ONE-HUNDRED YEAR EVOLUTION OF PRANDIAL INSULIN PREPARATIONS: FROM ANIMAL PANCREAS EXTRACTS TO RAPID-ACTING ANALOGS

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SUMMARY

The first insulin preparation injected in humans in 1922 was short-acting, extracted from animal pancreas, contaminated by impurities. Ever since the insulin extracted from animal pancreas has been continuously purified, until an unlimited synthesis of regular human insulin (RHI) became possible in the ‘80s using the recombinant-DNA (rDNA) technique. The rDNA technique then led to the designer insulins (analogs) in the early ‘90s. Rapid-acting insulin analogs were developed to accelerate the slow subcutaneous (sc) absorption of RHI, thus lowering the 2-hour post-prandial plasma glucose (PP.PG) and risk for late hypoglycemia as comparing with RHI. The first rapid-acting analog was lispro (in 1996), soon followed by aspart and glulisine. Rapid-acting analogs are more convenient than RHI: they improve early PP-PG, and 24-h PG and A1C as long as basal insulin is also optimized; they lower the risk of late PP hypoglycemia and they allow a shorter time-interval between injection and meal. Today rapid-acting analogs are the gold standard prandial insulins. Recently, even faster analogs have become available (faster aspart, ultra-rapid lispro) or are being studied (Biochaperone lispro), making additional gains in lowering PP-PG. Rapid-acting analogs are recommended in all those with type 1 and type 2 diabetes who need prandial insulin replacement.

INTRODUCTION

We are already in the 100th year of the insulin era, but only quite recently has insulin therapy been based on the physiology of the normal pancreas to mimic the dynamics of insulin secretion. The modern era of physiological insulin therapy began in the ‘80s, after clarification of the complex regulation mechanisms of the glucose homeostasis system [reviewed in (1)], which opened the door to the milestone studies DCCT/EDIC and the first solid evidence of the benefits of long-term near-normoglycemia (2, 3).

Insulin replacement is a challenging task both in the inter-prandial fasting state (controlled by “basal” insulin), and at mealtime when rapid-acting insulin must to be provided. Improving post-prandial glucose (PP-PG) control with rapid-acting insulin, however, is especially difficult, more difficult than optimizing the fasting state with basal insulin. At mealtime the sc tissue delays the absorption of injected insulin, thus slowing down its physiological, rapid increase in serum, the key factor in normal PP-PG. In addition, other confounding factors, such as the quantity, quality and composition of the food ingested, gastrointestinal motility, and the dynamics of enteric glucose absorption are complex variables [reviewed in (4)] which make sc insulin replacement at mealtime especially challenging.

Rapid-acting insulin analogs are important tools to optimize PP-PG control because they accelerate the sc insulin absorption at mealtime. In addition to efficacy, these analogs offer greater flexibility and safety, i.e., lower risk for late PP hypoglycemia than regular human insulin (RHI). Today they are the gold standard insulins for mealtime.

This review focuses primarily on rapid-acting insulin analogs as ideal insulin preparations at mealtime and discusses the need to optimize at the same time the replacement of basal insulin to improve the full 24 hour PG and A1C.

FROM RAPID-ACTING INSULIN EXTRACTED FROM ANIMAL PANCREAS TO SYNTHESIS OF HUMAN INSULIN

The insulin extracted from animal pancreas in Toronto in 1922 for use in humans was “short-acting”. The main problem at that time was the stringent need to expand insulin production for the millions of people who needed it for survival and to purify insulin preparation to avoid toxic, sometimes severe, reactions.

On 11 January 1922, in Toronto, Banting’s pancreatic extract was injected sc into Leonard Thompson, a 14-year-old boy with recent-onset diabetes and in poor conditions (cachexia, DKA). Two separate administrations of 7.5 mL were made sc, with only marginal PG decrease, however, and followed causing induration at the site of one of the injections. It was the pioneering work of James Bertram Collip in purifying the pancreatic extract which made it possible to inject Leonard Thompson again on 23 January, this time with success. Since then, purification of animal-extracted insulin by removing contaminants, primarily protein material, has been a continuously improving process over decades, until in the early ‘80s recombinant-DNA (rDNA) technology led to the synthesis of human insulin (HI).

In 1922 early collaboration with the Eli Lilly Company led to improved extraction procedures. George Walden, the head chemist of the company, discovered iso-electric precipitation which greatly contributed to both yield and purity. Abel in 1926 was the first to crystallise insulin, thereby improving its purity (5). Then Scott, on realising that crystallisation occurs in the presence of zinc ions, introduced zinc into the extraction

process, with several re-crystallisation steps increasing purity to 80-90% (6). However, the advent of radio-immunoassays (RIA) at the end of ‘50s revealed the continuing presence of insulin antibodies in all patients receiving re-crystallised insulin (7, 8). Although insulin antibodies were rarely responsible for clinically relevant insulin resistance, they still interfered with the pharmacokinetics (PK) and pharmacodynamics (PD) of injected insulin and contributed to poor glycemic control and the risk of hypoglycemia (9-11). Impurities, and not insulin itself, were later demonstrated to be primarily responsible for generating insulin antibodies (12, 13). Consequently, gel filtration and anion-exchange chromatographic purification steps were introduced, and in the ‘70s the resulting highly purified monocomponent (MC) insulins reached levels of contaminants at or below detection limits.

Inadequate supplies of animal insulin for therapeutic purposes stimulated attempts to synthesise HI beginning in the 1970s using a variety of methods. In 1978 HI was synthesized from porcine insulin using a transpeptidase reaction which substituted the B^{30} alanine residue at the C-terminus of the porcine insulin with a threonine residue characteristic of the HI molecule (14-16). This manufacturing process was abandoned with the development of the rDNA technology that has the main advantage of being an unlimited supply process of HI production (17). The rDNA technique was at the same time instrumental in enabling selectively modification of the HI molecule at critical points to alter the kinetics of sc absorption after injection. These modified HI molecules, named “designer insulins”, gave rise to the insulin analogs in use today- the rapid-acting analogs for mealtime and the long-acting analogs for the insulin needs in the fasting state (18). The availability of monocomponent (MC) insulins and ultimately HI resulted in a continuing decline and virtual disappearance of insulin allergy and titers of insulin antibodies among insulin treated patients.

PHYSIOLOGY OF PRANDIAL GLUCOSE HOMEOSTASIS

Insulin is produced and released by the beta-cells of the pancreatic islets which sense the ambient arterial glucose, the primary driver of secretion, in a highly sensitive manner. In the prandial state, in addition to other factors, primarily glucose from a meal and the incretin system sustain insulin secretion (1). Fine tuning of insulin secretion over 24 h maintains the PG concentration within a narrow range, usually not above 7.0 mmol/L (126 mg/dL) after meals, even if large and rich in CHO, and not below 3.9 mmol/L (70 mg/dL), after an overnight fast (Figure 1) (19).

