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REVIEW ARTICLE

INSULIN FORMULATIONS FOR BETTER SUPPLY

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Abstract

Since insulin was first used in 1922 for the treatment of diabetes, the inability to mimic normal physiological secretion has been a major limitation for achieving optimal glycemic control. Therefore, considerable research has been undertaken to develop insulin formulations that have more physiological time-action profiles to cover basal and prandial needs. Although human insulin formulations produced by recombinant DNA technology were a significant advance over animal insulin isolated from pancreatic tissue, therapy still resulted in a delayed onset and prolonged duration of action of bolus insulin. To overcome these limitations, insulin and premixed insulin analogs formulations have been developed in an attempt to mimic the insulin secretion patterns found in individuals without diabetes. This interest has led to the development of insulin formulations that are characterized by flexible, predictable and physiological action profiles along with freedom from the fear of hypoglycemia.

Introduction

Insulin

Insulin produced by the beta cells of the pancreas, which are located in the islets of Langerhans

Biosynthesis

Insulin participates in carbohydrate and fat metabolism. Along with insulin like growth factors (IGF1 and IGF2), it regulates a variety of metabolic and growth-related effects in target tissues, including stimulation of glucose transport, glycogen synthesis, lipogenesis, gene transcription, and deoxyribonucleic acid (DNA) synthesis. Moreover, the insulin/IGF signaling system promotes cell growth and survival, and is essential for normal reproductive capacity.

Human insulin is composed of 51 amino acids with a molecular mass of 6000. It is synthesized from the proinsulin gene located on human chromosome 11 (11p15.5). The proinsulin gene encodes a protein called pre-proinsulin, which is targeted to the endoplasmic reticulum where the amino-terminal signal peptide is removed to produce proinsulin. Proinsulin displays only 10% of the biological activity of insulin. The A-chain and the B-chain that form insulin are produced by removal of the C-peptide (residues 33–63) from the middle of proinsulin. The C-peptide facilitates proper folding of proinsulin so that three disulfide bonds form correctly between the A- and B-chains to stabilize the shape of insulin needed for biological activity. No biological function has been assigned to the C-peptide, although it is packaged and secreted with insulin in equimolar amounts.

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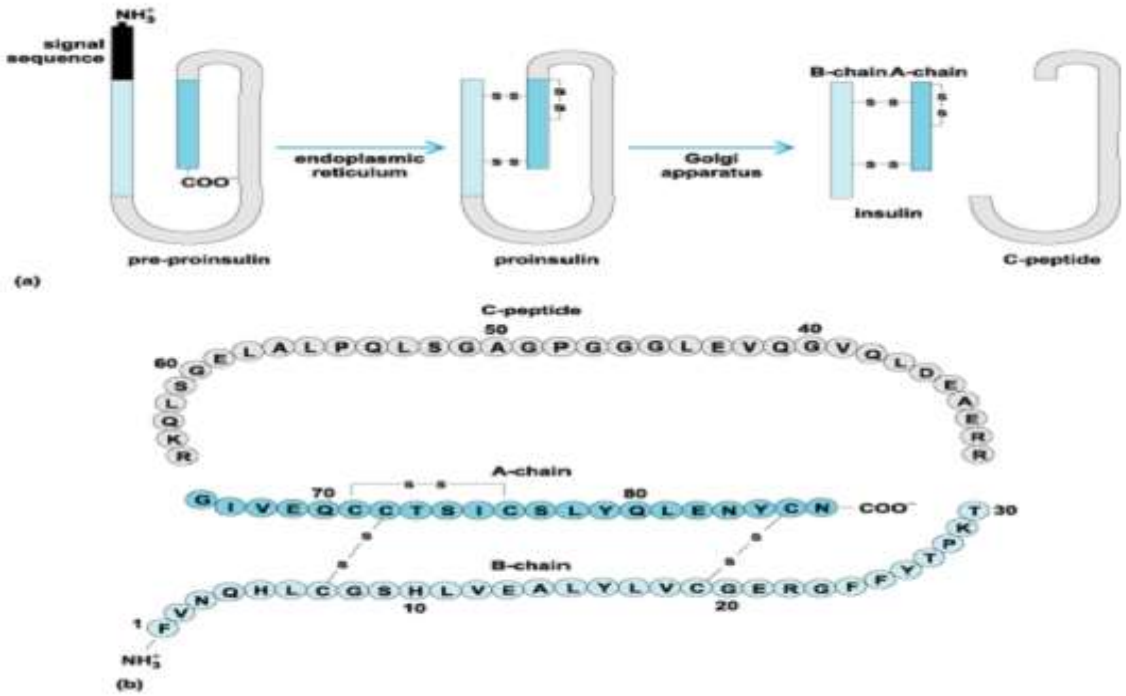

Figure 1: Insulin biosynthesis. (a) Schematic diagram. Pre-proinsulin is synthesized from the insulin gene and directed into the endoplasmic reticulum by the signal sequence, where it folds into a proper conformation that is stabilized by three disulfide bonds (S S). The signal sequence is removed, and proinsulin is further processed in the Golgi apparatus, where the C-peptide is removed and packaged with insulin for secretion. (b) Primary amino acid sequence of human insulin and the C-peptide. (Adapted from [1])

Physiological activity

The consumption of a carbohydrate meal causes an immediate rise in blood glucose concentration from 80 mg/dl to 130 mg/dl. Immediate insulin secretion then lowers blood glucose to the original level within 90 min. In healthy people, the blood contains about 0.2–1 ng/ml insulin before a meal and 1–5 ng/ml after the ingestion of a carbohydrate meal. Antibodies that specifically bind to insulin are used to determine precisely the concentration of insulin in blood. Similar methods measure the concentration of C-peptide in blood, which is used as an indicator that insulin is being produced in the beta cells, especially in people receiving insulin injections.

The blood glucose level is an important signal for insulin secretion. Glucose enters the pancreatic beta cells and increases the production of adenosine triphosphate (ATP), which directly stimulates insulin secretion. Other hormones released from cells in the small intestine during a meal travel to the beta cells to further promote insulin secretion. Upon secretion from the beta cells, insulin passes through the liver on its way to the peripheral tissues. Insulin-degrading enzyme is abundant in the liver, destroying about 50–60% of the secreted insulin as it passes through: the "half-life" of insulin in the body has been estimated to be 10–30 min. Insulin inhibits the production of glucose by the liver and increases the uptake of glucose by muscle and adipose tissues. Depending upon the tissue type, the glucose is used for energy (ATP production) or stored [1].

Diabetes

Diabetes mellitus, often referred as diabetes, is caused by decrease in insulin secretion by pancreatic islet cells, leading to increase in blood glucose level (hyperglycemia). Diabetes mellitus is characterized by excessive weight loss, increased urge for urination (polyuria), increased thirst (polydipsia), and an excessive desire to eat (polyphagia) [2]. Diabetes mellitus has been classified as (i) type 1, or insulin-dependent diabetes, (ii) type 2, or non–insulin-dependent diabetes, and (iii) gestational diabetes. Type 1 diabetes mellitus is characterized by the loss of insulin-producing β cells of islets of Langerhans in the pancreas, thereby leading to deficiency of insulin. The main cause of this β-cell loss is T-cell–mediated autoimmune attack. Type 1 diabetes in children is termed as juvenile diabetes. Type 2 diabetes mellitus is caused by insulin resistance or reduced insulin sensitivity combined with reduced insulin secretion. The defective responsiveness of body tissues to insulin almost certainly involves the insulin receptor in cell membranes. Gestational diabetes occurs in women without previously diagnosed diabetes who exhibit high blood glucose levels

during pregnancy. No specific cause has been identified, but it is believed that the hormones produced during pregnancy reduce a woman's sensitivity to insulin, resulting in high blood sugar levels. Controlling the blood sugar level through modified dietary sugar intake, physical exercise, insulin therapy, and oral medications has been advised for control of type 1 diabetes mellitus. The goal of insulin formulation is to mimic the physiologic pattern of insulin secretion, which under normal conditions consist of a basal secretion and meal related short peaks and is discussed in the following sections [3].

