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SYMPOSIUM

Ultrafast-Acting Insulins: State of the Art

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Abstract

Optimal coverage of prandial insulin requirements remains an elusive goal. The invention of rapid-acting insulin analogs (RAIAs) was a big step forward in reducing postprandial glycemic excursions in patients with diabetes in comparison with using regular human insulin; however, even with these, the physiological situation cannot be adequately mimicked. Developing ultrafast-acting insulins (UFIs)—showing an even more rapid onset of action and a shorter duration of action after subcutaneous (SC) administration—is another step forward in achieving this goal. The need for UFIs has been gradually recognized over the years, and subsequently, a number of different approaches to cover this need are in clinical development. A rapid increase in circulating insulin levels can be achieved by different measures: modification of the primary structure of insulin molecule (as we know from RAIAs), addition of excipients that enhance the appearance in the monomeric state post-injection, or addition of enzymes that enable more free spreading of the insulin molecules in the SC tissue. Other measures to increase the insulin absorption rate increase the local blood flow nearby the insulin depot in the SC tissue, injecting the insulin intradermally or applying via another route, e.g., the lung. The development of these approaches is in different stages, from quite early stages to nearing market authorization. In time, daily practice will show if the introduction of UFIs will fulfill their clinical promise. In this review, the basic idea for UFIs will be presented and the different approaches will be briefly characterized.

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Background

When a healthy individual is presented with a meal and begins to ingest it, a rapid release of insulin from the beta cells of the Langerhans islets in the pancreas ensues. Upon neural, hormonal, and/or metabolic stimulation, the normal mammalian islet beta cell releases preformed insulin extremely rapidly, enabling superb glucose tolerance. In comparison with the modest amounts of basal insulin secreted between meals and overnight, insulin secretion rates increase dramatically in healthy individuals, with up to 1000-fold higher rates following a carbohydrate

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Abbreviations: (AP) artificial pancreas, (ARIA) alternative routes of insulin administration, (CGM) continuous glucose monitoring, (CSII) continuous subcutaneous insulin infusion, (FDA) Food and Drug Administration, (GIR) glucose infusion rate, (GV) glycemic variability, (HbA1c) hemoglobin A1c, (HGP) hepatic glucose production, (ID) intradermal, (IMI) injection-meal interval, (NDA) new drug application, (PD) pharmacodynamic, (PK) pharmacokinetic, (PPG) postprandial glycemic excursion, (RAIA) rapid-acting insulin analog, (RHI) regular human insulin, (rHuPH20) recombinant human hyaluronidase, (SC) subcutaneous, (T1DM) type 1 diabetes mellitus, (T2DM) type 2 diabetes mellitus, (TI) Technosphere insulin, (UFI) ultrafast-acting insulin

Keywords: insulin absorption, insulin action, insulin analogs, insulin pharmacodynamics, insulin pharmacokinetics, insulin therapy

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challenge. The physiologically induced rapid increase in circulating insulin levels prepares the body for the expected influx of carbohydrates. Following a mixed meal, insulin levels circulating in blood reach half of maximal concentration in approximately 16–18 min and peaks within 30–45 min.[1] The flux of glucose from the gut to the bloodstream is handled proficiently in healthy subjects by means of a very rapid reduction in hepatic glucose production (HGP), followed by an increase in peripheral cellular glucose uptake (mainly muscle and adipose tissue), with the result that only a moderate increase in postprandial glycemic excursion (PPG) is seen, even after ingestion of a large carbohydrate load. The liver is sensitive to the rate of change of the insulin concentration in blood, and thus the rapidity and extent to which the HGP is reduced depends on the insulin kinetics.

This complex and highly tuned regulation system is disrupted in patients with diabetes: no insulin is secreted at all in patients with type 1 diabetes mellitus (T1DM), and in patients with type 2 diabetes mellitus (T2DM), the early phase of insulin secretion is deficient. In both cases, there are profound metabolic consequences, and PPG is higher than in healthy subjects. This prandial hyperglycemia is a major contributor to the overall hyperglycemia of diabetes; this is especially the case for subjects with good overall control as compared with moderate or poor control.[2] An important reason for higher PPG in patients with diabetes is that that exogenous insulin is administered in the subcutaneous (SC) tissue, a less than ideal place for rapid absorption. The old statement that insulin is applied in clinical practice at the wrong site, at the wrong time, and in the wrong dose is still valid. Unfortunately, it is not practical to circumvent this issue by administering the insulin routinely into the portal vein.

Clinical experience shows that good PPG control after breakfast helps to set the stage for glucose control during the rest of the day adequately. Optimizing PPGs helps to improve long-term glycemic control.[2] At hemoglobin A1c (HbA1c) levels of ~10%, the relative contributions of fasting and PPG to HbA1c are ~70% and 30%, respectively, versus nearly the opposite relative contributions with HbA1c levels near 7%. For HbA1c values < 8.5%, PPG contributes more to overall glycemic control than fasting glucose levels. Thus, it is of clear relevance to address not only fasting glucose, but also postprandial and postabsorptive levels as well in order to achieve optimal metabolic control in a given patient.

In addition, avoidance of swings in glycemia [i.e., glycemic variability (GV)] has been the subject of intensive discussion with respect to the impact of GV on the development of both microvascular and macrovascular complications.[3,4] Epidemiological studies have also suggested an association between increased PPG and an increased macrovascular disease and mortality risk.[5,6] However, it is by no means proven that either GV or PPG independently contributes to adverse long-term outcomes.[7–10] Studies to date have been inadequate to separate out mean glycemic exposure from intraday variability, including PPG;[11] one can be certain that there will be more discussion on this topic.

The differences between the physiological and the pathological situation and their (potential) clinical implications explain why optimization of postprandial metabolic control is regarded to be of high clinical relevance.

Insulin in the Subcutaneous Tissue

The rate of insulin absorption from the SC insulin depot is determined by several factors (see **Table 1**). Spread of injected materials through in the extracellular matrix of the SC tissue is affected by the presence of structural macromolecules that limit the rate by which injected drugs permeate the interstitium before being absorbed into the bloodstream. The attempts to increase the rate of insulin absorption described here rely on influencing one or the more of these factors, e.g., by altering the properties of the SC interstitium through which the insulin molecules must spread to reach the capillaries or by reducing the size of the insulin molecule complex (from hexamers to dimers/monomers) to enhance permeation through the capillary pores into the bloodstream.

Table 1.
Factors Known to Influence Absorption and Action of Subcutaneously Injected Insulin

Insulin preparation	Differences between injection sites	Changes on the injection site
• Dose • Physical status (soluble or suspension) • Concentration • Volume • Species • Shaking	• Injection site (intramuscular versus SC) • Injection depths • Anatomical region of injection • Lipodystrophy	• Temperature • Physical activity • Substances known to increase local blood flow • Massage • Hypoglycemia • Ketoacidosis • Smoking • Age • Metabolic control • Local degradation

The commercially available prandial insulin formulations have high insulin concentration, and these insulin molecules self-assemble into hexamers in the presence of zinc, similar to the way in which they are stored in the beta cells of the pancreas. Thus, the insulin molecules generally exist in solution as a dynamic equilibrium of hexamers, dimers, and monomers, the proportions of which depend on the concentration and pH of the solution. In commercial insulin formulations at neutral pH, this equilibrium strongly favors the zinc-stabilized hexamer, which is advantageous for shelf life stability. As described by Supersaxo and coauthors,[12] molecular weight of water-soluble compounds is directly related to the proportion of the dose that is absorbed lymphatically. The size of the insulin hexamers (as a large polypeptide with a molecular weight of ~36,000 Da and a mean diameter of 5.6 nm) thus limits rapid capillary absorption from the SC insulin depot. As described in 1990 by Brange and colleagues,[13,14] dilution of these insulin formulations in the SC insulin depot must occur before these hexamers dissociate into dimers (mean diameter 3.5 nm) and monomers (mean diameter 2.5 nm) that are more readily absorbable. As stated earlier, the macromolecular interstitial barrier in the SC tissue limits the spread of injected drugs and thus retards the concentration-dependent dissociation of multimers, retarding their absorption. The delay in insulin dilution is regarded as the major factor for the delayed absorption of SC-injected prandial insulin formulations.

