Insulin - Evolution of insulin formulations and their application in clinical practice over 100 years.

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Journal: Acta Diabetologica

Word count: 6982

Abstract word count: 210

No. of references: 161

No. of figures/tables: 4/1

Declarations

Funding

This work was supported by Sanofi, Paris, France. Editorial assistance was provided by Arthur Holland, PhD, of Fishawack Communications Ltd., part of Fishawack Health, and funded by Sanofi.

Conflict of interest

Geremia Bolli: has received honoraria or consulting fees for Menarini, Sanofi; and has received research support/speaker's bureau from Sanofi

Alice Cheng: has served on an advisory panel for Abbott, AstraZeneca, Bayer, Boehringer Ingelheim, Dexcom, Eli Lilly, HLS Therapeutics, Janssen, Merck, Medtronic, Novartis, Novo Nordisk, Sanofi; and has served as a co-investigator in trials supported by Applied Therapeutics, Boehringer Ingelheim, Sanofi, Novo Nordisk; and has

received honoraria for speaking from Abbott, AstraZeneca, Bayer, Boehringer Ingelheim, Dexcom, Eli Lilly, Janssen, HLS Therapeutics, Medtronic, Merck, Novo Nordisk, Sanofi

David Owens: has received lecture fees/honoraria from Sanofi, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, and Roche Diagnostics

Availability of data and material

Not applicable

Code availability

Not applicable

Author contributions

All authors contributed to the interpretation, writing and editing of this review, and had final responsibility for approving the published version

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

All authors contributed to the interpretation, writing and editing of this review, and had final responsibility for approving the published version

Abstract

The first preparation of insulin extracted from a pancreas and made suitable for use in humans after purification, was achieved 100 years ago in Toronto, an epoch-making achievement, which has ultimately provided a life-giving treatment for millions of people worldwide. The earliest animal-derived formulations were short-acting and contained many impurities that caused adverse reactions, thereby limiting their therapeutic potential. However, since then insulin production and purification improved with enhanced technologies, along with a full understanding of the insulin molecule structure. The availability of radio-immunoassays contributed to the unravelling of the physiology of glucose homeostasis, ultimately leading to the adoption of rational models of insulin replacement. The introduction of recombinant DNA technologies has since resulted in the era of both rapid- and long-acting human insulin analogues administered via the subcutaneous route which better mimic the physiology of insulin secretion, leading to the modern basal-bolus regimen. These advances, in combination with improved education and technologies for glucose monitoring, enable people with diabetes to better meet individual glycaemic goals with a lower risk of hypoglycaemia. While the prevalence of diabetes continues to rise globally, it is important to recognise the scientific endeavour that has led to insulin remaining the cornerstone of diabetes management, on the centenary of its first successful use in humans.

Keywords

Glycaemic control, hypoglycaemia, insulin, insulin analogues, 100 years insulin use

1. Introduction

Diabetes is characterised by increased glucose levels in the blood, with symptoms and signs of hyperglycaemia having been documented thousands of years ago in ancient Egyptian, Indian and Chinese literature, including descriptions of sweet or honey-like urine.[1,2] The earliest known detailed description of diabetes was made by the Greek physician, Aretaeus of Cappadocia, in the 2nd–3rd century AD[1-3] Through scientific endeavour, we now understand that diabetes is caused by impairment of insulin secretion and/or action resulting in dysregulation of glucose and lipid metabolism. Following the first description of the pancreatic islets by Paul Langerhans in 1869,[4] the important role of the pancreas in carbohydrate metabolism was hypothesized in 1877 by Lanceraux,[5] and demonstrated in 1889 by Joseph von Mering and Oscar Minkowski, who extirpated the pancreas of a dog, resulting in polyuria and glycosuria.[6] Subsequently, in 1901 the concept that an internal secretion of the pancreas regulated blood glucose was supported by the histological observations of Eugene L. Opie that diabetes was associated with hyaline degeneration in the islets of Langerhans.[7] These discoveries stimulated the search for the active principle secreted by the pancreas that controlled glucose metabolism. In the two decades preceding the successful use of a pancreatic extract in humans in Toronto, several researchers obtained crude pancreatic extracts that reduced hyperglycaemia and glycosuria, predominantly in animals.[8,9]

Eugène Gley at the end of the 19th century was perhaps the first to demonstrate the efficacy of pancreatic extracts, using sclerosed/degenerated pancreas, having excluded the exocrine pancreas by obstructing the glandular ducts weeks prior, and a two-stage complete pancreatectomy as described by Hédon[10,11]. Between 1900 and 1905, Gley observed consistent reductions of glycosuria in pancreatectomized dogs after intra-abdominal and intra-peritoneal injections of his early aqueous pancreatic extracts.[12,13] This experiment was, in fact, similar to those made more than two decades later in Toronto by Banting and Best,[1,2] although Gley only published his pioneer observation in 1922.[2,13-16] Georg Ludwig Zülzer, in Berlin, carried out research on pancreatic extracts between 1905 and 1914.[5] He developed a pancreatic extract by 1906,[17] and studied its effects in dogs and a small number of people with clinically severe diabetes during 1906 and 1908.[5,18] Positive glucose lowering effects were observed in some individuals, but these were accompanied by toxic side effects and consequently financial support was withdrawn by the Schering Co. in Berlin in 1908 [5]. Nevertheless he patented his extract (*Acomatol*), and persisted in improving its purification with the aid of Camille Reuter, a

chemist from Luxembourg working for Hoffman La Roche, eventually producing a highly effective extract in 1914.[14,18] However, research was discontinued due to loss of interest from Hoffman-La Roche, and the onset of World War 1.[18] In 1911, Ernest Lyman Scott, a Master's student in Chicago, proved the consistent efficacy of his pancreatic extract in pancreatectomised dogs. However, Scott left Chicago in 1911 and his results were published in 19112 by Anton Carlson, the director of the laboratory.[5,19] Similar experiments were conducted by Israel Kleiner in New York and published in 1919.[21] Nicolae Paulescu eventually published in 1921 the results of his earlier and successful experiments conducted in 1916 later interrupted by the war in Europe. Paulescu injected intravenously his pancreatic extract into pancreatectomized dogs, demonstrating both its glucose lowering effects and the suppression of ketones and urea.[22] Paulescu's initial extract, patented as "pancréine" in April 1922, caused adverse local reactions at the site of injection.[23] Later Paulescu's extract was refined with acid precipitation of proteins and alcoholic extraction in 1923[24] and administered to two people with diabetes, but with limited effect.[23,25] Although Paulescu had plans for more research with the goal of application to humans,[26] he was forced to terminate his work due to the lack of further support.