The administration of rapid-acting insulin at mealtime to people with diabetes should mimic that physiology. Insulin is secreted in cycling pulses (20) directly into the portal vein primarily to enrich the liver. The portal insulin-to-glucagon ratio continuously modulates minute-to-minute endogenous glucose production in the fasting state. This is also the case in response to a meal, when the large amount of insulin secreted increases the portal insulin-to-glucagon ratio, with timely suppression of endogenous glucose output, the key mechanism of PP-PG homeostasis (21). Because about 50% of the insulin secreted is extracted by the liver both in the fasting and PP state, there is a porto-systemic insulin gradient, with serum insulin concentration 2.4-4-fold lower in peripheral circulation as compared to portal circulation (22-24) (Figure 2). Thus, physiologically, hyperinsulinemia in portal plasma reflects the high insulin need of the liver when modulating endogenous glucose output.

The fast increase of insulin concentration we see in response to meal ingestion culminates in an early peak (Figures 1 and 2) which highlights the physiological importance of rapid insulinization of the liver and peripheral muscle, the two organs which harmoniously cooperate to limit PP-PG.

THE LIMITATIONS OF PRESENT INSULIN REPLACEMENT

Today the strategy of insulin replacement benefits from our advanced knowledge of physiology. After 100 years of the insulin era, however, insulin therapy is still limited, primarily by the route of insulin delivery, in addition to other factors.

Peripheral rather than portal insulin delivery

We still inject insulin with a needle in a non-physiological site, the sc tissue, which is a barrier to insulin absorption and drains into peripheral, non-portal circulation. With peripheral injection, we aim at providing insulin to the most insulin-avid organ of the body, the liver. This is possible only via systemic circulation and the hepatic artery, and only as long as the arterial insulin concentration is increased 3.0-4.5 times higher to the values of the portal blood (22-24). This leads to therapeutic hyperinsulinemia in insulin-treated people with diabetes, which may have adverse metabolic effects, such as excessive suppression of lipolysis with fat deposition and adiposity, and insulin resistance, primarily in the muscle.

Subcutaneous rather than intravenous insulin delivery

Purified pork and HI molecules have a high tendency for self-association to form hexamers in the presence of the ligand zinc in vials and pens. However, insulin is absorbed sc primarily as dimers and to a greater extent, as monomers. Thus, after sc injection, insulin absorption initiates after the hexamers dissociate as a consequence of lower insulin concentration into monomers. This process is slow and evolves over several hours. As a result, with sc injection of RHI at mealtime, insulin increases in serum slowly and plateaus with no peak (25). This under-insulinization of the liver and

muscle in the early PP phase leads to an excessive increase in PP-PG (18, 25). Hours later post-injection, continuing insulin absorption from the sc site, maintains serum insulin at too high level when meal glucose is nearly completely absorbed, creating a risk of hypoglycemia. These events are illustrated in Figure 3 (25) when pork insulin (with a high propensity to self-aggregate in hexamers similar to that of RHI) was injected at mealtime. Insulin injection 30 and 60 min before a meal, resulted in an increase in serum insulin before intestinal glucose absorption so that PP-PG was lower and the risk for late hypoglycemia reduced. This pioneering observation was the basis for the recommendation in the ‘80s to inject RHI 10-40 min before the meal, an approach that is neither practical nor convenient for people (26, 27).

THE FIRST-GENERATION RAPID-ACTING INSULIN ANALOGS

Physiologically, the tendency of insulin to self-associate seems appropriate in a normal beta cell, since it facilitates proinsulin transportation, conversion and intracellular storage of insulin microcrystals (28), this is not the case with insulin given sc at mealtime (25).

Biotechnology in the late ‘70s and early ‘80s opened a new area of research with the introduction of insulin analogs (29). One amino-acid modification could lead to changes in the tridimensional structure of the insulin molecule and to major alterations in its biological properties (30). The fact that insulin in concentrated neutral solution associates into dimers and hexamers (31) combined with the observation of a lag-phase in the sc absorption of soluble insulin (32, 33) gave rise to the hypothesis that a reduced propensity to self-associate might lead to faster absorption (29). The approaches used in the creation of insulin molecules with low propensity to self-associate were charge repulsion (AspB28; AspB9,GluB27; GluB28,AspA21),

decrease interface hydrophobicity (GluB16,GluB27) and interference with hydrophobic contacts and beta-sheet formation (LysB28,ProB29) (29, 34, 35).

There are three rapid-acting analogs on the market with similar characteristics of PK and PD. Insulin lispro is obtained by inverting the sequence B28,B29 of the amino-acids (proline and lysine) of HI into B28-lysine and B29-proline. Insulin aspart is obtained by replacing the proline in B28 of HI with the negatively charged aspartic acid. Glulisine has two amino-acid substitutions in the B chain of HI, one in in the B3 position (lysine in place of asparagine) and the other in B29 (glutamic acid in place of lysine).

Insulin lispro (LysB28, ProB29-human insulin)

An important clue for the modification that led to insulin lispro was inspired by the work with insulin-like growth factor-I (IGF-I) (36). This hormone does not self-associate even though it is highly homologous to insulin. In IGF-I, the normally occurring Pro-Lys sequence in insulin at position B28 and B29 is reversed to Lys-Pro. The hypothesis was raised and then experimentally proven that if the Lys-Pro sequence renders IGF-I incapable of self-association, the inversion of the Pro-Lys sequence in insulin would generate an insulin analog also incapable of self-association (35). In fact, lispro has a weakened propensity to self-associate into dimers. Contrary to the prevailing understanding of monomeric analogues, both RHI and lispro exist in their respective pharmaceutical formulations as hexamers. The lispro formulation, however, differs in that its hexamer complex dissociates into monomers nearly instantaneously upon injection in the sc tissue, resulting in a plasma absorption profile indistinguishable from that of a pure monomeric insulin (37).

Insulin Aspart (Asp-B28-human insulin)

The degree of self-association of insulin aspart into hexamers in the presence of the ligand zinc decreases rapidly upon sc injection, and fast dissociation into dimers and monomers is similar to lispro. In fact, the main subcutaneous mechanism of rapid dissociation of both lispro and aspart products is the sudden decrease of insulin concentration which weakens the propensity to self-aggregate of the two analogs. The absorption rate of insulin aspart is not different from a purely monomeric analog such as AspB9,GluB27 (38). AspB28 replacement removes contact between ProB28 and GlyB23 at the monomer-monomer interface (38), and the charge repulsion contributes to the rapid dissociation into monomers (18).