Insulin Therapy

Modern insulin regimens are focusing on reducing PPG by trying to mimic the endogenous insulin secretion pattern, i.e., to match insulin absorption and action to the dynamics of baseline HGP and food-related rises in plasma glucose. The use of basal–bolus insulin therapy allows separate coverage of the basal and prandial insulin requirements. However, the delayed absorption of regular human insulin (RHI) from the SC tissue into the bloodstream led to a pattern of circulating insulin levels that is clearly different from the physiological situation in the prandial state. Nevertheless, also by using such conventional insulin formulations, it is possible (but not easy) to minimize postprandial hyperglycemia while simultaneously avoiding late postprandial hypoglycemia when using an optimum injection-meal interval (IMI; discussed later) coupled with carefully chosen meal size and content along with appropriate size and timing of snacks.

Rapid-Acting Insulin Analogs

Injection of insulin formulations into the SC tissue in which the multimers are destabilized, i.e., that dissociate into dimers and monomers more rapidly (i.e., by requiring less dilution), led to a more rapid insulin absorption and improved PPG. One strategy to reduce the intermolecular forces that hold the insulin molecules in the hexamer status is to modify the primary structure of the insulin molecule (i.e., insulin analogs). A number of rapid-acting insulin analogs (RAIAs) were developed in 1996, and this was a big step forward to optimize PPG.[15] The three RAIAs that are on the market (insulin lispro, insulin aspart, and insulin glulisine) do not substantively differ in their pharmacokinetic (PK) and pharmacodynamic (PD) properties. Although, in contrast to insulin lispro and insulin aspart, insulin glulisine is commercialized in a zinc-free formulation and thus does not form zinc-stabilized hexamers, insulin glulisine behaves in a functionally similar manner. It has absorption kinetics that are very similar to those of the zinc-containing RAIAs. Sometimes RAIAs are also called short-acting insulin analogs to highlight that they also have a shorter duration of action compared with RHI.

Such "modern" insulins are now widely used in patients with T1DM and some insulin-requiring patients with T2DM. Their usage facilitates achievement of good metabolic control without undue risk of hypoglycemia when used in an appropriate combination with basal insulins. Ideally, the dosage of the latter is selected in a manner that suppress HGP over the whole day, while the dosage of the prandial insulin is modified in a manner to allow optimal coverage of the carbohydrate content of a given meal and in relation to the preprandial glycemia and inducing euglycemia prior to the next meal.

Invention of RAIAs was a big step forward in optimizing PPG; however, the time-action profiles of the currently available RAIAs do not adequately mimic the physiological postmeal insulin secretion pattern in healthy subjects. Thus, the RAIAs offer a clinical advantage when compared with RHI, but more recent studies have shown that both types of prandial insulin formulations are still absorbed too slowly from the injection site to achieve optimal control of PPG if no IMI is used.[16,17] In the study by Luijf and associates,[17] the optimal bolus dose to meal interval in patients using continuous subcutaneous insulin infusion (CSII) showed that, even with RAIA, an interval of 20 min is necessary to optimize PPG following a standardized meal.

Many patients choose not to bother with the inconvenience of meal delay; this is particularly true in pediatric care, where it is difficult or impossible to accurately predict how much a child will actually eat at given meal. Thus, there is a medical need for an even faster onset of action after insulin administration to optimally control PPG.

In addition to the importance of rapid onset of action, the duration of action of prandial insulin is also a critical factor. Rapid-acting insulin analogs have a significantly shorter duration of action, and this contributes to the reduced risk of hypoglycemic events that these products afford compared with RHI. Even so, it is likely that there is room for further improvement in prandial insulin profiles by reducing both time of onset of action and duration of action. By having an insulin formulation that mimics the normal physiologic availability of insulin to an even greater extent than is possible with the currently available RAIAs, it should be possible to achieve PPG goals in a greater percentage of patients.

Ultrafast-Acting Insulins

How can prandial insulin be made faster? This does not mean that it must be the insulin *per se* that must be modified to become an ultrafast-acting insulin (UFI); it can also be that, by using other routes of insulin administration or altering the insulin absorption *per se*, an UFI with a further improved time-action profile can be achieved.

Historically, a number of approaches were studied to improve the rate of absorption of RHI from the SC insulin depot by more "mechanical" measures, e.g., application of ultrasound, addition of Trasylol (a basic pancreatic trypsin inhibitor), or usage of a sprinkler needle. However, after the invention of RAIAs, practically all these developments were halted. To be fair, one has to acknowledge that, when RAIAs were developed, there was hope that these would have properties to enable optimal control of PPG. Since this did not prove to be the case, a number of investigators and companies are now exploring approaches to improve insulin PK/PD properties.

The new approaches to UFIs must show not only significant differences in their PK and PD properties in comparison with RAIAs, but also that these differences are clinically relevant. This not only means a faster onset of action, but also a shorter duration of action (but not too short, as discussed later) to reduce the risk of late postprandial hypoglycemic events. However, it has to be studied in adequately designed meal studies and clinical studies if a shorter "duration of action" compared with RAIAs is really of benefit. In other words, it has to be proven that the e.g., shorter duration of action as measured under glucose clamp conditions translates in a relevent change in hypoglycemia frequency. It might be that, in reality, the "tail" seen with RAIA is appropriate (on average) in case absorption of carbohydrates continues up to several hours after a meal. As illustrated by RHI, "optimal" duration of action needs to be seen in the context of the timing of the peak effect and the overall shape of the curve. Another concept is that, e.g., in patients with T2DM, replacement of the early insulin response by just applying one standard dose of UFI might induce a considerable improvement in PPG by reducing the HGP; however, this also remains to be proven.

We will briefly describe the different approaches for UFIs that are in clinical development and discuss critically the current clinical development stage of the different products: A more rapid insulin absorption can be achieved by introducing certain changes in the primary structure of the insulin molecule itself (i.e., novel insulin analogs) or adding excipients that promote monomerization of insulin in the pharmaceutical formulations or that have an impact of the absorption properties in the SC tissue. Other measures to achieve a rapid appearance of insulin molecules in the blood stream are using alternative routes of insulin administration (ARIA), e.g., the pulmonary route; increasing the local blood flow in the vicinity of the SC insulin depot by, e.g., heating the site; or distributing the insulin to a wider area in the SC tissue by using enzymatic alteration of the SC matrix or a jet spray injection. One can categorize the latter approaches as *mechanical* and the former as *formulation* attempts to improve insulin pharmacology by modification of the insulin molecule itself (**Table 2**). As usual, each approach has its pros and cons.

Table 2.
Approaches That Are in Clinical Development for Ultrafast-Acting Insulins

Mechanical	Formulation
• Increase of local blood flow • Inhalation of a rapidly absorbed insulin • Intradermal application • Spreading the insulin into a wider area in the SC tissue (either mechanically or • enzymatically)	• Adding excipients that promote monomerization of insulin molecules • Adding excipients that increase local blood flow • Novel RAIAs

The regulatory hurdles vary for the different approaches, e.g., modifications of the primary structure of the insulin molecule require a full pharmaceutical development program, whereas local warming of the skin will face lower regulatory hurdles. Even more important is the question of which product will achieve a high acceptance by the patients with diabetes. In reality, many factors have an impact on the market acceptance of such products; uptake is driven by patient and provider perceptions of efficacy and safety, convenience, cost, public awareness, and barriers to access.

Mechanical Attempts

Increase of Local Blood Flow

The Israel-based company InsuLine (see the article by Freckmann and associates[18] in this special theme issue) has developed small patch devices to apply mild heat (38 °C) locally to the skin at the time of a SC insulin injection or bolus from an insulin pump.