The discovery of insulin in 1921 by Banting and Best was a quantum leap in terms of therapy for patients with type 1 diabetes initially, and then for other types of diabetes. The use of insulin has proved life saving for many patients with diabetes. From the early attempts at isolating insulin from animal pancreatic extracts, great strides have been taken in terms of purification of insulin to the development of human short-acting soluble insulins, intermediate- and long-acting insulins, human premix insulins, and now analogue insulins in a variety of formulations including rapid-acting, long-acting and premixed insulins [4].

In non diabetic individuals, ingestion of food results in a rise of serum insulin concentration to a maximum after 30 to 45min, followed by a decline to basal levels after 2 to 3h. The pharmacokinetic characteristics of the currently available rapid-, intermediate-, and long-acting preparations of human insulin make it almost impossible to achieve sustained normoglycemia. The onset of action of subcutaneous (s.c) injected regular insulin is too slow, and the duration of its action is too long to mimic the insulin secretion pattern of a healthy individual during a carbohydrate containing meal [5]. As a result, early postprandial hyperglycemia followed by an increased risk for hypoglycemia before the next meal are present. Similarly, the available intermediate/long-acting insulin preparations are unable to provide a stable, continuous baseline insulin level. Instead, they cause peak serum insulin levels at 3 to 4h after S.C. injection and show considerable inter-and intra subject variations in their bioavailability. The Diabetes Control and Complications Trial confirmed the link between glycemic control and the complications of diabetes (DCCT Research Group, 1993) [6].Therefore, to achieve improved glucose control, the need for new insulin preparations with a faster onset and shorter duration of action, and long-acting preparations with a more flat time-action profile and less variable bioavailability became apparent in the 1990s [7]. Until recently, however, improvements in insulin formulations were seriously limited, because advances were only achieved in insulin purity, species, and characteristics of the retarding agent. The availability of molecular genetic techniques opened new windows to create insulin analogs by changing the structure of the native protein and to improve the therapeutic properties of it.

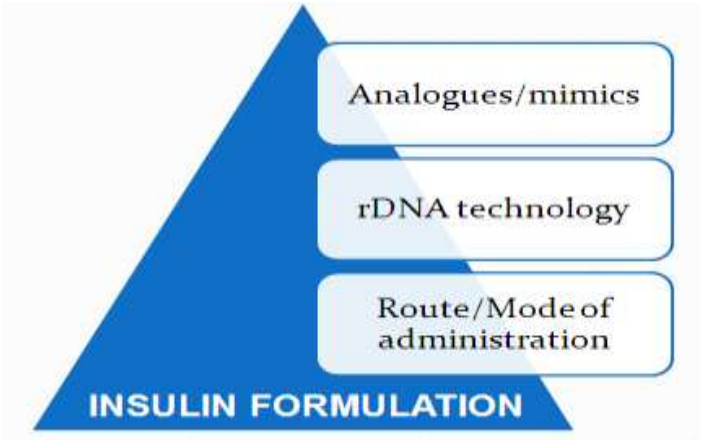

Figure 2: Insulin formulation and its multidisciplinary approach

Factor considerations during insulin formulations

A wide range of factors influence blood glucose and free insulin levels following insulin delivery. These include: (1) Delivery site (inter- and intra-site variations). Insulin delivered in the abdomen is absorbed 86% faster than that delivered in the thigh and in turn the thigh is 30% faster than in the arm [8]. Duration of insulin action varies with delivery site. (2) Liver and kidney function [9] (3) Skin temperature and fat thickness at administered site [10] (4) Presence and degree of lipodystrophy. The prevalence of lipodystrophy in diabetic patients has been assessed at as high as 52% [11]. (5)Exercise: Absorption is affected in the region of an exercising muscle. (6)Temperature of administered insulin: Insulin peak also occurs earlier when the insulin is stored in a fridge [12].

Simple insulin formulations

Until 1996, soluble formulations of "regular" human insulin, such as Humulin® R (human insulin [rDNA origin] injection), Novolin® R (human insulin [rDNA origin] injection), were the most rapid-acting formulations available and were used as bolus insulin before meals. Unfortunately, regular human insulin crystallizes in a hexameric form, which is physiologically inactive (cannot enter circulation) until it dissociates into monomers or dimers following subcutaneous injection. The required dissociation into monomers is slow and delays the absorption of insulin from the subcutaneous site of injection [13].

Regular insulin

Regular insulin is available as a clear solution at neutral pH. 0.4% of zinc is added to allow the insulin molecules to self associate into hexamers. For the

prevention of growth of micro-organisms phenol or m-cresol is added. Regular insulin has its onset of action within 15-30 min after subcutaneous injection, maximum activity peaks at 120-150 min while the action lasts for 6-8 hours. In order match the peaks of glucose and insulin, subcutaneous injection is advised to be taken 30-40 min prior to meals [14].

Table 1: Physical and chemical characteristic of some insulin formulations (Adapted from [15])

Type	Zn Content (mg/100 units)	pH	Buffer	Modifying Protein
Regular Insulin	0.01-0.04	2.5-3.5 (acidified) 7.0-7.8 (neutral)	-----	None
NPH Insulin	0.01-0.04	7.1-7.4	Phosphate	0.3-0.6 mg Protamine
PZI	0.15-0.25	7.1-7.4	Phosphate	1.0-1.5 mg Protamine
SemiLente Insulin	0.2-0.5	7.2-7.5	Acetate	None
Lente Insulin	0.12-0.25	7.2-7.5	Acetate	None
UltraLente Insulin	0.12-0.25	7.2-7.5	Acetate	None

NPH or isophane insulin

Isophane insulin is known as NPH insulin as it was developed in Denmark at the Hagedorn Laboratory in 1940s. In order to prolong the action of insulin, a positively charged protein, protamine is added in a molar ratio of 1:6 to regular insulin. It binds with the negatively charged insulin at neutral pH. Neutral pH value is achieved by use of phosphate buffer. Zn and m-cresol are also added. NPH insulin is slowly absorbed from subcutaneous tissue with peak at 5-7 hours and the action lasts for 12-15 hours. This insulin is most commonly used at bedtime to control fasting blood sugar [14].

Lente insulin

If zinc is added in excess amount (10 times that added in NPH), at neutral pH and if acetate is used as a buffer instead of phosphate, it forms insoluble insulin-zinc complexes [16]. This property is exploited for the production of lente insulins. The action profile of these preparations depends upon the physical conditions of insulin. Semilente is amorphous and has biphasic absorption kinetics with short duration of action. Ultralente is long acting crystalline suspension. These insulins cannot be mixed with regular insulin due to their zinc content and are not very popular.

Premixed formulations

Regular and NPH insulin are available in a premixed formulation with 30:70, 50:50, 25:75 proportions. These preparations are very popular as there is no mixing involved. Patients of type 2 diabetes on split mix regime may be shifted to premixed preparation if they are on approximately similar proportion [17].