Insulin Glulisine (Lys-B3, Glu-B29-human insulin)

As compared to lispro and aspart, the unique characteristic of the rapid-acting analog glulisine is the absence of the ligand zinc. Zinc is added to lispro and aspart (and RHI) as a stabilizing agent, with resultant hexamer formation. Glulisine monomers are stabilized by Polysorbate 20 (Tween 20) (40), and as a result glulisine exists in monomeric, not hexameric, form. This explains its faster sc absorption compared to lispro and aspart (40-43).

The safety and immunogenicity of rapid-acting insulin analogs

Any manipulation, even of a single amino-acid, of the HI molecule might potentially introduce risks of deviation from physiology. Great attention has been dedicated to the safety of insulin analogs after the observation that the rapid-acting analog AspB10 increased binding to the IGF-I and and insulin receptors, as well as a reduced off-rate from the insulin receptor (44-46). The finding of a higher induction of

mammary gland tumors with high doses of AspB10 to a sensitive female rat strain (the Sprague Dawley rat) (47) raised concern about potential carcinogenicity (44), causing this analog to never reach the market (48). Since then, all the subsequent insulin analogs have been carefully tested for binding to the IGF-I r and insulin receptors, and for the off-rate from the insulin receptor as compared to HI (41, 49). No difference in the off-rate is today considered as the *sine qua non* condition for safe use in humans of rapid-acting (46) and long-acting (49-51). Lispro, aspart and glulisine are analogs which do not interfere with binding to the receptor of IGF-I and to the insulin receptor.

The immunogenicity of lispro, aspart and glulisine is no different from that of HI (18, 41, 42).

Pharmacokinetics, pharmacodynamics and post-prandial PG control with mealtime first generation rapid-acting insulin analogs

From the mid '90s the more physiological PK/PD profile of rapid-acting analogs compared with RHI led to the availability of a new class of insulin, more specific and suitable for mealtime use. It was expected that a more selective and specialized insulin preparation for prandial use, with earlier and more robust activity within the first 60-90 min post-injection, would also have a shorter duration of action, and contribute less to inter-prandial insulin need ("basal" insulin). In fact, this had already been indirectly suggested by phase 3 studies of insulin lispro which showed reduced PG at 1 and 2 hours after meals, but resulted in more elevated PG by the next meal and overnight, with marginal, if any, effects on A1C (52-54). The clear demonstration by the rapid-acting analogs of the relationship between the PK/PD benefits in the initial 2 hours post-meal as compared to a later PP phase was clarified in 1996 with lispro (55). Given the similarity between aspart and lispro, and glulisine and lispro (41, 56-59), the results

obtained with lispro may well represent a “class” effect of all rapid-acting analogs. This was also the case for glulisine [reviewed in (41)].

With sc lispro, serum insulin concentration increased earlier, peaked two times higher in less than half of the time but also decreased earlier than RHI (55) with an overall left-shift of insulin PK, mimicking the physiology of endogenous insulin secretion (Figure 4). The more physiological PK of lispro translated into better PD than RHI, with lower PP-PG after a standard solid meal (55), an effect mediated primarily by earlier hepatic insulinization (60). In that study (55), RHI was injected 30 min before the meal to maximize its effects (25) while lispro was given at mealtime. Other studies compared RHI and lispro both given at mealtime (27), thus indirectly favoring lispro.

Figure 4 also shows the shorter duration of action of lispro as compared to RHI, which becomes evident 2-3 hours post-injection when PP-PG increased earlier and to greater a peak with lispro, in parallel with the earlier decrease in serum insulin concentration as compared to RHI. This observation was made possible by a study done in people with T1DM and virtually absent endogenous insulin secretion (55). Other studies of lispro and RHI had not reported the comparative late waning of PK/PD of lispro, because of confounders, either the endogenous insulin secretion of non-diabetic people (61), or the continuation of iv insulin infusion after the lispro bolus (27).

The observation of the shorter duration of action of lispro as opposed to RHI suggested that when rapid-acting insulin analogs are used in place of RHI, the replacement of basal insulin should be optimized at the same time to prevent late post-meal insulin deficiency and hyperglycaemia prior to the next meal and at night. Addition of basal insulin [at the time of study only the historical NPH was available (55)] to lispro in a tiny amount (about 1/3 of the lispro dose) to prolong the lispro effect

by a few hours, improved late PG up to 7 hours after lispro injection by increasing late post-meal serum insulin (Figure 4).

Optimized replacement of basal insulin with rapid-acting analogs

The novel information revealed by the meal study (Figure 4) (55) opened the door to the idea that optimization of basal insulin was an important, perhaps essential step when using rapid-acting insulin analogs at mealtime. The goal was to improve not only the PP-PG but also the inter-prandial and nocturnal PG, with ultimate benefits for the full 24 hour PG and A1C. In the '80s and '90s, the only available basal insulin NPH was given generally once/day (2, 62), with relatively high risk for nocturnal hypoglycemia (2). Because the PK of NPH does not make it suitable for one injection per day (63), the idea was to use small doses of NPH multiple times a day, in combination with lispro during the day (separate injections) and once again (NPH) at bedtime. Under these conditions, the co-administration of lispro and NPH improved early and late PPG. The 24-hour PG and A1C decreased by 0.35% compared with RHI after three months (64) while after one year patients had lower risk for hypoglycemia and greater satisfaction despite at least seven daily insulin injections (65). The availability of the first long-acting insulin analog glargine 100 U/mL a year later in 2000 approved for once/daily dosing (63, 66), ended the brief, but successful, experience with 4 daily NPH administrations (67).

Optimized replacement of basal insulin while using lispro as a mealtime insulin was easier with pumps, with immediate benefits as to A1C and hypoglycemia (68). Since then, pumps use rapid-acting analogs instead of RHI.

Today rapid-acting insulin analogs are universally accepted as mealtime insulins, both with multiple daily insulin injections (MDII) and continuous subcutaneous

insulin infusion (CSII). Yet, until recently, these analogs have been questioned because of lack of evidence on outcomes such as decrease in A1C and risk of hypoglycemia (62, 69-71). However, since most of the studies examined did not optimize basal insulin when lispro was used in place of RHI, the conclusions of those rigorous investigations (61) and meta-analyses (69-71) were not surprising considering earlier data (55), and the conclusions not applicable to the clinical reality where people optimizes basal insulin with either MDII or CSII. Today, after learning how to optimize basal insulin using long-acting analogs and pumps, we know that rapid-acting analogs can reduce A1C, reduce the risk of hypoglycemia, and most important, contribute to a better life-style for patients over RHI.

