insulin therapy and the attainment of optimal glycemic targets.

## INSULIN PREPARATIONS IN CLINICAL USE

Insulin replacement and supplementation strategies aim to replicate endogenous stimulated and basal insulin release by the healthy pancreas (see Fig. 3), such that excursions in postmeal blood glucose are minimal and hepatic glucose production between meals is appropriately suppressed, respectively. Thus, insulin replacement regimens comprise 2 components: a basal (fasting) and bolus (meal/prandial) insulin preparation. Commercially available insulin formulations are classified as rapid-, short-, intermediate-, or long-acting products based on their pharmacokinetic properties, including onset, peak, and duration of action, summarized in Table 2. Accordingly,

### Table 1

<table>
<thead>
<tr>
<th>Organization</th>
<th>HbA1c (%)</th>
<th>FPG mmol/L</th>
<th>mg/dL</th>
<th>Postprandial PG mmol/L</th>
<th>mg/dL</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDA</td>
<td>&lt;7.0</td>
<td>4.0–7.0</td>
<td>72–126</td>
<td>5.0–10.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90–180</td>
<td>177</td>
</tr>
<tr>
<td>ADA</td>
<td>&lt;7.0</td>
<td>3.9–7.2</td>
<td>70–130</td>
<td>&lt;10.0</td>
<td>&lt;180</td>
<td>178</td>
</tr>
<tr>
<td>AACE</td>
<td>&lt;6.5</td>
<td>&lt;6.1</td>
<td>&lt;110</td>
<td>&lt;7.8</td>
<td>&lt;140</td>
<td>179</td>
</tr>
<tr>
<td>ESC-EASD</td>
<td>&lt;6.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>180</td>
</tr>
<tr>
<td>IDF</td>
<td>&lt;6.5</td>
<td>5.5</td>
<td>&lt;100</td>
<td>7.8</td>
<td>&lt;140</td>
<td>181</td>
</tr>
</tbody>
</table>

Abbreviations: ADA, American Diabetes Association; AACE, American Association of Clinical Endocrinologists; CDA, Canadian Diabetes Association; ESC, European Society of Cardiology; EASD, European Association for the Study of Diabetes; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; IDF, International Diabetes Federation; PG, plasma glucose.

<sup>a</sup> Target of 5.0 to 8.0 mmol/L if HbA1c targets are not being met.
the rapid and short-acting insulin formulations are used as the bolus component of insulin therapy, whereas intermediate-acting and long-acting preparations act to replace endogenous basal insulin secretion. The unique pharmacokinetic parameters of individual insulin products depend primarily on rate and extent of absorption into the systemic circulation following subcutaneous injection.

