

SUBCUTANEOUS INSULIN THERAPY – END OF THE ROAD AFTER 80 YEARS?

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SUMMARY

Subcutaneous (SC) insulin therapy has been a mainstay of pharmacological diabetes management from the moment insulin was successfully developed as treatment. Insulin formulations have become more refined and less allergenic over time, and ancillary technologies such as injection devices and glucose measurement tools have evolved to

the extent of permitting closed-loop therapy. However, investigations have continued exploring alternative routes of administration with the ultimate goal of implantable islet replacements, whether cell- or “silicon”-based. Progress on these lines of research, however, has been slow to present patients with viable options: alternative delivery routes have failed to deliver insulin reliably and with commercially viable efficiency, while beta cell transplantation continues to struggle with tissue availability and in vivo viability. In the meantime, SC insulin formulations have advanced for rapid- and long-acting formulations, to better meet typical insulin requirements across the day. Thus, SC insulin will likely remain a key technology for the fore-

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seeable future in order to address the needs of an ever larger number of insulin-dependent patients with diabetes.

Key words: Insulin – Hyperglycemia – Type 2 diabetes – T2DM – Type 1 diabetes – T1DM

INTRODUCTION: PROGRESS SINCE 1922

Physiologically, only the monomeric form of human insulin is designed to rapidly penetrate into target tissues and exert biological effects. Within beta cell granules, however, insulin forms stable hexamers in the presence of zinc, calcium or phenol, allowing long-term intracellular storage. A rapid decrease in granular pH release upon fusion with the cell membrane facilitates the release of large amounts of insulin. Once released from beta cells, insulin continues to rapidly dissociate into dimers and monomers as concentrations drop quickly under the turbulent and high volume blood flow conditions in pancreatic islets (1). These favorable conditions are not usually found in the tissues considered for the administration of exogenous insulin, such as subcutaneous fat, thus significantly hindering the rate and efficiency of systemic insulin delivery. Also, the need to offer stable formulations with sufficient shelf life, even under adverse conditions (e.g., exposure to destabilizing glass or plastic surfaces, mechanical stress in injection devices or variable temperature) forces compromise. Thus, to achieve sufficiently long shelf-life, most pharmaceutical formulations of human insulin, until the 1980s, were designed to facilitate the formation of dimers, and in the presence of zinc ions and at higher insulin concentrations, stable hexamers (6:2, insulin/zinc). This notwithstanding, the efficacy of subcutaneous (SC) insulin therapy ultimately depends upon the timely dissociation of insulin from hexamers to bioactive monomers (2-4). Unfortunately, injection of insulin into the less well-vascularized (certainly compared to islets) subcutaneous tissue results in absorption kinetics that are both too slow to meet post meal demands, and too rapid to meet fasting requirements equally well. Thus, one approach to developing more clinically attractive insulin formulations relies on controlling insulin self-association by specific alterations to the amino acid sequence of human insulin (5-8). As a result, both shorter- and longer-acting analogues of human insulin emerged with pharmacokinetic profiles that better adapt to help patients cope with the vastly different prandial and fasting insulin requirements by more closely mimicking physiological insulin release. For the purpose of this review, these analogue insulins will be discussed only briefly as excellent reviews of their

detailed pharmacokinetics and clinical efficacy are available elsewhere (8-11).

SHORT- AND LONG-TERM SEQUELAE OF INSULIN DEFICIENCY: EVIDENCE FOR BENEFIT OF SC INSULIN THERAPY

Hyperglycemia defines diabetes, and glycemic control is fundamental to the management of diabetes. To date, approximately 382 million patients have diabetes, and this number is expected to rise to 592 million by 2035 (12). Thus, widespread, affordable and safe treatment options will be paramount to cope with this growing crisis, and ensure that patients can maintain acceptable glycemic control. Insulin plays a central role in achieving near-normal glycemic targets as reflected by HbA_{1c} levels of approximately 6.5-7%. However, recent studies have also highlighted that glycemic targets need to be carefully defined for individual patients, based on the ability to manage short-term changes in insulin requirements and prevent hypoglycemia, individual manifestations of micro- and macrovascular complications, and the option to treat associated metabolic disorders such as dyslipidemia or hypertension, with other safe medications.

The tenet of hyperglycemia causing much of the specter of microvascular complications was established for type 1 diabetes by the Diabetes Control and Complications Trial (DCCT) (13) and its follow-up study, the Epidemiology of Diabetes Interventions and Complications (EDIC) study (14, 15). For type 2 diabetes, the relatively small Kumamoto Study in approximately 110 patients with a 5- to 6-year disease duration (16) and the much larger UK Prospective Diabetes Study (UKPDS) in > 4,800 recently (3 months) diagnosed patients (17, 18) similarly demonstrated the benefits of intensive glycemic control, whether achieved by SC insulin or oral antihyperglycemic agents (in UKPDS, 15% of subjects were treated with sulfonylurea or insulin as initial pharmacotherapy, 33% with metformin as initial pharmacotherapy). The DCCT and UKPDS trials (13, 19) showed a curvilinear relationship between HbA_{1c} and microvascular complications, implying that the greatest impact on the prevention or reduction of the incidence of microvascular complications should be achieved by focusing on patients in very poor control, as further lowering of HbA_{1c} from "slightly abnormal" (7-8%) to normal (< 7%) values is returning to ever lesser degrees of protection from complications. Both EDIC and UKPDS also demonstrated that microvascular benefits in previously intensively treated subjects could persist for decades following in-

tial tight glycemic control, in spite of levels of good control not being maintained during follow-up, suggesting that a “metabolic memory” may play an important role in the evolution and/or prevention of microvascular complications (14, 15, 20). Furthermore, the EDIC study participants previously randomized to the intensive treatment group had a significant 57% reduction in the risk of nonfatal myocardial infarction (MI), stroke or cardiovascular disease (CVD) death compared with those previously in the standard arm (21). During the UKPDS trial, there was a 16% reduction in CVD events (combined fatal or nonfatal MI and sudden death) in the intensive glycemic control arm albeit without quite reaching statistical significance ($P = 0.052$). There was, however, no apparent benefit on other CVD outcomes such as stroke. After 10 years of follow-up, those subjects originally randomized to intensive glycemic control still showed reduced risk for MI (15% with sulfonylurea or insulin as initial pharmacotherapy, 33% with metformin as initial pharmacotherapy) and all-cause mortality (13% and 27%, respectively) (21). Ray et al. (22) summarized the effect of intensive glycemic control on adverse cardiovascular outcomes and came to the conclusion that intensive compared with standard glycemic control significantly reduces coronary events without an increased risk of death. However, the optimum treatment, and the speed and extent of HbA_{1c} reduction, might need to be identified for different populations.

