Expert Opinion

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Delivery of insulin to the buccal mucosa utilizing the RapidMist™ system

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Background: The burgeoning number of people with diabetes mellitus is a global problem. Simple but effective treatment with minimal side effects will be required to reduce the risk for macro- and micro-vascular complications. A big part of this goal can be achieved by the ready administration of insulin with or without other medications. *Methods*: The RapidMist™ drug delivery system places human recombinant insulin in a liquid formulation so as to be delivered to the buccal mucosa with an asthma-like device. As with nitroglycerin, the insulin PK-PD is very fast, thus affording flexibility. *Conclusion*: Serial data show clinical efficacy in Type 1 and Type 2 diabetes with at least non-inferiority to regular insulin.

Keywords: buccal mucosa, diabetes mellitus, insulin

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1. Overview of the problem

The basic problem is the effect of the synthesis of diabetes mellitus, obesity and cardiovascular disease on the world's population and international health. Put simply, diabetes mellitus is a syndrome of altered glucose metabolism resulting from a number of different disorders. The emerging common denominator other than the impact of elevated glucose is inflammation. About 5 – 10% of the diabetes population has Type 1 diabetes. This is an autoimmune disorder occurring in those people at risk because of abnormalities on the sixth chromosome at the DR3 and DR4 loci. It occurs mostly in children but is now recognized much more frequently in adults. The autoimmune process, precipitated by a yet to be determined antigen or antigens, results in an inflammatory process at the insulin producing Beta cells of the pancreas, resulting in a progressive destruction of those cells. The process is more accelerated in children. These patients must take insulin to survive.

Ninety percent of the diabetic population is Type 2. This is a multimolecular disorder resulting from molecular failure at a number of sites. It is likely that as the evolutionary process advanced, the human body morphed into a calorie-saving metabolism to account for periods of famine and the sudden need for energy to fuel muscles to escape predators. Humans have not readjusted to a new paradigm of relative plenty and as a result there is a worldwide 'epidemic' of obesity (except in areas of starvation). Insulin, a very old and primitive molecule, produced by the beta cells of the pancreas in response to varying glucose levels, works by attaching to its receptor on a peripheral cell surface. The insulin receptor is equally as old and is found in the earliest of animal species, such as a 700-cell worm. The insulin receptor complex then triggers a cascade of messages down three pathways. One of these tells the cell to allow glucose to enter. There are many proteins involved so it is likely that over hundreds of thousands of years many mutations occurred that compromised this message. The expression of these mutations did not occur because the three factors that require more insulin and

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more insulin action – too much food, not enough physical activity and ageing – simply did not exist in the long distant past. Type 2 diabetes, once thought to be a disease of adults, now occurs at all ages and is increasing in children.

The consequence of the failure of the insulin–insulin receptor system, or in Type 1, the production of insulin, is hyperglycemia, an elevation of blood glucose. It was thought for many years that an elevated glucose level was associated with the development of diabetes complications. It was not until two landmark studies in the 1990s that this suspicion was confirm. In 1993 the Diabetes Control and Complications Trial (DCCT) [1] was cut short after 10 years, its half-way point. The data showed that Type 1 patients receiving aggressive, tight control of blood glucose had reduced risk for the microvascular complications (which continued at the study's close, despite recidivism) as compared to a group with casual treatment and higher blood sugars. The United Kingdom Prospective Diabetes Study, a 20-year look at comparable data in Type 2 more or less came to the same conclusion [2].

However, the issue is not so simple. A large body of data has now evolved also showing an impact, a risk or an association of elevated blood glucose with macrovascular disease, such as heart attack and stroke. In the natural history of Type 2 diabetes this risk has been associated with elevated postprandial glucose (impaired glucose tolerance [IGT]), preceding the risk for microvascular disease by as much as 10 years. Those doing research in this area feel it is a form of oxidative stress on the endothelium, the lining of the blood vessels. Finally, the molecular biology of obesity, including the ability of the adipocyte to have reactive monocytes that set up an inflammatory milieu, is also relevant. In a nutshell obesity, hyperglycemia, hyperlipidemia, hypertension and physical inactivity are all capable of causing an inflammatory cascade at the endothelium, resulting in local damage and an increased risk of lipid deposits.