The preliminary steps leading to the first successful treatment of humans, happened in the summer 1921 in the Department of Physiology of Toronto University. The story is fascinating, although the role of individual researchers involved in this extraordinary achievement is still debated.[27] An orthopaedic surgeon, Frederick G. Banting, got credit for his ambitious research plans from John J.R. MacLeod, who had meanwhile moved from Cleveland to become Professor of Physiology and head of the department at the University of Toronto. Macleod offered Banting research facilities and the help of a medical student Charles H. Best, who had decided to skip summer vacation. Banting finally obtained a pancreatic extract from a dog several weeks after ligation of the pancreatic duct, and injected it intravenously into other pancreatectomised dogs.[27] Hyperglycaemia was reduced following administration of the pancreatic extract every 4 hours.[28] Banting believed that the prior degeneration of the exocrine pancreas (ligation of the duct) was essential to recover "the principle of internal secretion" from the islets of Langerhans. However, this hypothesis was soon to be proven wrong, and several steps of his research programme in 1921 were criticised along with the contribution of his assistant Charles Best.[29-31] On 11 January 2022, Leonard Thompson, a young boy with diabetic ketoacidosis, received the first injection of Banting's pancreatic extract into his buttocks, however the treatment produced only a modest reduction in blood and urine glucose, whilst resulting in a sterile abscess at one of the injection sites.[22,25] In fact, the adverse local reaction to Banting's extract was perhaps not dissimilar to that observed by Zülzer and

Paulescu.[5] The final step leading to the first truly successful treatment of a person suffering from severe diabetes was due to the skill of the biochemist James B. Collip, invited and supported by MacLeod to join the research team, which allowed improved purification of the pancreatic extract based on alcohol treatment.[29,32] Collip's extract proved to be efficacious on 23rd January 1992, with a dramatic reduction in blood glucose and disappearance of ketonuria with little or no toxic reactions following its subcutaneous administration.[27] This was the first demonstration of the new era of insulin therapy. Treatment continued over several days with significant clinical improvement. It was therefore Collip who played a key role in the preparation of the extract ultimately suitable for use in humans in Toronto.[30]

Overall, there were twenty three investigators who endeavoured to extract a glucose lowering principle from the pancreas of animals from 1892 to 1922.[33] However, only the stubborn research of the team in Toronto and the support of the University made it ultimately possible to obtain a preparation suitable for humans. However, large scale production was beyond the capabilities of the University of Toronto laboratory. Under the leadership of the chemist, George H.A. Clowes, research director at Eli Lilly & Co., the company's resources were subsequently mobilized to allow for mass production of insulin.[22]

As was the case 100 years ago, insulin replacement remains an absolute requirement to sustain life in people with type 1 diabetes (T1D) and is also required by many people with type 2 diabetes (T2D) due to diminishing insulin secretion and/or responsiveness to insulin as the disease progresses.[34] Worldwide, in 2021 it was estimated that approximately 537 million people have diabetes (of whom approximately 90% have T2D), with the prevalence predicted to rise to 781 million by 2045.[35] It is estimated that up to 40% of people with diabetes (150–200 million) globally who require insulin therapy,[336] include approximately 30 million people with T1D. The remaining population either have T1D misdiagnosed as T2D (antibody positive), or T2D with significant beta cell deficiency.[36] On the centenary of the availability of insulin that led to the successful use in humans, we look at how insulin formulations, related technology, and clinical applications have evolved over the last 100 years, whilst recognising also the key scientific achievements (**Figure 1**) that have been instrumental in the understanding of human physiology and the therapeutic use of insulin.

2. The evolution of insulin over 100 years

Although the early insulin preparations were truly life-saving, much improvement was needed, such as further purification, increased yield and production capacity, improved time-action profiles, reducing the risk of hypoglycaemia, and simplifying modes of delivery, efficacy and ease of glucose monitoring. A major limitation during the initial decades of the insulin era included the difficulty in measuring blood glucose and therefore a lack of understanding about the time-action characteristics of the available insulin preparations, representing two major obstacles to titrate insulin effectively. The later introduction of radio-immunoassays[37-39] provided a greater understanding of the physiology of glucose homeostasis, whilst also providing invaluable pharmacokinetics data for the different insulin formulations. The advent of recombinant DNA technology in the 1970s,[40,41] allowed synthesis of human insulin, soon to be followed by the introduction of insulin analogues, designed to better mimic both basal and prandial insulin secretion.

2.1. Evolution of insulin formulations

From animal insulin to human insulin

In addition to insulin itself, early pancreatic extracts contained impurities that caused toxic reactions, both at the injection site (abscesses) and systemically (e.g. fever), limiting clinical use in humans.[8,9] Early efforts to optimise the extraction of insulin focussed on improving yield by placing bovine pancreas immediately into an acidic alcohol solution to inhibit the activity of pancreatic enzymes,[32] although this was not really necessary.[30] Early commercial production by Eli Lilly of insulin derived from porcine pancreas suffered from low yields and early deterioration of the extract. It was George B. Walden, head chemist at Eli Lilly, who developed the isoelectric precipitation method in 1922, which increased the yield 10-100 times as compared to previous methods, and greatly improved the stability and purity of insulin.[9,27] However, despite these advancements, the presence of allergic reactions remained (albeit to a lesser degree), highlighting the need to achieve further purification.[42] The amorphous insulin then underwent a two-step crystallisation process, in the presence of certain metal ions to secure crystallisation,[43] which helped to reduce the allergic reactions in most patients.[44] Early insulin preparations, referred to as regular/soluble (bolus) insulins, had a short time-action profile (peak action at 1-2 hours with a duration of approximately 6-8 hours following subcutaneous injection[2]), necessitating administration multiple times a day. Thus, for approximately 25 years following the first

administration of insulin to humans in 1922, all formulations were short-acting/bolus soluble/regular insulins, until the advent of the first basal insulins that had a longer duration of action.

Development of insulin preparations with protracted action

Early attempts to prolong the time-action profiles of insulins included the addition of gum solutions, oil suspensions, lecithin emulsion and hormones which met with little success.[45] In 1936 Hans Christian Hagedorn and colleagues (Nordisk Company) introduced protamine insulinate, a neutral protamine insulin [46,47] that was soon followed by protamine zinc insulin (PZI), developed by Scott and Fisher, where a surplus of protamine and a small amount of zinc stabilised the insulin.[48] Charles Krayenbuhl, in Hagedorn's laboratory, then discovered the optimal relationship, the 'isophane point', i.e. the pH value at which there is no excess insulin or protamine after precipitation.[49] Neutral Protein Hagedorn (NPH) was developed as a modification of PZI involving zinc in the crystallisation of protamine and insulin (in stoichiometric proportions) at neutral pH, resulting in an insulin preparation that was fully mixable with soluble insulin.[48] NPH insulin was made available for clinical use in 1950[50] and became the first widely used basal insulin (BI), almost 25 years after insulin first became available. Once NPH insulin is injected subcutaneously, the insulin crystals slowly dissolve resulting in a peak action at approximately 5–6 hours and a duration of action of approximately 13 hours, which is dose related.[51,52] However, the appropriate use of NPH requires its careful re-suspension prior to injection.[53] An injection of NPH with insufficient, or no resuspension results in a significant change in its pharmacokinetic/pharmacodynamic profile,[53,540] which may put patients at risk of hypo- or hyperglycaemia. Indeed, during the NPH era, the need for adequate NPH resuspension prior to injection, was often underestimated by people with diabetes.[55]