Table 2: Premixed fixed-combination insulins (Adapted from [4])

Premixed insulin	Mixture components
Humulin 30/70®	30% regular/70% NPH
Actraphane 30/70®	30% regular/70% NPH
Humulin 50/50®	50% regular/50% NPH
Insuman Comb 30/70®	30% regular/70% isophane
Humalog Mix 50®	50% lispro/50% lispro protamine
Humalog Mix 25®	25% lispro/75% lispro protamine
Novomix 30®	30% aspart/70% aspart protamine

NPH = Neutral Protamine Hagedorn

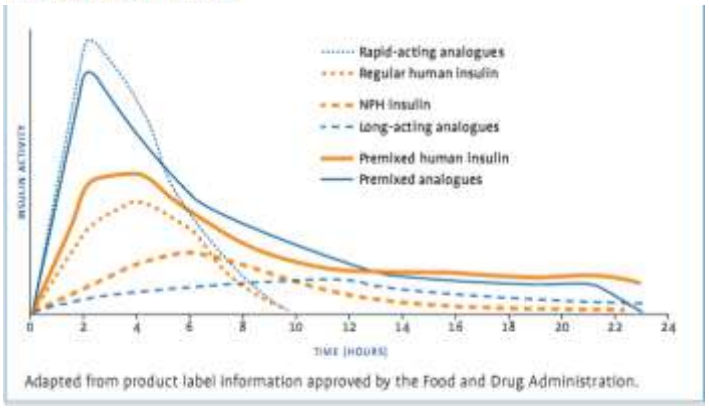

Figure 3: Activity of insulin preparations (Adapted from [18])

Development of insulin analogs formulations

It has become apparent that the pharmacokinetics of insulin formulations can be altered by bioengineered modifications of the natural amino acid sequence to produce "insulin analogs." Selective modifications of the native insulin molecule alter insulin pharmacokinetics following subcutaneous injection. This can either decrease (rapid-acting insulin analogs eg, aspart, glulisine, lispro), or increase (long-acting insulin analogs eg, detemir, glargine) the absorption time from the subcutaneous tissue with the corresponding changes in the time-action profile [19].

NovoLog® (insulin aspart [rDNA origin] injection) is a human insulin analog that is a rapid-acting, parenteral blood glucose-lowering agent. NovoLog is homologous with regular human insulin with the exception of a single substitution of the amino acid proline by aspartic acid in position B28 which introduces an additional negative charge within the insulin molecule. The change removes an important bond site between 2 insulin monomers, making the molecule less likely to self-associate. The aspart analogue has a slightly lower affinity for the insulin receptor compared to regular human insulin; however, this does not appear to affect potency, and is produced by rDNA technology utilizing *Saccharomyces cerevisiae* as the production organism. Insulin aspart has the empirical formula $C_{256}H_{381}N_{65}0_{79}S_6$ and a molecular weight of 5825.8. NovoLog is a sterile, aqueous, clear, and colorless solution, that contains insulin aspart (B28 asp regular human insulin analog) 100 Units/mL, glycerin 16 mg/mL, phenol 1.50 mg/mL, metacresol 1.72 mg/mL, zinc 19.6 µg/mL, disodium hydrogen phosphate dihydrate 1.25 mg/mL, and sodium chloride 0.58 mg/mL. NovoLog has a pH of 7.2-7.6. Hydrochloric acid 10% and/or sodium hydroxide 10% may be added to adjust pH [21-22].

Apidra® (insulin glulisine [rDNA origin] injection) is human insulin analog that is a rapid-acting, parenteral blood glucose lowering agent. Insulin glulisine is produced by rDNA technology utilizing a non-pathogenic laboratory strain of *Escherichia coli (K12)*. Insulin glulisine differs from human insulin in that the amino acid asparagine at position B3 is replaced by lysine and the lysine in position B29 is replaced by glutamic acid. It has the empirical formula $C_{258}H_{384}N_{64}O_{78}S_6$ and a molecular weight of 5823. APIDRA is a sterile, aqueous, clear, and colorless solution. Each milliliter of APIDRA contains 100 IU (3.49 mg) insulin glulisine, 3.15 mg m-cresol, 6 mg tromethamine, 5 mg sodium chloride, 0.01 mg polysorbate 20, and water for injection. APIDRA has a pH of approximately 7.3. The pH is adjusted by addition of aqueous solutions of hydrochloric acid and/or sodium hydroxide [23].

Table 3: Pharmacokinetic profiles of some insulin formulations (Adapted from [20])

Class of Insulin	Formulation*	Onset (hr)	Peak (hr)	Duration (hr)	Appearance	How Supplied‡
Rapid-acting analogs	Insulin aspart (NovoLog)	0.17 to 0.33	1 to 3	3 to 5	Clear	NovoLog FlexPen 3 mL, PenFill 3 mL cartridges
	Insulin lispro (Humalog)	0.25 to 0.5	0.8 to 4.3	4 to 6	Clear	Humalog Pen 3 mL, Humalog 3-mL cartridges
	Insulin glulisine (Apidra)	0.25	1 to 1.5	3 to 5	Clear	Opticlik 3-mL cartridges
Short-acting human	Regular human (Humulin, Novolin†)	0.5 to 1	2 to 3	3 to 6	Clear	Humulin R: vials only Novolin R InnoLet 3 mL, PenFill 3-mL cartridges
Intermediate-acting human	NPH (Humulin N, Novolin N†)	1 to 2	6 to 14	16 to 24	Cloudy	Humulin N Pen 3 mL Novolin N InnoLet 3 mL
Long-acting analogs	Insulin detemir (Levemir)	0.8 to 2	6 to 8	~24	Clear	Levemir FlexPen 3 mL, Levemir InnoLet 3 mL, PenFill 3-mL cartridges
	Insulin glargine (Lantus)	1.1	2 to 20	~24	Clear	Opticlik 3-mL cartridges
Premixed analogs	Insulin protamine aspart/aspart 70/30 (NovoLog Mix)	0.17 to 0.33	1 to 4	~24	Cloudy	NovoLog Mix FlexPen 3 mL, PenFill 3-mL cartridges
	Insulin protamine lispro/lispro 75/25 (Humalog Mix 75/25)	0.25 to 0.5	2	>22	Cloudy	Humalog Mix 75/25 Pen 3 mL 75/2
	Insulin protamine lispro/lispro 50/50 (Humalog Mix 50/50)	0.25 to 0.5	2 to 5.5	~24	Cloudy	Humalog Mix 50/50 Pen 3 mL
Premixed human	70% NPH/30% regular (Humulin 70/30, Novolin 70/30†)	0.5	1.5 to 12	12-16	Cloudy	Humulin 70/30 Pen 3 mL, Novolin 70/30 Innolet 3 mL, PenFill 3-mL cartridges
	50% NPH/50% regular (Humulin 50/50)	0.3	2 to 5.5	12 to16	Cloudy	Vials only
Human inhaled	Insulin human inhalation powder (Exubera)	<0.5	1 to 2	6 to 8	Powder	Blister tabs for inhalation

Lispro® Mix50/50™ is a mixture of insulin lispro solution, a rapid–acting blood glucose–lowering agent and insulin lispro protamine suspension, an intermediate–acting blood glucose–lowering agent. Chemically, insulin lispro is Lys (B28), Pro (B29) human insulin analog, created when the amino acids at positions 28 and 29 on the insulin B–chain are reversed. This change alters the 3-dimensional structure of the insulin protein and hinders formation of dimers in solution .Affinity of lispro for the insulin receptor remains similar to that of regular insulin despite these

structural changes. Affinity for the insulin-like growth factor insulin receptor is slightly higher for lispro compared to regular insulin; however, regular insulin and lispro have the same effect on cell growth. Insulin lispro is synthesized in a special non–pathogenic laboratory strain of *Escherichia coli* that has been genetically altered to produce insulin lispro [24]. Insulin lispro protamine suspension (NPL component) is a suspension of crystals produced from combining insulin lispro and protamine sulfate under appropriate conditions for crystal formation. Insulin lispro has the empirical formula $C_{257}H_{383}N_{65}O_{77}S_6$ and a molecular weight of 5808, both identical to that of human insulin. Each milliliter of Humalog Mix50/50 injection contains insulin lispro 100 units, 0.19 mg protamine sulfate, 16 mg glycerin, 3.78 mg dibasic sodium phosphate, 2.20 mg Metacresol, zinc oxide content adjusted to provide 0.0305 mg zinc ion, 0.89 mg phenol, and Water for Injection. Humalog Mix50/50 has a pH of 7.0 to 7.8. Hydrochloric acid 10% and/or sodium hydroxide 10% may have been added to adjust pH [25].

