Reproducibility and variability in the action of injected insulin

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SUMMARY
Insulin-treated patients are generally taught to adapt their doses of insulin according to the glycemic level obtained during self-tests. They usually adhere to medical recommendations, but are often confused by the results, which may not correspond to expectations. Patients have to contend with variability and a certain degree of unpredictability in the results. Our knowledge of the factors involved in this variability is often imprecise. We review here the factors depending on the preparation of insulin itself, not only with regard to its crystallization but also the speed at which the hexamers dissociate into dimers. The development of fast and slow-acting analogues is discussed along with their value in improving glycemic predictability. In addition to these factors, we mention those stemming from the injection technique itself, which are directly related to the instructions given to the patients. For crystalized insulin preparations, shaking the bottle is an important element that the development of slow-acting analogues should eliminate, but the time lapse before withdrawing the needle, the anatomical site of the insulin injection, and the depth of the injection are also factors for variability. Greater predictability in the action of insulin will be obtained from a combination of progress in manufacturing procedures and better patient education.

Key-words: Glycemic predictability · Insulin effect · Insulin diffusion.

Résumé
Reproductibilité et variabilité de l’action de l’insuline injecté
Les patients diabétiques traités par insuline sont habituellement éduqués pour adapter leurs doses d’insuline en fonction des contrôles glycémiques qu’ils ont eux-mêmes réalisé par auto surveillance. D’une façon générale ils adhèrent aux recommandations médicales mais sont parfois perturbés par les résultats qu’ils obtiennent, qui ne correspondent pas avec leur attente. Les patients sont donc confrontés à un certain degré de variabilité, voire d’imprédictibilité dans leurs résultats, qui peut les perturber dans leur adhésion thérapeutique ; en effet, leur connaissance des facteurs impliqués dans la variabilité de l’action de l’insuline est assez souvent imprécise. La revue présentée essaie d’analyser les facteurs qui dépendent de la préparation insulinique elle-même, non seulement fonction du degré de cristallisation de cette préparation mais aussi de la vitesse avec laquelle les hexamères se dissocient en dimères et monomères. Le développement des analogues aussi bien rapides que lents, permet probablement de s’affranchir de l’étape de la cristallisation, et il est discuté de l’apport de ces analogues dans la prédictibilité du niveau glycémique. Aux facteurs directement liés à la préparation insulinique, viennent s’ajouter les facteurs dépendant de la technique d’injection elle-même ; celle-ci dépend directement des recommandations et de l’éducation données aux patients. Pour les insulines cristallisées, l’agitation du flacon est un élément important, voire majeur, que le développement des analogues lents devrait éliminer ; cependant, le temps laissé entre l’injection et le retrait de l’aiguille, le site anatomique d’injection, la profondeur de l’injection sont aussi des facteurs de variabilité de la réponse : ils peuvent être réduits par une bonne éducation et bien sûr un respect de celle-ci. Une plus grande prédictibilité et une moindre variabilité des résultats obtenus peuvent donc être obtenus par la conjonction des progrès réalisés par l’industrie pharmaceutique dans la bio disponibilité des médicaments et par la qualité de l’éducation apportée aux patients pour leur technique d’injection.

Mots-clés : Prédicibilité glycémique · Effets de l’insuline · Diffusion de l’insuline.
In healthy individuals, blood glucose levels remain within narrow limits from one day to the next. On strict fasting, this regulation depends on the relationship between insulin secretion and hepatic glucose production; in postprandial conditions, more factors are involved: food ingestion, carbohydrate load and its nature (glycemic index), gastro-intestinal hormones, and also the capacity of insulin to completely eliminate the hepatic glucose production in post-prandial conditions (hepatic insulin sensitivity). Only the large capacity of the insulin secretory reservoir and the speed of the physiological action of insulin ensure glycemic stability and its reproducibility from one day to the next. In non-diabetic individuals, the ingestion of a meal is followed 15 minutes later by a rapid increase in glycemia, reaching a peak 45 minutes later of 40 mg/dl above the basal level. Levels then return to baseline and remain stable until the next meal; insulin levels follow a similar pattern [1].