In the 1950s the lente family of insulins (semi-lente, lente, ultralente) were first introduced by Novo and subsequently by Eli Lilly and Hoechst. These formulations were also insulin suspensions, produced by combining animal-derived insulin with variable amounts of zinc, with a duration of action dependent on physical state, size and zinc content of the zinc-insulin particles, as well as different solubilities of porcine and bovine insulin at neutral pH.[48,56,57] The chemical properties of zinc-insulin preparations, including the impacts of zinc concentration and species of insulin, were developed and studied extensively by Jorgen Schlichtkrull and colleagues.[58,59] The original lente insulin comprised a 3:7 ratio of amorphous porcine and crystalline

bovine insulin, with a duration of action similar to that of NPH. In contrast, ultralente consisted of relatively large rhombohedral bovine insulin crystals and was considered to be the first "long-acting" basal insulin.[48,56] Other lente-type insulins also took advantage of the differences in solubility between porcine and bovine insulin to modify duration of action. Novo produced Monotard (purely porcine insulin), and Rapitard which contained 25:75 mixture of porcine and bovine insulin.[48,56]

Development of human insulin preparations and insulin analogues

After the success of Frederick Sanger to fully sequence the primary structure of bovine insulin in 1955,[60] the first chemical synthesis of animal insulins took place in the 1960s, followed by chemical synthesis of human insulin in 1974.[61] In the following years, semi-synthesis of human insulin was also achieved, by several groups, via enzymatic conversion of porcine insulin.[62]

The 1980s saw the commercial introduction of the first biosynthetic human insulins using recombinant DNA technology,[2] which would come to supersede animal insulins as the primary choice for insulin replacement relinquishing the need for animal pancreases. Theoretical advantages of human insulin (semi-synthetic and biosynthetic), such as more physiological pharmacokinetics/pharmacodynamics and lower immunogenicity over purified animal insulin, were initially not demonstrated, and the benefit of routinely using human insulin was challenged.[63] The logical scientific achievement of human insulin proven to be slightly less immunogenic than porcine (but much less than bovine insulin), possessed only minimal pharmacokinetic differences and consequent negligible metabolic benefits especially to porcine insulin.[64] However, mass conversion from animal to human insulins occurred in the UK and elsewhere in Europe between 1983 and 1989. During this period Teuscher and Berger reported that conversion from porcine to human insulin resulted in a diminished awareness of hypoglycaemia[65] and in 1989 at a British inquest investigated the causes of sudden death in a small number of persons with type 1 diabetes who had changed over to human insulin. The question was raised as to whether human insulin was to blame, and a heated debate and threat of litigation lasting many years began. Unfortunately, media coverage fuelled a major crisis of confidence in human insulin necessitating Diabetic Associations world-wide to offer statements of reassurance. Many small studies in normal subjects and persons with diabetes provided conflicting evidence for a change in the counter-regulatory response with human insulin, to explain the reported increase in hypoglycaemic unawareness resulting in severe

hypoglycaemia and possibly death. The majority observed no difference in either the hormonal or symptomatic response to hypoglycaemia induced by human and porcine insulin.[66] There was also little evidence to implicate the species of insulin as a factor in the deaths of persons with type 1 diabetes taking human insulin at the time of death.[67] A meta-analysis of clinical studies also found no difference in the incidence of hypoglycaemia or hypoglycaemic symptoms between the two species of insulin.[68] Of note, in the 1980s human insulin was used primarily for intensification of insulin therapy, as suggested by the DCCT,[69] a strategy which itself leads to several-fold increase in the rate of severe hypoglycaemia[70] and the vicious circle of unawareness of hypoglycemia, impaired counterregulation and additional risk for severe hypoglycemia.[71] Thus, most likely it was intensification of treatment and not human insulin per sè to account for the observed reduction in the awareness of hypoglycaemia with human insulin.[72] However, historically, with better understanding of the function of specific amino-acids in the insulin molecule,[73] the use of recombinant DNA technology opened the possibility of modifying human insulin and creating a variety of insulin analogues with tailored properties.[48,74] By doing so, human insulin analogues were developed with improved time-action profiles, creating a new generation of both bolus and basal insulin formulations. Furthermore, with today's insulin analogue formulations, injection site and immunological reactions are rare.[42,75-78] **Figure 2** summarises the modifications of insulin analogues and the impact on their mechanisms of action.

Prandial (bolus) insulin analogues

The first human insulin analogue was insulin lispro (Eli Lilly), which was designed to replicate the sequence of lysine and proline at B28, B29 in insulin-like growth factor 1 (IGF-1) which does not self-associate. The low propensity of lispro to self-associate leads to a rapid dissociation into monomers after injection into the subcutaneous tissue.[79] This translates into a more rapid onset of action compared with regular human insulin (RHI) so that it could be administered closer to mealtimes, with its quicker peak effect better able to blunt post-prandial glucose peaks, while also possessing a shorter duration of action minimizing post- and inter-prandial hypoglycaemia.[74,80] Subsequently, aspart (NovoNordisk) and glulisine (Sanofi) were developed, also possessing an earlier onset and shorter duration of action compared with RHI.[74] The rapid action of aspart was achieved through amino acid modifications that promoted a more rapid dissociation of hexamers after subcutaneous injection similarly to lispro. Glulisine was the only insulin without zinc (substituted with polysorbate 20 as stabilizer). The absence of zinc allows for more rapid adsorption of glulisine, while its amino acid modifications provide molecular stability and increase solubility at physiological pH (**Figure 2**).[74,81]

The more recent faster-acting mealtime insulins, namely faster aspart (NovoNordisk), ultra-rapid lispro (Eli Lilly), and Biochaperone lispro (Adocia), benefit from added excipients that increase subcutaneous blood flow and/or vascular permeability to speed up absorption, and by the inclusion of the Biochaperone to insulin lispro that increases diffusion and the rate of hexamer dissociation (**Figure 2**).[82] These mechanisms result in an even earlier and higher peak serum insulin concentrations, with shorter durations of action than earlier rapid-acting insulin analogues, although none have been compared directly against either glulisine or each other.[82]

Basal insulin analogues

Basal insulin (BI) analogues were initially developed to have flatter and more stable action profiles and longer duration of action when compared with NPH insulin,[79] more closely reflecting the consistent, low levels of serum insulin that results from endogenous insulin secretion during the fasting state (**Figure 2**). [83] The first-generation BI analogues included insulin glargine 100 U/mL (Gla-100) (Sanofi), which became available in 2000,[77] 50 years after NPH, and followed in 2004 by insulin detemir (IDet) (NovoNordisk).[75]

Gla-100 was developed by replacing the asparagine at A21 on the A-chain of human insulin with glycine while retaining the two arginine molecules at the amino terminal of the B chain in the final intermediate stage from proinsulin to natural human insulin.[56,84,85] The amino acid changes increased the iso-electric point of the molecule from a pH of 5.4 (native insulin) to 6.7, a value at which glargine molecule is less soluble. Gla-100 is soluble in the acidic pH of the vial/pen, but after subcutaneous injection, glargine is exposed to a change of pH towards neutrality close to its iso-electric point, which results in micro-precipitation (an amorphous crystalline depot). There is then a slow dissociation into hexamers, into dimers and finally monomers prior to its entry into the systemic circulation.[56] In addition, rapid local enzymatic transformation results in A21-Gly-human insulin, which is the predominant active metabolite found in the circulation (M1). [85-88] These mechanisms explain the flatter, more stable and consistent action-profile of Gla-100 compared with NPH.[51]