Levemir® (insulin detemir [rDNA origin] injection Insulin detemir is long-acting basal insulin analog, with up to 24 hours duration of action, produced by a process that includes expression of rDNA in *Saccharomyces cerevisiae* followed by chemical modification. Insulin detemir differs from human insulin in that the amino acid threonine in position B30 has been omitted, and a C14 fatty acid chain has been attached to the amino acid B29. Insulin detemir has a molecular formula of $C_{267}H_{402}O_{76}N_{64}S_6$ and a molecular weight of 5916.9 [26]. LEVEMIR® is a clear, colorless, aqueous, neutral sterile solution. Each milliliter of LEVEMIR® contains 100 U (14.2 mg/mL) insulin detemir. Each milliliter of LEVEMIR® 10 mL Vial contains the inactive ingredients 65.4 mcg zinc, 2.06 mg m-cresol, 30.0 mg mannitol, 1.80 mg phenol, 0.89 mg disodium phosphate dihydrate, 1.17 mg sodium chloride, and water for injection. Hydrochloric acid and/or sodium hydroxide may be added to adjust pH ~7.4

Lantus® (insulin glargine [rDNA origin] injection) is produced by rDNA technology utilizing a strain of *Escherichia coli* (K12) as production organism. Insulin glargine differs from human insulin in that the amino acid asparagine at position A21 is replaced by glycine and two arginines are added to the C-terminus of the B-chain. These modifications shift the isoelectric point from a pH of 5.4 to 6.7 and make the molecule soluble in acidic solutions. However, when insulin glargine is injected into a neutral environment, such as SC tissue, the molecule is no longer soluble and forms micro-crystals. The amino acid sequence changes in insulin glargine also promote more stable hexamers in solution [27].These processes prolong absorption from the injection site and result in a basal supply of insulin similar to the normal basal release patterns of insulin from the pancreas. It has the empirical formula $C_{267}H_{404}N_{72}O_{78}S_6$ and a molecular weight of 6063.Lantus consists of insulin glargine dissolved in a clear aqueous fluid. Each milliliter of LANTUS contains 100 IU (3.6378 mg) insulin glargine. Inactive ingredients for the 10 mL vial are 30 mg zinc, 2.7 mg m-cresol, 20 mg glycerol 85%, 20 mg polysorbate, and water for injection. The pH is adjusted by addition of aqueous solutions of hydrochloric acid and sodium hydroxide. pH~4[28].

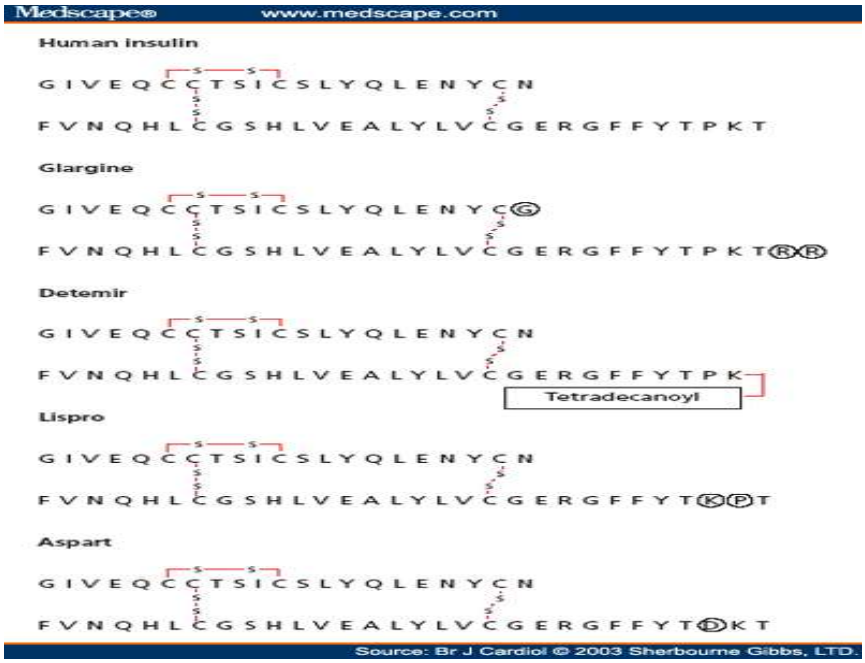

Figure 4: Modification in the insulin chain for the analogue formulations. (Adapted from [29])

Velosulin BR is a clear solution of insulin in a phosphate buffer. The concentration of this product is 100 units of insulin per milliliter. This human insulin is structurally identical to the insulin produced by the pancreas in the human body. This structural identity is obtained by rDNA technology utilizing *Saccharomyces cerevisiae* as the production organism. When a U-100 insulin syringe is used to deliver the insulin, the effect of Velosulin BR begins approximately $^{1}/_{2}$ hour after the injection. The effect lasts up to approximately 8 hours with a maximal effect between the 1st and 3rd hour [30].

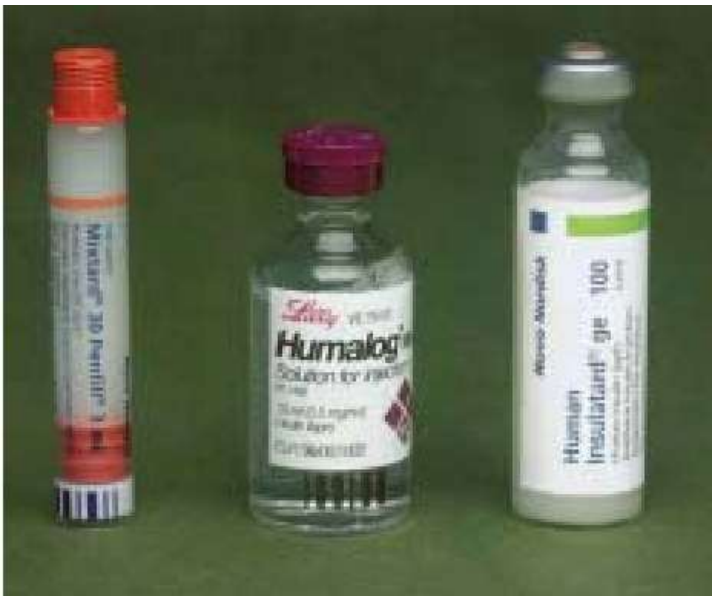

Figure 5: Some insulin formulations (Adapted from [31])

Formulations based on different routes of insulin delivery

The most unpleasant thing about insulin is that it needs to be given through an injection repeatedly which involves lots of pain and hence patient compliance is not there. Therefore there is a search for a non-injectable preparation of insulin, which could be administered by oral. This is the most preferred route & most of the drugs available today are in the form of tablets or capsule. But insulin's tablet is still a dream. The basic problem with insulin administration via oral route is its inherent instability in the harsh condition of the gastrointestinal tract (pH; Enzymes; adsorption to solids) and also absorption through GIT [Gastro Intestinal Tract] is not guaranteed [32]. Therefore for improved oral absorption, it's important to protect the formulation from degradation and increase its absorption through GIT [Gastro Intestinal Tract].