### Table 2

<table>
<thead>
<tr>
<th>Insulin Type</th>
<th>Trade Name</th>
<th>Manufacturer</th>
<th>Action Profile (h)</th>
<th>Onset</th>
<th>Peak</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rapid-acting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lispro (Humalog)</td>
<td>Eli Lilly</td>
<td></td>
<td>0.2–0.5</td>
<td>0.5–2</td>
<td>3–4</td>
<td></td>
</tr>
<tr>
<td>Aspart (Novorapid)</td>
<td>Novo Nordisk</td>
<td></td>
<td>0.2–0.5</td>
<td>0.5–2</td>
<td>3–4</td>
<td></td>
</tr>
<tr>
<td>Glulisine (Apidra)</td>
<td>Sanofi-Aventis</td>
<td></td>
<td>0.2–0.5</td>
<td>0.5–2</td>
<td>3–4</td>
<td></td>
</tr>
<tr>
<td><strong>Short-acting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>Humulin R</td>
<td>Eli Lilly</td>
<td>0.5–1</td>
<td>2–4</td>
<td>6–8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Novolin ge Toronto</td>
<td>Novo Nordisk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intermediate-acting</strong></td>
<td>Isophane insulin (NPH)</td>
<td>Eli Lilly</td>
<td>1.5–4</td>
<td>4–10</td>
<td>Up to 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Humulin N</td>
<td>Novo Nordisk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Novolin N</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Long-acting</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Glargine (Lantus)</td>
<td>Sanofi-Aventis</td>
<td></td>
<td>1–3</td>
<td>No peak</td>
<td>Up to 24</td>
<td></td>
</tr>
<tr>
<td>Detemir (Levemir)</td>
<td>Novo Nordisk</td>
<td></td>
<td>1–3</td>
<td>No peak</td>
<td>Up to 24</td>
<td></td>
</tr>
<tr>
<td><strong>Premixed human insulins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPH/regular*</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70%/30%</td>
<td>Humulin 70/30</td>
<td>Eli Lilly</td>
<td>0.5–1</td>
<td>3–12</td>
<td>Up to 24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Novolin 70/30</td>
<td>Novo Nordisk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%/50%</td>
<td>Humulin 50/50</td>
<td>Eli Lilly</td>
<td>0.5–1</td>
<td>2–12</td>
<td>Up to 24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Novolin 50/50</td>
<td>Novo Nordisk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Premixed insulin analogues</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPL/Lispro</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75%/25%</td>
<td>Humalog Mix 75/25</td>
<td>Eli Lilly</td>
<td>0.2–0.5</td>
<td>1–4</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Novo Nordisk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%/50%</td>
<td>Humalog Mix 50/50</td>
<td>Eli Lilly</td>
<td>0.2–0.5</td>
<td>1–4</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Novo Nordisk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAP/Aspart</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70%/30%</td>
<td>Novolog Mix 30</td>
<td>Novo Nordisk</td>
<td>0.2–0.5</td>
<td>1–4</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** IAP, insulin aspart protamine; NPH, neutral protamine Hagedorn; NPL, neutral protamine lispro.

* Mixtures with different proportions of NPH and regular insulin are available in Europe.


Human Insulins

**Short-acting human insulin**

Regular human insulin, the first insulin product generated using recombinant DNA technology, has traditionally been used as a bolus insulin to mimic the response of endogenous insulin to a meal and also to correct for premeal and intermeal hyperglycemia. Human insulin exists as a monomer at low concentrations and is best absorbed into the bloodstream following subcutaneous injection in this form. Although
the peptide sequence and tertiary structure of regular recombinant human insulin is identical to that of its endogenous counterpart, the former tends to self-aggregate into quaternary structures of dimers and hexamers in solutions of higher concentration containing zinc ions. This propensity to self-associate delays the absorption of regular human insulin, which must first dissociate into dimers and monomers in the subcutaneous space to effectively diffuse into the general circulation and exert its glucodynamic action. Consequently, the latter is reflected in its pharmacokinetic profile, which consists of a delayed onset of action (30–60 minutes after administration), relatively late peak effect (2–4 hours postinjection) and a longer duration of action (6–8 hours) compared with the sharper peak secretion of endogenous insulin that occurs following meal ingestion. Thus, regular human insulin does not adequately replicate the documented physiologic postprandial insulin secretion, which may lead to early postprandial hyperglycemia and late hypoglycemia. Given its delayed onset of action, regular insulin should be administered 30 minutes before meals. This recommendation is difficult to comply with in real life, as meal timing can vary substantially and thus premeal doses are often administered immediately before meals. As a result, the conventional use of regular human insulin as a bolus or mealtime insulin has decreased in favor of the novel, more rapid-acting insulin analogues.

