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Insulin absorption: a major factor in apparent insulin resistance and the control of type 2 diabetes mellitus

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Abstract

Our experience over many years from 2 diabetes clinics with large patient populations indicated that, apparently, excessive doses of intermediate-acting insulin preparations (150-300 U of NPH insulin), alone or in combination with rapid-acting insulin, generally did not result in acceptable control of fasting blood glucose. We hypothesized that insulin resistance at the tissue level and the known variability of insulin absorption were not satisfactory explanations. To deal with the ambiguities of available data on insulin absorption, we elected to measure insulin bioavailability via a different approach. Thirteen publications provided plasma insulin concentrations after the subcutaneous administration of defined doses of insulin. These data were then analyzed by noncompartmental analysis and by standard pharmacokinetic methods. Analyses required only knowledge of the areas under the plasma insulin curve and the metabolic clearance rate of insulin. Both of these are parameters measurable with considerable accuracy. Quantitative pharmacokinetic analysis of published insulin absorption curves for insulin administered subcutaneously revealed mean absorption levels for regular and lispro insulin of 70 to 80%, 30% or less for NPH insulin, and 30 to 40% for lente insulin. In conclusion, poor absorption of intermediate-acting insulin preparations, or combinations of intermediate- and rapid-acting insulin preparations, explains the difficulty in lowering blood glucose in patients with type 2 diabetes mellitus who have had long-standing disease, are insulin resistant, and have a flat insulin response to a glucose load.

1. Introduction

This investigation was undertaken based on the hypothesis that the unsatisfactory response of large numbers of patients with advanced type 2 diabetes mellitus to available insulin formulations is the result of poor bioavailability. Type 2 diabetes mellitus progresses over many years from a stage of excessive insulin secretion to one of islet cell insufficiency. In parallel with these events, treatment initially consisting of oral medications then progresses to the use of increasing amounts of insulin, which ultimately often become very large, but nevertheless inadequate. An understanding of the factors involved in this situation is confounded by data indicating that widely used intermediate-acting formulations of insulin are generally well absorbed. Our clinical experience in the treatment of large numbers of patients with advanced type 2 diabetes mellitus is not compatible with this information.

The patient population of the Texas Diabetes Institute and its predecessor, the Diabetes Clinic of the University of Texas Health Science Center at San Antonio, consists largely of overweight Hispanic individuals with a history of disease of 15 years or longer, with average fasting blood glucose of more than 200 mg/dL and glycosylated hemoglobin of 10% or higher. Such patients generally have end-stage islet cell function and a flat insulin response to a glucose load [1]. Conversely, the intravenous administration of 5 U/h of regular insulin to a group of these patients consistently produced a fall in blood glucose at a rate of 50 mg/dL per hour (SJ Friedberg, unpublished observations, 1983). This difference in the effect of subcutaneous vs intravenous insulin has been frequently noted [2-7]. Therefore, it was felt that insulin resistance alone, or the

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known large variability in the effect of subcutaneously administered insulin, might not entirely explain this conundrum. Although there is little doubt that some of these patients include individuals who have had increasing insulin resistance, have gained weight, or are noncompliant, these factors cannot explain our overall experience.

In contrast to our experience, many studies [8-10], notably the UK Prospective Diabetes Study, which enrolled patients with newly diagnosed disease, report good results in many patients with 30 to 40 U of insulin. The total insulin requirement of a patient with type 2 diabetes mellitus with insulin resistance is about 120 to 160 U/d [11,12]. If residual insulin capacity is severely impaired, this amount of insulin must be effectively supplied exogenously. Conversely, if considerable endogenous secretion of insulin remains, the putative effect of poor insulin bioavailability may not be apparent.

The Physicians' Desk Reference (PDR) on page 1852 of the 2005 edition indicates that the absolute bioavailability of lispro and regular insulin is 57% to 75% for both, but no information on the bioavailability of NPH, or of its various formulations, or of lente is provided, nor was this forthcoming when 2 major manufacturers were contacted. With the exception of one publication [13], bioavailability information, as opposed to time-action course, could not be found in 2 major texts on diabetes, 3 on internal medicine, 2 on endocrinology, nor in a well-known textbook of pharmacology.