By contrast, patients with long-standing and not optimally controlled type 2 diabetes experience only modest benefits with regards to microvascular complications when intensified treatment is used to aim at normal HbA_{1c} levels, as three more recently conducted outcomes studies revealed:

- The Veterans Affairs Diabetes Trial (VADT), where approximately 50% of subjects were using SC insulin, showed significant reductions in albuminuria with intensive compared to standard glycemic control but no difference in retinopathy or neuropathy changes (23, 24). The rate of CVD events was lower (but not significantly) in the intensive arm (23). An ancillary study of the VADT demonstrated that intensive glycemic control significantly reduced primary CVD outcomes in individuals with less atherosclerosis at baseline (as assessed by coronary calcium) but not in individuals with more extensive baseline atherosclerosis (25). A post hoc analysis showed that those subjects with a diabetes duration of less than 15 years had a mortality benefit in the intensive arm, while

those in the same study arm with a diabetes duration of ≥ 20 years had a higher mortality rate (26).

- The Action in Diabetes and Vascular Disease: Preterax and Diamicon MR Controlled Evaluation (ADVANCE) study of intensive versus standard glycemic control in type 2 diabetes had low SC insulin use at baseline (1.4-1.5%) rising to approximately 40% in the intensive and approximately 24% in the standard arm. There was no difference in overall or cardiovascular mortality between the intensive compared with the standard glycemic control arms (27).
- The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial where the use of SC insulin doubled from approximately 34% at baseline to approximately 77% in the intensively treated cohort (vs. 55% in the control cohort) showed lower rates for onset or progression of early-stage microvascular complications in the intensive glycemic control arm compared with the standard arm (28, 29).
- The Outcome Reduction with an Initial Glargine Intervention trial (ORIGIN) assessed cardiovascular outcomes with insulin glargine (Lantus®) treatment in 11,081 insulin-naïve patients with diabetes, and 1,456 with prediabetes. Fifty-nine percent of study participants had a prior CVD event, 80% had hypertension, 15% had albuminuria and 12% smoked cigarettes. The primary composite endpoint included myocardial infarction, stroke or cardiovascular death. There was no difference in event rates between those randomized to insulin, whose mean HbA_{1c} was 6.1%, and those allocated to standard of care (mean HbA_{1c} 6.4%) over a median follow-up of 6.2 years, suggesting that early treatment with insulin in a population with increased CV risk is not harmful (30).

ADVERSE EVENTS OF SC INSULIN THERAPY

Hypoglycemia

Despite more accessible technology for the monitoring of blood glucose (31, 32), better training of patients, computer-assisted interpretation of serial glucose measurements (33-35) and insulin formulations that more closely mimic postprandial or postabsorptive, “basal”, insulin exposure, hypoglycemia remains the most significant adverse event with SC insulin therapy. Post DCCT, the combination of lower glycemic targets and “intensified”, higher dose and multiple injection insulin therapy appears to be the primary driver of serious hypoglycemia (36, 37), especially in children in whom rates appear to

be even higher than in adults (38). As a result, emergency department visits for the treatment of hypoglycemia have also risen continuously over time (Fig. 1). Furthermore, recent outcomes trials like ADVANCE demonstrated an increased mortality risk, possibly associated with the increased risk for hypoglycemia that was observed in intensive glycemic control subjects (39). In this study, baseline use of insulin was approximately 35% but more than doubled to 77% in the intensive glycemic control group while use in the standard arm rose to only 55%. Exploratory analyses were unable to identify a clear explanation for the excess mortality in the intensive treatment arm. The ACCORD investigators subsequently published additional epidemiological analyses showing neither an increase in mortality in those intensive arm subjects who achieved HbA_{1c} levels < 7%, nor in those who lowered their HbA_{1c} quickly after entering the trial. However, intensive arm subjects whose HbA_{1c} remained highest also had the highest mortality risk (40). However, unlike in the DCCT trial, every 1% decline in HbA_{1c} from baseline to 4 months into the trial

was associated with a significant decrease in the rate of severe hypoglycemia in both arms (41). Moreover, while severe hypoglycemia was associated with excess mortality in both study arms, the association was stronger in those subjects randomized to the standard arm, and excess mortality in the intensive versus standard arms was only significant for participants with no severe hypoglycemia, not for those with one or more episodes (42). Thus, severe hypoglycemia may not have been the sole driver for excess mortality, and the inability to lower glycemia, through whatever mechanism, may be an important area to investigate with respect to risk for mortality. This notwithstanding, ACCORD and subgroup analyses of the VADT suggest that hypoglycemia may increase mortality in some patients with particular risk factors, such as higher age, long diabetes duration, propensity for severe hypoglycemia or advanced CVD. Thus, severe or frequent hypoglycemia remains a strong reason to raise glycemic targets, by modification of pharmacological treatment (43) or more widespread prescription of insulin formulations with proven reduced risk

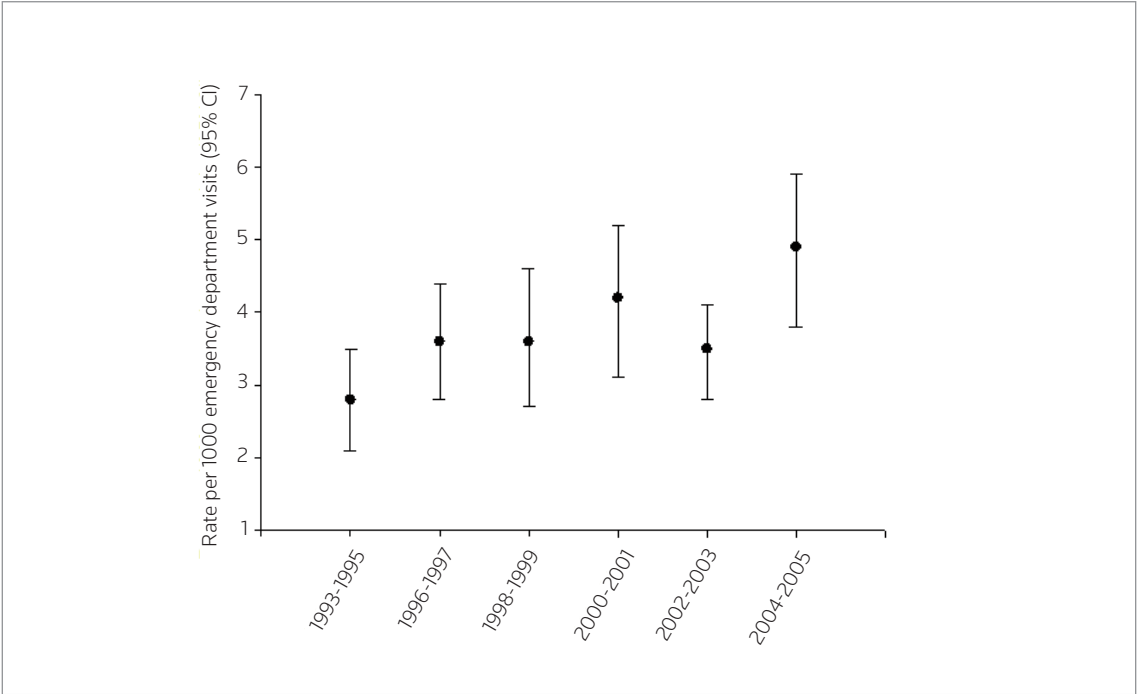


Figure 1. Rise of emergency department for hypoglycemia visits in U.S. hospitals between 1993 and 2005. Based on data from Trends and Disparities in U.S. (192).

for hypoglycemia, especially nocturnal episodes, such as insulin glargine. Apart from improving quality of life and reducing the potentially fatal consequences, reduction of hypoglycemia can also yield a substantial health economic benefit (44).