THE SECOND GENERATION RAPID-ACTING INSULIN ANALOGS

The sc absorption of the rapid-acting analogs lispro, aspart and glulisine may sometimes be still not sufficiently rapid, and further PP-PG may remain elevated. In an attempt to further accelerate sc absorption and improve PPG control, the “second-generation” rapid-acting insulin analogues have been developed, with faster onset and faster end of action than their predecessors (72). The “second generation” rapid-acting analogs are the fast-acting aspart (hereafter called faster aspart), ultra-rapid lispro (both approved and on the market), and BioChaperone lispro (phase 3 studies announced to be initiated in 2022).

The second generation rapid-acting analogs do not differ from the respective molecules of the first generation in terms of primary structure, but differ in the mechanism of faster absorption (Table 1) (72)**.** As compared to aspart, faster aspart contains two additional excipients: niacinamide, which increases sc blood flow, and L-

arginine which stabilizes the analog (73). Ultra-rapid lispro (LY900014) contains the excipients citrate and treprostinil. Citrate enhances vascular permeability at the injection site, while treprostinil increases local vasodilation (74, 75). BioChaperone lispro contains the excipients citrate and BioChaperone BC222 (76). BioChaperone BC222 reduces enzymatic degradation while increasing the rate of hexamer dissociation and monomer absorption from the sc tissue.

An additional line of research being explored at present to improve PP-PG is the add-on to rapid-acting insulin analogs of pramlintide, an analog of amylin, a hormone co-secreted with insulin from the pancreatic β-cells which slows down gastric emptying and contributes to satiety and loss of body weight (1). Following the observation with separate sc infusions of lispro and pramlintide (pumps) which reduced PPG more than lispro alone in T1DM (77), the second generation insulin analog BioChaperone lispro is currently being tested as co-formulation with pramlintide in a phase 1 pump study by Adocia (Lyon, France). It is expected that the continuous pump infusion of pramlintide will prolong over 24 hours the beneficial effects observed only in the initial 2 hours PP-PG with a prandial bolus of pramlintide co-formulated with rapid-acting insulin (78, 79).

Pharmacokinetic/pharmacodynamic studies

PK/PD of each of the three second-generation rapid-acting insulin analogs have been compared with the corresponding first-generation insulin analogs, either aspart or lispro. The results have recently been presented in detail in a review (72).

PK/PD with sc injection

There are common PK/PD characteristics to all three second-generation, rapid-acting insulin analogs as compared to the first generation (Figure 5). The sc injection of either faster aspart, ultra-rapid lispro or BioChaperone lispro, results in earlier onset and offset of action (by ~10 min, with small differences between the analogs) as compared to controls (72). This difference of only a few minutes may appear modest, but it does translate into an important gain in insulin exposure over the 30 min post-injection by 2-3 fold (72), which limits the increase in PP-PG by at least 1.0-1.5 mmol/L (Figure 6). In fact, because of the high insulin sensitivity of the liver (1, 80), faster aspart's earlier increase in portal insulin by even a few µU/mL, timely suppresses hepatic glucose production at meal time (81). A similar mechanism is thought to exist in the whole class of second-generation analogs. It should be noted that the results of Figure 6 have been obtained with experimental liquid meals which favor the early effect of more rapid-acting insulin analogs. Additional studies with the solid meals of the real lives of people with diabetes are needed.

PK/PD studies in pumps with CSII confirm the differences between the second- and first-generation rapid-acting insulin analogs (72). However, the second-generation analogs have been reported to be more frequently with episodes of unexplained hyperglycemia and possible catheter occlusion in pumps (see below). None of these analogs has been compared against insulin glulisine, which has been shown to have a slightly faster onset of action than both aspart and lispro (40-43).

Phase 3 clinical trials

Phase 3 trials have reported the efficacy and safety of faster aspart and ultra-rapid lispro as compared to their respective predecessors (72).

In people with T1DM, faster aspart has been studied in adults with basal insulin detemir (Onset 1) and degludec (Onset 8) with modest effects on A1C but lower PPG increments (82, 83). A single trial (Onset 7) in children and young persons (1-17 years) compared faster aspart with aspart in a basal-bolus regimen (84), with results on HbA1C and hypoglycemia similar to those observed in adults. Ultra-rapid lispro had an effect similar to those of faster aspart in a basal (glargine)-bolus regimen, with lower PP-PG (85).

In T2DM, two studies compared mealtime faster aspart with aspart in a basal-bolus regimen with insulin glargine 100 U/mL (glargine-100) (Onset 2)(86) and degludec as basal insulin (Onset 9)(87). No significant difference in HbA1C at the end of treatment was observed in either trial, while the PP-PG increment at 1-hour post-meal in meal tests at the end of each trial was reduced with faster aspart. In Onset 9 an increase in early (0-2-hour) post-meal hypoglycaemia was observed (87). A single trial (PRONTO-T2D) has compared mealtime ultra-rapid lispro with lispro in a basal-bolus regimen (88). The effect on HbA1C was similar, but the PP-PG increment at 1- and 2-hours post-meal was reduced with ultra-rapid lispro.

Trials in pumps and closed loop systems

An interesting application of the second generation rapid-acting analogs is pumps and closed loop systems. Pump use has been approved, but so far general experience is limited. New data are expected in the near future.

Faster aspart was tested in two pump trials (Onset 4 and 5) (89, 90). In Onset 4 there was a trend toward better glycemic control with faster aspart and a similar risk of hypoglycemia, but unexplained hyperglycemia and premature infusion set changes (<72 hours) were more common (89). In the larger Onset 5 trial, the change from baseline in HbA1C after 16-weeks was slightly in favour of aspart (ETD 0.09%, p<0.02) with a comparable rate of severe or overall BG-confirmed hypoglycemia (90). Nocturnal, pre-meal and 4-hour post-meal (especially the evening meal) glucose levels (CGM) were higher with faster aspart. Hypoglycemia within the first hour from meal initiation was increased with faster aspart. Similar to Onset 4, a higher number of participants on faster aspart rather than aspart experienced events related to infusion-site reactions. Two small trials have reported on the use of faster aspart in closed-loop delivery systems. The first trial in 20 participants with T1DM showed a similar proportion of time in target range (primary endpoint) as with aspart (91). A further study in 15 adults with T2DM achieved comparable time in target range with faster aspart, although a higher dose of faster aspart was required to achieve comparable glucose control (92).

Two trials (PRONTO-Pump and PRONTO-Pump 2) have evaluated ultra-rapid lispro use via CSII pumps (93, 94). PRONTO-Pump was designed to determine the safety of ultra-rapid lispro in pumps. Although there was no difference in the rate of infusion, set failures between the two treatments and premature infusion set changes (≤72 hours) were more common with use of ultra-rapid lispro (92). PRONTO-pump 2 was designed to establish the non-inferiority of ultra-rapid lispro in pumps in a 16-week cross-over study. A trend towards better glycemic control with ultra-rapid lispro during the 16-weeks was observed with no difference in the risk of hypoglycemia (94).