IDet is produced by acylating insulin with a carbon 14 fatty acid chain following the removal of the C-terminal B30 threonine amino acid.[56] In contrast to Gla-100, IDet is soluble at physiological pH so does not precipitate after subcutaneous injection. The acylated insulin analogue facilitates self-association at the injection site, and reversibly binds to albumin in the subcutaneous tissue and the circulation, which is the main mechanism of its protracted action (**Figure 2**).[56] Although neither have pronounced peaks, Gla-100 and IDet have different PK/PD profiles, with IDet possessing a shorter duration of action compared with Gla-100 with a reduced

glucose-lowering effects in the second 12 h post-dosing.[89] IDet has a lower potency than IGlar-100 necessitating four times more moles of insulin per unit of insulin than NPH and Gla-100 .[75,77,90] These differences explain the higher dose requirements and more frequent need of twice-daily regimens with IDet versus Gla-100, especially in people who are obese where the effectiveness of detemir is reduced reflecting its enhanced lipophilicity.[91]

The second-generation BI analogues, insulin degludec (IDeg) (NovoNordisk) and insulin Gla 300 U/ml (Gla-300) (Sanofi), were developed to provide an even flatter, more prolonged and reproducible insulin profile compared with the first-generation BI analogues.

Following the removal of threonine at B30, the second-generation acylated BI analogue IDeg has a 16-carbon fatty diacid attached at B29 via a glutamic acid spacer (**Figure 2**).[56] The absorption from the site of subcutaneous injection is delayed by the formation of multiple hexamers following the initial loss of phenol residues, and the subsequent loss of zinc ions allows further dissociation into dimers and monomers that then enter into the blood and bind to albumin, further delaying its activity.[56]

Gla-300 comprises the same insulin molecule glargine as Gla-100, but Gla-300 is three times more concentrated (300 units/mL). This means that the same unitage of glargine in Gla-300 is contained in only one-third of the volume compared with Gla-100. The smaller volume of Gla-300 leads to the precipitation of a smaller, more compact subcutaneous depot which results in a slower, more gradual and prolonged absorption compared with Gla-100 (**Figure 2**).[56,92] The flatter, more prolonged (i.e. more physiological) PK/PD of Gla-300 vs Gla-100 are evident in a study comparing the two BI analogues at the same dose.[93] Similar findings have been observed when Gla-300 has been studied in clinically relevant conditions where slightly higher doses are required in people with T1 diabetes to match the glucose-lowering effect of Gla-100.[94] In fact, due to the more prolonged residence time in the subcutaneous tissue, Gla-300 undergoes greater degradation by proteolytic enzymes, resulting in the lower bioavailability than Gla-100[95] which explains the non-bioequivalence vs Gla-100[93-95] as well as IDeg.[95] In the only study comparing head-to-head the clinical doses of Gla-300 and IDeg required to reach similarly good glycaemic control in people with T1 diabetes, doses were ~25% higher while the within-day variability was ~23% lower with Gla-300.[96] Higher doses of Gla-300 than Gla-100 have also been seen (~10–15%) in extensive studies in people with T2D.[97]

2.2. Evolution of insulin delivery technology

Over the years, several potential routes of insulin delivery have been evaluated. There are many significant challenges with each of these routes[98] but research is ongoing to overcome these limitations. For example, the attractive potential of oral insulin is limited by the fact that insulin is a peptide hormone, and as such is destroyed by gastric acids and pancreatic enzymes, and suffers from low permeability through the intestinal membrane. Employing polymer coatings, protease inhibitors and permeability enhancers to protect insulin from gastric acids and improve absorption through the intestinal membrane show promising results,[99-102] although much larger doses of insulin may be required compared with subcutaneous injection and there are concerns about the absorption of potentially toxic excipient molecules.[103,104] An additional limitation with the oral route of insulin delivery is of course the large variability in absorption depending on the presence of food in the intestine. Intranasal insulin could overcome the hurdle posed by gastric acids, but is also limited by low bioavailability due to the reduced permeability of insulin through the nasal mucosa. Furthermore, the use of excipients can improve absorption and bioavailability but may cause damage to the nasal mucosa.[105] Another alternative is inhaled insulins of which to date, two have reached the market; Exubera®, launched in 2006 but withdrawn in 2007, and Afrezza®, which is still available.[98,106] Concerns about long-term lung safety and the very short duration of action (pre- and post-prandial dosing is ideally required) limit the practical application of inhaled insulin. Transdermal delivery would overcome the pain and fear patients may experience with injections, but the insulin protein is unable to penetrate the outermost layer of skin without assistance by topical enhancers.[98] However, microneedle patch systems that can painlessly pierce the skin to deliver insulin are in development, and may also employ biopolymer technology to moderate the rate of insulin delivery according to the levels of glucose, i.e. glucose responsive insulins.[107,108] Currently, the most common method for administering insulin remains via the subcutaneous tissue, either using syringes, insulin pens, implantable devices or continuous subcutaneous infusion (CSII). Incorporating the delivery method with improved glucose monitoring and computer algorithms has resulted in more automated systems that can further reduce the burden of diabetes.[109]

Insulin syringes

In 1923, the first insulin commercially available was in concentrations of 3–5 units/mL. With the advent of continual process improvements, concentrations of insulin formulations increased rapidly to 20 units/mL administered using a syringe designed with 20 division marks per mL, then to be followed by 40 (1924) and 80 unit/mL (1925) concentrations that led to confusion and dosing errors.[110] As a result, 100 unit/mL insulin became the standard concentration, with two syringe sizes for injection of up to 50 or 100 units.[110] The original glass vials and reusable syringes with large-bore needles have since been replaced by disposable syringes with smaller, finer-gauge needles, which improved convenience, safety and reduced injection pain.[111]

Insulin pens

The introduction of insulin pens comprising of an insulin cartridge, a dose-adjustment dial and a needle, increased simplicity, convenience, discretion of administration and improved dosing accuracy.[111,112] Such insulin pens can either be pre-filled and disposable, or reusable with insulin cartridges, with high-capacity pens providing higher insulin doses without the need for multiple injections. Half-unit pens have also been developed for children and other people with low insulin requirements.[111,113] Connected insulin pens can communicate with Bluetooth enabled glucose meters and diabetes apps, providing data on injections (e.g. timing, dose, insulin-on-board, missed dose reminders), and provide dosage recommendations.[111,113]

Insulin pumps and artificial pancreas technology

CSII was originally introduced in the 1970s for T1D when it was demonstrated to improve blood glucose control with less variability, especially at night versus multiple daily injections.[114] Until few years ago, several barriers contributed to low numbers of people with T1D using pumps, primarily the higher cost, the need for greater patient and clinician input. However in recent years there has been an increasing popularity of CSII especially because of the advent of more reliable continuous glucose monitoring (CGM),[111] while the introduction of software that allows cross-talk between sensor and pump has successfully minimized the risk of hypoglycaemia, and partially “closed the loop”.[111] Currently, hybrid closed-loop systems require the patient to input carbohydrate counting and agree to the bolus insulin amount determined by the automated system throughout the day and night.[115]

