Glucose lowering strategies with insulin

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Abstract

People with type 1 diabetes must use insulin and a large fraction of those with type 2 condition also do so. Many therefore struggle with the unpredictable balancing of insulin dose with calorie intake and utility. A healthy pancreas makes meticulous adjustment on a continuous basis that present therapeutic insulin administration cannot match. However, much progress has been made to make it simpler to inject both background and fast-acting boost insulins with a view to better mimicking normal pancreatic output. The present fast insulins are reviewed with accent on the primary amino acid structures of the biosynthetic types that diffuse more quickly than regular insulin that associates in hexamers. This makes boost doses kinetically and clinically more effective, allowing people to inject better estimated boost and corrective doses. Formulation advances are discussed for their present and potential contributions. The newer slow-acting insulins are also described and compared, their advantage also being kinetic with a lower likelihood of inducing overnight hypoglycaemia when used optimally. Finally, the appreciation of the advantages of alternative routes of administration such as oral and peritoneal are included in this review because of the possibility of altering the hepatic to peripheral ratio, the reasons for which are more effective but less obesogenic insulin activity. The logistics of oral insulin are summarised in terms of the risks to the insulin structure, the facilitation of paracellular uptake at the apical surface and the paradoxically advantageous hepatic first pass. Other non-invasive routes are also included in the review.

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Key words: new insulins, insulin amino acid modifications, conjugations, formulatory changes, unfolding and aggregation protection, closed loop, delivery routes, hepatic to peripheral ratio

Introduction

Many patients with type 2 diabetes (T2D), all patients with type 1 diabetes (T1D) and eventually almost all those with latent autoimmune diabetes of adulthood (LADA) need insulin, the latter – as with conventional T1D – to prevent ketoacidosis. For all insulin users, the aim should be to match insulin delivery to the pattern found physiologically in healthy counterparts, in order to keep the blood glucose within the target range for most of the time. For T1D, success is often only partial and so HbA_{1c} values are commonly higher than target. This is true even for children for whom preventive measures for complications are so important.[1–3] The reason is that the risk of hypoglycaemia is commonly perceived to outweigh the risk of long-term complications in the day-to-day management of many users, perhaps particularly for children. This may relate to concomitant glucagon and/or glycogenolysis failures or glucagon resistance.[4] It is the case, therefore, that a significant fraction of the diabetic patient population needs improvements in the methods used to deliver insulin in order to improve safety and efficacy. Here, we chart progress in the design of approaches from the conventional to the innovative and futuristic. We will review various strategies including formulations to deliver, stabilise and protect the insulin molecule, improve comfort, convenience and compliance with the ultimate aim of optimising glycaemic control.[5]

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Open and closed loop

Open loop and tight control

Since the demonstration that tight control of blood glucose (BG) is beneficial for the prevention of diabetes complications, multi-dose injection (MDI) with basal and bolus (prandial and corrective) insulin accompanied by frequent BG testing has become the standard for T1D and not uncommon for T2D. Structured education programmes such as DAFNE and DESMOND have enabled people to take advantage of these approaches. The tools for the job include the increasingly improved BG meters, flash and continuous glucose monitoring systems (CGMS), smart phones for capturing, logging and manipulating data, internet guidance, improved injection devices and new insulins.

Open loop treatment of diabetes is the term used when the judgement of quantity and timing of insulin is left to the users, carers and/or prescribers.[6] This may be as unsophisticated as to impose a relatively constant daily macronutrient regimen (content and timing) while prescribing a fixed, matched quantity of insulin. The adjustment is usually made by healthcare providers on behalf of the individual in an attempt to keep BG within reasonable limits. This is no longer regarded as a suitable approach for the majority of people with T1D, for whom a more intensive MDI or insulin pump regimen is recommended which is sufficiently flexible to achieve tight glucose control despite variable amounts and timing of food, physical activity etc, thus placing the decision with the user rather