In type 2 diabetic patients, glycemic control is deteriorated because of the progressive loss of $\beta$ cell function and the increase in resistance to the action of insulin. However, these parameters remain constant from one day to the next, and although secretory capacity is reduced it does not fluctuate. Glycemic control is impaired, but remains reproducible and predictable from one day to the next, so long as nutritional supplies are controlled; pancreatic function is disrupted, but is globally reproducible from one day to the next. The main factors in variability are therefore the potential variations in carbohydrate intake and the caloric content of meals. Thus provided nutritional factors are controlled, there is glycemic reproducibility in type 2 diabetic patients and consequently a degree of predictability from one day to the next. Another factor is physical activity, which, if intense, can lead to a rapid decline in glycemia, but is also capable of affecting sensitivity to insulin over a longer period of time. Here again this is a factor that is susceptible to control and can help predictability when patients have full knowledge of their disease.

In insulin-treated diabetics, the same elements of variability in glycemia are present, and there will also be some degree of day to day predictability. Although the elements are as comprehensible and controllable as those in type 2 diabetes, two other major factors may be involved: the loss of pancreatic function and the individual’s sensitivity to exogenous insulin (dose, diffusion rate from subcutaneous cell tissue, the reproducibility of this diffusion rate and the kinetics of the injected insulin itself). We will attempt to analyze here the influence of each of these factors on the variability of blood glucose level and thus on the predictability of glycemia in diabetics.

Such an analysis would be of value as the less consistent the treatment of diabetic type 1 patients, the more they are likely to be confused with consequent loss of compliance with the treatment. The practitioner will have taught the patients to adapt their dose of insulin. If the expected results are not obtained, the patient may become anxious and feel that he/she did not do the right thing and so may eventually refuse treatment.

It is thus important to define the factors giving rise to poor reproducibility, and hence poor predictability. Technological or pharmacological advances that would reduce the degree of uncertainty of the effect of insulin would help in the management of type 1 diabetics and enhance compliance with their treatment.

A subcutaneous injection of an identical dose of insulin does not always lead to the same glycemic result from one day to the next. Furthermore, a number of episodes of hypoglycaemia may stem from this variability.

### Variability in the action of insulin

The variability in the action of insulin is related to several factors; some depend directly on the insulin preparation, some on the injection conditions and others on factors inherent to the organism, which may modify the interaction between insulin and its receptor. This variability can be measured by the pharmacokinetics of insulin (evolution of plasma insulin levels following injection) and by their pharmacodynamics (the hypoglycemic action), the latter corresponding to the true effect of insulin.

### Factors depending on the preparation of insulin itself

These effects are directly dependent on the physicochemical properties of insulin that may interfere with its diffusion and absorption in the subcutaneous tissue. In daily practice, insulin is injected into the subcutaneous tissue where it forms a deposit or reservoir from where it is resorbed. To reach the blood stream it must be present in the form of dimers or monomers, which are the only elements capable of diffusing in the interstitial fluid and crossing the blood barrier. Once in the blood stream, insulin can reach its receptors located on the surface of target organs.

The time lag for entry into the blood stream will depend on the the insulin formulation (fast-acting, fairly slow or delayed action). In general, the more complex the physicochemical preparation, the greater the variability in diffusion from the subcutaneous reservoir between individuals and in the same individual from one day to the next.

### Slow and semi-slow protamines or zinc-based insulins

In both cases, the insulin is presented in crystalline form (as can be observed in the vial, which requires adequate shaking to create a suspension). Once injected in the subcutaneous tissue, the crystalline structure must be destructured in order to release the insulin complexes followed by the
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hexamers, which, in turn, dissociate into dimers and monomers (Fig 1). The speed of this process varies from one patient to the next (which is not a significant problem), but it also varies in the same patient from one day to the next. The intra-individual variability in pharmacokinetic parameters has been studied by many authors and is around 20% under strict experimental conditions (subcutaneous injections by the same operator on three consecutive days in the same site with the nature of the injection and its depth controlled by scan) [2]. Under less controlled conditions, the coefficient of variation in the pharmacodynamic parameters is greater: 20 to 40% for NPH [3, 4] and 38 to 55% for ultralente [4], or even greater in current clinical practice [5].

Fast-acting insulin

Of non-crystalline structure, this is the regular insulin. It is in the form of a solution (the liquid is clear), but it tends to form hexamers spontaneously, which is related to the concentration in the vial. Following subcutaneous administration of a fast-acting insulin, the rate of absorption increases progressively and stabilizes after a varying period of time, corresponding to the constitution of the insulin reservoir. Once the reservoir has been formed, insulin is resorbed at a constant rate. The time lapse before obtaining a reservoir is related to the time required for dissociation of the hexameric insulin into dimers and monomers. This will give rise to some delay in the effect of insulin, and is a source of intra-individual variability from one day to the next [6]. This variability is at least 20% [3, 7].