Solid oral formulations

a) Micro spheres as oral delivery system for insulin

Microspheres prepared with Eudragit L-100, sodium glycocholate and aprotinin. Eudragit L-100 was used as carrier for micro spheres to give a site-specific release of insulin in the upper intestine; sodium glycocholate was used as penetration enhancer and aprotinin as a protease inhibitor. The in-vivo hypoglycemic effect study carried out using alloxan induced diabetic rats showed that the micro spheres prepared with Eudragit L-100, 1% aprotinin and 1% sodium glycocholate showed prolonged hypoglycemic effect for 4hours which was not even observed with subcutaneous bovine insulin injection [33].

b) Chitosan capsules for insulin delivery

Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon but also for its potential for the delivery of proteins and therapeutic peptides such as insulin. In vitro drug release experiments from chitosan capsules containing 5(6)-carboxyfluorescein (CF) were carried out by rotating basket method with slight modifications. The intestinal absorption of insulin was evaluated by measuring the plasma insulin levels and its hypoglycemic effects after oral administration of the chitosan capsules containing insulin and additives. Little release of CF (5(6)-carboxyfluorescein) from the capsules was observed in an artificial gastric juice (pH= 1), or in an artificial intestinal juice (pH= 7). However, the release of CF was markedly increased in the presence of rat caecal contents [34]. Cyclodextrin, which has the unique properties of solubilization and reducing enzymatic degradation, was used to increase the bioavailability of insulin in an alginate–chitson microsphere formulation.

c) Water-in-oil type of emulsion

Human insulin was incorporated into a w/o emulsion by high-pressure homogenization [35]. A fine stable dispersion of the aqueous phase was achieved and the emulsion was able to protect insulin against gastric degradation in vitro without further encapsulation.

d) Biocarrier insulin

In this system insulin was first en trapped in Liposome's. The preparation was developed using ghost erythrocytes as bio carriers for intra duodenal administration of insulin because proteolytic enzymes in duodenal region break erythrocytes. From such a system insulin was absorbed and showed its glucose lowering effects [36, 37].

e) Nanocapsules for insulin delivery

Nanocapsules using biodegradable Polymer Poly (isobutyl / Cyanoacrylate) [mean size 220nm] were

developed. When ad-ministered orally by force-feeding to diabetic rats, insulin nanocapsules (12.5, 25, and 50 U/kg) decreased fasted glycemia 50-60% by day 2. This effect was maintained for 6 or 20 days with 12.5 or 50 U/kg, respectively. Only the dose of 100 U/kg decreased fed glycemia by 25% in diabetic rats. In normal rats, hyperglycemia induced by an oral glucose load was reduced by 50% with the same dose of oral insulin nanocapsules [38].

Insulin in ceramic nanoparticles prepared from hydroxyapatite and encapsulated these particles in sodium alginate. In vitro release profile of insulin was carried out in simulated gastric (pH 1.2) and intestinal fluids (pH 7.4). 100 mg of insulin loaded nano particle was introduced into 10 ml of respective medium. 0.1 ml of sample was withdrawn at various time intervals and evaluated for insulin using Lowry's method for protein estimation [39]. Present investigation show that insulin loaded HA (hydroxyapatite) nanoparticle encapsulated in sodium alginate can effectively release almost 100 % of the drug in SIF [Simulated Intestinal Fluid] during a period of 2 hours. However, during the same period only 24-28 % of insulin was released in SGF [Simulated Gastric Fluid]

The protease inhibitors that improved insulin absorption in the intestine were, in order of increasing improvement, leupeptin, sodium glycocholate, bacitracin, bestatin, and cystatin. Novel strategies were used as covalently attached inhibitors (Bowman–Birk inhibitor–carboxymethylcellulose and carboxymethylcellulose–elastinal) was developed in which the enzyme inhibitors (Bowman–Birk inhibitor and elastinal). The use of these continuous protein inhibitors often showed a disturbance in digestion.

Table 4: Many companies have been trying to develop solid oral forms of insulin (Adapted from [40])

	PRODUCT	GASTROINTESTINAL TRANSPORT	LAST REPORTED STAGE OF DEVELOPMENT (YEAR)
Access Pharmaceuticals	Cobalamin-based insulin	Nanoparticle/vitamin B-12	Preclinical (2010)
Biocon	IN-105	Conjugated molecule	Phase III (2010)
Diabetology	Capsulin	Axcess absorption enhancers	Phase II (2009)
Diasome Pharmaceuticals	HDV-I	Hepatic-targeted liposomes	Phase II (2009)
Emisphere Technologies	Unnamed	Eligen carrier technology	Phase II (2008)
Merrion Pharmaceuticals	NN1952	GIPET enhancer	Phase I (2010)
NanoMega Medical	Unnamed	Chitosan/poly(glutamic acid) nanoparticle	Preclinical (2009)
NOD Pharmaceuticals	Nodlin	Bioadhesive nanoparticles	Phase I (2009)
Oramed Pharmaceuticals	ORMD-0801	Absorption enhancers	Phase II (2010)
Transgene Biotek	Unnamed	Polymeric nanoparticles	Preclinical (2010)

SOURCE: Company reports

Chitosan, sodium salicylate, polyoxyethylene-9-lauryl ether, some synthetic polymers like sodium carboxymethyl cellulose, polyacrylic acid, natural polymers like alginate, pectin, lectin, Gelatin etc., these are known to have good bio-adhesive properties & showed a good influence in enhancing the absorption of insulin.

f) Entrapment in liposomes

Liposomes prepared from phosphatidylcholine and cholesterol (1:9). No change in blood glucose levels was noted in normal animals but a significant reduction was obtained with diabetic rats with the maximum effect observed within 3 hrs [41].

Nasal formulations

Clinicians have attempted to administer insulin through the nasal mucosa. However, the bioavailability of nasally administered insulin was <10 % [42-44], a value that could only be achieved with the help of "absorption enhancers"[42-47]. Such agents increase the absorption rate of relatively large peptide such as insulin through the epithelial barrier. Only few of the many agents studied, increased the absorption rate without irritating the nasal mucosa or having other side effects [47, 48] .Absorption enhancers reported for transmucosal insulin delivery include saponins, sodium caprylate, sodium laurate, polyacrylic acid, fusidic acid and bile salts [49].

The pH of a nasal formulation is important for the following reasons:

- To avoid irritation of nasal mucosa;
- To allow the drug to be available in unionized form for absorption;
- To prevent growth of pathogenic bacteria in the nasal passage;
- To maintain functionality of excipient such as preservatives; and

• To sustain normal physiological ciliary movement. Lysozyme is found in nasal secretions, which is responsible susceptible to microbial infection. It is therefore advisable to keep the formulation at a pH of 4.5 to 6.5 keeping in mind the physiochemical properties of the drug as drugs are absorbs in the unionized form. Most nasal formulations are aqueous based and need preservatives to prevent microbial growth. Parabens, benzalkonium chloride, phenyl ethyl alcohol, EDTA and benzoyl alcohol are some of the commonly used preservatives in nasal formulations.

Aqueous solubility of drug is always a limitation for nasal drug delivery in solution conventional solvents such as glycols, small quantities of alcohol, Transcutol (diethylene glycol monoethyl ether), medium chain glycerides and Labrasol (saturated polyglycolyzed C_8-C_{10} glycerides) can be used to enhance the solubility of drugs. Other options include the use of surfactants or cyclodextrins such as HP-β-cyclodextrin that serve as a biocompatible solubiliser and stabilizer in combination with lipophilic absorption enhancers.

Recently, chitosan, a polysaccharide, has been investigated to increase insulin absorption with no evidence of toxic manifestation [50]. Both liquid and powder systems have been evaluated in humans. Chitosan is a bioadhesive material and therefore slows mucocilliary clearance allowing more time for the therapeutic material to be absorbed. Chitosan also causes a transient and reversible effect whereby the tight junctions of the nasal

Nasal insulin gel

The gel consisted of Carbopol 934P (0.7%) and HPMC (1.3%). Required quantities of polymers were weighed and blended thoroughly. The polymers were then suspended in insulin solution and allowed to swell. Triethanolamine was added drop wise with gentle stirring till a clear, smooth, and translucent gel was obtained at a pH of 7.2 to 7.4. Sodium deoxycholate (1%) was added to the gel and mixed until a complete blend was obtained. Sodium metabisulphite (0.01%) and methylparaben (0.05%) were finally incorporated and mixed well to obtain a uniform gel. The insulin content of the gel was 100 IU/g.