RAPID-ACTING INSULIN ANALOGS 25 YEARS LATER

It was 1996 when the first rapid-acting analog lispro came to the market for use as mealtime insulin in place of RHI. Aspart and glulisine followed soon afetr. Twenty-five years later, it is possible to make some consolidated comments.

The move from RHI to rapid-acting analogs as more suitable mealtime insulin preparations was a good one as to physiology. Of note, the long awaited “ideal” insulin formulation, HI, ultimately obtained with the sophisticated rDNA technology in the ‘70s, had to be soon modified upon understanding the too slow sc absorption. A second observation is that, with the exception of the use in pumps delivering optimal basal insulin, it was unfortunate that lispro became available in 1996 without any reliable basal insulin available at that time, except for CSII. The simple substitution of RHI with lispro, with no modification of the inadequate NPH regimen (63, 95, 96), did not improve the 24 hour PG and A1C, despite lower PG at 1 and 2 hour PP. It was only the understanding that rapid-acting analogs unmask the greater need for basal insulin that led to optimized use of basal insulin (52, 60, 61, 64, 65), with ultimately better 24 hour PG, lower A1C, and lower risk for hypoglycemia [reviewed in (18)].

Today no one would any longer dispute the use of the rapid-acting analogs as the favored mealtime insulin over RHI, with MDII and CSII. This was not the case in the years when lispro was introduced. At that time the predominant view was quite conservative, and the idea of modifying the natural insulin molecule of HI was opposed on the basis of potential risks for safety given the negative experience with AspB29 (44-49), the lack of experience of bioequivalence with HRI, and the unknown long-

term effects. Even an illuminated person like Michael Berger, a pioneering paladin of a “patient-centered approach”, at that time was not convinced of the need to prefer lispro to RHI, and designed himself the popular study “Pizza, cola and tiramisu” (27), to disprove the claimed greater efficacy of lispro. But, that study, as we know, demonstrated the efficacy and convenience of lispro even against an unusual oral load of CHO (140 g) (27).

The experience of 25 years indicates that rapid-acting analogs have the advantage of flexibility of injection timing and dose which can be adapted to the carbohydrate (CHO) content and size of the meal better than with RHI. That flexibility plus the earlier onset and offset of action make it easier to adapt prandial insulin treatment even in complex circumstances (slow gastric emptying, need for postprandial treatment in case of erratic nutrition).

The most important advantage of rapid-acting analogs over HRI, is the convenience for people who need prandial insulin for an improved life-style. This fundamental aspect is not usually evaluated by payors which instead emphasize endpoints such as A1C and risk for hypoglycemia. The popularity of rapid-acting analogs is such that RHI has virtually disappeared as a mealtime insulin, today it is is limited primarily to in-hospital use (i.v. route).

The recent availability of the second-generation rapid-acting analogs poses new questionsas to when, how, and for which patient they might be better indicated rather then first-generation analogs. Although the PK/PD characteristics of these more recent analogs have moved in the right direction, it will take some time and consolidated experience to answer these questions. Fast aspart, ultra-rapid lispro and possibly BioChaperone lispro appear quite similar to each other as to efficacy, but

each of these new prandial insulin formulations has been tested against its own comparator, all different from the others. It would be useful to see in a head-to-head study the differences, if any, between these three second-generation insulin analogs. The efficacy of mealtime analogs has to be proven with solid, not liquid meals, and in the presence of more versus less CHO, protein and fat rich meals. The need to optimize basal insulin should not be forgotten, since the second-generation have a shorter duration of action than first generation insulin analogs. The second-generation analogs might be particularly useful in pumps and closed-loop systems (97), as long as proper attention is paid to safety due to the greater risk for catheter occlusions (93). T2DM patients who maintain residual endogenous insulin secretion would be ideal candidates for second-generation analogs, given their liver and muscle insulin resistance. However, phase 3 studies suggest that care should be taken to avoid early PP hypoglycemia, perhaps due to slower glucose absorption from the meal as compared to faster insulin absorption from sc in some individuals.

FUTURE DIRECTIONS

Optimal regulation of PG in people with insulin-requiring diabetes remains an unmet need, and more so in the prandial as compared to the fasting state. The rapid-acting analogs of both the first and second generation, improve PP-PG better that RHI, but a perfect match between the PK/PD of sc injected insulin and glucose absorption from the meal remains difficult. New strategies to improve PP-PG are needed, for example the use of a sc dual prandial bolus at the beginning of the meal and again two hours later. However, studies with meals different for size and composition are needed to clearly define the strategy.

A long awaited goal has been and still is, oral insulin. The oral route of administration would have the advantage over sc insulin, not only of better compliance, but also of better physiological effect, primarily at the level of the liver and less in peripheral circulation. Recent, sophisticated technologies have made the enteric absorption of insulin possible, both for protracted-acting (98) and rapid-acting (99-102) insulin formulations. However, for prandial insulin, at present several barriers make the objective of oral administration a distant goal: low bioavailability, the uncertainty of the timing of administration in relation to meal ingestion and the high variability of the glucose lowering effect when insulin, a hormone with narrow therapeutic window, is absorbed with the oral meal.

A more promising approach appears to be the exploration of combined administration of rapid-acting insulin and pramlintide (76).

CONCLUSIONS

Rapid-acting analogs allow better control of PP-PG as compared to HRI when combined with optimized basal insulin replacement. Rapid-acting analogs are the gold standard of mealtime insulin replacement in people with type T1 and T2 DM. However, successful regulation of PP-PG remains a difficult task which is dependent also on the meal (composition and size) and gastric motility, in addition to the PK of injected prandial insulin. A meal richer in fat requires a type of insulin substitution more complex than a single pre-meal bolus, since there might be an end of action earlier than complete glucose absorption. Rapid-acting insulin analogs have provided more selective, specialized insulins for mealtime, and more so the most recent second-generation rapid-acting insulin analogs. These new, effective agents require, however,

careful optimization of basal insulin replacement, along with new strategies for use, such as greater flexibility in adapting the dose to CHO content and composition, as well as delivering boluses post-meal in addition to pre-meal, both with MDII or CSII, as needed, to consistently improve the PP-PG and 24-hour PG and A1C.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this article. G.B.B. has received honoraria for lectures and advisory boards from Sanofi and Menarini; F.P. has received honoraria for lecturing and consultations from Sanofi, Lilly, Novo Nordisk, MSD; P. L. declares no conflict of interest; C.G.F. has received honoraria for lecturing and consultations from Sanofi, Lilly and travel grants from Menarini; D.R.O. has received honoraria for lectures and advisory board activities from Boehringer Ingelheim, Novo Nordisk, Roche Diagnostics and Sanofi.