Figure 1. The primary structure of human insulin

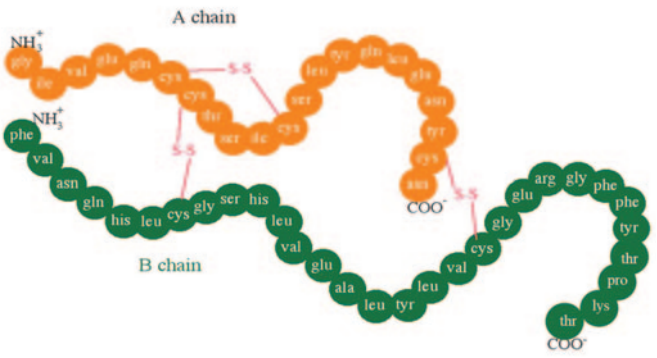
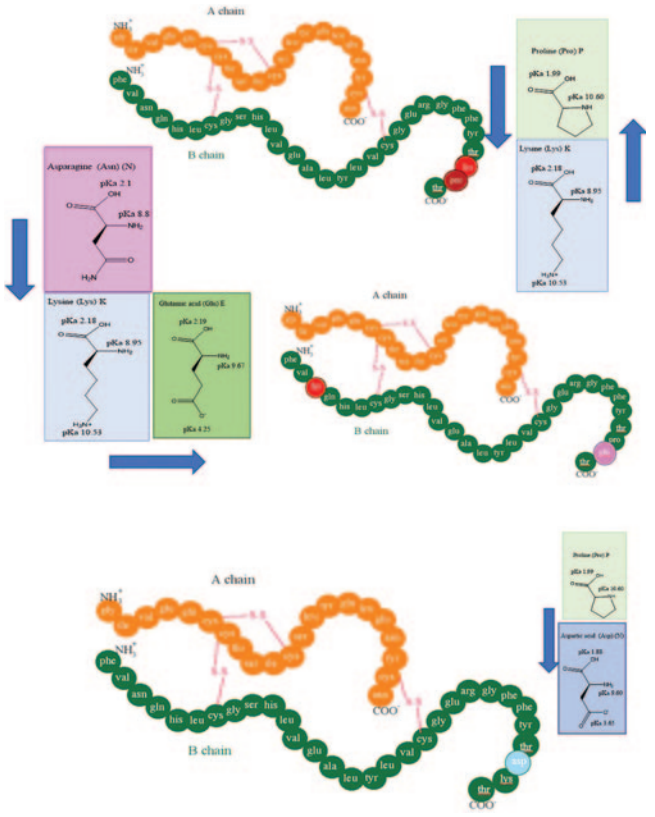


Figure 2. The biosynthetic short-acting insulins in current use: lispro, lysine and proline reversed at B28 and 29 (top); glulisine, asparagine at B3 and glutamic acid at B29 (middle); aspart, aspartic acid at B28 (bottom)



than the prescriber. Intensive control therefore requires the user to measure the BG fairly frequently and to calculate and administer bolus doses before food and as corrections. This method clearly relies on education and accurate BG information, often entering the latter on bolus advisor meters or phone apps that are available to help cope with the complexity and continual nature of the demands. The advent of flash and CGMS, that measure tissue fluid glucose rather than BG, gives information that is collected many times per hour, albeit with a potentially significant time lag in comparison with BG. Achieving tight control in order to reduce HbA_{1c} to the T1D optimum 48 mmol/mol (6.5%) is challenging because, not only is the BG a moving target, but a distorted sympathetic neuronal control of the glucagon-based counter-regulatory system may complicate the effects of imposition of harsh antihyperglycaemic doses/pump dose rates. Resulting hypos are common,[7] estimated at CGMS-measured 2.1 events per 24 hours that often go unnoticed even in hypo-aware people. This mitigates against safe glucose control despite the likely lower HbA_{1c} results and is especially true in people with hypoglycaemia unawareness. This has led to the concept of glucose targets being set for 'time in range' rather than HbA_{1c}.

Development of injectable insulins for intensive control regimens

A variety of innovative fast- and slow-acting insulins are now available, including the ultra-long-acting degludec and fast-acting insulins such as glulisine and fast aspart. Their purpose is to make tight control achievable by providing the tools for imposing sustained background control and tailored fast-acting doses around mealtimes to prevent post-meal hyperglycaemia without delayed hypoglycaemia. Over the last decades, specific insulins have been marketed that claim to have advantages when used in MDI regimens.