**Intermediate-acting human insulin**

At present, the only conventional intermediate-acting human insulin in clinical use is isophane NPH insulin. Originally developed in the 1940s in an attempt to prolong the biological action of regular insulin preparations to better approximate basal insulin profiles and minimize insulin dosing frequency, NPH was initially composed of an animal-derived insulin suspended in a neutral pH solution of protamine and zinc. This unique suspension allows for a significant delay in the absorption of insulin from the subcutaneous tissue, resulting in an onset of action 1.5 to 4 hours after injection, a pronounced peak 4 to 10 hours after administration, and a duration of up to 24 hours (see Table 2). Of note, NPH insulin was subsequently reformulated using recombinant human insulin during the 1980s. However, NPH does not constitute an appropriate surrogate for endogenous basal insulin production, for several reasons. From a pharmacokinetic perspective, an ideal exogenous basal insulin product should be “peakless,” whereas NPH displays a pronounced peak action profile. Moreover, the fact that NPH must be evenly suspended before administration has led to wide interindividual and intraindividual variability in its absorption from the subcutaneous tissue depot. Taken together, this often results in a failure to obtain consistent adequate glycemic control, with a range in blood glucose excursions from hypoglycemia (eg, particularly nocturnal hypoglycemia when injected in the evening) to hyperglycemia (eg, given its short duration of action). Despite these findings, NPH has retained clinical utility as a basal insulin when administered twice daily and in a premixed insulin preparation combined with regular human insulin. Its main attribute at this time is its low cost compared with the newer basal analogues.

**Human Insulin Analogues**

Insulin analogues were designed to improve on the subcutaneously administered pharmacokinetic and pharmacodynamic inadequacies of conventional human insulin products that limit their clinical efficacy. Through targeted structural manipulation of the human insulin molecule (eg, amino acid substitutions, inversions, or additions) using recombinant technology protein bioengineering techniques, several insulin analogues with modified biochemical properties resulting in altered rates of self-aggregation and subsequent subcutaneous absorption into the bloodstream have
been recently developed, without adversely affecting biological function (ie, affinity for the insulin receptor and subsequent signaling events).\textsuperscript{29,30} In general, rapid-acting and long-acting insulin analogues have been modified to possess a weaker or stronger ability to self-associate and hence, a faster or slower diffusion rate following subcutaneous tissue injection, respectively. Thus, the pharmacodynamic properties of analogues of native insulin better approximate endogenous insulin secretion and ultimately would be expected to result in to superior clinical end points, including improved glycemic control with reduced hypoglycemic episodes alongside greater treatment satisfaction and overall enhanced quality of life from the patient perspective.

\textbf{Rapid-acting insulin analogues}

At present, there are 3 commercially available rapid-acting insulin analogues approved for clinical use as bolus or mealtime insulins, namely lispro, aspart, and glulisine. The $\beta$ insulin chain of each rapid-acting insulin analogue has been structurally altered to impede self-aggregation of insulin molecules into multimeric complexes (Fig. 2B). With respect to insulin lispro, the first rapid-acting analogue developed in 1996, transposition of amino acids proline and lysine at positions B28 and B29 leads to a conformational change that augments steric hindrance between interfaces implicated in dimerization.\textsuperscript{61,62} Substitution of proline at B28 by a negatively charged aspartic acid residue in the case of insulin aspart results in repulsion of monomers.\textsuperscript{63,64} Regarding insulin glulisine, replacement of lysine at B29 for glutamine and aspartic acid at B3 for lysine simultaneously provides stability and a reduced ability to self-associate.\textsuperscript{65–67} The ensuing pharmacokinetic profile, that is, a faster onset of action (10–30 minutes postadministration), peak of action (0.5–2 hours), and a shorter duration of action (3–5 hours) in comparison with regular human insulin, are comparable among the rapid-acting insulin analogues and more compatible with physiologic time-activity profiles of meal-stimulated insulin release.\textsuperscript{68,69} Moreover, less variability between injection sites has been described for insulin analogues in comparison with regular insulin.\textsuperscript{64,68,70}