Peripheral hyperinsulinemia

Physiologically, endogenous insulin passes through the hepatic circulation first before peripheral tissues are exposed. Even though hepatic glucose uptake is not insulin dependent, the liver will take up to 50% of pancreatic insulin, thus playing an important role in regulating peripheral insulin exposure. Furthermore, selective infusion of exogenous insulin has shown discrete metabolic effects (45), although none of these are thought to raise clinical concern. Nonetheless, peripheral hyperinsulinemia is suspected to not only contribute to hypoglycemia but also increase the risk for CVD directly or indirectly by increasing peripheral insulin resistance (46-48) and, possibly, the risk for essential hypertension (49-52).

Antigenicity

Immunological reactions against contemporary SC insulin are very rare, and detectable antibody titers are infrequent and low, whether in patients with type 1 or type 2 diabetes (53). This scenario is in stark contrast to the times when insulin was extracted from animal pancreas and presented in less pure formulations, which led to insulin resistance and immediate or anaphylactic type hypersensitivity, likely as a result of co-presentation of other proteins (animal contaminants, protamine sulfate) (54). Later, ultrapure animal insulin formulations indeed showed reduced antibody generation equivalent to human insulin (55). Even modern, ultrapure human insulin administered via other routes of administration, such as with pulmonary delivery, can induce antibodies, although these are mostly of the IgG type and do not cause clinical sequelae or changes in insulin dose (56-58). Current SC insulin formulations including analogues have very low antigenicity (59-61), and may even be used to treat patients with antecedent insulin allergies (62, 63). Thus, the antigenicity of current SC insulin appears to be very low and of little clinical consequence, even with amino acid substitutions that are extensive compared to formerly used animal insulin, or with added moieties such as fatty acids or polyethylene glycol (PEG), designed to prolong duration of action.

CURRENT TRENDS IN SC INSULIN DEVELOPMENT

Extended-action insulin analogues for SC injection are designed to provide a basal level of insulin in between meals and overnight. Four products are currently approved:

- Neutral protamine Hagedorn (NPH) insulin made by a Eli Lilly, Novo Nordisk
- Glargine insulin (Lantus®), made by Sanofi
- Detemir insulin (Levemir®) made by Novo Nordisk
- Degludec insulin (Tresiba®) made by Novo Nordisk

NPH was developed in 1936 by adding a naturally occurring protein, protamine, and zinc to insulin, both facilitating the formation of stable crystals and retardation of dissolution post injection. Glargine differs from human insulin by a substitution of asparagine for glycine at A21, and the addition of two arginine residues to the C-terminus of the B-chain. As a result, human insulin's isoelectric point is shifted from approximately 5.4 to a more neutral pH, reducing solubility and causing precipitation under the physiologic pH conditions such as those found in the subcutaneous tissue. Thus, glargine precipitates post injection, resulting in slower absorption and extended duration of action compared to unmodified human insulin (64, 65). The value of pharmacokinetic data on glargine is compromised by the fact that commercially available antibodies against human insulin have only 56% crossreactivity for glargine. Thus, plasma insulin concentrations after SC glargine injection need to be corrected when comparing with human insulin (65).

The modification of detemir involves covalent acylation (C14 fatty acid, myristic acid) of the ϵ amino group of LysB29 and deletion of the adjacent ThrB30 amino acid residue (Fig. 2) which promotes reversible binding of insulin to albumin, delays its resorption from subcutaneous tissue because of slow dissociation post absorption and reduces the rate of transendothelial transport (66). Importantly, the fatty acid side chain at the C-terminus of the B-chain does not alter aggregation properties of the molecule and thus supports formulation stability. Unfortunately, the current pharmacokinetic data for detemir are of only limited value as it is difficult to distinguish between free and albumin-bound compounds (67).

Rapid-acting insulin

Rapid-acting insulin is used to control the postprandial glucose. Several strategies have been employed in order

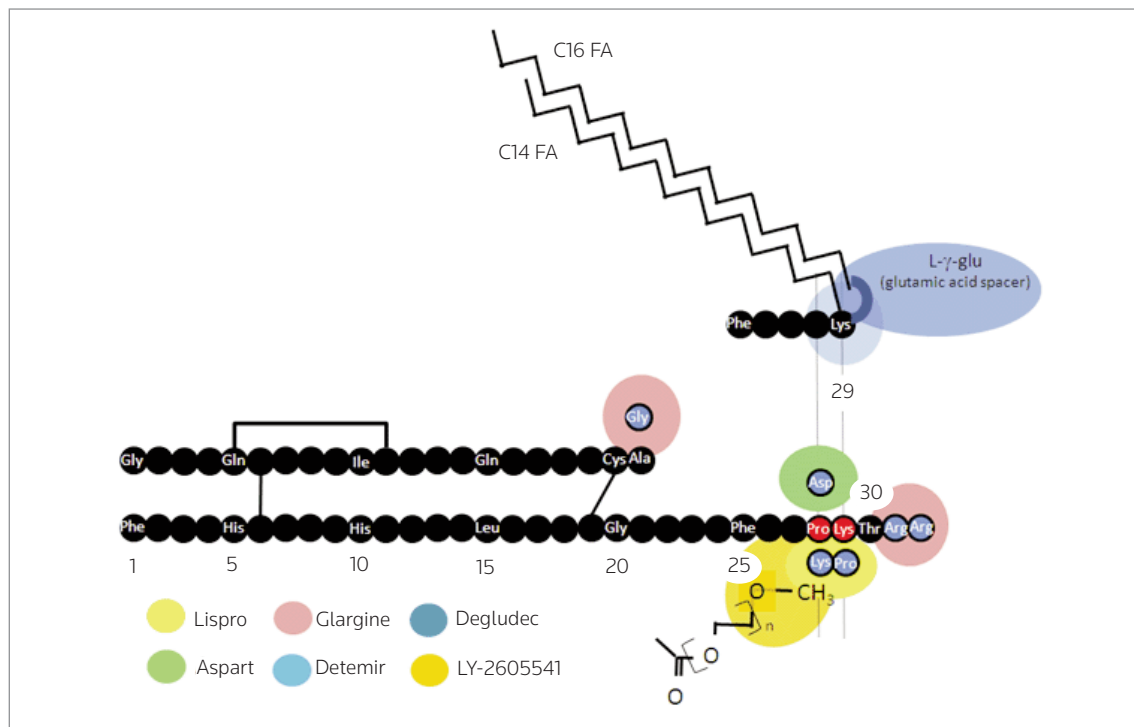


Figure 2. Modifications of human insulin to achieve pharmacokinetic profiles that can facilitate safer and more convenient subcutaneous insulin therapy.

to accelerate absorption from the subcutaneous tissue to match the fast-rising glucose levels post meals. These include removal of zinc by incorporating human insulin into a diketopiperazine or competitive complexing agent (Technosphere™/Afresa™; MannKind Corp.) (68); amino acid substitutions facilitating charge repulsion (such as those in insulin aspart [AspB28; Novolog®; Novo Nordisk]) (69, 70); reduction of interface hydrophobicity (e.g., insulin glulisine [LysB3, GluB29; Apidra®; Sanofi]) (71); replacement of metal binding sites (e.g., AspB10; Novo Nordisk, development discontinued); or steric manipulation at the B-chain terminus to decrease non-polar contacts and β sheet interactions between insulin monomers in the dimer (e.g., insulin lispro [LysB28, ProB29; Humalog™; Eli Lilly] (72) (Fig. 2).