Insulin analogues

Insulin analogues are regular insulin modified by genetic engineering to reduce the tendency to aggregate in the form of hexamers by the addition or alteration of a single amino acid [6, 8, 9]. This explains the faster rate of diffusion from the subcutaneous cell tissue with a reduced inter-individual variability [10]. Absorption of a monomeric insulin analogue leads to a mono-exponential dispersion of labelled insulin with an immediate acute slope, in contrast to the three-slope profile obtained with human hexameric insulin and the two slope profile with dimeric insulin [6]. With rapid insulin analogues, this contributes to a more reproducible profile since the initial latency observed with human insulin, the duration of which is largely dependent on the volume injected, the site of injection and heat, is reduced. As an example, on doubling the dose of insulin injected the T max is prolonged by 62% in the case of regular insulin, and by 27% for an analogue [11]. The improved reproducibility in the kinetics of fast-acting analogues is concomitant with a reduction in the coefficient of variation in intra-individual pharmacodynamic parameters, such as the postprandial glycemic excursion or the time before obtaining a maximum insulin effect [7, 12].

Slow-acting analogues are of more recent development and are represented by glargine and detemir. These two slow-acting insulins are presented in non-crystalline form, i.e., completely soluble as indicated by the absence of crystals in the vial. Glargine remains soluble at acid pH, and precipitates at neutral pH. Injection into subcutaneous tissue produces an homogenous and diffuse precipitate which subsequently releases its monomers. The conditions of preparation of glargine reduce intra-individual variability compared with crystalline insulins. However, no improvement has been shown in the coefficient of variability of glargine versus NPH even when the injection is made under optimal, well controlled conditions.

The other slow-acting insulin analogue, detemir, consists of insulin bound to a short-chain fatty acid. Once injected, this insulin does not pass through a micro-crystalline or precipitate stage. It remains in the soluble phase, diffusing across the interstitial tissue, capillary barrier and hence the blood stream. It tends to form dimers and binds to albumin on the binding site with albumin fatty acids. It
would appear that the absence of an intermediate phase, crystalline or precipitate, confers a lower intra-individual coefficient of variation. Indeed, it has been demonstrated [13-15] that the coefficient of variability for detemir was only of 16%, compared to 26% for NPH. Detemir insulin therefore has a lower variability than does NPH in both pharmacokinetics and pharmacodynamics [16]. The only study comparing the intra-individual variability of the NPH insulins, detemir and glargine, on the pharmacodynamic parameters found a significantly more predictable effect of the detemir insulin [17] (Tab I).

**Effects of concentration and dose.**

Independently of the physicochemical conditions of the preparation, the concentrations and doses of insulin also appear to play a part; for the same type of insulin, the higher the concentration, the slower the diffusion. Furthermore, with increase in dose, there is a reduction in rate of diffusion together with an increase in the coefficient of intra-individual variability [3]. For example, the T 50% for an NPH-based insulin was 7.5 hours for a dose of 6 units, and 10.6 hours for a dose of 24 units. A similar variation was found with fast-acting human insulin [9]. Thus, when the dose of insulin is greatly increased in an individual, it gives rise to a variation in kinetics. However, the dose response appears to be reduced with the analogues [18, 19]. Nevertheless, the relationship is not linear; a 3-fold increase in the dose of insulin only gives rise to a 2-fold increase in the quantities of insulin absorbed. Moreover, the increase in dose prolongs the absorption time and hence the duration of the effect of the injected insulin [20]. This shows that further variability is induced by large variations in injected dose.

**Factors depending on the injection conditions**

The injection process itself can also affect variability. However, neither the speed of injection nor the temperature of the insulin appear to affect the variability in diffusion. However, the depth, anatomical site, delay before withdrawing the needle, and blood flow in the subcutaneous tissue vary from one day to the next, giving rise to poor reproducibility [3, 21].