In addition to superior pharmacokinetic parameters, rapid-acting insulin analogues also demonstrate enhanced clinical efficacy, including improved normalization of postprandial glucose excursions, a decrease in hypoglycemic events, with either modest or no significant reductions in hemoglobin A\textsubscript{1c} ($\text{HbA}_{1c}$) levels in patients with type 1 and 2 diabetes, respectively, when compared with traditional human insulin.\textsuperscript{71–81} However, the lack of a significant reduction in $\text{HbA}_{1c}$ in patients using these analogues may be dependent, to some extent, on the accompanying basal insulin product used and on the initial level of glycemic control before treatment. In addition, most of the studies were not double blinded but rather the regular insulin was given as per protocol, 30 minutes before the meal, with the rapid-acting analogue being administered immediately before the meal. In fact, in real life most patients take their meal insulin just before the meal regardless of whether it is regular or a rapid-acting analogue, so the differences in pharmacokinetics and pharmacodynamics would be even greater. It is also likely that the reduction in very low values of glucose, that is, hypoglycemia with rapid-acting analogues, would tend to raise $\text{HbA}_{1c}$, masking its beneficial effect on overall glucose control.

Patients who use rapid-acting insulin analogues as opposed to regular insulin also report an enhancement in their quality of life.\textsuperscript{82–85} For instance, from a practical standpoint, the rapid onset of action of insulins lispro, aspart, and glulisine allows for greater flexibility and convenience in the timing of administration, that is, either at mealtime or even immediately postprandially. The latter option also allows for better matching of
insulin dose to carbohydrate load consumed, therefore improved control of postmeal glycemic fluctuations may be attained.

Potential clinical disadvantages related to the kinetic profile of rapid-acting insulin analogues include the risk of early postprandial hypoglycemia and preprandial hyperglycemia, compared with regular insulin.86

Long-acting insulin analogues
The long-acting insulin analogues currently licensed for basal insulin replacement and supplementation include insulins glargine and detemir. As shown in Fig. 2, the structure of each analogue has been uniquely altered to achieve prolonged absorption following subcutaneous injection and a relatively peakless 24-hour time-action profile, which is much more analogous to physiologic basal insulin release when compared with NPH insulin. In the case of insulin glargine, the first long-acting analogue of insulin introduced in 2000, modifications to both the α and β insulin chains, specifically the replacement of asparagine with glycine at position A21 and the addition of 2 arginine residues at position B30, respectively, results in a molecule that is less soluble at neutral, physiologic pH yet stable in the acidic pH of its storage solution (Fig. 2C).87–89 Thus, when injected into the neutral milieu of the subcutaneous tissue, glargine forms an amorphous precipitate from which insulin molecules are slowly released into the circulation.87–89 Insulin detemir, on the other hand, features 2 alterations within the β insulin chain: deletion of amino acid threonine at position B30 and acylation of a 14-carbon aliphatic fatty acid (myristoylative acid) to the ε-amino group of lysine at position B29, which enhances its affinity for albumin.90,91 This process results in an insulin product with protracted action, due to sustained release from multimeric complexes within the subcutaneous tissues postinjection as well as from albumin to which it is reversibly bound.91–93

The ensuing pharmacokinetic profiles of insulins glargine and detemir are comparable.94 Both long-acting analogues have an onset of action within 1 to 3 hours of administration and a relatively peakless, dose-dependent, mean duration of action of approximately 24 hours.58,94–98 Accordingly, these analogues represent better surrogates for basal insulin replacement and supplementation. Despite the controversy, clinical experience tends to suggest that there are some patients who require twice-daily dosing with glargine and detemir to provide full basal coverage. This situation appears to be more evident with insulin detemir.

Furthermore, both glargine and detemir demonstrate less intersubject variability in rates of absorption at different injection sites along with a reduced incidence of hypoglycemia when compared with NPH insulin (Fig. 4).78,99–110 Not surprisingly, in a treat-to-target study design, similar HbA1c levels were achieved with long-acting insulin analogues in comparison with NPH.86,109–111 However, this effect can only be achieved at the expense of increased nocturnal hypoglycemia with the NPH insulin. Similarly, several recent clinical trials comparing basal-bolus insulin regimens comprising only insulin analogues (eg, detemir/aspart) versus traditional insulin preparations (eg, NPH/regular) have failed to show consistent reductions in HbA1c with the use of regimens comprising insulin analogue only.76,112,113 Regarding insulin detemir, this analogue displays reduced within-subject variability in glycemia versus glargine97,99,104 and is associated with less weight gain than NPH105,114,115 and glargine116,117 in patients with type 2 diabetes. However, these differences are small and are generally not regarded as being of great clinical significance.