These aberrations and mutations appear to have affected the majority of the world's population, accounting for the continuous increase in numbers of affected people. This means that hundreds of millions will have Type 2 diabetes or IGT in the next quarter century. Therefore there is a market beyond anything seen in the past, including TB, malaria, trachoma, etc.

2. Current treatments

Treatment of this group of diseases requires lifestyle changes and polytherapy. A core therapy concerns regulating the blood glucose to as close to normal physiology without over-shooting and causing hypoglycemia. When insulin was discovered over 80 years ago it was prepared as a short-acting preparation lasting 6 – 8 h. The crystals were made with zinc and they dissolved into a clear solution. Over time techniques for longer action were developed. In the 1970s human insulin was produced utilizing *Escherichia coli* to synthesize the A and B chains in a recombinant technology. Now we have synthetic analogs providing a shorter acting insulin and a basal insulin. In the 1940s the first oral agents for Type 2 diabetes were developed. Initially only sulfonylures were developed, but now there are many categories. Type 1 patients must take insulin to live and must take many injections per day or use a pump to maximize control. Type 2 patients generally take insulin as a last resort. The latter is changing and the action of insulin beyond glucose has been recognized. Insulin has been found to be anti-inflammatory and protective of the endothelium. The move to use insulin when postprandial glucose exceeds guidelines is growing. Regulation of postprandial elevations as in IGT is not easily accomplished with oral agents. The challenge is how do we get Type 1 patients to take insulin more often and Type 2 patients to use it as a prandial control agent [3].

It was recognized early on that resistance to taking injections would emerge. Alternative delivery of insulin was attempted as early as 1925. The difficulty is moving a large molecule like insulin across a variety of membranes. It is doable but in impractical amounts. Vaginal, rectal and colonic delivery have obvious limitations. Oral pills or liquids failed when delivered through the stomach to the duodenum. Nasal was not approved because of local membrane effects, amongst other factors. Pulmonary insulin was doomed from the beginning as there was resistance to the product by the majority of endocrinologists. Four companies have withdrawn their inhaled pulmonary insulin from the marketplace as a number of cases of lung cancer were reported in smokers using inhaled insulin.

That leaves the buccal mucosa as a delivery site. That should not be surprising, as there are many medications such as nitroglycerin, nifedipine and fentanyl delivered via this route, not to mention nefarious substances such as amylnitrite and cocaine.

3. Technology

The advantages of the buccal mucosa are straightforward. It is visible and easily accessible. It has a very rich array of blood vessels, which accounts for the rapid action of drugs absorbed through it. It is extremely resistant and is exposed to trauma all day during the chewing process, self biting and hot foods. Yet it heals quickly. The main difficulty would be the size of molecules one attempts to pass through it. There is a substantive literature on peptides and proteins and buccal absorption [4]. Other factors are charge, lipo- and hydro-philicity. In addition a variety of additives facilitate absorption of proteins through the buccal mucosa. The anatomy is basically a layer of epithelium, a layer of lamina propria and submucosal area with its rete of blood vessels. Transport is either transcellular or paracellular. It would appear that insulin traverses the epithelium via the paracellular route. A little more than 10 years ago human