The structure, design strategies and formulation are comprehensively reviewed by various authors[4,8,9] but briefly, have mainly involved the biosynthetic alteration of the amino acid content, sequence and/or conjugation, resulting in changes in charge, hydrogen bonding and hydrophobicity. These modifications create the required pharmacokinetics either by solubility, competitive binding or changes in quaternary association, although modified insulins are generally inherently less resistant to physical destabilisation than the native molecule that normally groups in dimers and hexamers in concentrated solution (Figures 1 and 2).[10]

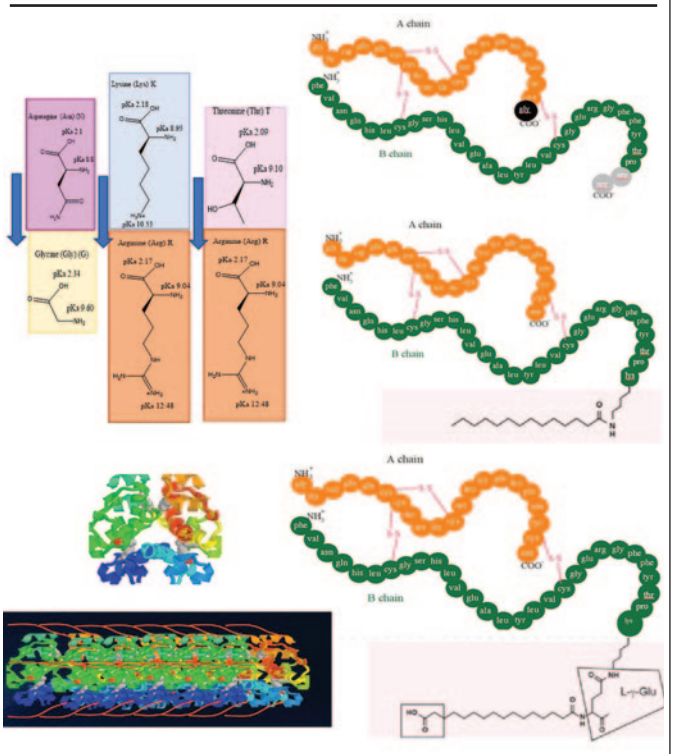
Formulatory changes in the solvent medium for insulins are additional common approaches,[11,12] with polyethylene glycol (PEG), glycerol and ethanol having been tried for their effects on electrostatic interactions in the highly polar insulin molecule. Chelating agents, antioxidant sugars, surfactants and amino acids such as lysine, arginine and glycine have also featured in developmental work, often to create the monomeric form. Linjeta (Viaject) was formulated with EDTA and citric acid to chelate zinc, for example, but has not been pursued. Two agents have been incorporated with aspart (Novorapid®, Novo Nordisk), a monomeric insulin that is given as a preprandial bolus and is commonly used in insulin pumps[13,14] because it may be cleared from the subcutaneous tissue quickly enough to transmit pump rate changes to appropriate modification of BG adjustment. Loss from skin to plasma is a more blunted process with soluble insulins that persist in hexamers, because the diffusion coefficient is much lower. Fiasp® (Novo Nordisk), like the normal version of aspart, contains glycerol, metacresol, phenol, zinc and pH adjustment by phosphate, hydroxide and hydrochloric acid.

However, it achieves an even more prompt onset of action and physiological profile by the addition of niacinamide (nicotinamide, niacin, vitamin B3) as an absorption enhancer that works not only as a localised vasodilator, but by increasing the fast diffusing monomer fraction by about 35%.[15] L-arginine is an additional agent working as a refolding protector and thus a stabiliser against aggregation. In this context, aggregation means an unwanted grouping in unspecified numbers of large molecules, usually peptides and proteins. It is different either from association into quaternary structuring such as hexamers or amorphous precipitation such as occurs with excess zinc or at the isoelectric zwitterionic pH point (pI), which can each preserve activity, as does crystallisation, even if some reversible unfolding happens. In aggregation, however, the normal tertiary and quaternary structures are lost because it involves irreversible unfolding. Correct folding is accomplished in the beta cell's endoplasmic reticulum and is normally vital for activity whether the insulin is endogenous or biotechnically synthesised. Unfolding is therefore a serious degradative change that may facilitate further permanent transformation, often involving amyloid fibrous structures that are inactive, potentially antigenic and can accumulate at injection sites. An aggregation protector is therefore an important formulation success. In the past, soluble Hoe21PH insulin for pump use was stabilised against aggregation by the poloxamer micellisation agent Genapol, in line with surfactant strategies for proteins stabilised in general.[16,17] In the case of Fiasp®, the combined changes to the previous aspart formulation halve the time of appearance in plasma with 74% greater insulin action within the first 30 min, the clear aim being better postprandial control.[14,18]