In solution, lispro, aspart and glulisine exist as inherently destabilized hexamers which rapidly dissociate into monomers post SC injection. As a result, lispro, aspart and glulisine can act within 5 to 15 minutes of injection compared to 30–60 minutes for regular insulin. Again,

changes in the primary amino acid sequence may confer some difficulties in interpreting pharmacokinetic data of these analogues based on standard immunoassays requiring the use of correction factors (70) unless analogue-specific immunoassays are used (73–77). However, these assays are not widely available. Although the above insulin analogues have a more rapid onset and shorter duration of action than regular insulin, synchronization between time-action profile and glucose absorption from a meal is still less than ideal, in part because size and composition of meals, exercise-induced changes in blood flow at the injection site and changing circadian insulin sensitivity are difficult to predict or integrate into accurate dosing (78). In general, the pharmacokinetics of currently marketed rapid-acting analogues fall in between pancreatic insulin secretion typically seen after an oral glucose tolerance test or a mixed meal (Fig. 3), with $t_{\max}$ varying between 40–60 minutes post injection, depending on the site of injection (79–83). Moreover, multiple daily injections of insulin or insulin pumps are

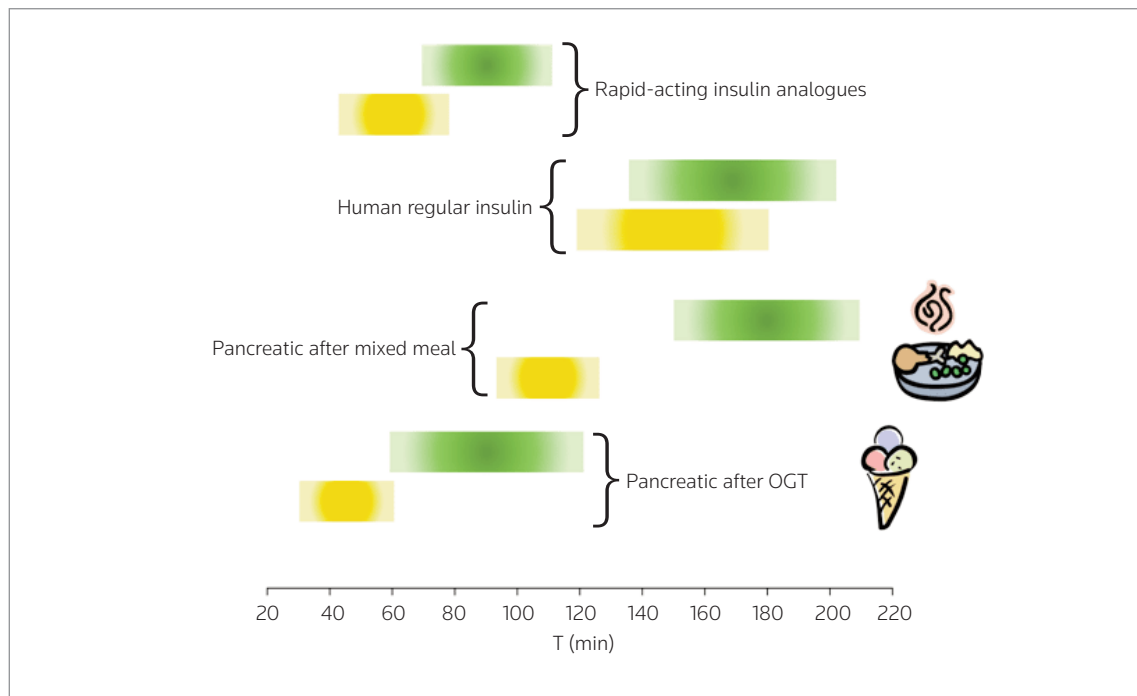


Figure 3. Comparison of ranges for $t_{\max}$ (yellow), $t_{1/2}$ (green) between pancreatic insulin secretion after oral glucose test (OGT) or mixed meal challenges versus pharmacokinetics data observed after subcutaneous injection of human regular insulin or analogues of human insulin. Shading intensity indicates probability of observed data based on observed mean and 95% confidence interval.

not accepted by all patients despite advances in needle and device technology (84, 85). Thus, efforts continue to develop SC insulins that further reduce hypoglycemia risk and are either even faster acting than the current generation of rapid-acting analogues to better mimic postprandial pancreatic insulin release, or smoother (little or no post injection peak) and longer acting (24-hour duration) “basal” insulin analogues compared to the current “gold standard”, insulin glargine.

EMERGING SC INSULIN FORMULATIONS

To minimize hypoglycemia, a number of new short- and long-acting insulin formulations as well as ancillary technologies are in development. For insulin formulations, the strategy appears to be to make rapid-acting insulin “peakier” than the current short-acting analogues, while reducing initial exposure and extending overall duration of action to 24 hours of long-acting insulin. At the same time, working with existing insulin, efforts continue to either build into the insulin formula-

tion glucose-sensitive elements that would reduce and increase bioavailability of injected insulin depending on ambient glucose, or use data from continuous glucose measurements to more accurately predict dosing of insulin, whether administered continuously via pumps or discretely via conventional injections.

Long-acting insulin

Insulin peglispro

Insulin peglispro (LY-2605541) is a novel, long-acting insulin that consists of insulin lispro that is modified with a 20-kDa PEG moiety (Fig. 2) and has a hydrodynamic size large enough to delay insulin absorption and reduce renal clearance, resulting in prolonged duration of action. The increase in functional molecular size also appears to alter the distribution of this insulin to tissues: hypothetically, the fenestrated hepatic sinusoidal endothelium may facilitate greater transport of LY-2605541 into the liver relative to peripheral tissues (i.e.,

muscle and fat), potentially providing a more preferential hepatic action akin to normal physiology (86-89). In a 3-month phase II trial, LY-2605541-treated patients had modest increases in triglycerides and LDL cholesterol compared with baseline and to insulin glargine-treated patients, while the HDL cholesterol with LY-2605541 decreased from baseline and compared with insulin glargine. LY-2605541 improved glycemic control but at the cost of increased daily hypoglycemia rates (even though nocturnal hypoglycemia was decreased), reduced body weight and lowered mealtime insulin doses (87). One will have to await further studies, though, to better understand whether the undesirable metabolic effects of LY-2605541 impact CV risk sufficiently to outweigh possible benefits from improved overall glycemic control, lowered nocturnal hypoglycemia rates and reduced body weight.