Pulmonary formulations

Delivery of medication to the respiratory tract for the localized therapy of respiratory diseases has been practiced for several decades. The lungs are an attractive site for the systemic delivery of insulin because lung has some inherent advantages for insulin administration. These includes,

for destroying certain bacteria at acidic pH. Under alkaline conditions lysozyme is inactivated and the tissue is mucosal cells open to allow the passage of insulin [51]. Increasing the time of contact with the nasal mucosa can increase the nasal absorption of insulin. The clearance half-life can be increased from 15 min with nasal solution to 240 min using starch micro spheres (SMS) [52].

When a surfactant such as saponin, sodium glycocholate or BL-9 was added to the preparation, the absorption of insulin from the nasal mucosa was enhanced independent of pH. The effect of different bile salts at various concentrations on intranasal insulin absorption in man was studied [53]. Maximum increase in serum insulin was obtained using deoxycholate followed by chenodeoxy-cholate and cholate at 1% w/v. Blood glucose concentration reduced by approximately 50% with deoxycholate and peak serum insulin concentration was reached in 10 min after administration of the spray [54]. Absorption enhancing effect of different cyclodextrins on intranasally administered insulin in rats and rabbits were determined. Dimethyl-beta-cyclodextrin was found to be most potent [55, 56]. Nasal absorption is also promoted by medium chain fatty acid salt [57] glycyrrhetinic acid derivatives in rat [58] sodium tauro-24, 25-dihydrofusidate in sheep [59] .The blood glucose remained low for 3 h after lunch when insulin was used intranasally with 1% deoxycholate in type1 diabetics[60].

• a vast (in humans 50-140 m2, ~500 millions of alveoli) and well-per fused absorptive surface (~5 l blood/min, pulmonary capillary blood volume ~0.25 l),
• the absence of certain peptidases that are present in the gastrointestinal tract
• no immediate degradation of the absorbed insulin by the liver ("first pass metabolism")
• a thin alveolar-capillary barrier
• marginal variance in the amount of mucus production [61]

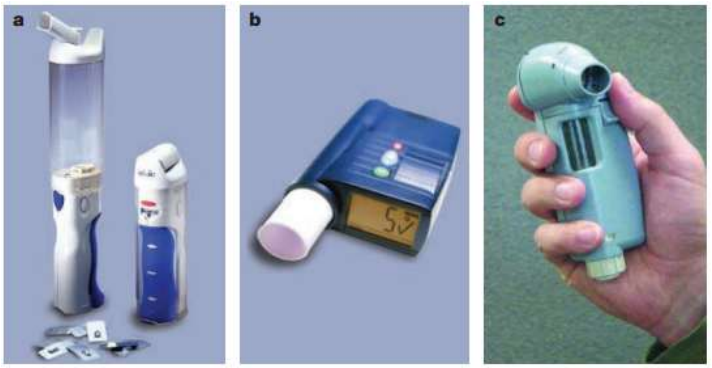

Figure 6: Pulmonary insulin-delivery devices: a) The Inhale Therapeutic Systems/Pfizer/Aventis Exubera inhalation system. b) The Novo Nordisk/Aradigm delivery system. c) The Aerodose/Aerogen delivery device. (Adapted from [62])

Type of compound	Enhancer/promoter	Example	F_{ab}/F_{rel} (%)[1]	Reference	Possible mechanism of action of each class
Absorption enhancers					
Bile salts (and derivatives)	Sodium deoxycholate/ -glycocholate/ -taurodihydrofusidate	1% SDC spray 1% SGC spray 4% SGC spray 1% STDHF spray 1% STDHF spray	20 12-12.5 67.5 11.4 7.1-9.2	Pontiroli A.E., Br. Med. J. 284 (1982) 303-306 Moses A.C., Diabetes 32 (1983) 1040-47 Pozza G., Clin. Pharm.17 (1989) 209-307 Nolte M.S., Horm. Metab. Res. 22 (1990) 170-174	Disrupt membranes, open tight junctions, enzyme inhibition, mucolytic activity
Surfactants	Sodium lauryl sulphate, saponin, polyoxyethylene-9-lauryl ether	0.8% Laureth-9 spray	21.6	Pontiroli A.E., Diabet. Metabol. 13 (1987) 441-443	Disrupt membranes
Chelating agents	Ethylenediaminetetraacetic acid, salicylates		-	Aungst B. J., Pharm Res. 5 (1986) 305-308	Open tight junctions
Fatty acids (and derivatives)	Sodium caprylate, sodium laurate, phospholipids (e.g. didecanoylphosphatidylcholine, lysophospatidylcholine)	2% DDPC spray 2% DDPC spray	6.4-11.2 / 20 8.8-13.2 / 9.9-14.8	Drejer K., Diabet. Med. 9 (1992) 335-340 Jacobs M.A., Diabetes 42 (1993) 1649-55	Disrupt membranes
Enzyme inhibitors	Bestatin, amastatin, aprotinin		-	Morimoto K., Int. J. Pharm. 113 (1995) 1-8	Enzyme inhibition
Miscellaneous	Cyclodextrins, N-acetyl cystein,	DM-βCD powder (0.25 mg/kg)	3.4-5.1	Merkus F.W., J. Control. Rel. 41 (1996) 69-75	Disrupt membranes, open tight junctions
Bioadhesive materials					
Powders	Carbopol, starch microspheres, chitosan, albumin, starch	Starch microspheres Starch microspheres Chitosan powder Maize starch/ Carbopol 974P powder	30 4.5 17.0 9.9-14.4	Bjork E., Int. J. Pharm. 47 (1988) 233-238 Illum L., Int. J. Pharm. 57 (1989) 49-54 Dyer A.M., Pharm. Res. 19 (2002) 998-1008 Callens C., J. Control. Rel. 66 (2000) 215-220	Reduce nasal clearance, open tight junctions,
Liquids, Gels	Chitosan, carbopol, carboxy-methylcellulose, hydroxypropyl-cellulose	Carbopol gel	20.6	Najafabadi A.R., Drug Deliv. 11 (5) 295-300 (2004). Illum L., Pharm. Res. 11 (1994) 1186-9 Morimoto K., J. Pharm. Pharmacol. 37 (1985) 134-136	

Nasal absorption promoting systems

[1] F_{ab}: absolute bioavailability, F_{rel}: relative bioavailability (to subcutaneous route)

Table 5: Nasal absorption promoting systems (Adapted from [51])

The pharmacodynamic effects of insulin formulations administrated via the lung are comparable to or even faster than, those of subcutaneously injected regular insulin or rapid acting insulin analogues [63]. The relative biopotency of inhaled insulin in most cases is approximately 10% i.e. the dose of insulin administered must be tenfold higher than with subcutaneous application [64]. Most experience with inhaled insulin has been obtained using either dry powder formulation in the Nektar Pulmonary Inhaler/Exubera device (Nektar Therapeutics Inc., San Carlos, CA, Aventis, Bridge- water, NJ, Pfizer, NY) or a liquid aerosol formulation in the AERx Insulin Diabetics Management System (AradigmCorp. Hayward, CA, NovoNordiskA/S, Copenhagen, Denmark). EXUBERA® consists of blisters containing human insulin inhalation powder, which are administered using the EXUBERA® Inhaler. EXUBERA blisters contain human insulin produced by recombinant DNA technology utilizing a non-pathogenic

laboratory strain of Escherichia coli (K12). Chemically, human insulin has the empirical formula $C_{257}H_{383}N_{65}O_{77}S_6$ and a molecular weight of 5808. 1 milligram of blister contains insulin human, sodium citrate, mannitol, glycine and sodium hydroxide [64]