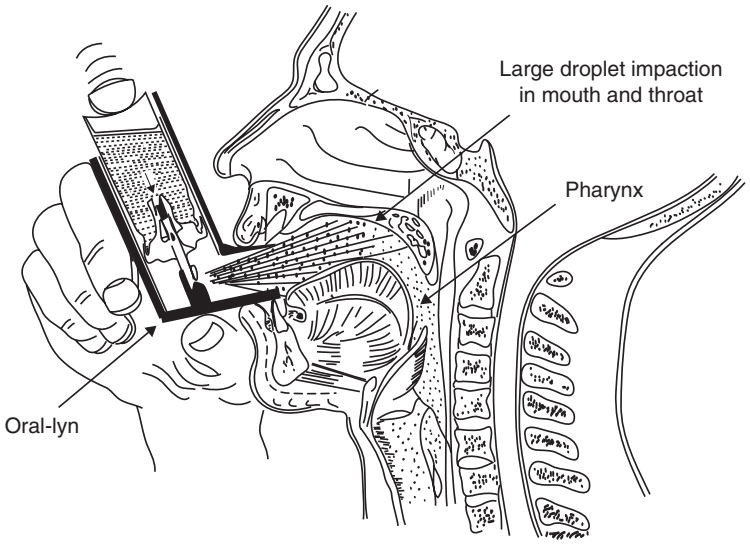

Figure 1. Proposed mechanism of absorption.

recombinant insulin was successfully put into a liquid formulation with very small amounts of generally regarded as safe (GRAS) ingredients, and when delivered to the buccal mucosa with a slightly modified asthma-like spray, successfully penetrated the mucosa (**Figure 1**). The formulation and device have matured and are heavily patented. The formulated insulin is stable at room temperature (North America) for 6 months or more. The micelles that are formed, containing the insulin, are > 7 microns and cannot enter the deep lungs regardless of effort. This was initially confirmed utilizing the Anderson cascade impactor [5]. It is tasteless and some feel the coldness of the propellant but adapt quickly. As formulated, one spray contains 10 units of insulin. The insulin is delivered in a metered dose so the first and last sprays of the container are identical. There is 10% absorption so one unit is delivered to the bloodstream with each puff. This allows dosing rather than approximating. It is important to remember that only 20 – 40% of subcutaneous injection is absorbed. As will be mentioned below, insulin appears in the blood within 5 min, peaks at 30 min and is back to baseline at 2 h [5]. This narrow window is unique to buccal insulin. It is possible because of the rich vascularity below the buccal epithelium. The lack of a 'tail' of insulin activity would favor less hypoglycemia.

4. The studies

The following safety studies were performed on dogs at the University of Guelf. Forty dogs received the insulin formulation by spray four times daily for two years while 10 dogs received the same spray without insulin four times daily for two years. Neither set of animals suffered any adverse events. Scrapings and biopsies of the buccal cavity were taken at intervals and examined by an oral pathologist. No changes were seen in the buccal epithelium of either set of dogs. Radio-labeled insulin was administered to adults [5]. The tracer was followed and was seen to only go down the esophagus, with no radio label appearing in the lung (**Figure 2**). This is in contrast to pulmonary insulin, which only fills the alveoli of the lungs. In the ensuing years no visible pathology has been seen in the buccal mucosa of hundreds of patients studied. In a study performed by Pozzilli and associates [6], the response to regular insulin by injection at breakfast was compared to a comparable amount of buccal insulin at breakfast on another day. Equivalent responses were obtained. They concluded that the buccal route was equally as effective as the subcutaneous route. A group of Type 1 patients were studied utilizing the glucose clamp technique [3]. They were dosed with 5, 10 and 20 puffs. The Glucose Infusion Rate (GIR) progressively increased, showing a proportional dose response (**Figure 3**). In a separate study a group of Type 1 patients were given a test meal and either an injection of human regular insulin or an equivalent amount of buccal insulin by spray. Blood levels of insulin and glucose were taken over a period of 4 h. The test meal and buccal insulin were repeated weekly for 3 weeks. The results showed a very high degree of reproducibility of the action of buccal insulin in individuals over the 3 weeks (**Figure 4**).