Degludec

Insulin degludec is based on the same technology originally developed for insulin detemir. It also shares the human insulin amino acid sequence (Fig. 2) except for

the deletion of ThrB30 and the addition of a longer (compared to detemir) 16-carbon fatty diacid attached to LysB29 via a glutamic acid spacer (90-91). Degludec provides glycemic control in a manner similar to insulin detemir but reduces nocturnal hypoglycemia compared to insulin detemir or premixed aspart/detemir insulin; it is also similar to insulin glargine (Fig. 4) (92-94). However, an FDA advisory panel advised against approval of degludec in November 2012 based on the agency's meta-analysis of CV events in the phase III program, which showed an increased hazard ratio of 1.67 (1.01, 2.75) for "core" major adverse coronary events (MACEs) and 1.30 (0.88, 1.93) for "expanded" MACEs (95). Subsequently, the FDA requested that the company conduct a CV outcomes study per FDA's 2008 guidance to demonstrate noninferiority versus standard of care. By contrast, the EMA approved degludec in October 2012, based on the same data submitted to the FDA (96) after agreeing to the sponsor's argument that a CV analysis that included the events observed in the extension period of the phase III program could be adversely influenced by the increased drop-out numbers for study subjects in that period (97). In Europe, degludec

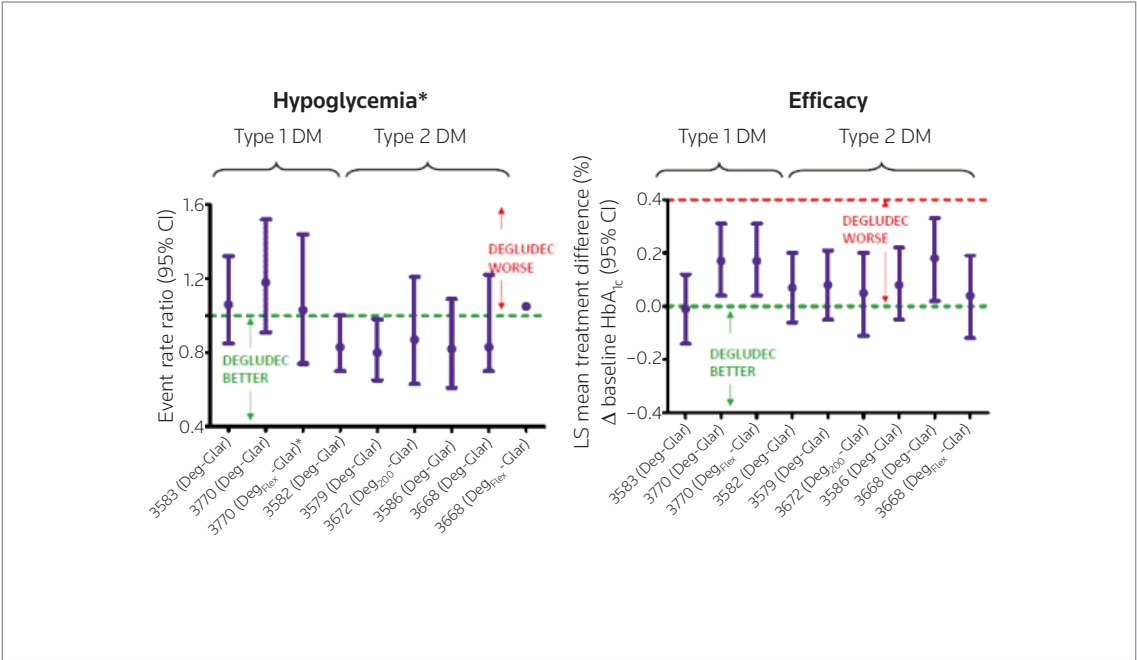


Figure 4. Modifications of human insulin to achieve pharmacokinetic profiles that can facilitate safer and more convenient subcutaneous insulin therapy. *Novo "confirmed" episodes.

is also available as a 200 IU/mL formulation, in a pre-filled pen only, to reduce accidental overdose. The higher concentration is meant to help patients with high insulin requirements keep the injection volume small.

HOE901-U300

HOE901-U300 is a new, not yet approved formulation of insulin glargine. It has the same composition as the current commercial formulation of insulin glargine 100 U/mL (Lantus) with adjustment of three-times the amount of active pharmaceutical ingredient and corresponding zinc content. The difference between HOE901-U300 and Lantus rests in the pharmacokinetic/pharmacodynamic profile which, for HOE901-U300, is without an observable maximum concentration and suggests a lower risk of hypoglycemia as compared with Lantus, at least shortly after injection. A comprehensive phase III program in more than 5,000 subjects with type 2 and type 1 diabetes is underway to evaluate the efficacy and safety of HOE901-U300. Results from the phase III EDITION I and II studies have already been published. EDITION I evaluated once-daily evening HOE901-U300 versus Lantus in 807 patients with type 2 diabetes (98). The basal insulin was titrated to achieve a fasting plasma glucose target of 80-100 mg/dL. Reductions in HbA_{1c} from baseline to 6 months (primary endpoint) were similar: the least squares mean change was -0.83% (0.06) in both groups. In addition, approximately 40% of subjects who had uncontrolled glycemic levels despite combined therapy (oral antidiabetic agents plus basal and prandial insulins) achieved glycemic targets. HOE901-U300 was associated with a 21% reduction in severe or confirmed nocturnal hypoglycemia between months 3 to 6 (36.1% vs. 46.0%; RR 0.79; $P = 0.0045$), and a lower occurrence of nocturnal hypoglycemic events during the entire 6-month study period (45.3% vs. 59.7%; RR 0.76; 95% CI 0.66 to 0.87). EDITION II evaluated HOE901-U300 in 811 patients with type 2 diabetes treated with basal insulin plus oral antidiabetic therapy. Top-line results reported that HOE901-U300 achieved similar blood sugar reduction while fewer patients experienced night-time hypoglycemic events compared to Lantus (99).

Short-acting SC insulin formulations under investigation

VIAject®

Biodel Inc. is developing an "ultrafast" insulin (VIAject®) which, unlike previous analogues of human insulin, does

not modify surface charges that contribute to self-association, such as exchanging amino acids, but by adding EDTA and citric acid to the formulation: EDTA captures the zinc ions away from insulin hexamers while citric acid masks charges on the surface of the insulin molecule. This causes the insulin hexamer to dissociate faster and prevents re-association to hexamers state once injected subcutaneously (100). The net effect is faster onset of action compared to lispro insulin: time to early half-maximal glucose infusion rate (GIR) is 25 minutes with VIAject versus 44 minutes with lispro insulin (101). The company has conducted two phase III studies with the promise to reduce postprandial hypoglycemia and weight gain without sacrifice of glycemic control. Biodel reported results from an open-label phase II study versus lispro in September 2013, noting that VIAject was equivalent to lispro with regards to HbA_{1c} and hypoglycemia incidence while subjects on VIAject had less weight gain (approx. 0.5 kg) compared to those on lispro (102).