Table 6: Pulmonary route insulin formulations (Adapted from [65])

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Dosage Form	Trade Name	Company	Development Stage
Solution	AERx iDMS	Novo Nordisk, Aradigm	Phase III clinical trial in progress
	Aerodose	Aerogen	Phase II clinical trial completed; development halted in January 2003
Powder	Exubera	Pfizer, Aventis, Nektar	Phase III clinical trial completed; long-term safety studies ongoing; marketing authorization application filed with European Medicine Evaluation Agency in March 2004; new drug application filed with FDA in 2005
	ProMaxx	Epic Therapeutics	Preclinical studies
	AIR	Eli Lilly, Alkermes	Phase II clinical trial in progress
	Spiros	Eli Lilly, Dura Pharmaceuticals	Discontinued after merger between Dura Pharmaceuticals and Elan Pharmaceuticals
	Technosphere Insulin System	MannKind Corporation	Late Phase II clinical trial ongoing; Phase III clinical trial initiated in Europe

Source: Am J Health-Syst Pharm © 2005 American Society of Health-System Pharmacists

Transdermal route formulations

The skin of a man provides a good barrier. Novel approaches to "driving" Insulin across the skin, such as iontophoresis and ultrasound have been explored but clinical trials have been disappointing [66, 67]. A possibility is studied of the transdermal delivery of insulin by using a mixture of synthetic analogues of phosphoglycerides (SAP); as a potential activator of insulin diffusion through the skin. Experimentally in vitro; it was proven that the diffusion of insulin through the skin of two types of transdermal therapeutic form (TTF)-matrix-type and matrix- hydrogel-type is possible only in presence of activator SAP-M-99[68] . The detected optional composition of insulin matrix TTF with the area of 40 sq cm collagenous sponges enabled to increase the insulin diffusion upto 0.54 units/hour[69]. Transferosomes are ultra-flexible liposomes with low pore resistance (Cevc) [69]. Insulin incorporated in this system transported therapeutic amounts of peptide across intact mammalian skin. Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation [70].

Buccal route formulations

By buccal delivery drugs are absorbed rapidly into the reticulated veins, which lie under the oral mucosa and enter the systemic circulation directly, by passing the liver [71]. A mucosal adhesive delivery system was developed for the buccal delivery of insulin. It was found that the systemic delivery of insulin through the buccal mucosa was significantly affected by the formulation composition used [71]. Insulin could not be effectively absorbed by a simple disk-shaped dosage form prepared by direct compression of insulin in a mixture of hydroxypropyl-cellulose [HPC] and carbopol-934 [72]. Buccal absorption was achieved using a dome shaped, two phased mucosal adhesive device prepared by dispersing insulin crystals with sodium glycocholate, an absorption promoter, in an oleaginous core and then overlapping the medicated core with an adhesive dome [73].

Rectal formulations

Insulin can be absorbed through this route in the presence of absorption enhancers, the co-administration of absorption enhancers such as sodium glycholate; has been reported to enhance the rectal absorption of insulin [74]. The effectiveness of insulin administration by rectal suppository was examined in normal and non-insulin-dependent non obese diabetic subjects. A 100-U insulin suppository (mean 1.8 U/kg) given to the diabetic subjects caused four times as great a fall in plasma glucose compared with the normal subjects given the same dose

(mean 1.6 U/kg). Diabetic subjects given a 100-U insulin suppository (mean 1.7 U/kg) 15 min after meals three times daily showed a significant (P less than 0.05) improvement in postprandial hyperglycemia accompanied by a reduction of urinary glucose from 26+/-5.9 to 2.0+/-1.0g/day [75].

Conclusion

The advanced methods of insulin delivery systems would gradually progress toward physiological insulin replacement and reduce the long-term complications of diabetes mellitus. Thus feasible alternative formulations for insulin are likely to emerge in the future. This new millennium promises a revolutionary change in the insulin formulations, which is not too far off for billions of sufferers who are reliant on subcutaneous administration. The approaches that seem to hold potential must be consolidated and converted to a working protocol. Among the various emerging formulations, each has their own set of favourable and unfavourable properties. Some unfavourable aspects have to be circumvented to make the alternative insulin formulation a reality and make them to reach the market.

References

1. Morris F. White, "Insulin", in Access Science @ McGraw-Hill, http://www.accessscience.com, DOI 10.1036/1097-8542.347200.
2. Kuzuya T, Nakagawa S, Satoh J, et al. (2002), Diabetes Res Clin Pract 55(1),65–85.
3. Scientific discussion for the approval of Novomix.1st Sep (2004); module 8B.
4. Joshi S, Joshi P. A review of insulin and insulin regimens in type 2 diabetes, CPD Article, Vol 51 No 2 SA Fam Pract 2009.
5. Heinemann L, Starke AAR, Hohmann A and Berger M (1992) Timing between the subcutaneous administration of insulin and consumption of a carbohydrate rich meal. Horm Metab Res 26 (Suppl): 137-139.
6. Diabetes Control and Complications Trial Research Group (1993). The effect of intensive treatment of diabetes on the development and the progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med **329**:977–978.
7. Berger.M, (1989); Towards more physiological insulin therapy in the 1990s. Diab Res Clin Pract 6:S25–S31.
8. VA Koivisto et al (1980) Alterations in Insulin Absorption and in Blood Glucose Control Associated with Varying Insulin Injection Sites in Diabetic Patients. Annals of Internal Medicine 92: 59-61.
9. A Hoffman, E Ziv (1997) Pharmokinetic Considerations of New Insulin Formations and Routes of Administration. Clinical Pharmacokinetics 33(4): 285-301.
10. G Sindelka et al (1994) Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous insulin absorption in healthy subjects. Diabetologia. 37:377-80.
11. L Saez-de Ibarra, F Gallego (1998) Factors related to lipohypertrophy in insulin-treated diabetic patients: role of educational intervention. Practical Diabetes International 15 (1): 9-11.
12. G Perriello et al (1998) Effect of storage temperature of insulin on pharmacokinetics and pharmacodynamics of insulin mixtures injected subcutaneously in subjects with Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 31(11): 811-5.
13. Burge MR, Schade DS: Insulins. Endocrinol Metab Clin North Am. 1997; 26:575-598.
14. Krayenbuhl C, Rosenberg T. Crystalline protamine insulin. Rep Steno Mem Hosp 1946; 1: 60-73
15. Jack De Ruiter, (2002), Insulin products: The number and types of insulin preparations.
16. Hallas-Mollor K. The lente insulins, Diabetes1956; 5: 7-14.
17. Shashank R. Joshi, Rakesh M. Parikh, A. K. Das, (2007), Insulin History, Biochemistry, Physiology and Pharmacology, Supplement of japi, vol. 55.
18. Johns Hopkins (2008) Comparative Effectiveness, Safety, and Indications of Insulin Analogues in Premixed Formulations for Adults with Type 2 Diabetes.
19. Hirsch IB (2005): Insulin analogues. N Engl J Med.; 352:174-183.
20. Triplitt C. (2007) How to initiate, titrate and intensify insulin treatment in type 2 diabetes. US Pharm; 32:10–16 http://www.medscape.com/viewarticle/583245_2
21. Novo Nordisk Inc. (2003) NovoLog® (insulin aspart). Prescribing information. Princeton, NJ.
22. Novo Nordisk Inc. (2002) NovoLog Mix 70/30 (70% insulin aspart protamine suspension and 30% insulin aspart [rDNA origin] injection). Prescribing information. November 1, Princeton, NJ.
23. Aventis Pharmaceuticals, Inc. (2004), Apidra (insulin glulisine [rDNA origin] injection). Prescribing information. April 1, Kansas City, MO.
24. Eli Lilly and Company. Humalog® insulin lispro injection. Prescribing information. June 1, 2004. Indianapolis, IN.