It is well-known from clinical practice that an increase in local blood flow induced by either exercising, massaging the injection site, or applying heat, be it local or general, will accelerate insulin absorption to a clinically meaningful degree.[19–23] Thus, insulin action can be accelerated by increasing the ambient temperature, such as in the summer, in tropical areas, or during sauna visits.[22] Elevations of skin temperature result in vasodilatation and increased tissue perfusion, which promotes absorption of SC-injected insulin.

One report describes a significant shortening of insulin T_{max} by 42% and reduction of postmeal glycemic excursions in patients with T1DM using CSII after elevating skin temperature to 38.5 °C for 30 min after an insulin bolus with either insulin lispro or aspart given directly before a liquid meal, although the numbers of subjects with complete data were limited.[24] Heating the skin resulted also in a smaller increment of PPG, with lower peak glucose and a significant reduction in postprandial glucose burden, as reflected by the 3 h area under the glucose curve.[24] The device and the heating were well tolerated by the patients.

Intradermal Application of the Insulin

The U.S.-based company Becton Dickinson (see the article by McVey and coworkers[25] in this special theme issue) is developing an insulin infusing product that applies the insulin by means of short insulin needles ("microneedles") into the dermis instead of the SC tissue. The microneedles (single needles with short length—1.25, 1.50, or 1.75 mm and a diameter of 260 μm) penetrate the stratum corneum and epidermis to reach the dense beds of capillaries and lymphatic vessels of the dermis. The dermis is highly vascularized and contains a dense network of lymph vessels. The regional capillaries also have thinner vessel walls and reduced endothelial barrier function, both of which promote absorption of small proteins, such as insulin.[26] The faster absorption observed (discussed here) is reported to be facilitated by the increased lymphatic absorption of the dermis layer.

The intradermal (ID) insulin application leads to improved PK/PD properties; an increased rate of absorption of RAIA in human volunteers was observed, as evidenced by reductions in the T_{max} of 28–44% in comparison with SC injection, also $tGIR_{max}$ was reduced by 14–18%.[26–28] A number of early phase clinical studies have been performed with this system.

There are also other approaches to develop smaller microneedles that allow ID insulin application.[29] The use of borosilicate glass microneedles that are 900 μm in length was studied in five subjects with T1DM. Also in this study, insulin was absorbed significantly faster (T_{max} 27 min) than with a 9 mm SC insulin infusion catheter (T_{max} 57 min). Peak insulin concentrations were not significantly higher compared with ID delivery (C_{max} 32 versus 25 μU/ml), and the area under the serum insulin profile did not differ. Blood glucose levels were lower with ID insulin delivery than with SC delivery but of uncertain statistical significance.[29]

Another option for applying insulin into a compartment that allows more rapid insulin absorption is the intraperitoneal route. The Swiss/German company Roche Diagnostics has a redesigned system in a late stage of development that is supposed to allow safe and efficient insulin application into this compartment by means of a special catheter (DiaPort).

Inhalation of Rapidly Absorbed Insulin

Among numerous attempts to develop insulin that is applied via ARIA, only one is left that is in the late phase of clinical development. The U.S.-based company MannKind is developing an inhaled insulin that differs from previous inhaled insulin offerings (see article by Boss and colleagues[30] in this special theme issue). Technosphere insulin (TI) is primarily intended for usage in patients with T2DM and has been submitted as a new drug application (NDA) to the Food and Drug

Administration (FDA). Initial regulatory review resulted in a requirement for additional studies.

Application of insulin via the lung to improve PPG is a rational option, because the lungs have a large absorption surface area and a thin epithelium, and they are richly perfused with blood. Optimal absorption from the lung can be achieved when insulin is deposited deep in the alveoli, which requires a particle size between 1 and 5 μm. Technosphere insulin is a dry powder inhaled insulin in which the insulin is encapsulated in microparticles. The substances that construct the microparticles support a rapid absorption by immediately dissolving after entering the alveolar space to release the insulin. The time-action profile of TI is characterized by a very rapid onset of action and a very short duration of action.[31–33] The uptake of TI is significantly faster than RHI, with a reported T_{max} of 12 to 17 min for doses of 25 to 100 U of TI (bioavailability relative to SC insulin was 21–25%) and glucose infusion rate (GIR) T_{max} 42–58 versus 174 min for RHI.[34] In a phase 3 study comparing glargine plus TI inhaled preprandially with biphasic (biaspart) SC injections twice daily, changes in HbA1c with TI were noninferior/equivalent, despite the much faster PKs. The TI was associated with a 1.6 kg lower weight gain.[35]

Technosphere insulin is inhaled through a specifically designed breath-activated handheld inhaler at a size smaller than that of an insulin pen. Apart from a dry cough after inhalation that usually abates over time and an initial small, reversible decline in pulmonary function, TI is generally well tolerated.

There are only a few remaining efforts to develop ARIAs, oral insulin being one that has drawn the most attention.[36] It appears that intranasal insulin is not being actively pursued at this time. One company (Bentley Pharmaceuticals/CPEX Pharmaceuticals) did develop a RHI formulated for nasal administration. When administered to healthy subjects, T_{max} was reported to be ~15–22 min, with relative bioavailability of 15–20%.[37,38] Subcutaneous insulin was not given as a control.

Spreading the Insulin into a Wider Area in the Subcutaneous Tissue

Jet injectors deliver insulin at a high velocity (typically >100 m/s) across the skin into the SC tissue, without penetration of the underlying muscle, and disperse the insulin over a larger tissue volume than insulin injected with a syringe. The resultant larger surface area and the increased diffusion of the insulin in the SC tissue both facilitate absorption into the bloodstream. The jet injection technique was first available in the 1960s as a needle-free alternative, primarily for patients with needle phobia or unwillingness to initiate conventional insulin therapy.[39,40]

Later, additional studies were performed using this technique.[41,42] A study published in 1981 by Taylor and associates[41] showed faster insulin action, and another study published 1986 by Malone and coworkers[43] showed an earlier insulin peak after jet injection compared with syringe injection of neutral protamine Hagedorn insulin. A study from 1991 of 10 young children with diabetes did not recommend the device for this age group due to side effects, and it never enjoyed wide use in diabetes therapy.[44]

Two studies have been published that investigated the use of jet injection for the administration of RAIAs. A small study among four subjects showed that 30 U of insulin lispro were absorbed approximately twice as fast using jet injection compared with injection with a syringe.[45] A crossover study in 18 healthy volunteers demonstrated significant shortening (~50%) of insulin T_{max} (31 ± 3 versus 64 ± 6 min, $p < .0001$) and $tGIR_{max}$ (51 ± 3 versus 105 ± 11 min, $p < .0001$) in a euglycemic clamp compared with conventional pen administration of insulin aspart.[46] The peak insulin concentration was also significantly increased (108 ± 13 versus 79 ± 7 mU/liter, $p = .01$) using the injector.

In summary, jet injection of insulin showed a remarkable reduction of time to peak insulin concentration compared with conventional SC administration of insulin but has not been widely adopted. Limitations are that proper training is needed and that it is still questionable if modern jet injectors were more successful than the old models in the hands of patients.

Another idea to distribute the idea in a wider area in the SC tissue was to use a "sprinkler needle"; this is an injection needle with a sealed tip and several holes in the wall. In the late 1980s, results from a meal tolerance test study showed increased absorption rate of RHI and reduced postprandial glucose levels when insulin was injected using a "sprinkler needle" compared with conventional needles. The total area of the holes was equal to the original outlet.[47] The increased absorption rate was attributed to the fact that insulin is divided between different insulin depots. This approach to UFI has not been available on the market.

Formulation Attempts

Adding Excipients That Keep Insulin Molecules as Monomers

The U.S.-based company Biodel (see the articles by Pohl and associates[48] and Krasner and colleagues[49] in this special theme issue) has been developing an UFI by using a novel combination of excipients (ethylenediaminetetraacetic acid and citric acid) to modify the insulin hexamer complex of RHI resulting in more rapid dissociation into monomers and dimers following SC injection. Ethylenediaminetetraacetic acid is a chelator of zinc, which destabilizes insulin hexamer formation by pulling out the zinc ions, whereas citric acid masks surface charges so that monomerization of insulin and subsequent absorption is facilitated. The excipients used in these formulations are used in other pharmaceutical preparations and have been demonstrated to be safe in long-term clinical trials [they are listed by the FDA as "generally recognized as safe" (GRAS)].