In another series of studies in Type 2 patients failing on oral agents, buccal insulin was added [7]. In the first, a group of patients on metformin and a sulfonylurea but with unsatisfactory A1c hemoglobins was divided; half had buccal insulin added to their regimen while the other group continued their regimen. The buccal insulin group dropped their A1c hemoglobin more than 1% in 90 days. Similarly, a group failing TZD treatment was handled in the same fashion and also had a drop in A1c of around 1%. Finally,

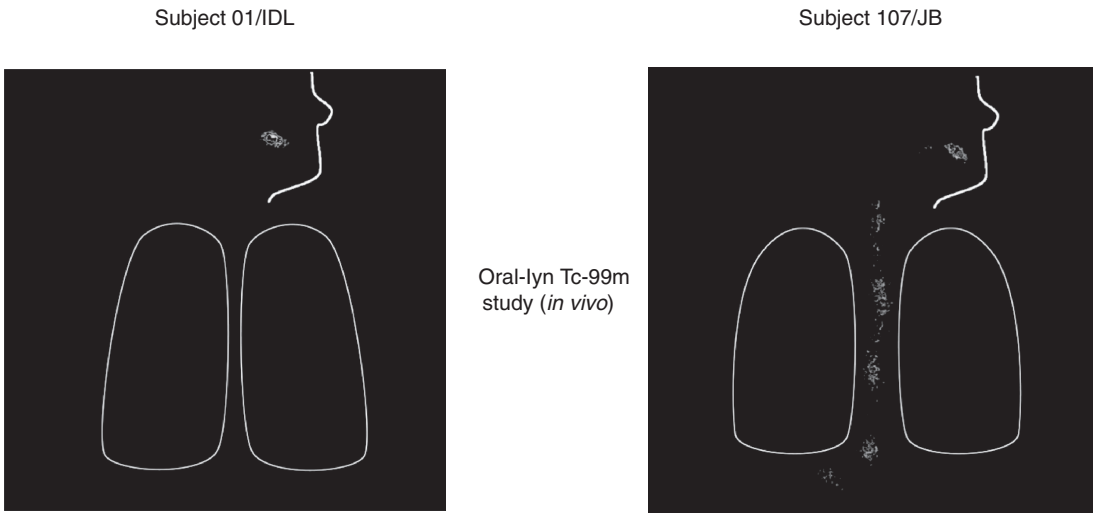

Figure 2. *In vitro* and *in vivo* radio-label oral-lyn study. *In vitro* and *in vivo* studies with radio-label Tc99 formulation in healthy volunteers completed to prove that the formulation is truly buccally absorbed (quantitative mouth deposition, i.e., absorbed through cheeks and tongue and back of the throat linings and not deposited in the lung (no absorption in the lungs).

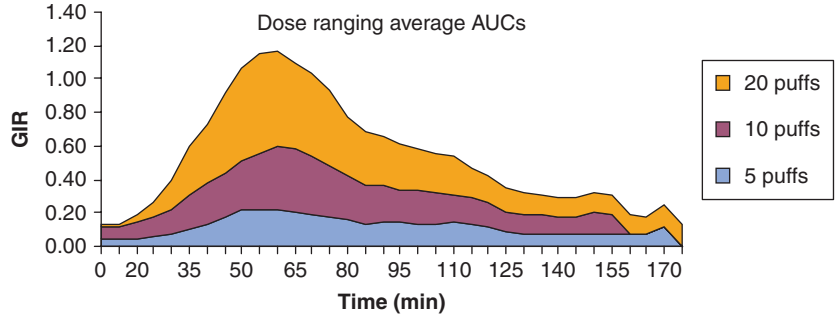

Figure 3. Glucose infusion rate in respone to Oral-lyn dose.