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Table 1: Current second-generation rapid-acting insulin analogues in development. [From Owens DR, Bolli GB (71), with permission]

Drug	Company	Core insulin structure	Added excipients	Mechanism of action
Faster aspart	Novo Nordisk	Insulin aspart	Niacinamide (vitamin B3), L-arginine	Increased subcutaneous blood flow
Ultra-rapid lispro	Lilly	Insulin lispro	Treprostinil, citrate	Enhanced vascular permeability and increased local vasodilation
BioChaperone lispro	Adocia	Insulin lispro	BioChaperone BC222[a], citrate	Enhanced diffusion

[a]An oligosaccharide modified with natural molecules.

LEGENDS TO FIGURES

Figure 1 – Plasma glucose and insulin concentrations over the 24 hours in normal, non-diabetic subjects before and after the three daily meals and at night From Ciofetta M. et al., with permission (19).

Figure 2 – Serum insulin concentrations in peripheral circulation (venous) and in portal vein (estimated from C-peptide) in the fasting state and during an oral glucose load. From Schade D. et al, with permission (23).

Figure 3 – The effect on post-prandial plasma glucose with delivery of a bolus of rapid-acting sc at meal-time (full squares), or 30 min before the meal (open circles) or 60 minutes before the meal (full circles). From Dimitriadis G. and Gerich J.E,, with permission (25).

Figure 4 – Effect of sc injection of lispro insulin (full circles, 5 min before the meal) or RHI (open circles, 30 min before the meal) (both 0.15 U/kg) on post-prandial plasma insulin and glucose concentrations. In two additional experiments, 0.07 U/kg NPH were added to either lispro (full squares) and RHI (open squares). From Torlone E. et al, with permission (55).

Figure 5 – Pharmacokinetics (serum insulin concentrations) and pharmacodynamics (glucose infusion rates in euglycemic clamp studies) of the second- as compared to first-generation, rapid-acting insulin analogs. From Owens DR and Bolli GB, with permission (72).

Figure 6 – Post-prandial blood glucose concentration after a liquid meal with the second- as compared to first-generation, rapid-acting insulin analogs. From Owens DR and Bolli GB, with permission (72).

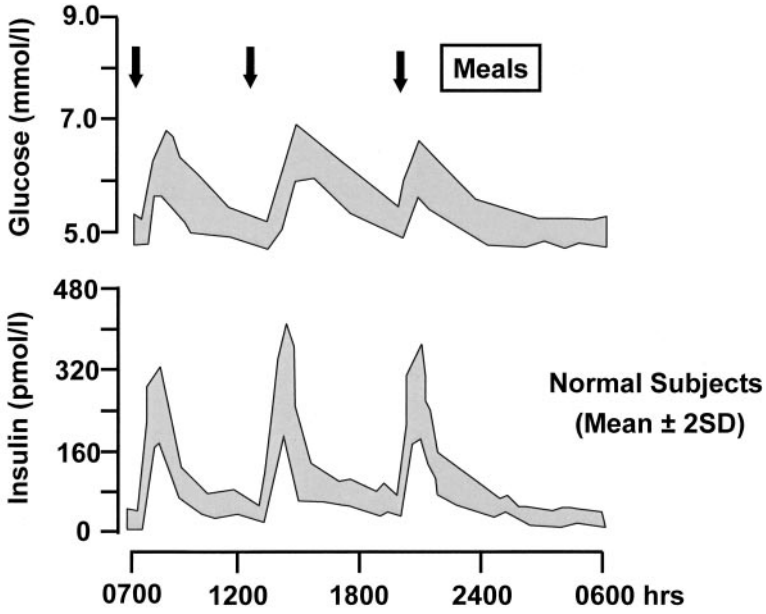

9.0
7.0
5.0
Glucose (mmol/l)
Meals
480
320
160
0
Insulin (pmol/l)
Normal Subjects
(Mean ± 2SD)
0700
1200
1800
2400
0600 hrs