Premixed Insulin Preparations
Two main classes of premixed insulin preparations are currently on the market: premixes of conventional insulin products and fixed-ratio mixes of insulin analogues.
With respect to the former, traditional short-acting and intermediate-acting human insulins have been combined in different ratios to form 2 preparations: Humulin 50/50 (comprising 50% NPH and 50% regular insulin) and Humulin or Novolin 70/30 (consisting of 70% NPH and 30% regular insulin). Regarding the latter, 3 formulations combining different ratios of rapid-acting insulin analogues are available: Humalog Mix 50/50 (a 50% neutral protamine lispro suspension with 50% insulin lispro), Humalog Mix 75/25 (75% neutral protamine lispro and 25% insulin lispro), and Novolog Mix 30 (70% protamine crystalline aspart and 30% insulin aspart). Of note, the protamine forms of lispro (neutral protamine lispro [NPL, insulin lispro protamine]) and aspart (protamine crystalline aspart or insulin aspart protamine) are functionally identical to NPH. The pharmacokinetic profiles of premixed insulin products are shown in Table 2.

Studies comparing the clinical efficacy of premixed human insulins and premixed insulin analogue preparations suggest that premixes of insulin analogues are superior in terms of controlling postprandial glucose excursions with less intrasubject variation, yet do not show consistent improvements in HbA1c levels.60,119–125 Premixed insulin products represent a convenient alternative to basal-bolus insulin therapy, with a decreased number of daily injections, and should be used only in patients with type 2 diabetes.60,126

CURRENT CONTROVERSIES IN THE USE OF INSULIN

Insulin Analogues and Cancer Risk

Despite the superiority in the clinical efficacy of insulin analogues over traditional insulin formulations, safety concerns have been raised regarding the putative carcinogenic potential of certain analogues. The rationale underlying the potential
mitogenicity of analogues of insulin is that structural modifications to the native insulin molecule may result in perturbations of receptor binding sites (particularly the B10 and B26–B30 regions of the β chain),\textsuperscript{127} and thus inadvertently lead to: (1) enhanced affinity toward the insulin-like growth factor (IGF-1) receptor, which shares greater than 50% amino-acid sequence similarity to the insulin receptor; and/or (2) altered binding kinetics (ie, prolonged occupancy time) of the insulin receptor, causing aberrant downstream signaling and promoting tumor development.\textsuperscript{128–130} However, with respect to the rapid-acting insulin analogues, there is currently no compelling evidence that insulins lispro, aspart, and glulisine have increased mitogenic potential compared with regular human insulin.\textsuperscript{86,131–133}

The main area of controversy revolves around the potential tumorigenic properties of long-acting insulin analogues, particularly insulin glargine. In vitro studies indicate that glargine has a higher affinity for the IGF-1 receptor and increased downstream proliferative and antiapoptotic effects in benign and malignant cell lines compared with human insulin and other analogues.\textsuperscript{132–134} Similarly, the serum of patients treated with insulin glargine displayed greater mitogenic potency on human breast cancer cells compared with the serum of those treated with regular insulin or insulin detemir.\textsuperscript{135} However, no difference in the mitogenic capacity of insulin glargine versus human insulin was reported in another study using normal and malignant breast epithelial cell lines,\textsuperscript{136} nor did long-term glargine administration increase tumor development in murine models in vivo.\textsuperscript{137} Recent data from observational studies and randomized control trials examining the risk of malignancy with insulin glargine are also equivocal. Several studies have identified an association between the use of glargine monotherapy and an increased incidence of malignancy, particularly breast cancer,\textsuperscript{138,139} compared with the use of insulin products with and without glargine,\textsuperscript{138–141} but were heavily criticized on methodological grounds.\textsuperscript{142,143} Other studies have failed to show this association.\textsuperscript{144–146} Furthermore, a recent cohort study by Suissa and colleagues\textsuperscript{147} demonstrated that the incidence of breast cancer does not increase with short-term use (ie, <5 years) of insulin glargine in women with type 2 diabetes, but that a trend toward increased risk may be apparent with extended use (>5 years), in the context of long-standing insulin therapy before initiating treatment with glargine.