In T1D, bi-hormonal artificial pancreas systems delivering both insulin and glucagon may prove to be more beneficial in avoiding hypoglycaemia in situations with rapidly changing glucose levels (e.g. during exercise or around daily mealtimes).[116] Adjustment in insulin administration alone may be sufficient at times when glucose levels are changing less rapidly, such as overnight.[116]

2.3. Evolution of hypoglycaemia - assessment and clinical relevance

Insulin has the greatest efficacy of any therapy in terms of blood glucose reduction, however, achieving target glycaemic control with insulin is limited by the risk of hypoglycaemia. From mealtime RHI to rapid-acting insulin analogues, the risk of late post-prandial hypoglycaemia has decreased,[78] although it is difficult to substantiate this result in rigorous meta-analyses.[117,118] Similarly, the transition from NPH and Lente insulins, to first- and now second-generation BI analogues, with improved PK/PD characteristics (**Figure 3**),[56] has reduced the risk of hypoglycaemia.[119,120 97,121,122]

The concerted effort to develop new insulin formulations with a lower risk of hypoglycaemia acknowledges the severe impact that hypoglycaemic episodes can have on people with diabetes. Older adults, those with longer diabetes duration, lower insulin reserves, and/or impaired kidney function, are at greater risk of hypoglycaemia, while pursuing lower glycaemic targets.[123] Non severe hypoglycaemia events (NSHE) are widely under-reported, but they can be associated with economic consequences due to lost productivity and out-of-pocket expenses, feeling of tiredness, fatigue, having a lower quality-of-life and emotional wellbeing, impaired cognitive and physical function, and an increased risk of cardiac events.[124] Experiencing hypoglycaemia is also a disincentive to adhere to treatment, and is associated with a higher likelihood of under treatment or even discontinuation.[125] Fear of hypoglycaemia, among people with diabetes but also their healthcare providers, can also lead to delays in insulin initiation and inadequate insulin titration,[126] all of which are likely to worsen long-term outcomes.

Recurrent events of hypoglycaemia can lead to hypoglycaemia unawareness, defined as the failure or suboptimal ability to sense the symptomatic drop in glucose levels below normal, increasing the risk of subsequent severe hypoglycaemia.[127,128] However, unawareness of hypoglycaemia is potentially reversible as long as the daily risk for hypoglycaemia is reduced with better diabetes management,[129] suggesting that more physiological mealtime and basal insulin preparations be employed to allow achievement of glycaemic targets, while minimising the risk of hypoglycaemia.

The importance of achieving glycaemic control without significant hypoglycaemia is highlighted as a treatment goal in clinical guidelines.[130,131] However, glucose targets should be individualized with less stringent targets for those at risk of severe hypoglycaemia.[130,132] To standardise the reporting of hypoglycaemia, recent guidelines have adopted a 3-level categorisation of hypoglycaemia. Level 1 is defined as BG <3.9 mmol/L (<70 mg/dL) and ≥3.0 mmol/mL (≥54 mg/dL), which is the threshold for counter-regulatory hormone release followed by appearance of specific symptoms, provides an alert value that allows time for corrective action to be taken. Level 2 is defined as BG values of <3.0 mmol/L (<54 mg/dL), the threshold at which neuroglycopenic symptoms begin to occur and immediate action is required.[130,133] Level 3 describes severe hypoglycaemia, and is not associated with a BG threshold but is characterised by an altered cognitive state and/or physical status that requires urgent third party assistance.[130] The thresholds for Level 1 and 2 hypoglycaemia are now reflected in time-in-range (TIR) and time-below-range targets for CGM,[130,134] although it should be remembered that hypoglycaemic symptoms will appear at lower plasma glucose concentrations after recent hypoglycaemia, but at higher concentrations in patients with inadequately controlled diabetes with infrequent hypoglycaemia. Therefore, putative TIR thresholds may require adjusting to accommodate these different scenarios.

2.4. Evolution of treatment practice

Insulin combinations

The ideal strategy for replacement insulin therapy is to mimic the normal physiological levels of insulin secretion. However, before the physiology of endogenous insulin secretion was fully understood it was difficult for clinicians to provide adequate insulin coverage for people with diabetes. Following the availability of NPH in the 1940s, a number of different regimens were used as a substitute for multiple daily injections of rapid-acting insulin. These included once- or twice-daily NPH for convenience, and the 'split-mixed' regimen of twice-daily combinations of rapid- and intermediate-acting insulins that ultimately led to the concept of a twice-daily 'premixed' insulin regimen (with fixed-ratio combinations of the longer- and shorter-acting constituent insulins) which was widely adopted by people with T1D and T2D. Only after the development of the radioimmunoassay by Yalow and Berson in 1960[39] and subsequent studies[37,38,135] could plasma insulin levels be accurately measured, leading to the understanding that to best mimic endogenous insulin secretion, a basal-bolus regimen was needed. In people with T1D a basal-bolus regimen is now the recommended

approach,[34] and premixed insulins are not generally recommended as a treatment given the inability to independently titrate the constituent insulins.[136] Basal-bolus insulin treatment can also be appropriate for people with more advanced T2D, typically as an intensification after basal insulin when glycaemic control is not achieved. In such situations premix insulin is still considered an option, but it is a suboptimal choice as compared to the basal-bolus approach[34] because the constituent therapies cannot be titrated separately thus limiting the potential of the insulin regimen to adequately control hyper- and hypoglycaemia.

In T1D, adjunctive therapies are less common as insulin replacement is an absolute requirement but those that target additional pharmacological pathways such as amylin analogues,[137] GLP-1 receptor agonists and SGLT2 inhibitors continue to be evaluated.[34,138] In T2D, the multifactorial and progressive nature of the disease often requires the combination of several therapeutic options to be considered,[34,131] and the positive results of combining of basal insulin and a GLP-1 receptor agonists have recently received much attention, especially when obesity is present.[130]

Towards self-management

With the increasing recognition of the importance of the patient voice and experience, treatment practices have evolved towards greater emphasis on diabetes self-management. In the 100 years since insulin was developed, treatment of diabetes has evolved from inpatient to outpatient settings, from solo physician-led care to multidisciplinary diabetes team-based care, and from exclusive specialty care to primary and shared care models.[139] Direct patient contact and self-management education provided by a multidisciplinary diabetes team remains a vital aspect of treatment.[140] This increasing focus on self-management and, more recently, the move towards virtual clinics has been particularly relevant during the Covid-19 pandemic.[141] Technological advances have been instrumental in the move towards self-management, providing greater access to data and guidance. Since the introduction of blood glucose meters in the 1970s, self-monitoring of blood-glucose has become the standard of care.[142] Subsequent advances in CGM technology allow for more frequent and accurate readings to be taken in real-time, with more detailed assessments of blood glucose profiles to inform appropriate goals and treatment.[142]