The subject of insulin pharmacokinetics has been extensively reviewed by Binder et al [14]. This review indicates that many studies show excellent absorption of insulin. However, these studies have been frequently carried out by means of external counting of radioiodinated insulin to measure the amount remaining at the injection site. Additional studies in sheep and pigs suggest that only a small percentage of the insulin is subject to local degradation. The reliability of using iodinated insulin to measure local clearance and time course of blood glucose and plasma insulin concentrations seemed to be indicated by strong covariance between the rate of disappearance of radioactivity, changes in plasma immunoreactive insulin, and blood glucose [14]. However, Berger et al noted that at a time when Actrapid insulin (Novo Nordisk, Princeton, NJ) reaches its maximum hypoglycemic effect, only one fifth to one quarter of the injected radioiodinated insulin had been cleared from the subcutaneous depot [15]. Using tritiated rather than radioiodinated insulin, it was shown that, contrary to previously accepted views, a considerable amount of insulin is degraded at the injection site [15-17]. Studies of insulin degradation in subcutaneous tissue in vitro on both control subjects and in subjects suspected of having very large subcutaneous destruction of insulin indicated similar rates of degradation of about 2% per milligram of tissue per minute [18]. Measured in hours, this rate would amount to considerable destruction. Nevertheless, studies showing extensive local degradation of insulin have been challenged [19]. It is also universally recognized that approximately 35% to 50% variation in insulin absorption exists within the same individual and between individuals [20,21]. It seems to us that this variability is not compatible with the conclusions indicating excellent absorption. This issue of the great inter- and intrasubject variability of absorption and action of subcutaneously administered insulin was recently discussed by Heinemann [20,22], but data on quantitative absorption kinetics are not specifically provided by this author.

Because of the controversial issues discussed above, we have elected to reevaluate the problem of insulin bioavailability in humans by a different method, one which is independent of any considerations or methodology discussed above and which appears to have been overlooked. We have analyzed insulin bioavailability by means of noncompartmental analysis and standard pharmacokinetics using 2 parameters, which can be measured with great accuracy and do not depend on any knowledge of the magnitude of the insulin space, the rate constant for the irreversible loss of insulin from the insulin space, or on the use of radioactive insulin preparations [22-25]. These 2 parameters are the area under the insulin absorption curve (AUC) after subcutaneous injection and the metabolic clearance rate (MCR) of insulin.

2. Methods

The fraction of insulin administered subcutaneously that reaches the insulin space can be determined by the following equation:

$$F = AUC \times MCR/D$$

where F is the fraction absorbed, AUC the area under the plasma insulin curve after the subcutaneous administration of insulin, MCR the metabolic clearance rate of insulin, and D the dose of insulin administered subcutaneously.

The derivation [22-25] is as follows:

$$df = k\ cV\ dt,$$

where f is the total insulin flux over time t, k the rate constant for the irreversible loss of insulin from the total insulin space, c the insulin concentration, and V the insulin space expressed as a volume.

The total insulin flux over time t from 0 to ∞ then is:

$$f = k\ V\int c\ dt,$$

Then, $\int c\ dt$ is the area under the insulin concentration curve (AUC) over time t, expressed as μU $\times$ minutes per mL. The equation may also be expressed as

$$I = AUC \times kV$$

where I is the total amount of insulin absorbed and AUC (in μU/mL $\times$ minutes) is the area under the insulin

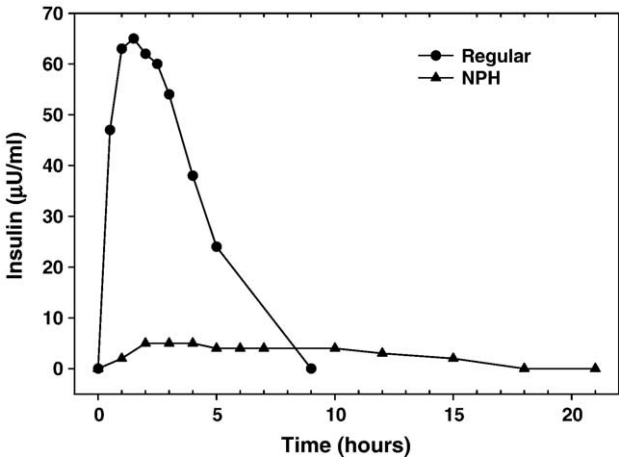

Fig. 1. Blood insulin concentration after the subcutaneous injection of regular and NPH insulin. Circles indicate insulin concentration after injection of 19.5 U of regular insulin [13]; triangles, insulin concentration after the subcutaneous injection of 25 U of NPH insulin [30].

absorption curve after subcutaneous administration of insulin. Because kV is equal to the metabolic clearance rate,

$$I = AUC \times MCR$$

This equation bypasses the need to know anything about the size of the insulin space or the rate constant for the irreversible loss of insulin from the insulin space.