The original Biodel formulation in its phase 3 studies contained insulin at a concentration of 25 U/ml at pH 4 and had a modest and variably but statistically significantly shorter T_{max} and $tGIR_{max}$ than insulin lispro and RHI, as shown by euglycemic clamps.[50,51] In a proof-of-concept clamp study with 10 healthy subjects, this formulation was absorbed twice as fast as RHI and slightly faster than a RAIA; maximal glucose-lowering action was also reached faster. In a meal study, use of this insulin resulted in lower PPG than RHI or RAIA.[52] The phase 3 studies were designed for noninferiority of metabolic control versus SC RHI; however, the NDA was not approved by the FDA. Since the insulin formulation used in these studies induced some side effects (pain at injection site, perhaps caused by increased injection volumes and/or the acidity of the product), newer formulations—U-100 insulin concentration at neutral pH—were developed and were shown to be bioequivalent to the original.[53] However, the frequency of injection site discomfort was still elevated in comparison with insulin lispro. Biodel was able to develop formulations that still have ultra-rapid absorption properties, but with significantly less pain (see the Krasner and coworkers[49] article). It will be of interest to see if using a RAIA instead of RHI will have an additional beneficial effect of the time-action profile of this UFI; preliminary animal experiments conducted by Biodel, Inc. (Pohl and associates[48] and Krasner and coauthors[49]) showed promise in this regard.

Adding Excipients to Enhance Absorption Kinetics

The Danish company Novo also has an UFI in development; however, only limited information about the status of this development and details of the approach used are publically available. Referred to as NN1218, this UFI is a reformulation of a marketed RAIA, insulin aspart that is supposed to increase local blood flow. No clinical results using NN1218 have been disclosed to date (*www.novonordisk.com/investors/rd_pipeline/rd_pipeline.asp?showid=18*).

In another attempt under development by the French company Adocia, a polymer is studied that forms a molecular complex with human insulin to accelerate insulin blood penetration. These polymers are designed to form a reversible molecular complex with therapeutic proteins in order to solubilize and stabilize these proteins and to control their delivery. HinsBet is a formulation comprising human insulin and one polymer of the BioChaperone platform patented by Adocia. This BioChaperone platform is a library of polysaccharides modified with naturally occurring molecules. In a phase 1 glucose clamp study with 12 healthy volunteers, the safety and clinical utility of HinsBet were evaluated in comparison with a RAIA and RHI (*http://www.adocia.com/Biochaperone-Insulin-Innovations,en,44.html*). HinsBet was similarly well tolerated in comparison with the commercial products tested. The onset of action of this UFI was as short as that of the RAIA and shorter than that of the RHI. Also the intersubject variability on glycemic control was lower with HinsBet than with the two other insulin formulations.

Adding Excipients That Facilitate Dispersion of Insulin Molecules

The U.S.-based company Halozyme is developing an UFI for SC injection and infusion (see the article by Muchmore and Vaughn[54] in this special theme issue). In this case, the idea is to reduce the diffusion barriers in the SC tissue by locally applying a small amount of recombinant human hyaluronidase (rHuPH20), a spreading factor that disrupts hyaluronan in the SC adipose layer.[55] The major support component of the SC extracellular matrix is collagen, but hyaluronan is the component that confers a gel-like consistency to the matrix, limiting the spread of injected materials to the process of diffusion. Recombinant human hyaluronidase is a soluble, neutral pH-active enzyme that rapidly, transiently, and locally acts on hyaluronan, promoting the permeation and absorption of SC-injected insulin by facilitating bulk

fluid flow (convection) away from the site of injection. This mechanism may be especially useful for high-dose insulin users.

In healthy volunteers, coadministration of rHuPH20 to RHI or RAIA reduced the time until peak plasma insulin levels by ~50%.[56] Subsequent meal studies among patients with T1DM and T2DM confirmed the faster PK of both insulins when coadministered with rHuPH20.[57,58] Coadministration with hyaluronidase was also associated with lower PPG and—at least in in one study—a reduced tendency toward hypoglycemia. Small studies have also been performed with CSII.[58,59] There is also evidence that usage of hyaluronidase reduces the intraindividual and interindividual variability of PK and PD parameters.[60] Longer-term studies on the effect of adding HuPH20 to the treatment with RAIAs in patients with T1DM or T2DM are ongoing.[59]

Novel Rapid-Acting Insulin Analogs

Thermalin has developed single-chain insulin analogs that demonstrate marked structural stability and resistance to fibril formation, possibly advantageous for CSII. The PKs and PDs remain to be shown in human studies; limited data in rats for one analog showed similar glucose-lowering activity as regular insulin.[61]

Injection-Meal Interval

One simple measure to adjust the timing of prandial insulin to the insulin requirements is employing an IMI, i.e., injection of the prandial insulin some minutes prior to the meal. The manufacturers of RHIs/RAIAs still have respective recommendations in their instructions for usage. The issue is that patients in real life tend to use no or only a short IMI.[62,63] It is not only more convenient if the injection is done shortly or directly before the meal is started, but also safer, e.g., in a restaurant situation. Some patients use an IMI according to the result of their preprandial glucose measurement result, e.g., they have elevated values to use an interval of 20–30 min to reduce the preprandial hyperglycemia.[64]

The use of UFIs might reduce the importance of using an appropriate IMI even further than was already possible by using RAIAs. This might also improve the response to injecting insulin after the meal.

Hypoglycemia Risk

Another potential advantage of UFIs (and RAIAs) is that they may also reduce the risk of late postprandial hypoglycemic events, i.e., that blood glucose declines toward low levels several hours after a meal due to the ongoing effect of the prandial insulin. In higher doses, the metabolic effect of, e.g., RHI can last for 6 or 8 h. This also increases the risk of insulin stacking; the metabolic effect of an insulin dose given, for example, along with breakfast adds to the effect of the dose applied for lunch. Patients counteracted such risky situations by eating a snack some hours after a meal. Such additional calorie intake might contribute to the weight gain associated with insulin therapy in a number of patients with diabetes.

What Is the Optimal Time-Action Profile of an Ultrafast-Acting Insulin?

This might sound trivial; however, in reality, this is a complex question. In the human body, insulin secretion is automatically adjusted essentially every second to the current requirements. These might change due to, for example, the meal composition; a meal consisting predominantly of rapidly absorbable carbohydrates not only requires more insulin for optimal coverage, but the timing is different in comparison with a situation in which a meal with less carbohydrates is eaten or the carbohydrates are complex and do not immediately influence blood glucose levels.

An insulin therapy that mimics the physiological insulin secretion profile in healthy subjects might be assumed to be ideal; however, this does not mean—which is stated quite often—that a plasma insulin concentration profile should be achieved as it is observed when collecting venous blood samples in healthy subjects after meals. These levels are 50–70% lower than the insulin levels that the liver sees in healthy subjects due to the fact that physiologic insulin is delivered into the portal vein and insulin exerts important metabolic effects during its "first pass" through the liver. Therefore, in patients with diabetes, not only do the systemic levels need to be higher to induce a comparable metabolic response of all insulin-sensitive organs in the body, but the timing has to be optimized. Thus, the onset of action should include a rapid signal to the liver to effect appropriate metabolic changes, followed by appropriately timed stimulation of peripheral glucose uptake.

The optimal duration of action is more tricky: If the duration of action is too short with a mixed meal, for example, blood glucose might be optimally covered in the first 90 to 120 min after insulin application/start of the meal; however, thereafter, the decline in metabolic activity might be so rapid that the ongoing glucose

absorption in the gut induces an increase in glycemia. This was at least an observation made in a meal-related study with nasal insulin.[65]

Thus, if the duration of action of a given UFI is too short, there might be a need for a second insulin administration with a given meal. This appears to be the case, for example, with TI (see the article by Boss and colleagues[30] in this special theme issue). **Table 3** includes PK and glucodynamic comparisons of RAIAs/RHIs and many of the UFIs discussed in this paper.