Hyaluronidases

Hyaluronidases increase the dispersion and absorption of co-injected regular human insulin and insulin lispro. No increased injection site pain, erythema or induration, and no other adverse effect has been observed thus far with a recombinant human hyaluronidase (rHuPH20; Enhance™, Halozyme Therapeutics Inc.) formulation (103, 104). Time to 50% exposure decreased from approximately 2 hours for the analogues injected by themselves to 75 minutes when injected with rHuPH20. Early (first hour) exposure to insulin was doubled with rHuPH20 and halved beyond 2 hours (105).

Similarly, nicotinamide and arginine are being considered to enhance and stabilize insulin aspart monomers, with the hope to shorten exposure time and reduce postprandial hypoglycemia.

"Smart insulins"

Biodel is also developing the BIOD-Smart Basal insulin formulation which is aimed at releasing insulin proportional to the subcutaneous glucose concentration. BIOD-Smart insulin (BIOD-620) is based on insulin glargine but adds glucose oxidase and peroxidase to the formulation (106): as interstitial glucose levels rise, glucose oxidase and peroxidase react to form gluconic acid. This further lowers pH and increases glargine's solubility. Conversely, low glucose would slow the formation of gluconic acid and maintain pH at a level that hinders glargine's dissolution into monomers.

Other companies developing “smart” insulins include Merck & Co., who acquired the original developer SmartCells in 2010 (107) and Sensulin LLC. SmartCells’ technology is based on a competitive binding strategy: human insulin is attached to a sugar group binding to a sugar-binding molecule in the formulation (108). When interstitial glucose rises, it competes with insulin to bind to the sugar-binding molecules, displacing insulin and enabling release of the latter into the bloodstream. Sensulin’s technology is based on microencapsulated insulin. The microspheres are linked together by a glucose-sensitive molecule into a lattice. In the presence of low glucose, the insulin-containing microparticles remain fixed in the lattice. Rising interstitial glucose levels disrupt the linker molecules freeing the microparticles from the lattice to dissolve and set insulin free. In vivo data from these technologies have not been publicly disclosed to date, however. Thus, it remains to be seen whether simple SC human insulin can be “smartened up” sufficiently to compete with closed-loop systems.

Ultrastable SC insulins

One of the historical challenges of SC insulin formulations has been their propensity for degradation and aggregation when exposed to higher temperatures or mechanical stress such as in pump devices. It is now recognized that parts of both the A and B chains contribute to this. Amino acid substitution, mainly at the B terminus, has already furthered development of more convenient insulin analogues for SC use. However, other modifications, whether adding stabilizing peptide sequences, A–B chain connecting peptide domains or a fourth disulfide bridge, may become novel development options to create products that could be stable long-term, even unrefrigerated and in harsh environments such as tropical climate zones (109).

Closed-loop SC insulin infusion

As early as 1959, the idea for an implantable artificial endocrine pancreas (110) was proposed but was only realized in the mid to late 1970s. Earlier prototype devices such as the Biostator® (Miles Laboratories) were large, desktop-sized apparatuses dependent on external power supply and intravenous insulin infusion (111, 112). However, insulin sensors were unreliable and required constant recalibrations, and chronic i.v. blood draws or drug infusions were simply impractical for outpatient use. Only recently, the advent of more reliable continuous glucose monitoring systems and the miniaturization

of SC insulin infusion devices has reinvigorated the idea of a mechanical device system as a long-term beta cell replacement. Current efforts focus on perfecting the computer algorithms necessary to compensate for the inevitable time delays between intra-arterial/intravenous and interstitial glucose levels, and between the rise and fall of arterial/venous insulin levels post SC insulin infusion (113).

COMBINATIONS OF SC INSULIN WITH OTHER PEPTIDE DRUGS IN DEVELOPMENT

SC insulin plus SC amylin

Amylin is another beta cell hormone that is co-secreted with insulin in response to nutrient stimuli (114). Amylin complements the effects of insulin on postprandial glucose metabolism through several centrally mediated effects, such as suppression of postprandial glucagon secretion and delayed gastric emptying, which reduce the influx of endogenous and exogenous glucose (115, 116). Clinical studies have demonstrated amylin’s potential to reduce HbA_{1c} and body weight in type 1 and type 2 diabetic patients without increased insulin use or rates of severe hypoglycemia. The most commonly observed side effects were mostly transient nausea. However, the duration of action of the commercially available amylin analogue pramlintide is short, and co-formulation with commonly used SC insulin is not being offered forcing patients to administer two injections before every meal.

SC insulin plus SC GLP-1

SC long-acting analogues of glucagon-like peptide 1 (GLP-1) have now been used for almost a decade as an adjunct to diabetes management in clinical practice, mostly in patients with type 2 diabetes. Like amylin, GLP-1 slows gastric emptying and the speed of nutrient absorption. In addition, GLP-1 increases satiety and promotes weight loss while amplifying nutrient-stimulated endogenous insulin secretion. Also like amylin, its adverse effects are mainly gastrointestinal in nature (nausea, vomiting). There may be also CV benefits although more clinical evidence is required to validate this nonclinical research-based hypothesis. Initially conceived to provide an option after failure of oral diabetes drugs and before initiation of insulin therapy, only a few studies have been published in which GLP-1 analogues have been used in combination with SC insulin (117–126). These published study results indicate that GLP-1 analogues are capable of improving glycemic control while

preventing weight gain and excessive rise of hypoglycemia when used in patients with insulin-dependent diabetes. Depending on treatment targets (better vs. same glycemic control), insulin dose up- or downtitration resulted in better HbA_{1c} at the expense of weight gain and increased hypoglycemia, or maintenance of HbA_{1c} while decreasing weight and hypoglycemia rates. Currently, fixed-dose SC GLP-1/insulin analogue combinations of lixisenatide/glargine (NCT01910194) and liraglutide/degludec (NCT01336023, NCT01392573, NCT01952145) are being tested in clinical studies.

COMPETITION FOR SC INSULIN – ALTERNATIVE ROUTES OF INSULIN ADMINISTRATION

Of the many possible alternative routes of insulin administration, only three remain today under active investigation: pulmonary, buccal and oral delivery.