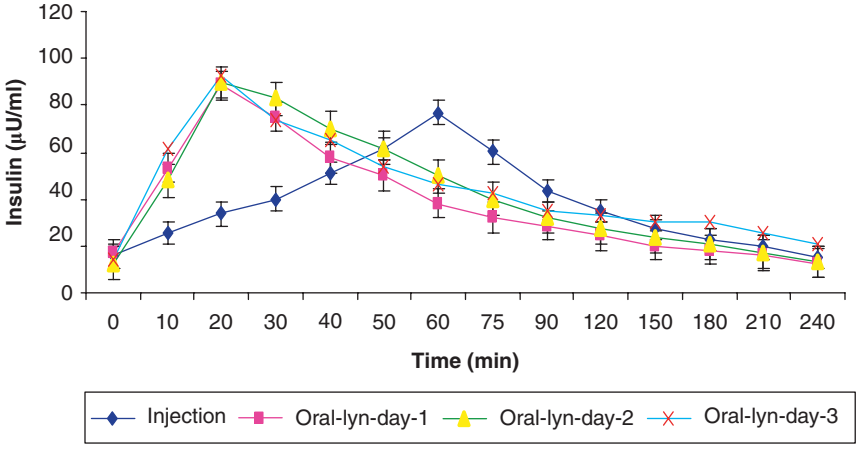

Figure 4. Response of Type 1 patients to Oral-lyn after a test meal performed on 3 separate occasions.

half of a group of patients who only managed their diabetes with lifestyle was given buccal insulin and once again A1c dropped significantly. The last Type 2 study involved 26 patients on metformin and sulfonylureas. Half also took buccal insulin at meal time. At the end of 12 weeks, post-prandial glucose was lower than the controls at every meal time.

5. The road to Phase III

In order to satisfy most regulatory groups, efficacy, safety, etc. had to be proven in Type 1 patients, with ultimately buccal insulin being non-inferior to human recombinant insulin. The first study looked at a group of 10 patients with Type 1 diabetes. They were stabilized on glargine and human regular insulin before each meal. At the point of stability they tested their blood 10 times daily: before and 1 h and 2 h after the meal and at bed time. After 72 h they switched to glargine and buccal insulin, again before each meal. They continued to test their blood glucose for nine more days. The mean glucose curves for individuals and the group were virtually identical (**Figure 5**) [8]. The next study looked at a group of adolescents using a similar run-in period. At the stability point they replaced human insulin with buccal insulin before lunch and continued for more than 3 months, serving as their own controls. Lunchtime for Type 1 adolescents is the most common meal for skipping insulin, for a variety of social and behavior factors. The end point was A1c hemoglobin [9]. The buccal insulin at lunch showed a continued fall in A1c hemoglobin. Guevara-Aguirre also studied a group of Type 1 adult patients for 99 days comparing NPH twice daily and either regular insulin pre-prandially or split doses of buccal insulin pre-prandially [10]. A1c was the end-point. The latter had dropped from a mean of 9 to 8.1 at the stability point and then a further fall to between 6 and 7 at 99 days (**Figure 6**).

Despite the popularity of analog basal insulins such as glargine and detemir, NPH insulin is still the most commonly used around the world. This is primarily related to cost. NPH is generic and is one-third or less than the cost of analogs. A series of studies now took a further look. A large group of Type 1 patients were stabilized over a few months on NPH and human regular insulins. At the stabilization point they were divided in two, with one group continuing the NPH and regular insulin and the second group changing to NPH and buccal insulin. A1c hemoglobin fell during stabilization and continued to fall during the study phase in both groups. The study phase lasted at least 6 months (**Figure 7**). The rate of decrease of A1c was roughly equal in both groups, but the buccal insulin group remained lower throughout. Finally glargine and a rapid-acting analog were compared to NPH and buccal insulin over a period of 1 year and showed non-inferiority of the NPH-buccal group [6]. Phase III is underway and is a simple, straightforward non-inferiority study of NPH and human regular insulin versus NPH and buccal insulin. The short-acting insulins are given at meal time and when required for isolated rises in blood glucose. The primary end-point is A1c hemoglobin. Aside from blood glucose measurements, the following will be studied: insulin antibodies, buccal mucosa by inspection and cytopathology and the usual general health parameters.