Other Implications of Ultrafast-Acting Insulins

A rapid onset of insulin action is also of high importance in a different setting. The development of an artificial pancreas (AP) that be used in daily life is hampered by the limitations of currently available insulin formulations. Beside other challenges in these investigations, including performance of today's continuous glucose monitoring (CGM) sensors (which measure interstitial, not capillary, glucose), the relatively slow (and inconsistent) kinetics of today's "rapid" insulin analogs—and the peripheral (versus intraportal) route of insulin administration—is a major one. In order to be able to establish good metabolic control by this approach, the applied insulin should induce ideally an instantaneous effect. In most attempts to develop an AP system, the insulin is applied in repeated small boluses by insulin pumps. It is questionable whether control algorithms can reproduce physiological metabolic control when SC analog insulin require up to 1 h to reach maximum concentration in the blood (T_{max}) and 90–120 min for maximum glucose-lowering effect ($tGIR_{max}$). There is a clear need for faster-acting insulin, for which the Juvenile Diabetes Research Foundation funded an UFI project in 2006 (*http://www.jdrf.org/files/General_Files/For_Scientists/Insulin_initiative_EOI.pdf*).

Discussion and Outlook

The presented list of attempts to develop an UFI is undoubtedly incomplete. Our level of understanding about the science behind these various developments differ; in some cases, a wealth of studies is available, also good review articles about the principle; in other cases, the available information is incomplete. Clearly, it is not only the time-action profile that may lead to widespread use of one or another of these potential offerings, but the issues related to regulatory approval and commercial aspects will play important roles in this evolving field. One of the reasons for the massive interest in UFIs by large insulin manufacturers is that their RAIA patents will expire in the not-distant future. Generic RAIAs may play an increasing role in the market as time passes.[76]

On the technical front, the reproducibility of the insulin effect after dosing of an UFI is also of relevance to optimize postprandial metabolic control. Interestingly enough, the number of studies about the intraindividual variability of absorption and action of insulin is relatively small. For example, no study has been performed to date to study the reproducibility of insulin bolus application with CSII. This is an aspect that is also of particular relevance for the development of a practically applicable AP. In some studies with UFIs, it was observed that the variability was smaller with a given UFI than with RAIA or RHI; however, it would be worth evaluating this more systematically.[60]

One thing is relatively simple to answer: Which is the best of the approaches? In other words, with which approach can the best optimization of postprandial metabolic control be achieved? The best way to demonstrate the benefits of UFIs in the short term is to employ good meal studies. Well-designed and well-executed meal studies, ideally in a head-to-head comparison of different UFIs will clearly answer this question. To perform such studies might look simple on the first glance; however, they are quite demanding in actual practice. Without taking care of all relevant aspects, the outcome of such studies is nearly meaningless. Unfortunately, most meal studies published thus far do not fulfill these expectations, e.g., they have not paid enough attention to the preprandial metabolic state. Without comparable (i.e., identical) glucose/insulin levels in the last hour(s) prior to the intervention (i.e., ingestion of the meal/insulin application), the validity of comparing the results obtained on different study days is compromised. With the UFIs presented here, some meal-related studies fulfilling these requirements have been performed demonstrating their benefits also in comparison with RAIAs (see the other related manuscripts[18,25,30,48,49,54]). One can envisage head-to-head comparisons involving two or more of the approaches presented in a given study under identical UFI conditions that would allow one to make clear statements about the pros and cons of the studied developments.

In clinical trials aiming to evaluate the relevance of an improvement in PPG by using a given UFI, the question is how to evaluate this in daily life. One option is to measure a 7- or even 10-point daily blood glucose profile; however, this might not be sufficient, as the best

Table 3.
Pharmacokinetic/Pharmacodynamic Characteristics of Prandial Insulins

Reference (first author)	Population (*N*)	Dose	Insulin exposure (PK, min)			Insulin action (GIR, min)		
			Onset (early $t_{50\%}$)	Peak (t_{max})	Offset (late $t_{50\%}$)	Onset (early $t_{50\%}$)	Peak (t_{max})	Offset (late $t_{50\%}$)
Non-UFIs (SC injection)								
Regular insulin								
Rave[66]	Healthy (17)	18 U		148		48	193	415
Heinemann[67]	Healthy (9)	0.2 U/kg		129		61	156	387
Becker[68]	T1DM (18)	0.15 U/kg	53 (Early $t_{10\%}$)	104	348 (Late $t_{90\%}$)	88 (Early $t_{10\%}$)	169	330 (Late $t_{90\%}$)
Becker[68]	T1DM (not specified)	0.2 U/kg		82		43	161	306 (Late $t_{80\%}$)
Lispro								
Rave[66]	Healthy (17)	18 U		148		41	137	313
Rave[69]	Healthy (20)	18 U		45		38	136	273
Rave[69]	Healthy (20)	12 U		45		38	112	248
Rave[69]	Healthy (20)	6 U		45		35	85	184
Heise[70]	Healthy (80)	0.2 U/kg	50 (early $t_{10\%}$)	76		87 (10%)	171	
Heise[70]	Healthy (80)	0.4 U/kg	54 (early $t_{10\%}$)	92		88 (10%)	198	
Becker[68]	T1DM (not specified)	0.2 U/kg		58		46	94	228 (late $t_{80\%}$)
Aspart								
Heinemann[67]	Healthy (10)	0.2 U/kg		70		41	104	264
Mudaliar[71]	Healthy (20)	0.2 U/kg		52			94	
Heinemann[67]	Healthy (10)	0.2 U/kg		48			104	
Glulisine								
Becker[68]	T1DM (18)	0.15 U/kg	31 (10%)	57	205 (90%)	45 (10%)	114	238 (90%)
Becker[68]	T1DM (not specified)	0.2 U/kg		51		34	98	218 (80%)
Becker[68]	Healthy (not specified)	0.1 U/kg		44		31	127	
Heise[70]	Healthy (80)	0.2 U/kg	44 (early $t_{10\%}$)	94		83 (early $t_{10\%}$)	190	
Heise[70]	Healthy (80)	0.4 U/kg	49 (early $t_{10\%}$)	100		85 (early $t_{10\%}$)	196	
Ultrafast prandial insulins								
Jet injection								
Lispro								
Sarno[45]	Healthy (4)	30 U		41			131	
Aspart								
Engwerda[46]	Healthy (18)	0.2 U/kg		31			51	
Heated patch								
Lispro								
Raz[24]	T1DM (17)	0.15 U/kg	20	45				

Continued →

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Pharmacokinetic/Pharmacodynamic Characteristics of Prandial Insulins

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			Onset (early $t_{50\%}$)	Peak (t_{max})	Offset (late $t_{50\%}$)	Onset (early $t_{50\%}$)	Peak (t_{max})	Offset (late $t_{50\%}$)
Hyaluronidase								
Regular insulin								
Morrow[60]	Healthy	0.15 U/kg	22	63	137			
Hompesch[57]	T1DM	Individualized		57				
Hompesch[58]	T2DM	Individualized (mean=28.7 U)	21	82	220			
Lispro								
Morrow[60]	Healthy	0.15 U/kg	14	40	88			
Hompesch[57]	T1DM	Individualized		30				
Hompesch[58]	T2DM	Individualized (mean=27.3 U)	19	43	124			
Excipients (citrate + ethylenediaminetetraacetic acid)								
Regular insulin								
Steiner[50]	Healthy (10)	12 U	26	66	170	51	152	295
Heinemann[53]	T1DM (43)	12 U	8	29	143	28	138	285
Inhaled (TI)								
Regular insulin								
Rave[72]	T2DM (12)	24 U		17	51		79	
Rave[33]	Healthy (12)	25 U		12	45		42	
Rave[33]	Healthy (12)	50 U		15	42		50	
Rave[33]	Healthy (12)	100 U		17	50		58	
Oral insulin								
Regular insulin								
Luzio[73]	T2DM (16)	150 U		156			198	
Luzio[73]	T2DM (16)	300 U		214			242	
Buccal insulin								
Lispro								
Cernea[74]	Healthy (6)	150 U		23		29	44	101
Cernea[75]	T1DM (6)	50 U		27		23	40	57
Cernea[75]	T1DM (6)	100 U		29		28	46	70
Cernea[75]	T1DM (6)	200 U		23		31	44	76
Microneedle								
Lispro								
Pettis[28]	Healthy (10)	10 U 1.25 mm needle	8	36	130	29	106	287
Pettis[28]	Healthy (10)	10 U 1.5 mm needle	9	41	134	31	108	282