The deeper the injection of insulin, the more rapid its diffusion. It has been demonstrated that an accidental intramuscular injection of NPH reduced the T 50 to 5.3 hours from the 10.3 hours noted after subcutaneous injection. When the insulin is injected too deeply, it has a more rapid rate of diffusion [22], and, consequently, a shortened duration of action [2]. Furthermore, the intra-individual coefficient of variation is significantly higher with an intramuscular injection than with a subcutaneous injection. Intramuscular injection is accompanied by faster absorption, shorter duration of activity and a greater variability in absorption than via the subcutaneous route. Inappropriate injections of insulin into muscle tissue are probably fairly frequent, notably in the thighs and arms of thin patients. The differences in insulin kinetics depending on subcutaneous or muscular administration indicate the need for care in the choice of needle length. The anatomic site also plays a part; insulin diffuses faster when injected in the abdomen, more slowly in the arm and even more slowly in the leg [23, 24]. For a given dose, absorption of 50% from the abdomen is quicker than absorption of 30% from the thigh. This variation from one site to another seems to be related to the degree of vascularisation of subcutaneous tissue, and perhaps also to degradation of insulin in certain subcutaneous sites. Patients should therefore vary the injection site, but also change location in the same tissue to minimise variability. A study assessing the elimination of the radioactivity of labelled glargine in the arm, the thigh and the abdomen did not show any significant difference in the profile of insulin elimination between these various sites [25].

Allowing a certain time lapse before withdrawing the needle is also an important element. Indeed, if the needle is withdrawn rapidly, there may be reflux from the subcutaneous tissue, which will tend to vary from one injection to the next giving rise to further variability. This has been demonstrated using insulin syringes [26]: in 25% of the patients, rapid withdrawal of the needle led to a loss of insulin ranging from 10% of the dose in 13.8% of the patients to 5-10% of the dose in 8.6% of patients. In all cases there was a loss of insulin administered that varied from one day to the next. There was no relationship between the amount lost and the anatomical site. This loss may be greater with insulin pens. With a syringe, the insulin is injected when the plunger is completely pushed in, but with a pen, the plunger is partially pushed in pressurising...
the liquid in the cartridge, and the insulin is only completely injected when the pressures in the cartridge and the subcutaneous tissue are equal. Thus as the plunger advances, there is no immediate equalisation of pressure, and if the needle is withdrawn too quickly, there is not only reflux of insulin from the subcutaneous tissue, but insulin will continue to flow from the pen. The technique of injection is particularly important, especially with respect to the time lapse before withdrawing the needle as this can lead to more than 10% variability in the injected dose.

Lastly, [27] the use of crystallised insulin (NPH, Zinc) requires adequate shaking to create a suspension before injection. It has been shown that almost one third of the patients did not shake the vials adequately, giving rise to a variation of around 20% of the concentration of insulin in the vial. The patients who shook their vials the least were those who described the greatest hypoglycaemia when changing the cartridge, whereas those who carefully followed the suspension technique were those with the least hypoglycaemia [28]. Lepore compared the pharmacokinetic and pharmacodynamic profiles following subcutaneous injection of NPH insulin using a pen in three experimental conditions: adequate insulin resuspension, absence of resuspension with the pen stored vertically with the needle upwards or downwards [28]. Depending on these different experimental conditions, a 100% variation in all the parameters was observed, in particular a duration of the action of insulin of around 15 hours in optimal conditions of resuspension, 10 hours with the needle upwards and 20 hours with the needle downwards. This gives an indication of the possible variation in the practices of resuspension of NPH, and may account for some of the difficulty in obtaining reproducible results from one day to the next. The availability of slow-acting insulin (glargine, detemir) in a clear solution in the vial would eliminate some of these pitfalls in injection technique.

Glycemic predictability appears to hinge largely on absorption in the subcutaneous cell tissue. It has been demonstrated that there is an inverse relationship between the speed of elimination of insulin in subcutaneous tissue and the area under the curve of the progression of glycemia [26]. The variability in diffusion of insulin is therefore a source of variation in glycemic predictability. The improvement in insulin preparations should improve predictability by the reduced tendency of the rapid insulins to form hexamers. For the slow insulin, the reduction in the subcutaneous cell tissue factors, or with detemir, their neutralisation will enhance predictability. However, despite these physicochemical improvements, the injection technique is still important. It has a crucial role and requires better diabetological education along with improvement in compliance.

Other local factors are involved, subcutaneous blood flow in particular. Increase in blood flow, recruitment of capillaries or increase in the exchange surface will result in a shorter distance for the insulin to cover before reaching the blood stream. The dependence of insulin kinetics on room temperature, physical exercise and the use of vasodilating or vasoconstricting drugs can be accounted for in terms of alterations in blood flow [29].