The equality between kV and MCR derives from the following: if insulin is infused to equilibrium at a constant rate, a, the total amount of insulin in the insulin space, Vc, is equal to a/k [22,23]. However, the MCR is equal to a/c. Because both equations contain the quantity a, MCR is equal to kV.

For our calculations, a mean value for the metabolic clearance of insulin of 10.2 mL per kilogram of body weight was obtained from data provided by Sherwin et al [26] and is in close agreement with calculations that we have made from data provided by Prager et al [27].

Insulin time-course absorption curves provided by 13 publications [13,19,28-37] obtained after subcutaneous injection of various insulin preparations from groups of individuals were reviewed and their respective AUC values determined by the trapezoidal rule. The average number of subjects in each study was 17 for a total of 221 subjects. For the purpose of our calculations, the relatively large number of subjects studied by each author should reduce the problem of variation between individuals and provide mean bioavailability data. The AUC values were then used to calculate the respective total insulin flux as described above. The total insulin flux was then compared with the dose administered. With one exception [13], none of these publications provided bioavailability data for intermediate-acting insulin.

In addition to the foregoing, it was of interest to obtain a value for k, the rate constant for the disappearance of insulin from the insulin space. This value was calculated from data obtained from Sherwin et al [26] from compartmental analysis of their model A. Analysis of this model by the authors indicates that the constant infusion of 10.2 mU of insulin per minute resulted in a total insulin compartment of 142.6 mU of insulin at equilibrium. The rate constant, k, is obtained by dividing the rate of infusion by the total amount of insulin in the insulin compartment at equilibrium [22,24]. Using these parameters, the rate constant obtained was on the order of 0.064 to 0.0715 per minute. The half-life of insulin in the insulin space was found to be 9.7 minutes

Table 1
Percentage of absorption of insulin formulations calculated by stochastic analysis from published plasma insulin concentration curves after subcutaneous injection

Insulin type/mixture	% Absorbed	Subcutaneous dose (U)	Reference[a]
NPH	12.5	25	Woodworth et al [28] (6)
NPH/regular 50/50	52	23.4	Woodworth et al [29] (18)
NPH/regular 70/30	36	23.4	Woodworth et al [29]
Lispro	77.3	21	Heise et al [30] (30)
Lispro/NPL 75/25	59.4	21	Heise et al [30]
Lispro/NPL 50/50	60.5	21	Heise et al [30]
Lispro /NPL 25/75	45.0	21	Heise et al [30]
NPL	36.3	21	Heise et al [30]
Glargine	40	23	Lepore et al [31] (20)
NPH	25	23.4	Lepore et al [31]
NPH	13.4	18.4	Thow et al [32] (8)
NPH 10 h	13.2	19.2	Galloway et al [13] (45)
Lente 10 h	40.0	19.5	Galloway et al [13]
Regular 10 h	65.2	19.5	Galloway et al [13]
Lispro/ NPH mixed	28.8	23.4	Joseph et al [33] (12)
NPH	34.0	23.4	Kølendorf et al [34] (10)
Novolog mix 70/30	27.4	23.4	PDR [35] (24)
Novolog	67.0	23.4	PDR [35]
Novolog isophane	10.3[b]	16.4	PDR [35]
NPH	26.7	25 and 67	Lauritzen et al [19] (8)
NPH /regular 69/23	25%[c]	100 (±24)	Henry et al [9] (14)
NPH	24.5	38.3	Danne et al [36] (11)
NPH	21	29.4	Heinemann et al [37] (15)

Data for glargine insulin were obtained by extrapolation of 24-hour data to zero at 41 hours [27].

[a] Numbers in parentheses indicate number of subjects used in the study for the reference provided.

[b] Obtained by subtracting Novolog component from Novolog mix 70/30 absorption.

[c] Obtained by calculating increase to AUC attributable to exogenous insulin, as described in the text.

(half-life = ln 0.5/−k). The magnitude of the insulin space was approximated from the following relationship: $Vk = a/c$ (where a is the rate of insulin infusion and the MCR is a/c), or approximately 141 to 159 mL/kg body weight. The values for k and for the metabolic clearance rate are the same for normal individuals, obese individuals, and in obese and nonobese patients with type 2 diabetes mellitus. These data were calculated from levels of insulin achieved by infusion of insulin at a constant rate [27,38,39]. In particular, we refer to Figs. 25 and 26 of reference [39].