Continued →

Table 3.
Pharmacokinetic/Pharmacodynamic Characteristics of Prandial Insulins

Reference (first author)	Population (*N*)	Dose	Insulin exposure (PK, min)			Insulin action (GIR, min)		
			Onset (early $t_{50\%}$)	Peak (t_{max})	Offset (late $t_{50\%}$)	Onset (early $t_{50\%}$)	Peak (t_{max})	Offset (late $t_{50\%}$)
Pettis[28]	Healthy (10)	10 U 1.75 mm needle	10	46	139	35	112	271
Pettis[28]	T1DM (29)	0.125 U/kg 1.5 mm needle	12	30	87			
Gupta[29]	T1DM (5)	Individualized 6-15 U, 0.9 mm needle		27				

time point to measure PPG is not clear. Most probably, there is no ideal point in time. One would like to catch the peak PPG; however, this depends on a number of factors. Composition of the meal, for example, might be different between certain patient groups (e.g., subjects with gastroparesis), so the simple recommendation to measure 2 h PPG would not reveal full truth. An alternate approach is the use of CGM systems. Usage of CGM systems blinded to the study subject allows observation of PPG in daily life, whereas the use of unblinded CGM itself has a marked effect on diabetes control.[77,78] In any case, the recorded 24 h glucose profiles are not easy to evaluate, and analytic tools are only now being refined and agreed upon[79] (see also the FDA guidance for the development of AP systems *http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/HomeHealthandConsumer/ConsumerProducts/ArtificialPancreas/default.htm*).

To discuss the optimal way to evaluate PPG and the effect different UFIs have from a scientific point of view is one thing; however, the question for the manufacturers is more: what is the view of the regulatory authorities on this topic, i.e., what kind of studies do they expect to see? It might also be that the regulatory view and the scientific view are different. Other end points that are of relevance in such clinical trials are clearly weight gain, reduction in hypoglycemic events, and quality of life. Additionally, safety aspects are of major concern, i.e., one of the major concerns with inhaled insulin was the risk of developing more lung cancer cases, and an important unanswered question is the relevance of PPG control on cardiovascular risk apart from overall HbA1c control. Adherence of a patient to the prescribed therapy in practice is also a major challenge, i.e., in the case of insulin, many patients are unwilling or reluctant to begin insulin therapy because of fear of side effects as well as the inconvenience of multiple daily injections.

Another option for improving PPG might be the invention of smart insulins. Bringing a large insulin depot into the human body that is attached to glucose-sensitive molecules might allow mimicking physiological insulin secretion in an ideal manner if the kinetics of glucose binding and subsequent insulin release is appropriate. However, all respective developments are in the early phases, and clearly the safety risks associated will need to be carefully evaluated in appropriate studies.

Another question is whether or not the use of UFIs benefits each patient group/indication. This is not of doubt in (most) patients with T1DM and when it comes to the use of an AP system; however, it might be that the use of UFIs is not of benefit in (many) patients with T2DM. For example, in obese subjects with T2DM, absorption of insulin from the SC depot might be so delayed, even with UFIs applied, that, with inhalation of a UFI, a better coverage of prandial insulin requirements is possible.

In summary, with the invention of "UFI," (further) optimization of postprandial metabolic control can be assumed to be possible by improving the synchronization between the postprandial action of prandial insulin and the PPG dynamics. This may be more readily achieved with the parallel introduction of improved, "flatter" basal insulins. However, it remains to be proven in appropriately designed randomized controlled trials and also in clinical practice whether the invention of UFIs is a true step forward in controlling PPG or not.

How long it will take for one of the different UFIs in development to become available as a product is difficult to say; at least for two, the dossiers have been submitted to regulatory authorities (MannKind, Biodel), and others are more or less advanced in the development process and can also become available in the next few years.

We have to acknowledge that good progress has been made with UFIs and that, most probably, the "portfolio" to cover prandial insulin requirements better than this was possible will become bigger quite soon. To what extent this can be converted into improved long-term metabolic control (i.e., reduced glycated hemoglobin) remains to be seen. It is also not clear right now what the market uptake/success of the different attempts will be; many factors such as costs (i.e., adequate health insurance coverage and access), safety, and patient preferences/acceptance (practicability, handling efforts) will have a major impact on that. To demonstrate a more appropriate time-action profile is not sufficient in this sense. We believe that UFIs can improve diabetes care, but only time will tell which UFI program will safely deliver better metabolic control and lower the risk of hypoglycemic events in patients with diabetes.

Disclosures:

Lutz Heinemann is consultant for a number of companies that develop novel diagnostic and therapeutic options for diabetes treatment. He is partner and scientific consultant of Profil Institut für Stoffwechselforschung in Neuss, Germany, and Profil Institut for Clinical Research in San Diego, CA. Douglas B. Muchmore is an employee of and stockholder in Halozyme Therapeutics, a company that is actively pursuing development of UFI products.

References:

1. Polonsky KS, Given BD, Van Cauter E. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. J Clin Invest. 1988;81(2):442–8.
2. Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). Diabetes Care. 2003;26(3):881–5.
3. Monnier L, Colette C, Boniface H. Contribution of postprandial glucose to chronic hyperglycaemia: from the "glucose triad" to the trilogy of "sevens". Diabetes Metab. 2006;32 Spec No2:2S11–6.
4. Siegelaar SE, Holleman F, Hoekstra JB, DeVries JH. Glucose variability; does it matter? Endocr Rev. 2010;31(2):171–82.
5. De Vegt F, Dekker JM, Ruhé HG, Stehouwer CD, Nijpels G, Bouter LM, Heine RJ. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. Diabetologia. 1999;42(8):926–31.
6. Temelkova-Kurktschiev TS, Koehler C, Henkel E, Leonhardt W, Fuecker K, Hanefeld M. Postchallenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis than fasting glucose or HbA1c level. Diabetes Care. 2000;23(12):1830–4.
7. McCarter RJ, Hempe JM, Chalew SA. Mean blood glucose and biological variation have greater influence on HbA1c levels than glucose instability: an analysis of data from the Diabetes Control and Complications Trial. Diabetes Care. 2006;29(2):352–5.
8. Kilpatrick ES, Rigby AS, Atkin SL. The effect of glucose variability on the risk of microvascular complications in type 1 diabetes. Diabetes Care. 2006;29(7):1486–90.
9. Kilpatrick ES. Arguments for and against the role of glucose variability in the development of diabetes complications. J Diabetes Sci Technol. 2009;3(4):649–55.
10. Kilpatrick ES, Rigby AS, Atkin SL. Effect of glucose variability on the long-term risk of microvascular complications in type 1 diabetes. Diabetes Care. 2009;32(10):1901–3.
11. Raz I, Wilson PW, Strojek K, Kowalska I, Bozikov V, Gitt AK, Jermendy G, Campaigne BN, Kerr L, Milicevic Z, Jacober SJ. Effects of prandial versus fasting glycemia on cardiovascular outcomes in type 2 diabetes: the HEART2D trial. Diabetes Care. 2009;32(3):381–6.
12. Supersaxo A, Hein WR, Steffen H. Effect of molecular weight on the lymphatic absorption of water-soluble compounds following subcutaneous administration. Pharm Res. 1990;7(2):167–9.
13. Brange J, Owens DR, Kang S, Volund A. Monomeric insulins and their experimental and clinical implications. Diabetes Care. 1990;13(9):923–54.
14. Brange J, Ribel U, Hansen JF, Dodson G, Hansen MT, Havelund S, Melberg SG, Norris F, Norris K, Snel L. Monomeric insulins obtained by protein engineering and their medical implications. Nature. 1988;333(6174):679–82.
15. Heinemann L, Heise T, Wahl LC, Trautmann ME, Ampudia J, Starke AA, Berger M. Prandial glycaemia after a carbohydrate-rich meal in type I diabetic patients: using the rapid acting insulin analogue [Lys(B28), Pro(B29)] human insulin. Diabet Med. 1996;13(7):625–9.
16. Cobry E, McFann K, Messer L, Gage V, VanderWel B, Horton L, Chase HP. Timing of meal insulin boluses to achieve optimal postprandial glycemic control in patients with type 1 diabetes. Diabetes Technol Ther. 2010;12(3):173–7.
17. Luijf YM, van Bon AC, Hoekstra JB, Devries JH. Premeal injection of rapid-acting insulin reduces postprandial glycemic excursions in type 1 diabetes. Diabetes Care. 2010;33(10):2152–5.
18. Freckmann G, Pleus S, Haug C, Bitton G, Nagar R. Increasing local blood flow by warming the application site: beneficial effects on postprandial glycemic excursions J Diabetes Sci Technol. 2012;6(4):780–5.
19. Dillon RS. Improved serum insulin profiles in diabetic individuals who massaged their insulin injection sites. Diabetes Care. 1983;6(4):399–401.
20. Kellogg DL Jr. In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. J Appl Physiol. 2006;100(5):1709–18.
21. Minson CT, Berry LT, Joyner MJ. Nitric oxide and neurally mediated regulation of skin blood flow during local heating. J Appl Physiol. 2001;91(4):1619–26.
22. Koivisto VA. Sauna-induced acceleration in insulin absorption. Br Med J. 1980;281(6240):621–2.
23. Sindelka G, Heinemann L, Berger M, Frenck W, Chantelau E. Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous insulin absorption in healthy subjects. Diabetologia. 1994;37(4):377–80.
24. Raz I, Weiss R, Yegorchikov Y, Bitton G, Nagar R, Pesach B. Effect of a local heating device on insulin and glucose pharmacokinetic profiles in an open-label, randomized, two-period, one-way crossover study in patients with type 1 diabetes using continuous subcutaneous insulin infusion. Clin Ther. 2009;31(5):980–7.
25. McVey E, Hirsch L, Sutter DE, Kapitza C, Dellweg S, Clair J, Rebrin K, Judge K, Pettis RJ. Pharmacokinetics and postprandial glycemic excursions following insulin lispro delivered by intradermal microneedle or subcutaneous infusion. J Diabetes Sci Technol. 2012;6(4):743–54.

26. Harvey AJ, Kaestner SA, Sutter DE, Harvey NG, Mikszta JA, Pettis RJ. Microneedle-based intradermal delivery enables rapid lymphatic uptake and distribution of protein drugs. Pharm Res. 2011;28(1):107–16.
27. Pettis RJ, Ginsberg B, Hirsch L, Sutter D, Keith S, McVey E, Harvey NG, Hompesch M, Nosek L, Kapitza C, Heinemann L. Intradermal microneedle delivery of insulin lispro achieves faster insulin absorption and insulin action than subcutaneous injection. Diabetes Technol Ther. 2011;13(4):435–42.
28. Pettis RJ, Hirsch L, Kapitza C, Nosek L, Hovelmann U, Kurth HJ, Sutter DE, Harvey NG, Heinemann L. Microneedle-based intradermal versus subcutaneous administration of regular human insulin or insulin lispro: pharmacokinetics and postprandial glycemic excursions in patients with type 1 diabetes. Diabetes Technol Ther. 2011;13(4):443–50.
29. Gupta J, Felner EI, Prausnitz MR. Rapid pharmacokinetics of intradermal insulin administered using microneedles in type 1 diabetes subjects. Diabetes Technol Ther. 2011;13(4):451–6.
30. Boss AH, Petrucci R, Lorber D. Coverage of prandial insulin requirements by means of an ultra-rapid-acting inhaled insulin. J Diabetes Sci Technol. 2012;6(4):773–9.
31. Heinemann L, Heise T. Current status of the development of inhaled insulin. Br J Diab Vasc Dis. 2004;4:295–301.
32. Richardson PC, Boss AH. Technosphere insulin technology. Diabetes Technol Ther. 2007;9 Suppl 1:S65–72.
33. Rave K, Potocka E, Heinemann L, Heise T, Boss AH, Marino M, Costello D, Chen R. Pharmacokinetics and linear exposure of AFRESA compared with the subcutaneous injection of regular human insulin. Diabetes Obes Metab. 2009;11(7):715–20.
34. Rave K, Heise T, Pfutzner A, Boss AH. Coverage of postprandial blood glucose excursions with inhaled technosphere insulin in comparison to subcutaneously injected regular human insulin in subjects with type 2 diabetes. Diabetes Care. 2007;30(9):2307–8.
35. Rosenstock J, Lorber DL, Gnudi L, Howard CP, Bilheimer DW, Chang PC, Petrucci RE, Boss AH, Richardson PC. Prandial inhaled insulin plus basal insulin glargine versus twice daily biaspart insulin for type 2 diabetes: a multicentre randomised trial. Lancet. 2010;375(9733):2244–53.
36. Heinemann L, Jacques Y. Oral insulin and buccal insulin: a critical reappraisal. J Diabetes Sci Technol. 2009;3(3):568–84.
37. Stote R, Marbury T, Shi L, Miller M, Strange P. Comparison pharmacokinetics of two concentrations (0.7% and 1.0%) of Nasulin, an ultra-rapid-acting intranasal insulin formulation. J Diabetes Sci Technol. 2010;4(3):603–9.
38. Stote R, Miller M, Marbury T, Shi L, Strange P. Enhanced absorption of Nasulin™, an ultrarapid-acting intranasal insulin formulation, using single nostril administration in normal subjects. J Diabetes Sci Technol. 2011;5(1):113–9.
39. Hingson RA, Hughes JG. Clinical studies with jet injection; a new method of drug administration. Curr Res Anesth Analg. 1947;26(6):221–30.
40. Weller C, Linder M. Jet injection of insulin vs the syringe-and-needle method. JAMA. 1966;195(10):844–7.
41. Taylor R, Home PD, Alberti KG. Plasma free insulin profiles after administration of insulin by jet and conventional syringe injection. Diabetes Care. 1981;4(3):377–9.
42. Bremseth DL, Pass F. Delivery of insulin by jet injection: recent observations. Diabetes Technol Ther. 2001;3(2):225–32.
43. Malone JI, Lowitt S, Grove NP, Shah SC. Comparison of insulin levels after injection by jet stream and disposable insulin syringe. Diabetes Care. 1986;9(6):637–40.
44. Theintz GE, Sizonenko PC. Risks of jet injection of insulin in children. Eur J Pediatr. 1991;150(8):554–6.
45. Sarno MJ, Bell J, Edelman SV. Pharmacokinetics and glucodynamics of rapid-, short-, and intermediate-acting insulins: comparison of jet injection to needle syringe. Diabetes Technol Ther. 2002;4(6):863–6.
46. Engwerda EE, Abbink EJ, Tack CJ, de Galan BE. Improved pharmacokinetic and pharmacodynamic profile of rapid-acting insulin using needle-free jet injection technology. Diabetes Care. 2011;34(8):1804–8.
47. Edsberg B, Herly D, Hildebrandt P, Kühl C. Insulin bolus given by sprinkler needle: effect on absorption and glycaemic response to a meal. Br Med J (Clin Res Ed). 1987;294(6584):1373–6.
48. Pohl R, Hauser R, Li M, DeSouza E, Feldstein R, Seibert R, Ozhan K, Kashyap N, Steiner S. Ultra-rapid absorption of recombinant human insulin induced by zinc chelation and surface charge masking. J Diabetes Sci Technol. 2012;6(4):755–63.
49. Krasner A, Pohl R, Simms P, Pichotta P, Hauser R, De Souza E. A review of a family of ultra-rapid-acting insulins: formulation development. J Diabetes Sci Technol. 2012;6(4):786–96.
50. Steiner S, Hompesch M, Pohl R, Simms P, Flacke F, Mohr T, Pfutzner A, Heinemann L. A novel insulin formulation with a more rapid onset of action. Diabetologia. 2008;51(9):1602–6.
51. Hompesch M, McManus L, Pohl R, Simms P, Pfutzner A, Bulow E, Flacke F, Heinemann L, Steiner SS. Intra-individual variability of the metabolic effect of a novel rapid-acting insulin (VIAject) in comparison to regular human insulin. J Diabetes Sci Technol. 2008;2(4):568–71.
52. Heinemann L, Hompesch M, Flacke F, Simms P, Pohl R, Albus K, Pfützner A, Steiner S. Reduction of postprandial glycemic excursions in patients with type 1 diabetes: a novel human insulin formulation versus a rapid-acting insulin analog and regular human insulin. J Diabetes Sci Technol. 2011;5(3):681–6.
53. Heinemann L, Nosek L, Flacke F, Albus K, Krasner A, Pichotta P, Heise T, Steiner S. U-100, pH-Neutral formulation of VIAject(®): faster onset of action than insulin lispro in patients with type 1 diabetes. Diabetes Obes Metab. 2012;14(3):222–7.
54. Muchmore DB, Vaughn DE. Accelerating and improving the consistency of rapid-acting analog insulin absorption and action for both subcutaneous injection and continuous subcutaneous infusion using recombinant human hyaluronidase. J Diabetes Sci Technol. 2012;6(4):764–72.
55. Bookbinder LH, Hofer A, Haller MF, Zepeda ML, Keller GA, Lim JE, Edgington TS, Shepard HM, Patton JS, Frost GI. A recombinant human enzyme for enhanced interstitial transport of therapeutics. J Control Release. 2006;114(2):230–41.
56. Vaughn DE, Yocum RC, Muchmore DB, Sugarman BJ, Vick AM, Bilinsky IP, Frost GI. Accelerated pharmacokinetics and glucodynamics of prandial insulins injected with recombinant human hyaluronidase. Diabetes Technol Ther. 2009;11(6):345–52.
57. Hompesch M, Muchmore DB, Morrow L, Vaughn DE. Accelerated insulin pharmacokinetics and improved postprandial glycemic control in patients with type 1 diabetes after coadministration of prandial insulins with hyaluronidase. Diabetes Care. 2011;34(3):666–8.
58. Hompesch M, Muchmore DB, Morrow L, Ludington E, Vaughn DE. Improved postprandial glycemic control in patients with type 2 diabetes from subcutaneous injection of insulin lispro with hyaluronidase. Diabetes Technol Ther. 2012;14(3):218–24.
59. Vaughn DE, Muchmore DB. Use of recombinant human hyaluronidase to accelerate rapid insulin analogue absorption: experience with subcutaneous injection and continuous infusion. Endocr Pract. 2011;17(6):914–21.