Towards individualization

Patient-centred care is now a key part of clinical guidelines, with choice of medications, dose and BG targets depending on factors such as age, activity, comorbidities and patient expectation.[34] As more evidence accumulates, guidance documents are providing more specific diabetes management recommendations for older adults,[143,144] children and adolescents. [145-147] European Association for the Study of Diabetes (EASD) / American Diabetes Association (ADA) guidelines stratify therapy options for people with T2D by the presence of atherosclerotic cardiovascular disease (ASCVD) risk factors or chronic kidney disease, or whether there is a compelling need to avoid hypoglycaemia and/or weight gain, or if cost is a major issue.[131] Insulin is not considered as a first-line therapeutic option for T2D,[34] with newer therapeutic options recommended owing to their lower risk of hypoglycaemia and proven CV benefits.[131,148] However, introducing insulin needs to be considered in people with CV disease/risk factors and/or renal impairment,[34,131,148] to support achievement and maintenance of the individual glycaemic targets. Insulin, a natural hormone, can be added to any other glucose lowering drug, including the GLP-1 RA and SGLT2 inhibitors, both of which have been shown to have cardio-renal benefits.[34,131] In those, perhaps many people with diabetes, in whom insulin is needed to keep HbA_{1C} at the target, insulin can exert a powerful CV/renal protective effect (the "hidden" protective effect of insulin). Thus, in those persons initiated to more recent therapeutic options, but remain above HbA_{1c} targets within a 3–6-month interval, insulin (basal and/or prandial as appropriate) should be introduced.[131] It is hoped that in the next international guidelines, basal insulin will be re-admitted as an earlier stage of treatment to more effectively reach and maintain better glycaemic control with strong CV benefits.

Diabetes is a heterogeneous disease, which complicates therapeutic management, but recent classification of specific subgroups of type 2 diabetes (such as severe insulin deficient diabetes [SIDD])[149] could help health care professionals (HCPs) to identify those individuals who would benefit most from insulin therapy. This move to more precision medicine in diabetes will be supported by the analysis of "big data" to not only accurately identify diabetes subtypes but also to predict responses to different therapies and integrating data from ongoing monitoring to optimise therapeutic management.[150]

The future of insulin

Further developments in both prandial and basal insulin formulations are ongoing. As well as the new generation of faster rapid-acting insulin analogues,[82] a once-weekly basal insulin, Icodec (NovoNordisk), was recently investigated in insulin-naïve people with T2D and shown to be non-inferior to once daily Gla-100.[151] However, the safe titration of a weekly insulin may be challenging, as suggested by the higher risk of Level 1 hypoglycaemia reported with Icodec versus Gla-100.[151] It will be important to include insulin-deficient and insulin-treated people in future studies, who are at greater risk of hypoglycaemia as compared to insulin-naïve people. It is also important to note that Gla-100 was used as the comparator.[151] Therefore, Icodec should be compared with the daily second-generation BI analogues IDeg and Gla-300 that provide improved hypoglycaemia profiles.[97,121] The potential for increased risk of hypoglycaemia may impact the clinical use of Icodec, given that the weekly dosing provides less flexibility in terms of titration versus second generation BI analogues. Consequently, additional studies are ongoing and being planned to explore the possible clinical application of Icodec and other once weekly insulins. A study with the once-weekly basal insulin, LY3209590 (BIF) (Eli Lilly), has been conducted in T2D and demonstrated similar reductions in HbA_{1c} to degludec, and a lower rate of hypoglycaemic events when targeting fasting blood glucose levels of <140 mg/dL.[152]

Other potential avenues of insulin evolution include the development of “smart insulins”, which refer to strategies involving glucose-responsive insulins (GRI) for the delivery of insulin in accordance with the ambient levels of glucose, and therefore mitigate the risk of hypoglycaemia.[153] This interesting but difficult concept has now been under investigation for some time.[154] The glucose-sensing system may be achieved either by embedding insulin within a matrix of biopolymers that regulate the release of insulin, or by conjugating the insulin molecule itself to motifs that are able to sense glucose levels.[153,155,156] Such glucose-sensing technologies have potential applications in various administration routes for insulin.[108,157]

Alongside such developments in insulin formulations and delivery systems, the advent of newer technology for insulin delivery with the support of artificial intelligence provides the opportunity to further optimise diabetes management. For example, algorithms that evaluate many sources of data, including physical activity, carbohydrate intake, and blood glucose levels, have been shown to effectively predict the risk of hypoglycaemia in individuals and improve glycaemic control by automatically providing insulin dosage recommendations.[158] Integrating these algorithms into the next generation of smart insulin pens may help

reduce the burden on individuals and HCPs in terms of data interpretation and insulin dose calculations whilst also limiting costs.[159] However, despite the enormous potential of these more recent advances to improve diabetes care,[160] current global access to insulin remains a significant concern in, but not exclusively, low- and -middle income countries.[161] While biosimilar insulins may help improve access to the drug itself, many of the barriers such as cost and lack of human resources for training and education, will similarly impact on the potential progress with newer technologies.

3. Conclusions

Since the pancreatic extract containing insulin was for the first time successfully injected in humans in 1922, efforts have continued to be made to improve insulin preparations in terms of purity and pharmacological properties, in an attempt to normalise the blood glucose levels in people with diabetes [144]. NPH and the Lente family of insulins developed in 1940s and 1950s, were the first insulins that could be considered to be basal insulins. Since then, many improvements in both basal and bolus insulin formulations have occurred. The first rapid-acting insulin analogue became available in 1996, and was soon followed by the first-generation BI analogues in the 2000s in the form of once-daily glargine Gla-100[77] and once- or twice-daily IDet.[75] The second-generation of longer-acting insulin analogues IDeg and Gla-300 appeared in the 2010s [76,78], along with the more rapid rapid-acting insulin analogs.[82] In 2020, third generation once-weekly BI, Icodec and BIF, have emerged and are currently being evaluated.[151,152] As insulins have evolved, so has the technology for insulin administration improved for both subcutaneous and parenteral routes and for the intermittent or continuous monitoring of glucose in blood and interstitial space, respectively. Today, insulin continues to be an essential life-saving medicine for approximately more than 30 million people with T1D globally,[36] and potentially for many more million others with advanced T2D.[162]

We should not forget that while insulins and other medicines can effectively manage diabetes, they do not cure the disease (as once noted by Elliott Joslin, “Insulin marked the end of one era in diabetes management, not the end of diabetes”). Research into treatments and strategies that may prevent or reverse diabetes is ongoing, with the hope that the ‘Flame of Hope’ outside Banting’s former residence can finally be extinguished in recognition of a cure for diabetes (**Figure 4**). During the last century, despite the introduction of many new

anti-hyperglycaemic medications and some recent ones with proven cardiovascular benefits, insulin has remained central in the treatment of diabetes. Insulin is indispensable for many people with diabetes to reach and maintain the desired glycaemic targets, and is expected to remain a vital part of diabetes management for the foreseeable future.