6. Safety

To date there have been no reported serious adverse events related to buccal insulin in any study.

7. The RapidMist system

Generex Biotechnology has created the RapidMist™ drug delivery system (**Figure 8**). The patented formulation is capable of putting 150 or more compounds into a solution similar to regular human insulin. Proof of concept has only been tested with morphine, fentanyl and low molecular weight heparin to date. For insulin the canister hold 400 units and delivers 10 units per puff in a precisely metered dose. The formulated insulin is called Oral-lyn™. It satisfies a number of criteria for effective clinical use. It is safe, simple, fast, flexible and very familiar to patients and physicians alike, as it resembles an asthma inhaler. Each puff delivers one unit of insulin to the body, a 10% absorption. All other alternative forms of delivery, mostly now gone, delivered approximate amounts. Its unique pharmacokinetics allow the dose to be split before and after meals. It may be taken just before the first bite and just after the last. This flexibility offers both Type 1 and Type 2 patients a unique opportunity to aggressively treat diabetes with a minimal risk of hypoglycemia.

8. Conclusion

There is an impending worldwide medical and economic catastrophe. Unless there is a dramatic change in the geologic fortunes, of the planet the number of people with clinical and preclinical diabetes mellitus will continue to grow. Already in the hundreds of millions, the number will easily exceed a billion. The cost is present regardless of action or inaction. If there is a major intercession, the cost of education, pharmacology and technology is and will be significant, but if there is no action the cost of human failure and the macro- and micro-vascular complications of diabetes will be even greater. A worldwide public health intervention must be mounted. Early and preventative detection and medication are a must. The uncomplicated delivery of Oral-lyn to the buccal mucosa with the RapidMist system at meal times with a simple device satisfies a major part of that need.