3. Results

Quantification by kinetic analysis of the amount of insulin transiting the insulin space after subcutaneous injection of various insulin preparations shows grossly defective absorption of intermediate-acting insulin and poor absorption of commonly used mixtures of intermediate- and rapid-acting insulin. Fig. 1 illustrates an example of the difference between the absorption of regular and NPH insulin. In Table 1, our calculated values for total insulin flux of commonly used insulin preparations are presented as a percentage of the administered dose. The results show that the absorption for NPH insulin obtained from the mean from 9 investigators is about 20%. Forty percent of lente insulin is absorbed over a 10-hour period. In agreement with information published in the PDR, regular and lispro insulin are about 70% to 80% absorbed. Percentage of absorption for a number of mixtures of intermediate- and rapid-acting insulin is also given in the table. The percentage of absorption increases in proportion to the amount of rapid-acting insulin present in various mixtures. Galloway et al [13], in a related study, determined the extent of insulin absorption by comparing AUCs after intravenous injection of regular insulin with those obtained after the subcutaneous injection of various insulin formulations and their mixtures. With our method of calculation and using AUCs obtained from their study [13] we obtained comparable values for percentage of insulin absorbed (Table 2).

Although Lauritzen et al [19] in a refutation of the data of Berger et al [15] indicate good absorption of NPH measured by means of external counting of radioiodinated insulin and measurement of absorbed insulin by radio immunoassay, our calculations indicate only 26.7% bioavailability. We also refer to the results from reference [35] which indicate, by inspection, that progressive addition of Novolog isophane insulin to Novolog results in a progressive reduction in AUC.

Table 2
Comparison of fraction of absorbed insulin obtained by Galloway et al [13] with data obtained by our calculations using their areas under the insulin curve

Insulin type	Area under curve from reference [33] (μU × h)	Fraction absorbed according to reference [35]	Fraction absorbed by present calculations
Lente	46	0.09	0.14
Lente	87	0.20	0.26
Isophane	74	0.19	0.22
Isophane	89	0.30	0.27

4. Discussion

We have analyzed published data from 13 articles to provide quantitative calculations derived by noncompartmental analysis and standard pharmacokinetics that indicate serious limitations in the use of intermediate-acting insulin formulations for the treatment of patients with insulin-resistant type 2 diabetes mellitus. These limitations are the result of variable and marginally adequate average absorption. The problem of poor absorption is most apparent in patients who have seriously impaired endogenous insulin secretion as indicated by fasting plasma glucose levels of more than 11 mmol/L (200 mg/dL).

Three lines of evidence validate our conclusions. First, our results for lispro and regular insulin agree with those provided by the 2005 PDR. Second, our results agree with those of Galloway et al [13] who used a different method. Third, examining Fig. 1 and assuming that regular insulin is 70% to 80% absorbed, comparison of the respective AUCs for regular vs NPH insulin indicates about 30% absorption for NPH insulin.

If the insulin requirement of a normal individual is about 40 U/d and that of an insulin-resistant individual unable to secrete endogenous insulin is 120 to 160 U/d [11,12], and NPH insulin is approximately 25% absorbed (Table 1), the administration of 200 U would result in the biologic availability of only 50 U. Therefore, the actual exogenous insulin requirement needed to provide 160 U of bioavailable insulin would be more than 400 U of intermediate-acting insulin per day. Similarly, the administration of 100 U of a 75/25 mixture of NPH and regular insulin can be calculated to provide only about 40 U of bioavailable insulin.

These conclusions are further supported by the observations of Cusi et al [40] who noted that the average intermediate-acting bedtime postabsorptive dose required to normalize fasting plasma glucose by the following morning was about 65 U. This amount of insulin is far greater than the amount needed to normalize fasting plasma glucose by intravenous administration. As calculated from the data of Eaton et al [41], basal prehepatic insulin secretion during an 8-hour overnight fast would be 7.2 U/70 kg man. Assuming a 3-fold increase in insulin requirement in an insulin-resistant individual, the basal requirement in such an individual would be 21.6 U over this period. This amount is considerably less than the amount of subcutaneously administered insulin required to achieve overnight normalization of fasting blood glucose as reported by Cusi et al [40], suggesting that only a portion of the subcutaneously administered insulin was absorbed. Twenty-one units of insulin secreted endogenously over an 8-hour overnight period is the equivalent of 2.7 U/h given intravenously. According to information relating to the

efficacy of intravenous insulin, this amount might well be expected to normalize the fasting plasma glucose over an 8-hour period in the fasting state.

Regarding the calculations we have made, it would be useful to know to what extent exogenous insulin reduces endogenous production. Available literature indicates no reduction [42], partial reduction [43,44], or in the case of insulin levels resulting in hypoglycemia, near complete reduction [45]. However, in the presence of type 2 diabetes mellitus, hyperglycemia may continue to provide stimulation of available or residual endogenous insulin secretory capacity. In any case, the data that have been provided here are based on insulin AUCs, which exclude baseline levels and represent the physiologically useful increment above baseline following exogenous insulin administration.