We hereby celebrate the epoch-making discovery and the first successful application of insulin to a person with diabetes[20] and the subsequent evolution of insulin therapy which, although not a panacea, has transformed the life of countless people with diabetes during this first centenary of use. However, in today's world, access to insulin remains beyond the reach of one in two people whose existence and quality of life relies on insulin.[163] This problem of affordability and availability of insulin is not restricted to low- and middle-income countries, being evident also in high-income countries where people forgo or economise their insulin use with dire short- and longer- term consequences.[161,164,165] It also remains to be seen whether the advent of biosimilar insulins will provide the anticipated benefits in terms of cost and availability. The challenges to ensure insulin is available and affordable to all those in need and not just for some[166] are complex, and require a range of different solutions.[161,164] Ensuring insulin and future innovations in insulin therapy with improved delivery of care and education become available to those in need is a priority for the coming centenary, especially when faced with an unprecedented, increase in diabetes world-wide.[35]

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Tables and Figures

Table 1: List of investigators who tried to isolate the principle of internal secretion of the pancreas between end of 19th century and 1922. From Owens D.R.[33]

Capparelli, 1892	Sjöquist, 1908
Comby, 1892	Lépine, 1909
Battistini, 1893	Pratt, 1910
White, 1893	Knowlton & Starling, 1911
Vanni, 1895	Scott, 1911
Hougounena & Doyou, 1897	Massaglia & Zannini, 1912
Blumenthal, 1898	Murlin & Kramer, 1913
Hédon, 1898	Clark, 1916
Zuelzer, 1903 – 1914	Kleiner & Meltzer, 1919
Gley, 1890 - 1905	Paulescu, 1916; 1920 – 1921
De Witt, 1906	Banting & Best, 1921 – 1922
Rennie & Fraser, 1907	

Figure 1: Timeline of key milestones in the history of diabetes and insulin

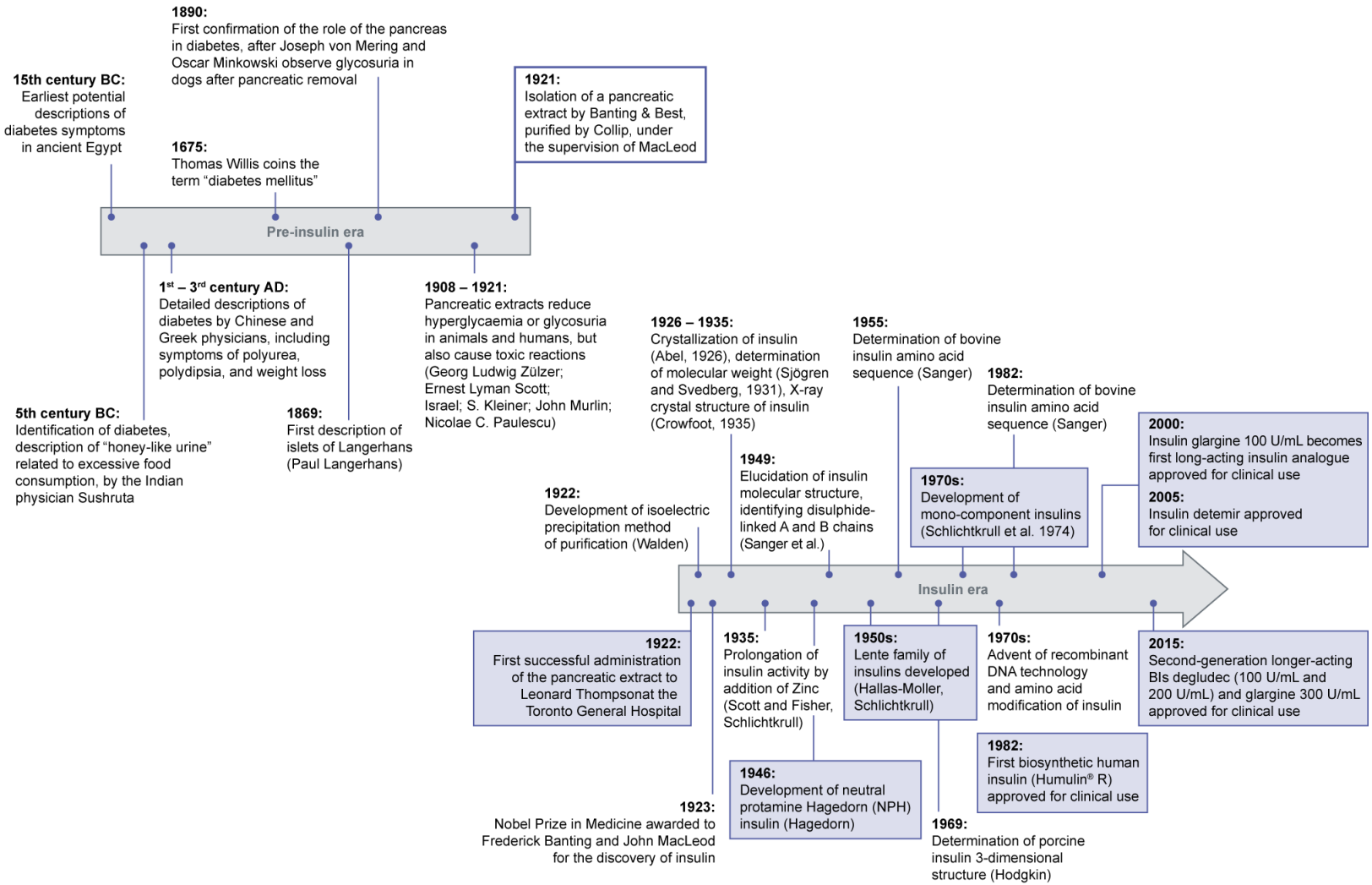
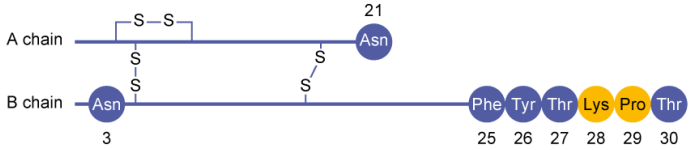


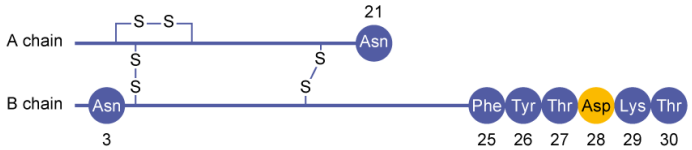
Figure 2. Modifications and mechanisms of action of insulin analogues

A) Rapid-acting insulin analogues

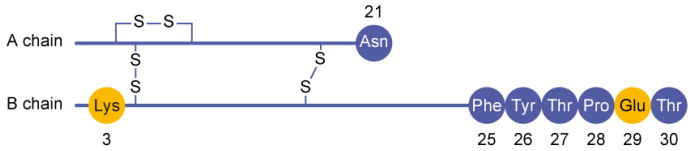
Lispro: Amino acid inversion results in rapid dissociation into monomers for faster absorption
Ultra-rapid lispro: Excipients treprostinil and citrate enhance vascular permeability and local vasodilation
Biochaperone lispro: Excipients BioChaperone BC222 and citrate enhance diffusion



Aspart: Amino acid substitution prevents self-association into insulin dimers and hexamers, increasing rate of absorption of monomers
Faster aspart: Excipients niacinamide and L-arginine increase s.c. blood flow