Pulmonary route

Early attempts to deliver insulin as an inhaled aerosol proved that, in principle, inhaled insulin was able to reduce (urinary) glucose (127, 128). However, significant progress was not achieved until the 1990s once the importance of engineering proper aerosolizable particles was recognized: Deposition efficiency into peripheral lung areas is only optimal for aerosol particles with a median mass aerodynamic diameter (MMAD) of 1-3 µm while larger particles with a MMAD of 5-10 µm or > 10 µm remain predominantly in the tracheobronchial or oropharyngeal regions while the smallest particles (< 1 µm) are being predominantly exhaled (129). Pulmonary administration is attractive because the lungs offer one of the largest (50-140 m²) surface areas available (only second to the gastrointestinal tract), and are well-perfused (approx. 5 L blood/minute). The importance of local insulin degradation is unclear at present but may add to drug delivery loss. The pattern of breathing (inspiratory flow rate, inhaled volume and 'breath-hold' time) (130, 131), the presence of airflow obstruction, asthma and interstitial lung disease (132, 133), smoking (134, 135), underlying lung diseases (136) and exercise (137, 138) all also impact the efficiency and reproducibility of inhaled insulin delivery. The performance of inhaler devices (precision of emitted dose; control of particle size, distribution and dispersion) and the breathing pattern during delivery add to the challenge to provide safe and effective insulin therapy via this route. Thus far, most of the clinical experience with inhaled insulin has been obtained using a dry-powder

insulin formulation such as in the Exubera™ device, which was originally developed by San Francisco-based Nektar Therapeutics, later in collaboration with Pfizer Inc. and Sanofi (139), and the Technosphere™/Afresa® device developed by MannKind Corporation (67, 140). Exubera was withdrawn from the market by Pfizer for lack of commercial success in late 2007. Thus, MannKind Corp. remains the sole developer of inhaled insulin at this time, having resubmitted an NDA to the FDA in October 2013. The onset of action after inhalation of regular human insulin is rapid and comparable to that rapid-acting SC analogue insulin (141-143). Interestingly, Afresa has an even shorter onset of action and duration than other inhaled insulin products. It may owe this to its particular formulation that allows faster disintegration of the hexa- and dimeric insulin to monomeric insulin. However, despite the optimized aerodynamic design of aerosol particles, loss of insulin in the upper airways remains a significant factor contributing to the relatively low bioavailability compared to SC insulin (10-25% vs. approx. 90%) (67).

Buccal/sublingual delivery

Administering insulin through the mucosa of the mouth is another potentially attractive option as the oropharynx also offers a relatively large surface for potential absorption (100-200 cm²). The route is also easily accessible, proteolytic enzyme activity is low, and the mucosal lining of the mouth is highly vascularized. On the other hand, the mucosal layer is relatively thick, and the coverage with and constant flow of saliva further impedes reliable insulin absorption (144, 145). RapidMist/Oral-Lyn® (Generex Biotechnology Corporation) is the only trans-buccal/oropharyngeal form of insulin in advanced stages of clinical development, and is currently approved in a few countries (Ecuador, India, Lebanon). Oral-Lyn shows rapid onset (t_{max} approximately 30 minutes) and disappearance from plasma (within approximately 60-80 minutes), and a modest bioavailability of approximately 5% (146).

Peroral delivery

Peroral delivery of insulin remains the most desirable alternative to injection, despite many setbacks. Apart from ease and broad acceptance of use, this route is one of the few options to restore physiological insulin exposure by providing a desirable hepatic first pass effect as blood from the upper GI tract is primarily drained into the portal circulation via the mesenteric vein (147-149). In

addition, the surface area available for absorption is also very large (approximately 200-500 m²) (150). However, some hurdles appear to be more difficult to overcome than with any other form of delivery:

- Bioavailability tends to be very low as a result of chemical (low gastric pH), biochemical (enzymes – pancreatic proteases, serine endopeptidases, brush-border peptidases [151]) degradation, and low permeability of the mucosal lining for peptides or proteins, especially for those that are charged, hydrophilic and of larger molecular weight such as insulin.
- Reproducibility of pharmacokinetics and pharmacodynamics is also poor, probably a result of variable gastric emptying and negative food interaction.
- Potential for food–drug interactions can diminish further bioavailability at a time when higher insulin availability would be desirable.

Thus far, development efforts have mostly targeted the improvement of bioavailability. To enhance the chemical stability of insulin and protect it against proteolytic enzymes, attempts have been made to incorporate insulin into liposomes (152), use surfactants or emulsions to increase the permeability of the intestinal mucosa for insulin (153, 154), or encapsulate insulin, with or without protease inhibitors, into enteric-coated and pH-dependent biodegradable synthetic (155-159) or natural polymer nanocarriers (160-164). More recently, modifications to the insulin molecule such as non-acylated amino acids (Emisphere Technologies) have been evaluated to facilitate the exposure of the hydrophobic side chains of insulin and its passive diffusion through lipid bilayers (165). A philosophically similar strategy attaches amphiphilic oligomers to insulin to enhance passage through membranes (Nobex Corporation) (166, 167). However, in the few studies that have been carried out in humans, only modest glucose-lowering effects have been observed while pharmacokinetic data show high variability (CV approx. 48-106%) and low bioavailability (approx. 2%, probably even less postprandially, relative to SC insulin) which, at the very least, makes this treatment modality very costly. This notwithstanding, other companies are continuing the development of oral insulin formulations: Oramed is currently conducting phase II studies with its encapsulated insulin formulation (168). Biocon Pharmaceuticals in Bangalore, India, has signed an agreement with Bristol-Myers Squibb to develop Biocon IN-105 as oral insulin. Capsulin™ (Diabetology Ltd.) is being developed in India and

reported to be in phase II clinical development. An interesting spin-off from oral insulin technology is the development of intestinal patches that aim to improve bioavailability by encapsulating insulin into polyacrylic patches that self-attach to the upper gastrointestinal mucosa and protect insulin from proteolytic enzymes, as insulin is only released to the contact surface between patch and gastrointestinal mucosa. A proof-of-principle study in rats showed relatively good bioavailability (5-10%) (169). However, further studies are required to establish long-term safety (high local concentrations of insulin, predictability of release and potential for hypoglycemia).

Implantable insulin pumps

Starting from the mid-1970s, bedside closed-loop IV insulin infusion systems emerged to test IV glucose-dependent insulin infusion algorithms and the potential to achieve near-normal glycemia, at least over short periods of time while awaiting technical progress on device size and component reliability, especially of the glucose sensor (170-172). Finally, more reliable and miniaturized glucose sensing and SC insulin infusion systems have emerged over the past decade (173-175), as well as refined algorithms to provide long-term automated glucose control with minimal needs for calibration or other use intervention (176-178). Notwithstanding the recent success with extracorporeal, closed-loop SC insulin delivery systems, many patients would prefer more discrete implanted devices. However, those devices face additional challenges: resupply of the insulin reservoir, and biocompatibility of materials. Importantly, the risk for infections and thromboembolism in case of intravascular placement of components, or excessive scarring when placing catheters or pumps intraperitoneally (179, 180) remains to be addressed satisfactorily before widespread use should be considered. Thus, efforts are being made to test novel materials, and embedded antibiotics are being designed to reduce infection risk (181-184), while concepts are being developed for refilling and recharging devices non-invasively (185). However, in vivo studies have not been reported, and widespread use of IV or IP pumps appears unlikely for the near future.

Islet cell replacement

Currently, islet or beta cell transplantation is not a viable option for the treatment for most potential recipients, largely due to limitations in sourcing suitable cells and

the requirement for life-long immune suppression. Xenografts (186-188) or stem cell products (189) may be more abundantly available but still require immunological protection, administration of immunomodulatory cell subsets or encapsulation. Even with corticosteroid-free immunosuppressive protocols such as the Edmonton protocol (190), longevity of islets is limited, and long-term sequelae remain unknown until larger trials have been completed. California-based ViaCyte (previously Novocell) previously reported positive nonclinical results with their Pro-Islet™ system (191). However, it remains to be seen whether this approach will fare better in the clinic than past attempts.