60. Morrow L, Muchmore DB, Ludington EA, Vaughn DE, Hompesch M. Reduction in intrasubject variability in the pharmacokinetic response to insulin after subcutaneous co-administration with recombinant human hyaluronidase in healthy volunteers. Diabetes Technol Ther. 2011;13(10):1039–45.

61. Phillips NB, Whittaker J, Ismail-Beigi F, Weiss MA. Insulin fibrillation and protein design: Topological resistance of single-chain analogs to thermal degradation with application to a pump reservoir. J Diabetes Sci Technol. 2012;6(2):277–88.

62. Heinemann L, Overmann H, Mühlhauser I. How do patients with diabetes mellitus type I handle the injection-meal interval in daily life? Diabetes. 1997;46 Suppl 1:340A.

63. Overmann H, Heinemann L. Injection-meal interval: recommendations of diabetologists and how patients handle it. Diabetes Res Clin Pract. 1999;43(2):137–42.

64. Heinemann L, Hohmann A, Starke AAR, Berger M. What is the correct timing of subcutaneous injections of regular insulin to cover carbohydrate rich meals? Horm Metab Res Suppl. 1992;26:137–9.

65. Coates PA, Ismail IS, Luzio SD, Griffiths I, Ollerton RL, Volund A, Owens DR. Intranasal insulin: the effects of three dose regimens on postprandial glycaemic profiles in type II diabetic subjects. Diabet Med. 1995;12(3):235–9.

66. Rave K, Bott S, Heinemann L, Sha S, Becker RH, Willavize SA, Heise T. Time-action profile of inhaled insulin in comparison with subcutaneously injected insulin lispro and regular human insulin. Diabetes Care. 2005;28(5):1077–82.

67. Heinemann L, Weyer C, Rauhaus M, Heinrichs S, Heise T. Variability of the metabolic effect of soluble insulin and the rapid-acting insulin analog insulin aspart. Diabetes Care. 1998;21(11):1910–4.

68. Becker RH, Frick AD. Clinical pharmacokinetics and pharmacodynamics of insulin glulisine. Clin Pharmacokinet. 2008;47(1):7–20.

69. Rave KM, Nosek L, de la Peña A, Seger M, Ernest CS 2nd, Heinemann L, Batycky RP, Muchmore DB. Dose response of inhaled dry-powder insulin and dose equivalence to subcutaneous insulin lispro. Diabetes Care. 2005;28(10):2400–5.

70. Heise T, Nosek L, Spitzer H, Heinemann L, Niemoller E, Frick AD, Becker RH. Insulin glulisine: a faster onset of action compared with insulin lispro. Diabetes Obes Metab. 2007;9(5):746–53.

71. Mudaliar SR, Lindberg FA, Joyce M, Beerdsen P, Strange P, Lin A, Henry RR. Insulin aspart (B28 asp-insulin): a fast-acting analog of human insulin: absorption kinetics and action profile compared with regular human insulin in healthy nondiabetic subjects. Diabetes Care. 1999;22(9):1501–6.

72. Rave K, Heise T, Heinemann L, Boss AH. Inhaled Technosphere insulin in comparison to subcutaneous regular human insulin: time action profile and variability in subjects with type 2 diabetes. J Diabetes Sci Technol. 2008;2(2):205–12.

73. Luzio SD, Dunseath G, Lockett A, Broke-Smith TP, New RR, Owens DR. The glucose lowering effect of an oral insulin (Capsulin) during an isoglycaemic clamp study in persons with type 2 diabetes. Diabetes Obes Metab. 2010;12(1):82–7.

74. Cernea S, Kidron M, Wohlgelernter J, Modi P, Raz I. Comparison of pharmacokinetic and pharmacodynamic properties of single-dose oral insulin spray and subcutaneous insulin injection in healthy subjects using the euglycemic clamp technique. Clin Ther. 2004;26(12):2084–91.

75. Cernea S, Kidron M, Wohlgelernter J, Modi P, Raz I. Dose-response relationship of oral insulin spray in healthy subjects. Diabetes Care. 2005;28(6):1353–7.

76. Heinemann L, Hompesch M. Biosimilar insulins: how similar is similar? J Diabetes Sci Technol. 2011;5(3):741–54.

77. Muchmore D, Sharp M, Vaughn D. Benefits of blinded continuous glucose monitoring during a randomized clinical trial. J Diabetes Sci Technol. 2011;5(3):676–80.

78. Bergenstal RM, Tamborlane WV, Ahmann A, Buse JB, Dailey G, Davis SN, Joyce C, Perkins BA, Welsh JB, Willi SM, Wood MA. Sensor-augmented pump therapy for A1C reduction (STAR 3) study: results from the 6-month continuation phase. Diabetes Care. 2011;34(11):2403–5.

79. Heinemann L. Continuous glucose monitoring and clinical trials. J Diabetes Sci Technol. 2009;3(4):981–5.