FIGURE 1

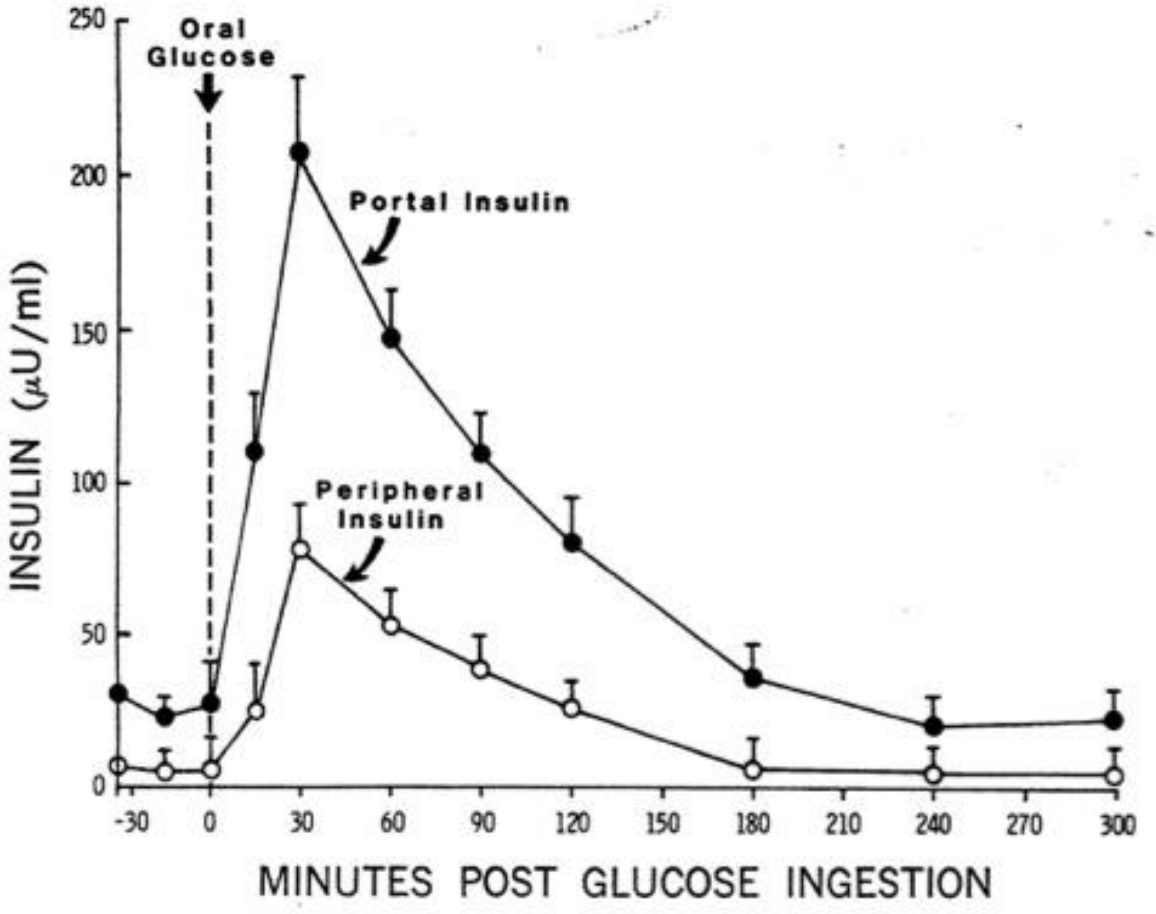

Oral
Glucose
Portal Insulin
Peripheral
Insulin
INSULIN (μU/ml)
250
200
150
100
50
0
-30
0
30
60
90
120
150
180
210
240
270
300
MINUTES POST GLUCOSE INGESTION

FIGURE 2

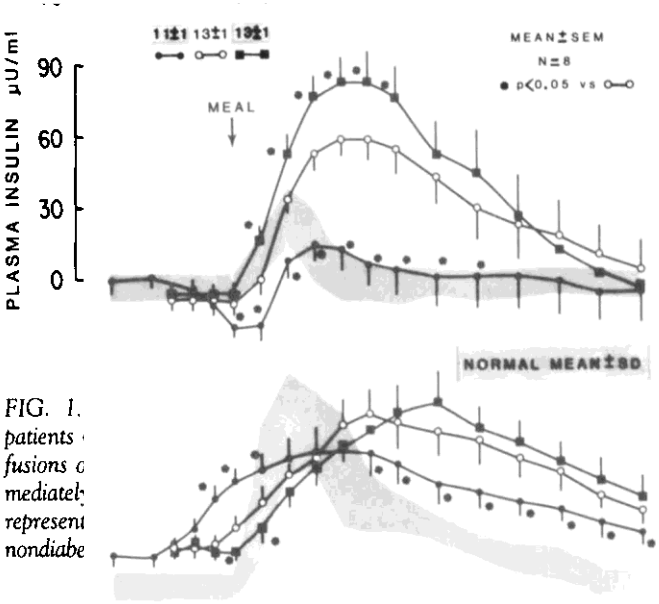
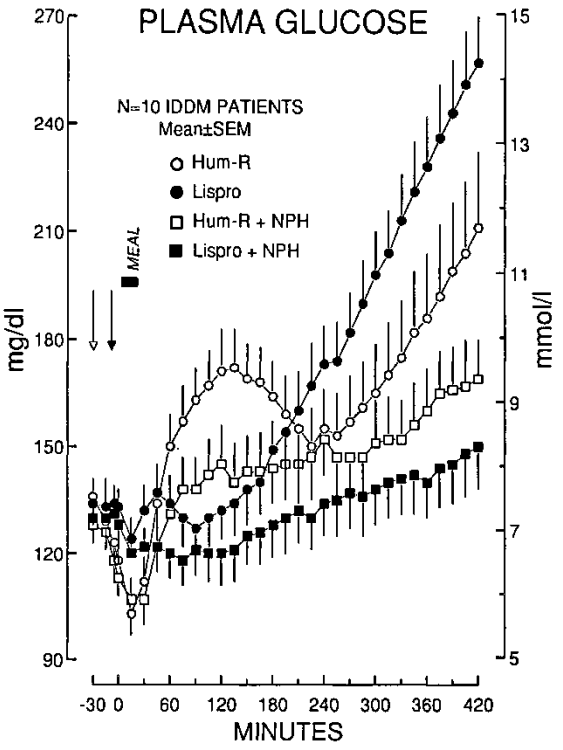


FIG. 1.
patients
fusions o
mediatel
represent
nondiabe

FIGURE 3

A


PLASMA GLUCOSE
N=10 IDDM PATIENTS
Mean±SEM
Hum-R
Lispro
Hum-R + NPH
Lispro + NPH
MEAL
mg/dl
mmol/l
270
240
210
180
150
120
90
15
13
11
9
7
5
-30 0 60 120 180 240 300 360 420
MINUTES

B

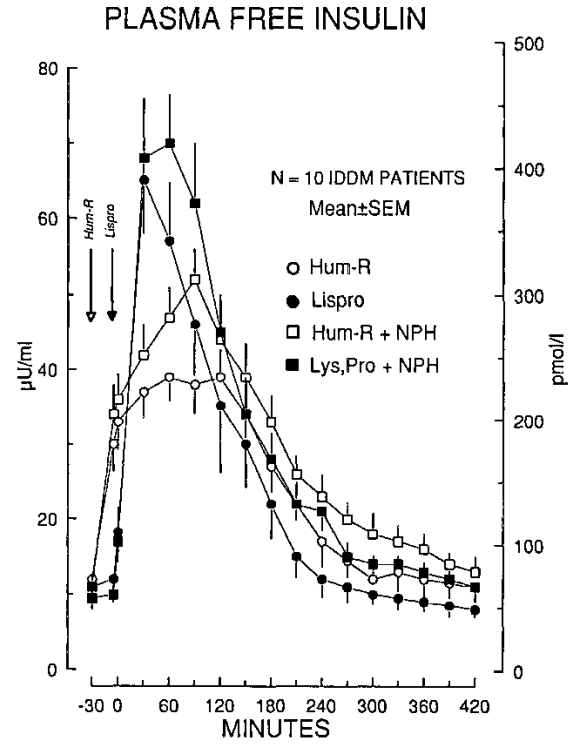

PLASMA FREE INSULIN
N = 10 IDDM PATIENTS
Mean±SEM
Hum-R
Lispro
Hum-R + NPH
Lys,Pro + NPH
Hum-R
Lispro
µU/ml
pmol/l
80
60
40
20
0
500
400
300
200
100
0
-30 0 60 120 180 240 300 360 420
MINUTES