25. Eli Lily and Company. Humalog Mix75/25 (75% insulin lispro protamine suspension and 25% insulin lispro [rDNA origin] injection). Prescribing information. May 1, 2002. Indianapolis, Ind.

26. Novo Nordisk Inc. (2005) NovoLog® (Insulin levemir). Prescribing information. Princeton, NJ.

27. Aventis Pharmaceuticals, Inc. Lantus (insulin glargine [rDNA origin] injection). Prescribing information. January 1, 2003. Kansas City, MO.

28. Dunn CJ, Plosker GL, Keating GM, McKeage K, and Scott LJ: Insulin glargine: an updated review of its use in the management of diabetes mellitus. Drugs. 2003;63: 1743-1778

29. Caroline Day, Helen Archer, Clifford J Bailey, (2003) Recent Advances in Insulin Therapy: Basal Insulin Analogues, Br J Cardiol. ; 10(5) © 2003 Sherborne Gibbs Ltd.

30. Novo Nordisk™, Velosulin © (1999) Novo Nordisk Pharmaceuticals, Inc.

31. Peter j watkins, (2003) ABC of diabetes, Fifth Edition, BMJ Publishing Group Ltd, ISBN 0-7279-16939.

32. Duckworth, W. C.; Bennett, R.G. and Hamel, F.G. (1998), Endocr.Rev. 19, 608-24.

33. Gowthamarjan, K.; Kulkarni, J.T.; Rajan S.D. and Suresh, B. (2003) Ind. J. Pharm. Sci. 65(2), 176-179.

34. Saffran, M. and Kumar, G.S. (1986) Science, 233, 1081.

35. Trenktrog, T. and Muller, B.W. (1995) Int. J. Pharm., 123, 199.

36. Al-Alchi, A. and Greenwood, R. (1993) Drug Dev. Ind. Pharm., 19(6), 673.

37. Al-Alchi, A. and Greenwood, R. (1993) Drug Dev. Ind. Pharm., 19(11), 673.

38. Roques, M.; Cremel, G.; Aunis, D. and Hubert, P. (1995) Diabetologia, 38, 180.

39. Damge, C. and Michael, C. (1998) Diabetes, 37, 246.

40. Company report (2009-10): solid oral forms of insulin

41. Stefanov, A.V.; Kononenko, N.I.; Lishko, V.K. and Shevshenko, A.V. (1980) Ukr. Biokhim. Zh., 52, 497.

42. Jacobs, M. A.; Schreuder, R. H.; Jap, A.J.K.; Nauta, J. J.; Andersen P. M. and Heine R. J. (1993) Diabetes, 42, 1649-55.

43. Drejer, K.; Vaag, A.; Bech, K.; Hansen, P.; Sorensen, A.R. and Mygind, N. (1992) Diabetic Med., 9, 335-40.

44. Hilsted, J.; Madsbad, S.; Hvidberg, A.; Rasmussen, M. H.; Krarup, T.; Ipsen, H.; Hansen, B.; Pedersen M.; Djurup, R. and Oxenbull B.(1995) Diabetologia, 38, 680-4.

45. Nolte, M. S.; Taboga, C.; Salamon, E.; Moses, A.; Longenecker, J.;Flier, J. and Karam, J. H. (1990) Horm. Metab. Res., 22, 170-4.

46. Coates, P. A.; Ismail, I. S.; Luzio, S. D.; Griffith, I.; Ollerton, R. L.; Volund, A. and Owens D. R. (1995) Diabetic Med., 12, 235-9.

47. Andersen, P. M. (1993) In: Frontiers in Insulin Pharmacology,

48. Berger M, Gries FA (Eds), Thieme Verlag, 126-36.

49. Olsen, C.L.; Liu, G. and Charles A. (1994) Novel routes of insulin delivery. In The Diabetes Annual /8. Marshall SM, Home PD, Eds.

50. Amsterdam, London, New York, Tokyo, Elsevier, p. 243-276

51. Richard, E.G., Lowerence S.O., Physiological determinants of nasal absorption, J.Cont. Rel. 1987, 6,361-366.

52. Ali, A. and Trehan, A. (1998) Drug Dev. Ind. Pharm., 24(7), 589-597.

53. Illum, L.; Farraj, N. F. and Davis, S.S. (1994) Pharm. Res., 11,1186-1189

54. Dodane, V.; Khan, M. A. and Merwin, J.R. (1999) Int. J. Pharm.,182, 21-32.

55. Farraj, N.F.; lllum, L.; Davis, S.S. and Johansen, B.R. (1989) Diabetologia, 32, 486.

56. Gordon, G.S.; Moses A.C.; Silver, R.D.; Flier, J.C. and Carey, M.C. (1985) Proc. Natl. Acad. Sci. USA, 82, 7419-23.

57. Moses, A.C.; Gordon, G.S.; Carey, M.C. and Flier, J.S. (1983) Diabetes, 32, 1040-7.

58. Merkus, F.E.W.; Verhoef, J.C.; Romeijn, S.G. and Schipper, N.G. (1991) Pharm. Res., 8, 588-92.

59. Watanabe, Y.; Matsumoto, Y.; Kawamoto, K.; Yazawa S. and Matsumoto, M. (1992) Chem. Pharm. Bull., Tokyo, 40, 3100-4.

60. Mishima, M.; Wakita, Y.; and Nakano, M. (1987) J. Pharmacobiodvn. , 10, 624-31.

61. Lee, W.A.; Narog, B.A.; Patapoff, T.W. and Wang, Y.J. (1991) J.Pharm. Sci., 80, 725-729.

62. Wall, D. A. (1995) Drug Delivery 2, 1-20.

63. Farr, S. J. et al., Diab. Technol. Therapeut., 2000, 2, 185–197.

64. Heinemann, L.; Pfützner, A. and Heise, T. (2001) Curr. Pharm.Design, 7, 1327-1351.

65. EXUBERA (insulin human [rDNA origin]) Inhalation Powder, (2008)[Pfizer Inc.]

66. Am J Health-syst pharm (2005), American society of Health-System Pharmacists

67. Langkjaer, L.; Branje, J.; Grodsky, G.M. and Guy, R.H. (1998) J.Control. Rel., 51, 47-56.

68. Mitragotri, S.; Blankschtein, D. and Langer, R. (1995) Science, 269, 850-853.

69. Sevastianov, V.L.; Salomantia, L.A.; Ruznetsova, E.G.; Iakaleva, N.V. and Shumakov, V.I. (2003) Med. Teck., 4, 21.
70. Cevc, G. (1996) Crit. Rev. Ther. Drug Carrier Syst., 13, 257-288.
71. Godin, B. and Touitou, E. (2003) Crit. Rev. Ther. Drug Carrier Syst., 20(1), 63-102
72. Sadikot, S.M. (1992) J. Diabetic Assoc. India, 32(1), 15.
73. Hussain, M.A.; Aungust, B.J. and Sheffer, E. (1986) Eur. J. Pharm.Sci., 75, 218.
74. Aungust, B.J.; Rogers, N.J. and Sheffer, E. (1988) J. Pharm. Exp.Ther., 23, 244.
75. Yamasaki, Y.; Shichiri, M.; Kawamori, R.; Kikuchi, M.; Yagi, T.;Arai, S.; Tohdo, R.; Hakui, N.; Oji, N. and Abe, H. (1981) Diabetes Care, 4(4), 454-45