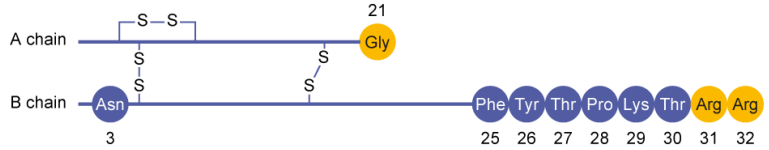


Glulisine: Amino acid substitutions result in enhanced molecular stability and lower isoelectric point (pH 5.1), increasing solubility at physiologic pH

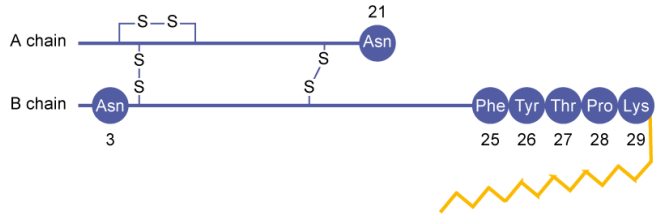


B) Basal insulin analogues

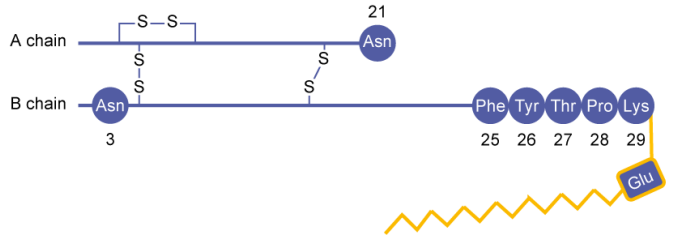
Glargine: Amino acid modification (retention of di-arginine) increases the isoelectric point to pH 6.7 (from pH 5.4 of human insulin). Micro-precipitates form after s.c. injection and are slowly released into the blood.
Gla-300 contains the same modifications as Gla-100 but is 3 times more concentrated, resulting in a smaller s.c. depot that slows the rate of absorption



Detemir: Acetylation with 14-carbon myristic acid results in self-association as di-hexamers and reversible binding to albumin in injection depot and in circulation slows rate of absorption

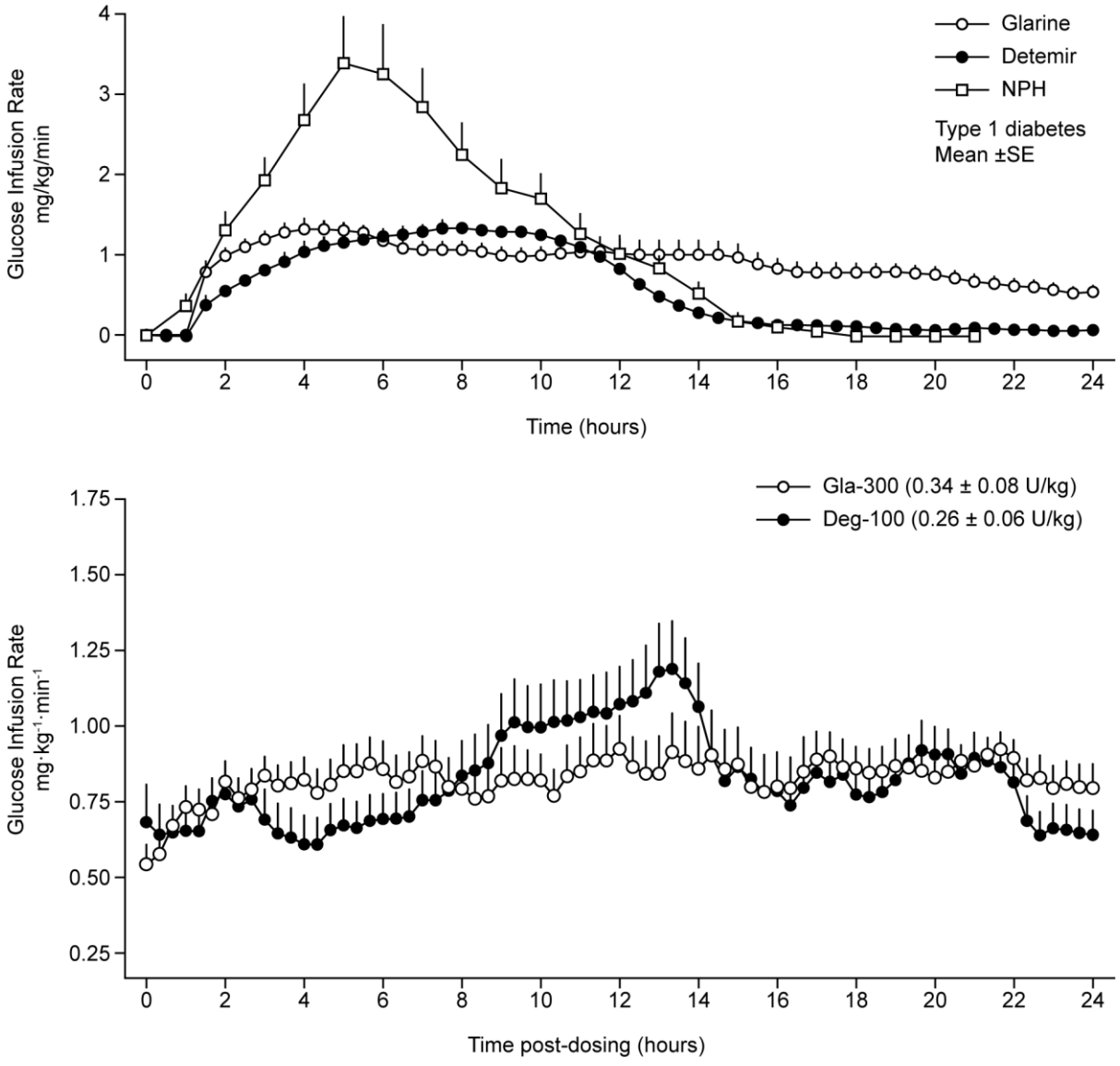


Degludec: Acetylation with 16-carbon fatty diacid via a glutamic acid spacer results in multi-hexamer formation at injection depot and albumin binding in circulation, which slows rate of absorption



Orange denotes modifications compared with human insulin

Figure 3. (A) Glucose infusion rates of NPH, Gla-100 and detemir in people with type 1 diabetes and (B) glucose infusion rates at clinical doses of Gla-300 and IDeg in people with type 1 diabetes.



A) Reproduced from Rossetti et al. Prevention of hypoglycemia while achieving good glycemic control in type 1 diabetes: the role of insulin analogs. Diabetes Care. 2008;31 Suppl 2:S113-20. © 2008, American Diabetes Association. B) Reproduced from Lucidi et al. Pharmacokinetic and Pharmacodynamic Head-to-Head Comparison of Clinical, Equivalent Doses of Insulin Glargine 300 units·mL^{-1} and Insulin Degludec 100 units·mL^{-1} in Type 1 Diabetes. Diabetes Care. 2021 Jan;44(1):125-132. © 2008, American Diabetes Association.

Figure 4. The Flame of Hope

Photograph by Ken Lund from Reno, Nevada, USA, CC BY-SA 2.0, via Wikimedia Commons

The Flame of Hope in London, Ontario, Canada, serves as a reminder that insulin manages but does not cure diabetes, and the flame will only be extinguished when a cure is developed.