By contrast, detemir and degludec were synthesised as soluble, long-acting products both involving the covalent addition of polymeric lipophilic side chains.[9,19] These alter the kinetics to produce low level basal dosage with a flattened plasma profile; this was an improvement on what could be achieved with suspension products like isophane (NPH) insulins and with insulin zinc suspension (IZS). The clinical value with these newer products in MDI regimens was the reduction in overnight hypoglycaemic events. Detemir has a myristic (C14) acid substitution at a terminal B29 lysine (no threonine), and its slow action is attributable to hydrophobic self-association in tissues and also to binding of the acyl chain to fatty acid binding sites on serum albumin.[6,20] A further flattened profile is associated with degludec ($t_{1/2}$ ~25 hours, duration >40 hours) and is enabled because, despite its superficial similarity to detemir, its des-30-structure (again no threonine) forms very long unique hexameric sequences stabilised by zinc, phenol and hydrophobic contact between covalently attached hexadecanoic (C16) diacid chains linked at B29 with glutamic acid.[9,19] These lipophilic agents therefore differ from an alternative soluble long-acting insulin, glargine, which has a replacement of glycine for asparagine at position A21 and addition of two arginine molecules at positions B29–30. This alters the pI such that precipitation occurs at pH 7.4 instead of 5.4 as with native insulin (Figure 3), thus creating a subcutaneous depot.

A variety of other new kinetic approaches included a liver-specific analogue that was an interesting departure. Peglispro

Figure 3. The biosynthetic soluble long-acting insulins in current use: glargine, terminal A chain asparagine to glycine and terminal B chain lysine and threonine to arginines (top); detemir, acyl group at B29 lysine (middle); degludec, hexadecanoic (C16) diacid chains linked at B29 lysine with a glutamic acid linker (bottom). Bottom left shows the insulin hexamer and its ability to form strings in degludec insulin

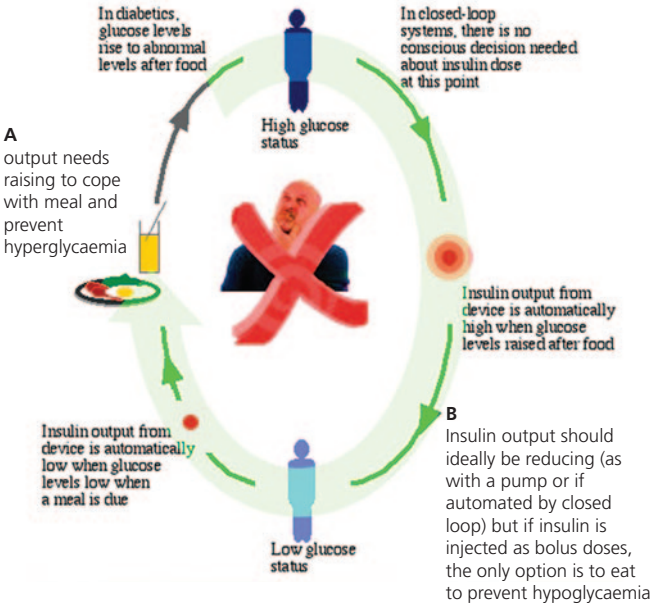


(LY2605541) was a developmental effort to slow the release but also to raise the ratio of hepatic to peripheral insulin, changing the ratio of hepatic glycogenolysis to peripheral glucose disposal and thus to suppress the weight gain associated with insulin medication. Other strategies such as oral insulin have a similar aim. The 5.8 kDa insulin lispro monomer had a 20 kDa linear PEG moiety covalently bound to the B28 lysine (not B29 as in native insulin), and promoting the binding of three water molecules thus giving a hydrodynamic radius equivalent to a globular molecule of about 78 kDa. This slowed the renal excretion as well as the absorption from the subcutaneous administration site. Peglispro binding to the insulin receptor is much reduced and thus the effective molar dose is greater. Madsbad[21] reviewed the evidence for the hepatic specificity and Hirose[22] pointed out the additional protective effect of the pegylation towards proteolytic enzymes. By 2015, however, this compound had failed its clinical assessment because of unacceptable lipid values and liver function tests.[23]

Closed loop: the automation of tight control

The closed loop system is the long-term aim of diabetes control using exogenous insulin because it takes decision-making by the

Figure 4. Closed loop control: implies the automation of insulin dosage, meticulous and frequent adjustment replacing the necessity of the user decision points A and B.



user out of the system[24,25] and, in the optimum embodiment, operates by several components including a frequent coupling with real-time sensing to correct BG in small steps, fast response for each corrective move and prompt catabolic mechanisms so that excess insulin is removed (Figure 4).