FIGURE 4

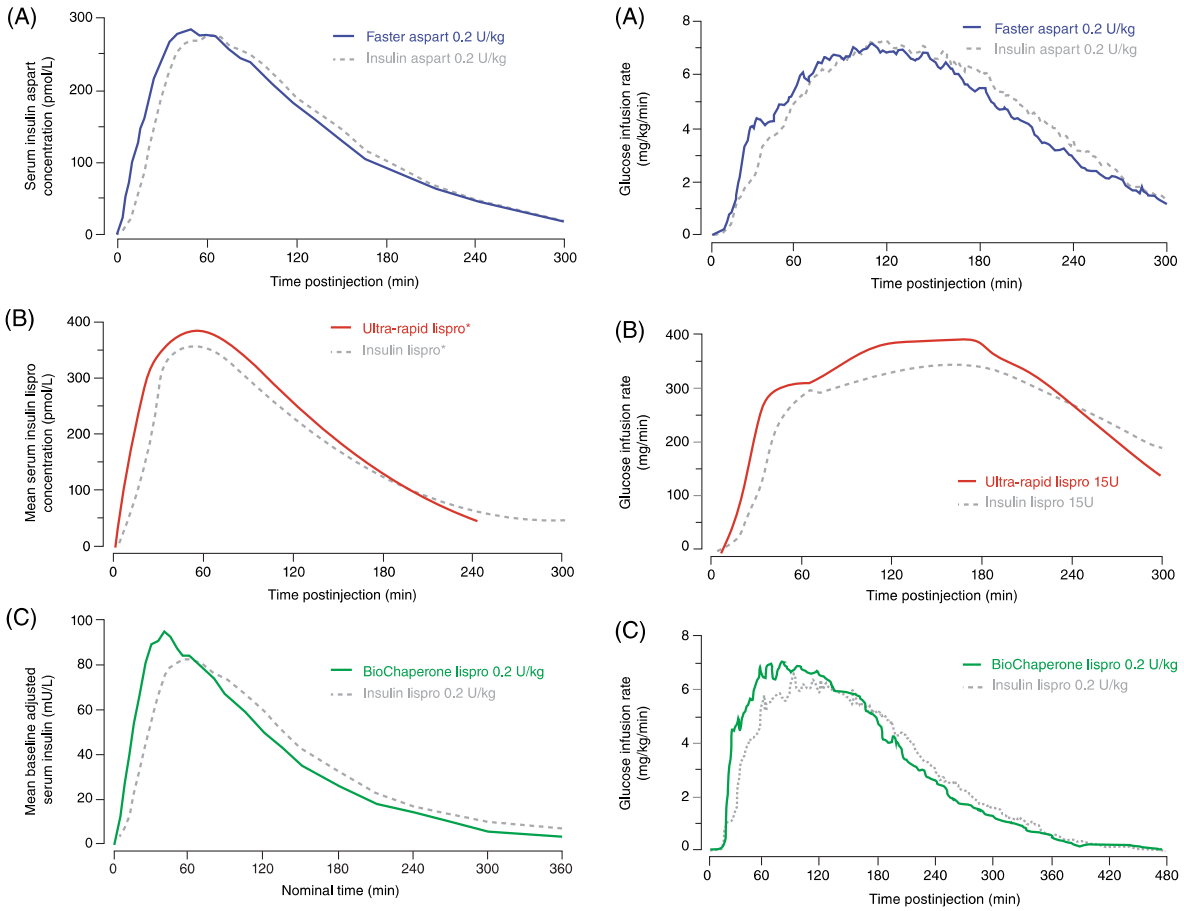

(A)
Serum insulin aspart concentration (pmol/L)
Faster aspart 0.2 U/kg
Insulin aspart 0.2 U/kg
Time postinjection (min)
(A)
Glucose infusion rate (mg/kg/min)
Faster aspart 0.2 U/kg
Insulin aspart 0.2 U/kg
Time postinjection (min)
(B)
Mean serum insulin lispro concentration (pmol/L)
Ultra-rapid lispro*
Insulin lispro*
Time postinjection (min)
(B)
Glucose infusion rate (mg/min)
Ultra-rapid lispro 15U
Insulin lispro 15U
Time postinjection (min)
(C)
Mean baseline adjusted serum insulin (mU/L)
BioChaperone lispro 0.2 U/kg
Insulin lispro 0.2 U/kg
Nominal time (min)
(C)
Glucose infusion rate (mg/kg/min)
BioChaperone lispro 0.2 U/kg
Insulin lispro 0.2 U/kg
Time postinjection (min)

FIGURE 5

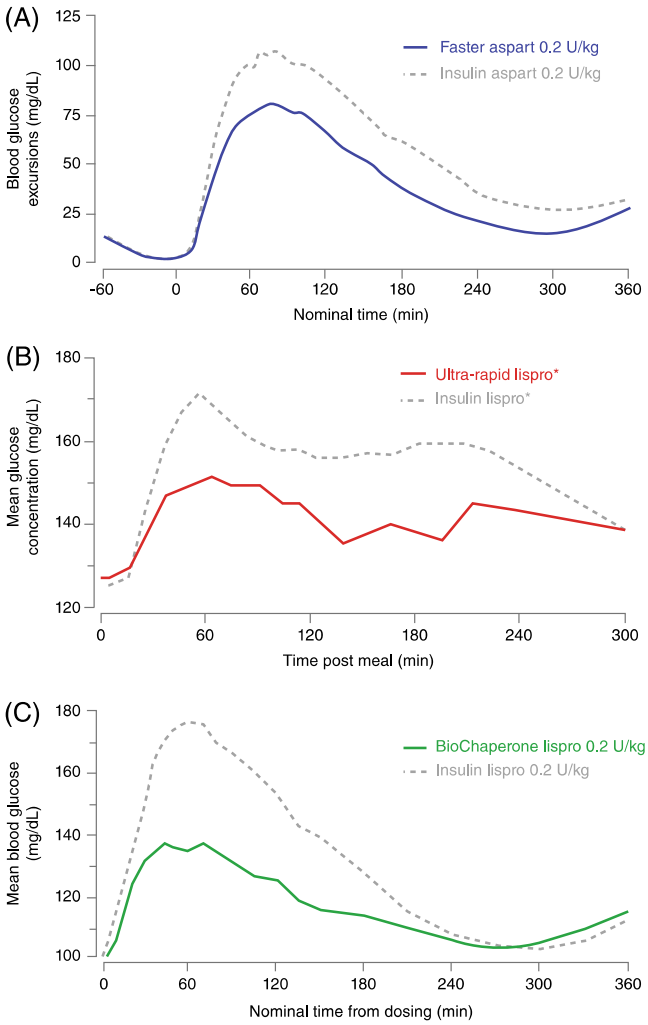

(A)
Blood glucose excursions (mg/dL)
125
100
75
50
25
0
-60
0
60
120
180
240
300
360
Nominal time (min)
Faster aspart 0.2 U/kg
Insulin aspart 0.2 U/kg
(B)
Mean glucose concentration (mg/dL)
180
160
140
120
0
60
120
180
240
300
Time post meal (min)
Ultra-rapid lispro*
Insulin lispro*
(C)
Mean blood glucose (mg/dL)
180
160
140
120
100
0
60
120
180
240
300
360
Nominal time from dosing (min)
BioChaperone lispro 0.2 U/kg
Insulin lispro 0.2 U/kg

FIGURE 6