The analyses presented here are relevant not only from a theoretical standpoint, but also from a practical standpoint. Because of the fear of inducing hypoglycemia, it is common practice to begin the insulin treatment of patients with type 2 diabetes mellitus with about 30 to 40 U of intermediate-acting insulin [8-10]. If the ultimate insulin requirement is more than 300 U, numerous clinic visits are generally needed to achieve the necessary upward titration. The result is that the required insulin level is seldom reached and 200 U of NPH insulin (2 full 1-mL syringes twice daily) seems to represent a psychologic barrier for patient and physician alike.

In recent years, the use of U500 insulin (500 U/mL human regular insulin) has been examined as a solution to the problem of supplying patients with adequate insulin [46]. Anecdotal information from the Texas Diabetes Institute suggests that U500 insulin has a time-course action similar to that of NPH. In agreement with Cusi et al [40], we also note that normalization of fasting morning glucose concentration results in significantly better control of glucose levels throughout the day [47]. It is also relevant that Garvey et al [48] noted that 150 U of regular insulin administered by continuous subcutaneous infusion was necessary to normalize fasting plasma glucose in type 2 diabetic patients. This amount would be expected in an insulin-resistant individual with seriously impaired insulin secretion, data that agree with our conclusions.

Although the results of Garvey et al are consistent with our conclusions, they are not in agreement with those of others [8-10]. For example, Henry et al [9] reported that treatment of 14 patients with type 2 diabetes mellitus by means of 86 to 100 U of a 75/25 mixture of NPH and regular insulin per day resulted in a reduction of hemoglobin A_{1c} from 7.7% to 5.1% over a period of 3 months. Hemoglobin A_{1c} of 7% is equivalent to a mean glucose over time of 9.2 mmol/L (166 mg/dL) [49]. When the data from their Fig. 3B were analyzed as described above, it became apparent that most of the insulin transiting the insulin space in these patients was endogenous and that the endogenous secretion improved greatly after treatment. Using the analytical methods described above and correcting for hepatic glucose extraction of 40%, we have calculated that endogenous insulin production in these patients increased from a baseline of 103 to 164 U/d and that only about 25% of exogenous insulin was absorbed. The superior results obtained by Henry et al [9] are attributable to improved endogenous insulin secretion, especially in the evening, resulting from exogenous insulin administration and endogenous insulin secretion late in the day, possibly because of a decrease in glucose toxicity, careful monitoring and support, or because of other unknown factors. Thus, patients with residual beta-cell function may increase endogenous insulin production with intensive insulin therapy. Reversal of "glucose toxicity" after a 3-week continuous subcutaneous insulin infusion led to a 60% increase in 24-hour insulin secretion [48]. It is no surprise that similar results using similar treatment methods were not obtainable in patients whose disease has lasted 15 years or more. Patients with type 2 diabetes mellitus who have had their disease for many years, with fasting plasma glucose of more than 11 mmol/L (200 mg/dL), generally have flat insulin responses in a glucose tolerance test and secrete little if any insulin even when baseline insulin levels are elevated [1]. Patients who lack the ability to mount a significant insulin response to a glucose load may be completely dependent on exogenous insulin. Inadequate absorption must be taken into account when attempts to supply sufficient insulin to overcome insulin resistance are carried out.

The problems of treating type 2 diabetes mellitus with insulin vs type 1 diabetes mellitus differ in significant ways. In type 1, the total insulin requirement usually averages 25 to 60 U/d, as opposed to 3 or 4 times as much in patients with insulin-resistant type 2 diabetes mellitus who have end-stage islet cell function. These requirements in patients with type 1 diabetes mellitus can be met by manageable quantities of insulin even in the face of impaired absorption. A greater proportion of better absorbed regular insulin is generally used in type 1 diabetes mellitus, whereas patients with type 2 diabetes mellitus are usually treated with larger amounts of poorly absorbed intermediate-acting insulin. In the treatment of brittle type 1 diabetes mellitus, the variability of absorption rather than the total amount absorbed produces one of the major stumbling blocks, so that constant juggling of insulin regimens is often necessary to achieve some sort of balance between hyper and hypoglycemia. It has been shown that insulin absorption capacity decreases with age [36]. This problem may be related to increasing amounts of subcutaneous fat. The population of patients with type 1 diabetes mellitus on average is younger and thinner than patients with type 2 diabetes mellitus.

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