**Intrinsic factors affecting variability**

Although many factors modulate the action of insulin and in turn insulin-sensitivity and insulin-resistance, some do not have any day-to-day influence and only contribute to a slow progressive modification in kinetics. For example, progressive renal failure reduces the metabolic clearance of insulin and also increases insulin-resistance [30]. Although this type of factor alters insulin kinetics, it does not amplify the intra-individual coefficient of variability from one day to the next.

By contrast, the hypo- and hyper-glycaemic effect is far more important. Indeed, hypoglycaemia triggers contra-regulation mechanisms that give rise to insulin-resistance, and the same quantity of insulin, or a smaller amount of insulin injected after hypoglycaemia, will always have a smaller effect than that predicted from effects on the preceding days, due to the transitory insulin-resistance induced by regulatory hormones. These are fine-tuned physiological mechanisms that no pharmacological property of insulin and no injection technique can effectively modify.

Lastly, the variation in tissue sensitivity to insulin induced by external phenomena (physical activity, for example) may modify the glycemic effect of insulin from one day to the next.

**Clinical consequences of the variability in action of insulin**

The variability in glycemic response to the insulin injection and the non-predictability from one day to the next may impact patients’ quality of life and their compliance to treatment, but it will also influence the progression of the diabetes itself and its complications.

Data are contradictory concerning the progression of diabetes and its complications. It has been claimed that the level of glycosylated haemoglobin is not itself affected by glycemic variability [31]. However, in the DCCT study, analysis of the glycosylated haemoglobin levels, not only at baseline but also during follow-up of the patients, could not account for all the differences in terms of microangiopathic complications. Although glycohaemoglobin levels are an index of the duration of exposure to chronic hyperglycaemia, in the DCCT study only 18.8% of the variance in glycemic variability [31]. However, in the DCCT study, analysis of the glycosylated haemoglobin levels, not only at baseline but also during follow-up of the patients, could not account for all the differences in terms of microangiopathic complications. Although glycohaemoglobin level itself [32]. Other factors need to be taken into consideration, such as individual sensitivity to
the genus inherent in the progression of complications, but also probably to the glycemic variability. This variability generates both hypo- and hyperglycaemia, the one compensating the other in terms of glycohaemoglobin. However, studies conducted on type 2 diabetic patients are now showing that at constant glycosylated haemoglobin, large post-prandial glycemic excursions are risk factors of degenerative complications. It is therefore likely that the glycemic variability induced by the low predictability and resorption of insulin participates in the progressive genus of degenerative complications.

Nevertheless, the greatest element of variability in insulin resorption appears to be the patients’ quality of life and compliance to treatment. Clinical practice reveals the dismay of the patients who cannot understand why their blood glucose levels vary so much despite adherence to the recommendations of their diabetologist (dietary rules, time schedule, regular physical activity). The explanation and response to these questions stem from the variability and predictability of the insulin effect. The physician’s response is often vague and leaves patients perplexed, resulting either in anxiety because they may have misunderstood or not done the right thing, or conversely, they may even abandon the basic principles of diabetology. To obtain the best possible compliance to treatment, patients need reproducible devices. The advent of slow and fast-acting analogues appears to represent a step in this direction.

Conclusion

The variability in the effect of insulin, a rather intuitive notion, can be analysed in terms of certain, more or less controllable factors. It is important to try and discriminate factors relative to the insulin itself from other factors in order to reduce variability over time and hence improve predictability. This in turn should improve the patients’ compliance with treatment. The choice of the insulin itself and the constant advances in insulin treatments should help reduce the lag time for subcutaneous diffusion, or even, with some analogues, avoid this problem altogether.

Nevertheless, proper use of the devices available to the patient is crucial: needle length adapted to the thickness of the subcutaneous skin, adequate resuspension of the insulin, allowing sufficient time before withdrawing the needle, and the use of the same anatomical site for the same time schedule are all important elements that will reduce glycemic instability. Likewise, the use of other devices, such as the portable insulin pump with fast-acting insulin (reduced deposit of exclusively rapid insulin, single injection site, calibrated depth of injection) or the intra-peritoneal pump (more physiological absorption route), will further reduce the variability in insulin diffusion.

In summary, optimal metabolic balance is the objective for diabetologists and patients, with predictability in the action of the injected insulin the ultimate goal. The results will hinge on the combined efforts of pharmaceutical companies to develop more predictable insulins, and medical and paramedical personnel to educate the patients in the optimal use of the available devices.

References

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