CONCLUSIONS: NO REPLACEMENT FOR SC INSULIN IN THE IMMEDIATE (NEXT 10-15 YEARS) FUTURE

The newer long-acting insulin products show reduced nocturnal hypoglycemia, decreased weight gain and comparable HbA_{1c} lowering when compared with Lantus in type 2 diabetes. However, larger phase III clinical trials are needed to confirm hepatic (LY-2605541) and cardiovascular (LY-260441, degludec) safety in patients. The newer short-acting insulin formulations appear to reduce postprandial hyperglycemia better, and offer the potential for more convenient dosing shortly before or even after meals. However, larger phase II-III trials are still required to confirm efficacy, site tolerability and cardiovascular safety. On the other hand, most alternatives to SC insulin injection investigated thus far have not met critical milestones based on pharmacokinetic presentation and bioavailability. In order to treat diabetes safely and effectively with insulin, patients need to rely on predictable parameters such as onset of action, particularly for prandial insulin injections, and duration of action. Bioavailability is critical because cost of goods is an important limitation to obtain patients' or payers' willingness to adopt the product, especially if safety and efficacy do not surpass that of SC insulin. Unfortunately, reported numbers for bioavailability are not based on a uniform definition and thus are difficult to compare. This review is also limited by the scarcity of reported intraindividual variability measures. This is a particular concern as variations in t_{max} from administration to administration may increase the risk for hypoglycemia. In general, novel administration routes need to use SC regular human insulin as benchmark, and meet or better exceed SC insulin's performance for intraindividual exposure variability. Although the efficiency of absorption across epithelial surfaces, such as of the mouth, gastrointesti-

nal tract, skin or nose, has increased somewhat using a wide variety of absorption-enhancing techniques, bioavailability remains too low to warrant commercial development and too erratic to be of clinical value. How important bioavailability and cost, but also comparative medical effectiveness and competition from concurrent non-insulin options, are is exemplified by the fate of inhaled insulin which, despite demonstration of safety and efficacy being comparable to that of SC insulin, failed to attract enough interest, possibly as a result of high price and persistent patient burden (frequent dose administration in combination with invasive glucose monitoring). Thus, while general clinical safety, precision of dosing, dose adjustments and reproducibility will remain key requirements, more attractive, longer-lasting formulations and attractive costs (based on a bioavailability of at least 20%) will be paramount to justify the nowadays very expensive clinical development for diabetes drugs. Thus, not too surprisingly, the majority of current development programs focus on novel SC insulin formulations and delivery systems, as well as on SC biosimilar products (Fig. 5). At the same time, research into improved treatments for diabetes continues on several fronts and directly competes with alternative insulin administration routes. There has been renewed interest in the use of external and implantable SC pumps which, in combination with non-invasive continuous glucose sensors as closed-loop systems may help to optimize insulin replacement therapy. Islet cell transplantation may also expand once new tissue sources and more tolerable immunosuppressive agents become available, with the promise to restore glycemia to near normal. A rapidly expanding array of new treatment modalities for patients with type 2 diabetes involving SC agents with potentially better durability of action, such as incretin analogues, may reduce the need for insulin therapy. Finally, SC "smart" insulin formulations may further increase protection from hypoglycemia and obviate the need for closed-loop control systems in many patients.

DISCLOSURES

E. Leifke is employed by Sanofi. The views and opinions expressed in this article by the authors do not necessarily reflect the position of Sanofi.

T. Strack is employed by Asubio Pharmaceuticals. The views and opinions expressed in this article by the authors do not necessarily reflect the position of Asubio.

This review includes information and discussions on non-approved use of therapeutics.

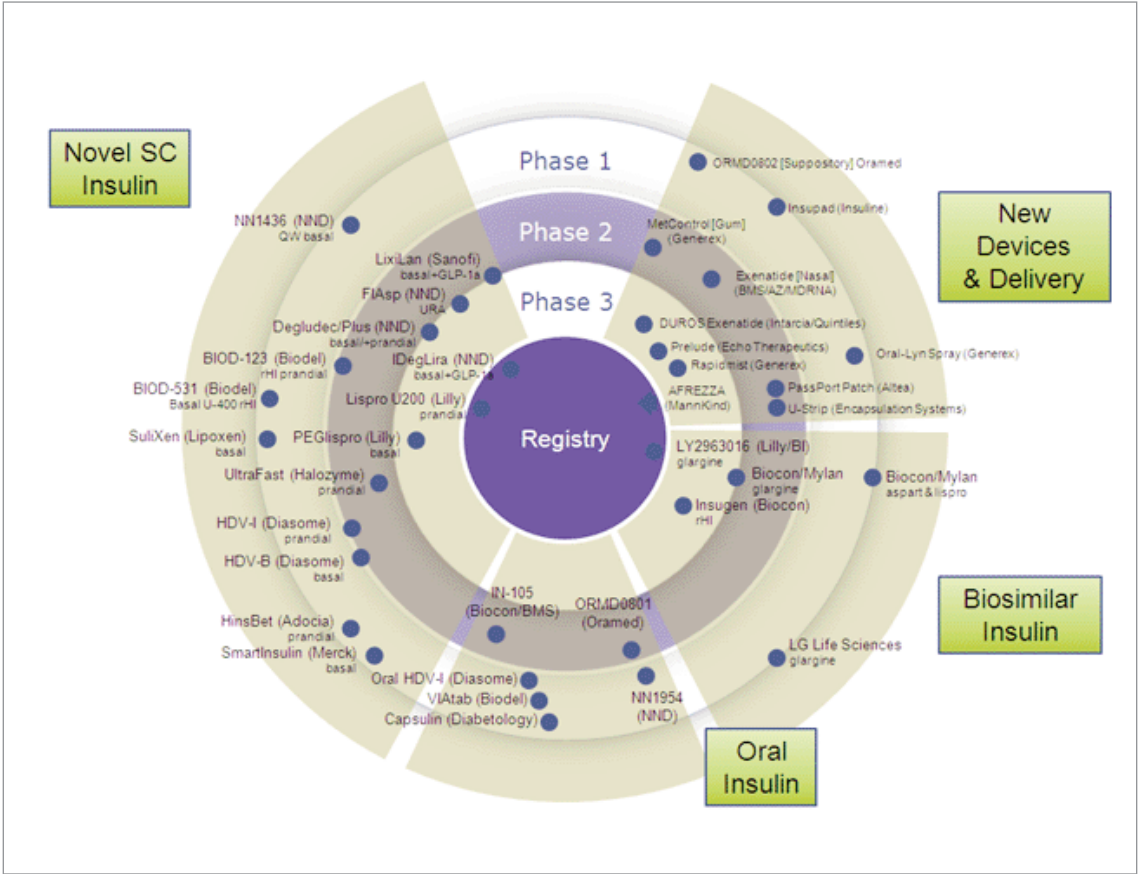


Figure 5. New insulin formulations and insulin delivery systems currently in development. Novel subcutaneous (SC) insulin formulations and analogues as well as SC biosimilars comprise the majority of ongoing development activity.

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