Of note, the other commercially available long-acting insulin analogue, detemir, displays a decreased affinity for IGF-1 compared with native human insulin, and hence does not possess increased mitogenicity in vitro.\textsuperscript{132,134} However, a recent meta-analysis of data from randomized controlled trials on insulin detemir suggests that this analogue is associated with a lower or comparable cancer risk compared with NPH or glargine, respectively.\textsuperscript{148}

Taken together, the evidence that long-acting insulin analogues promote cancer is limited. Indeed, this potential association may be confounded by the fact that diabetes and cancer share similar risk factors (eg, age, obesity) and comparable pathophysiologic mechanisms (eg, hyperinsulinemia, hyperglycemia, inflammation).\textsuperscript{130,149} A consensus report by members of the American Diabetes Society and the American Cancer Society has recently been published on this subject.\textsuperscript{149} In essence, further studies are warranted to establish the mitogenic potential and long-term safety of insulin analogues, so at present cancer risk should generally not be a major determinant when choosing appropriate therapeutic options for patients with diabetes.\textsuperscript{149}

**Insulin Analogues in Pregnancy**

Owing to concerns of adverse side effects, including potential teratogenicity, embryo toxicity, immunogenicity with transplacental transfer, and mitogenicity, the use of
insulin analogues for the management of hyperglycemia in pregnancy was initially limited. Subsequent studies have shown that in addition to conventional human insulin preparations, the therapeutic use of rapid-acting insulin analogues lispro and aspart in pregnancy is safe and clinically efficacious, with no adverse maternal or fetal consequences reported. To date, there are no published reports on insulin glulisine in pregnancy. Moreover, the safety of long-acting insulins glargine and detemir have not yet been extensively studied in this patient population and thus are not currently approved for use in pregnancy.

**FUTURE PERSPECTIVES**

To optimize glycemic control in patients with type 1 or 2 diabetes and thus prevent the development and progression of long-term complications, insulin replacement and supplementation strategies must aim to replicate physiologic insulin excursions. To this end, 2 main approaches have evolved over the past 85 years to modify the kinetics of injectable exogenous insulin products: manipulation of the pharmaceutical formulation (eg, addition of zinc, protamine) and/or alteration of the insulin molecule itself (ie, the insulin analogues). However, as current exogenous insulin preparations are not ideal, several novel rapid-acting and long-acting insulin formulations are presently under development, which may broaden the therapeutic options for the management of patients with diabetes in the near future (Table 3).

**Novel Insulin Formulations**

In an effort to attain optimal postprandial glycemic targets, 2 novel ultra–rapid-acting insulin preparations, insulin-PH20 and Linjeta (formally VIAject), are currently under development in phase II and III trials, respectively. The insulin-PH20 (Halozyme Therapeutics, San Diego, CA) formulation contains one of the commercially available meal-time insulin products mixed with recombinant human hyaluronidase (rHuPH20). A recent phase I study revealed that coadministration of insulin (either regular human insulin or insulin lispro) with rHuPH20 results in faster absorption of insulin into the circulation, accelerated pharmacokinetic and glucodynamic effects, and decreased intersubject and intrasubject variability of metabolic activity compared with each insulin formulation alone. Linjeta (Biodel Inc, Danbury, CT; www.biodel.com) is another unique insulin formulation comprising regular human insulin with ethylenediaminetetra-acetic acid and citric acid. The latter additives act to chelate zinc ions and prevent self-aggregation of insulin molecules into hexamers on injection into the subcutaneous tissue, thus maintaining insulin in a monomeric state. As anticipated, Linjeta displays a faster onset of action and peak effect, with reduced intraindividual variability of metabolic action compared with regular human insulin and/or insulin lispro in healthy subjects and patients with type 1 diabetes.