These points all emulate the physiological process and, to complete the best possible outcome, the insulin output should ideally be oscillatory with a frequency of minutes (ie, within the BG adjustment cycles).[26] Oscillatory release is thought to minimise the downregulation of tissue receptors and oppose resistance developing which is a risk even in T1D.[27] Closed loop systems working to these principles should avoid the danger of hypos yet be able to respond in real time to postprandial and counter-regulatory peaks, keeping BG within normal tolerances 100% of the time.

Currently there are three approaches to closed loop systems: biological (pancreas or islet transplants), electronic (artificial pancreas) and chemical (including smart insulin formulations), of which only the first two are in human development and use, and have been reviewed elsewhere.

The remainder of this article is the formulation of insulins that interact with tissue/BG such as to regulate insulin output.

Routes for insulin delivery in closed loop systems

Injectable and implantable

All people with T1D and about 25% of those with T2D are prescribed recipients of formulations intended mainly for subcutaneous injection. While this route is not the fastest route for absorption, it has the advantage of being an easily-learned technique for self-administration. Its exploitation therapeutically has been the norm, especially as it was safe for the longer acting suspension formulations that have been used for many years. However, some people with diabetes are needle phobic, develop injection site lipohypertrophy or localised allergy or bruise because of concomitant anticoagulant therapy. As a general consequence, efforts have been made to exploit alternative parenteral delivery paradigms such as microneedles, pumps, patch pumps and patches, as well as other routes of administration that are less invasive. Other considerations are important, so that the peritoneal route circumvents the delays due to the dense, fatty subcutaneous skin layer and can be built into viscerally administered insulin from devices, possibly with a similarly placed sensor.[25,28–32] This may solve the potential iatrogenic problem of the non-physiological effects of distorting the peripheral and hepatic concentration ratio of released insulin, as discussed also below. In a different context, Rhea *et al*[33] report access to the CNS mainly by intrathecal, cerebral, ventricular but also nasal and ocular routes, for idiopathic CNS insulin resistance that is inadequately treated by peripheral administration.

Oral

Alternatives to parenteral delivery feature high in diabetes pharmaceutical research.[34] The oral route has been a long-term goal for insulin delivery, not only for convenience and compliance but because the mesenteric to portal drainage implies a potentially normal ratio of hepatic to peripheral insulin, fostering normal liver regulation of hyperglycaemia and differing markedly from parenteral delivery.[35,36] However, the harsh environment of proteolytic digestive enzymes, food interaction and the obstacles to the absorption of large molecules has meant that development has been slow, waiting for tactics to overcome the problems. Protective strategies include chitosan, an aminopolysaccharide gel derived from crustacean shell.[37,38]

Access to gut vasculature via the apical aspect of the gut epithelium is an equal challenge. The transport of large polar molecules depends largely on the paracellular route, implying the traversing of a complex mixed environment gap between cells that is also size limited. Information about the paracellular gap size is variable. Anderson comments that the biology and pharmaceutics approaches differ, and typically drug delivery studies use a series of labelled tracers that may or may not be charged, while transcytosis may be ignored in the assessment.[39] Chitosan and its quaternised derivatives such as trimethylchitosan have the ability to influence permeation by accessing claudin and actin structures.[37,38,40–43] However, the balance of alkyl chain length and charge on the water solubility permeation effectiveness and tight junction molecular recovery is critical, as discussed by Benediktsdóttir *et al*.[44] Maher *et al* categorise both paracellular and transcellular mechanisms, highlighting sucrose laurate, a surfactant-like material, as well as the phosphatase inhibitor PIP 640 and some ionic liquids such as choline geronate (CAGE).[45] These may influence tight junctions to increase gut uptake of insulin, as may the formulation as nanoparticles.[45]

Biologically, it seems that two paracellular routes may co-exist,

Key messages

- The subcutaneous route for daily divided doses or pumped insulin comprises current therapy for most people who use insulin
- The delays in attempting to alter the dose and dose rates in multi-dose and pump systems, respectively, are due to the rate-determining step of the transfer of the large hydrophilic molecule from fatty tissue into capillaries
- A second non-physiological step when normal physiological delivery is replaced by subcutaneous delivery is the low concentration gradient in the liver leading to a reduced hepatic to peripheral ratio. New formulations may increase hepatic targeting even for subcutaneously delivered drug, but the oral and peritoneal routes may also accomplish this
- Closed loop delivery will eventually improve manual programmes for 'tight control' programmes, and the subcutaneous delays in both the insulin delivery and the glucose sensing are slowly becoming less of an obstacle to safe automatic upwards adjustment of insulin delivery for preventing postprandial glucose surges
- Much has improved in insulin management of diabetes, but new insulins, new routes and automated systems should achieve better control and improved outcomes