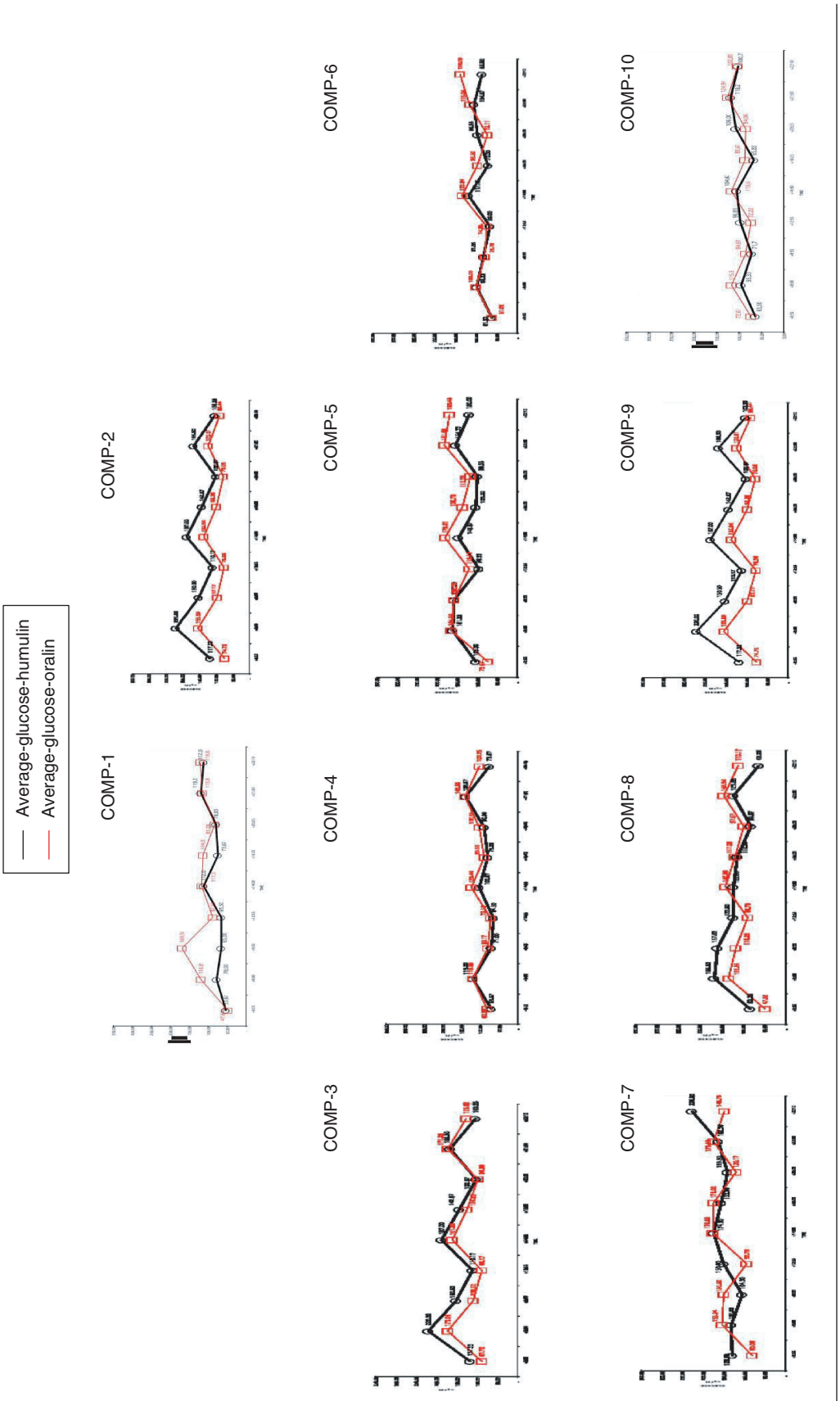

Figure 5. Comparison 10-point glucose testing after Oral-lyn™ or regular insulin by injection before meals.

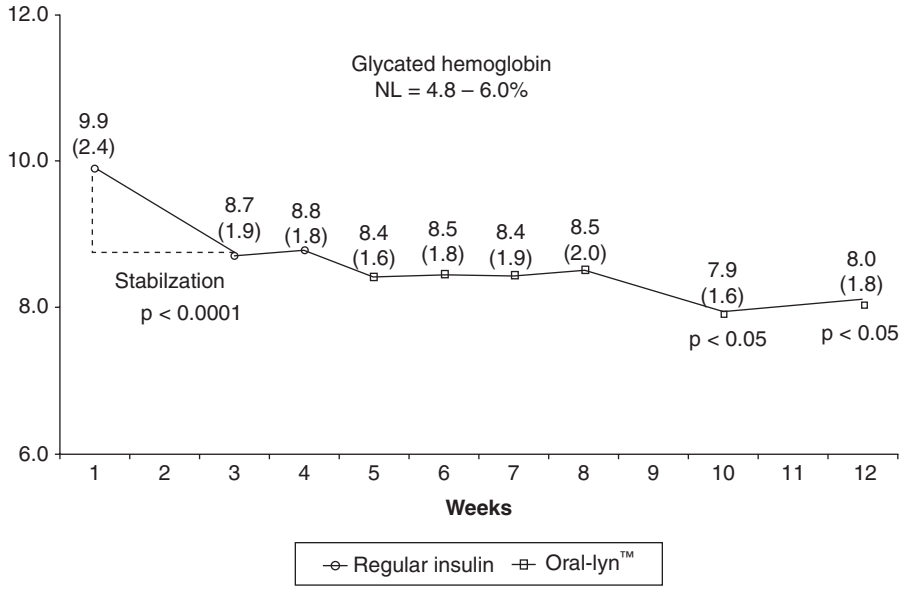

Figure 6. Adolescents with Type 1 before and after switching from regular to Oral-lyn™ at lunchtime.