With respect to basal insulin supplementation, 2 novel and potentially improved formulations of insulin glargine are currently in the pharmaceutical pipeline: LY2963016 (Eli Lilly, Indianapolis, IN) and BIOD-Adjustable Basal (Biodel). The latter is an altered preparation of glargine with a prolonged duration of action that can be premixed with other insulin products. A new basal insulin formulation by Sanofi-Aventis (Bridgewater, NJ; www.sanofi.com) is also under development. Moreover, insulin lispro protamine, the basal component of several premixed insulin analogue preparations, is being investigated as a stand-alone basal insulin analogue in patients with type 1 and 2 diabetes.

Another novel approach to delay insulin absorption involves chemically coupling the insulin molecule to poly(ethylene glycol) (PEG). A PEGylated form of insulin lispro,
with a flatter, extended duration of action, has been developed by Eli Lilly and is currently entering phase III trials.

A unique ultra–long-acting basal insulin product, FT-105, is under development by Flamel Technologies Inc (Washington, DC; www.flamel.com). In this formulation, insulin is noncovalently bound to a polymer consisting of a polyglutamate peptide backbone linked to vitamin E molecule within a hydrogel, which forms a depot of dense microparticles following subcutaneous injection, leading to the slow release of insulin molecules into the bloodstream. Preliminary results from a phase I clinical study indicate that FT-105 exhibits a prolonged duration of action, up to 48 hours,

### Table 3

**Novel insulin preparations in development**

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Manufacturer</th>
<th>Description</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Rapid-acting</strong></td>
<td></td>
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<tr>
<td><strong>Novel formulations</strong></td>
<td></td>
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<tr>
<td>Insulin-PH20</td>
<td>Halozyme Therapeutics</td>
<td>Prandial insulin products mixed with recombinant human hyaluronidase</td>
<td>Phase II</td>
</tr>
<tr>
<td>Linjeta</td>
<td>Biodel</td>
<td>Regular human insulin mixed with EDTA and citric acid</td>
<td>Phase III</td>
</tr>
<tr>
<td><strong>II. Long-acting</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Novel formulations</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LY2963016</td>
<td>Eli Lilly</td>
<td>Novel preparation of insulin glargine</td>
<td>Phase III</td>
</tr>
<tr>
<td>BIOD-Adjustable Basal</td>
<td>Biodel</td>
<td>Novel preparation of insulin glargine</td>
<td>Phase I</td>
</tr>
<tr>
<td>Insulin lispro protamine</td>
<td>Eli Lilly</td>
<td>Protaminated form of insulin lispro</td>
<td>—</td>
</tr>
<tr>
<td>PEGylated insulin lispro</td>
<td>Eli Lilly</td>
<td>Insulin lispro linked to poly(ethylene glycol)</td>
<td>Phase III</td>
</tr>
<tr>
<td>FT-105</td>
<td>Flamel Technologies Inc</td>
<td>Insulin attached to polymer (polyglutamate peptide backbone linked to vitamin E molecules)</td>
<td>Phase I</td>
</tr>
<tr>
<td>“Smart” insulins</td>
<td>SmartCells Inc</td>
<td>Glucose-responsive insulin preparations</td>
<td>Preclinical</td>
</tr>
<tr>
<td>SmartInsulin</td>
<td>Biodel</td>
<td></td>
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<tr>
<td>BIOD-Smart Basal</td>
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<tr>
<td><strong>Novel analogues</strong></td>
<td></td>
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</tr>
<tr>
<td>Degludec (IDeg)</td>
<td>Novo Nordisk</td>
<td>Two modifications of native insulin β chain: deletion of B30-threonine and addition of a 16-carbon fatty diacid B29-lysine</td>
<td>Phase III complete</td>
</tr>
<tr>
<td>LY2605541</td>
<td>Eli Lilly</td>
<td>No description given</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

Abbreviation: EDTA, ethylenediaminetetra-acetic acid.
decreased intrasubject variability, and reduced hypoglycemic episodes when compared with insulin glargine (www.flamel.com).