namely a high capacity one with steep size dependence, a cut-off of 4 Å and a larger gap that may occur in intact tight junctions under the influence of inflammatory cytokines. A gap size of 2–5 Å is thus often quoted but, depending on tissue, where normal transport can admit molecules of 200 Da such as mannitol (3.5 Å) but not usually the fructal inulin (molecular weight variable but possibly 3–5 kDa and about 11 Å, thus approaching insulin monomer size). However, Taverner holds that ileal tight junctions can exist in open and shut form, as a function of the phosphorylation of light chain myosin and its regulatory kinase system that maintains a circumferential tension. Several efforts have been made to set the default to open the junctions for insulin transport through the apical surface using kinase system blockers that have been shown to permit transport of labelled dextran of 70 kDa.[46] In another approach, chitosans have been used not only for their protective effect but because they can open tight junctions in a transient and reversible manner by a disruption of claudin-4, a tight junction protein.[43]

Other non-invasive routes

The use of other routes involving transport across mucous membranes is reviewed by Easa *et al.*[35] Briefly, inhaled insulin has undergone a revival as Afrezza since the abortive attempt with the unwieldy Exubera, because the theory is well-developed, as reviewed by Lin *et al*,[47] and the practicalities have been improved. Transdermal delivery can also include microneedles and, for completeness, it should be mentioned here that iontophoresis systems have been widely studied and sometimes combined with microneedles. Buccal and nasal routes are conceivable, so that micelle-associated insulin in Generex's Ora-lyn has been reported as beginning trials in 2019, but Aquestive's bioadhesive film associated with glycan and insulin aspart-bearing gold nanoparticles (1–2 nm) has failed because, although the particles were renally eliminated and despite apparent absorption through buccal tight junctions, the product demonstrated impractically low bioavailability.[35] Nevertheless, buccal formulations – as well as providing access paracellularly – could be developed to optimise as least basal dose.[48–50] Rectal and vaginal routes seem impractical and neither finds many recent citations except in reviews[51,52] or as a rectal instillation (for anti-inflammatory treatment of colitis rather than for its antihyperglycaemic properties).[53] The ophthalmic route may be effective, as shown in accidental systemic toxicity after an insulin eye mishap,[54] but predictability is an issue due to nasolacrimal loss and other dose impracticalities. The reality is also that administration of insulin by these alternative routes – or, indeed, by parenteral routes other than peritoneal – is unable to emulate the physiological hepatic to peripheral insulin ratio, except for the potential of oral delivery (see above). Ironically, in drug delivery generally, many alternative routes are developed to avoid hepatic first pass effects. However, for insulin, first pass would enable the advantageous liver effects that are suppressed with non-oral delivery. The major effect of hepatic targeting is to curb glycogenolysis and also gluconeogenesis compared with normal, and so glucose peaks are harder to control unless the liver is targeted. Hepatocentric activity would be an asset because a further factor is that glucagon is also often poorly regulated in diabetes so that hyperglycaemic peaks postprandially are potentially exaggerated for that reason also. Finally, the peripheral effects of excess insulin on adipose tissue are obesogenic, which affects treatment compliance.[52]

Conclusions

The quest for much more physiological insulin delivery has been ongoing for many decades, ever since the realisation that crudely dissolved insulin was life-saving but not as a long-term sustainable treatment. It is true that great strides have been made such that diabetic people can now be in possession of the information about their glucose status on a minute-by-minute basis. They have the tools to plan background dosage with bolus doses to cover meals and aim for low HbA_{1c} values yet avoid repeated hypoglycaemic crises. Eating and exercising variably are possible, thanks to education programmes to take advantage of these strategies. The advent of smart phone apps has made this easier, yet still BG and HbA_{1c} levels are too high. The problem is that the target moves, but the delivery of insulin cannot keep pace with the variable need. On the whole, people avoid hypoglycaemia at the expense of hyperglycaemia. A closed system is needed. The perception of a slow pace of commercial development has meant that some users themselves

have become skilled enough to be able to combine pump delivery and sensor to create fairly adventurous and sophisticated artificial pancreases, as described on the Nightscout webpage.[55] The physiological delays that limit the kinetics of skin sensors and delivery systems remain a barrier to development of this type, however, whatever the source. Since algorithm development cannot detect what has not yet happened as a meal is consumed, subcutaneous systems must undergo a change or replacement in the quest. Other systems such as oral, transdermal and pulmonary may ultimately prove to be superior to subcutaneous basal insulins.

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