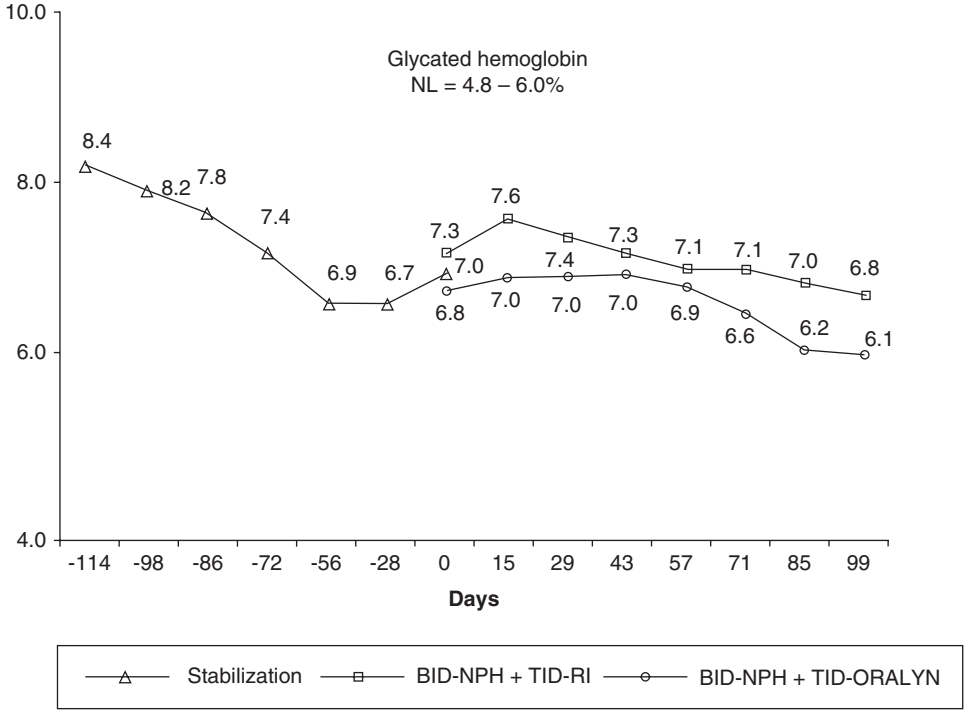

Figure 7. Oral-lyn™ and NPH compared to regular and NPH after stabilization.

Figure 8. Generex oral-lyn.

9. Expert opinion

If one were to design an ideal absorptive site for accurate delivery of medications it is likely that the most common site, the subcutaneous area, would not top the list. The buccal mucosa, in contrast, is ideal and would rank up at the top. Its value lies in its shallow lamina propria and rich vascularity. Once penetrated, molecules rapidly enter the bloodstream, in contrast to the lag time seen with subcutaneous injection. This allows great flexibility in pinpointing the time of action of medications such as insulin. Small molecules such as nitroglycerine cross with ease, but large molecules such as insulin need facilitation. Injection into the buccal mucosa is obviously not practical or desirable. The RapidMist system developed by Generex Biotechnology overcomes this limitation in a number of ways. Human recombinant insulin is put into a patented liquid formulation with minimal amounts of GRAS ingredients and delivered to the buccal mucosa utilizing a patented spray device, similar to that used in asthma. The use of a familiar system allows both patient and physician to accept the use of insulin more readily. This is critical as the number of people with diabetes mellitus worldwide will be over a billion in several decades. The insulin product is called Oral-lyn. Over 150 different molecules have been put into this formulation successfully. In addition, small molecules such as glucose in a pump spray or metformin in a gum carrier can be delivered to the buccal mucosa successfully. If large numbers of people are to benefit from glucose control using insulin, the safe, simple, fast, flexible and familiar RapidMist system is the answer.

Declaration of interest

GB is the Vice-President of Medical Affairs at Generex Biotechnology.

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