Lastly, 2 unique glucose-responsive injectable “smart” insulin preparations that release insulin in proportion to ambient subcutaneous glucose levels are currently undergoing preclinical evaluation. SmartInsulin, a polymer developed by SmartCells Inc (Beverly, MA; www.smartinsulin.com), is composed of insulin reversibly bound to a glucose-binding molecule. Insulin is released from the SmartInsulin polymer in the presence of glucose, which competes with insulin for the binding sites on the glucose-binding molecule.167 Another promising insulin preparation is BIOD-Smart-Basal, a formulation developed by Biodel that contains insulin glargine, glucose oxidase, and peroxidase. In the presence of glucose, glucose oxidase and peroxidase react to form gluconic acid, which lowers the pH and increases the solubility of insulin glargine, thereby promoting its release into the circulation.168 Owing to their novel glucose-responsive mechanism, the smart insulin products may theoretically result in tighter glycemic control and a lower risk of both hypoglycemia and hyperglycemia compared with current insulin formulations.

**Novel Insulin Analogues**

The molecular structure of human insulin has been gradually refined over the past 2 decades, yielding several unique rapid-acting and long-acting insulin analogues with pharmacokinetic properties that closely imitate endogenous insulin profiles. The most promising novel analogue currently in clinical development is insulin degludec (IDeg; Novo Nordisk, Bagsvaerd, Denmark), an ultra–long-acting, basal insulin preparation. This analogue was generated through modification of the native insulin β chain at 2 locations: deletion of threonine at position B30 and addition of a 16-carbon fatty diacid to the lysine residue at position B29 via a glutamic acid spacer (see Fig. 2C). As a consequence, insulin degludec is able to self-aggregate and form large multihexamer complexes upon injection in subcutaneous tissues, which subsequently slowly dissociate into monomers that enter the circulation.173 The result is a protracted action profile longer than 24 hours in duration.

Phase II clinical trials comparing once-daily insulin degludec to glargine indicate that both basal insulins provide comparable degrees of glycemic control; however, degludec is associated with lower rates of hypoglycemia in patients with type 1 diabetes174 and superior postprandial glucose control compared with glargine in those with type 2 diabetes.175 Another phase II proof-of-concept trial by Zinman and colleagues176 reveals that insulin degludec, administered 3 times a week, results in similar glycemic control but no benefit with respect to hypoglycemia risk compared with insulin glargine, when injected once daily in insulin-naive patients with type 2 diabetes. Degludec has also been studied in a premixed formulation with insulin aspart, denoted IDegAsp or Degludec Plus.175 At present, phase III clinical trials evaluating the safety and efficacy of insulin degludec in type 1 and 2 diabetes have been completed, with publication of the final data pending.168

An additional novel candidate basal insulin analogue, LY2605541, developed by Eli Lilly, is presently undergoing phase II trials.

**SUMMARY**

The last 90 years have witnessed tremendous progress in insulin therapy, from the initial crude, yet life-saving, animal insulin extracts to novel human insulin analogues. Although the complete physiologic replacement of insulin is inherently difficult to achieve with open-loop subcutaneously administered insulin, the continued
development of improved injectable insulin formulations with superior pharmacokinetics and pharmacodynamics will enhance glucose control, and represents important clinical advances in the treatment of both type 1 and type 2 diabetes.

REFERENCES


112. Murphy NP, Keane SM, Ong KK, et al. Randomized cross-over trial of insulin glargine plus lispro or NPH insulin plus regular human insulin in adolescents with type 1 diabetes on intensive insulin regimens. Diabetes Care 2003;26:799–804.


129. Gerstein HC. Does insulin therapy promote, reduce, or have a neutral effect on cancers? JAMA 2010;303:446–7.


