How to Accurately Establish Pharmacokinetics/ Pharmacodynamics of Long-Acting Insulins in Humans: Relevance to Biosimilar Insulins

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In this issue of *Diabetes Care*, Linnebjerg et al. (1) compare the pharmacokinetics (PK) and pharmacodynamics (PD) of a Lilly synthetized insulin glargine (LY IGlar) with the insulin glargine Lantus (IGlar) based on a series of clamp studies in healthy volunteers and conclude for PK/PD that there is similarity of the LY IGlar vs. IGlar. The strengths of the study should be discussed along with its limitations.

The strengths of the study are several. First, there are consistent rate ratios of geometric means of the several PK/PD parameters of LY IGlar and IGlar examined, which are all close to 1.0, with the 90% CIs contained in the prespecified acceptance limits of 0.80–1.25. Second, there is an elevated number of subjects studied with the technique of euglycemic clamp (N = 211) and the study design (randomized, double-blind, two-treatment, four periods, crossover) is strong. Third, the comparison between LY IGlar and IGlar is repeated twice (one time vs. European Union [EU]-approved IGlar, the other time vs. US-approved IGlar), and there is the unique comparison between EU-approved IGlar and US-approved IGlar. Of note, the authors not only demonstrate PK/PD similarity between LY IGlar and IGlar but also originally prove the intraindividual similarity (reproducibility) of different batches of IGlar (EU- and US-approved IGlar both originate from the same manufacturer, Sanofi in Frankfurt, Germany).

The limitations of the study by Linnebjerg et al. (1) derive from study design and population studied. The conclusions are correct in the specific conditions examined in this study, i.e., normal volunteers without diabetes, a single insulin injection, a dose of 0.5 units/kg (supra-therapeutic, at least for subjects with type 1 diabetes [T1D]), and an insulin dose given in the morning. It is our opinion that one should be cautious regarding the results when extrapolating to the general population of "users" of basal insulin, i.e., subjects with T1D or type 2 diabetes (T2D) who inject basal insulin every day (at steady state), usually at lower doses and nearly always in the evening.

The study by Linnebjerg et al. (1) opens a number of interesting and relevant questions. How can we best establish PK/PD of long-acting insulins, which today have duration of action beyond 24 h? Which is the correct methodology of the glucose clamp technique and how should it be used? In which category subjects should the clamp studies be done? The premise is that the clamp studies should be designed and executed not only to fulfill the requests of the regulatory agencies (2,3) but also to provide meaningful and objective evidence for doctors and patients on how the new candidate basal insulins work in clinical practice as compared with standard treatment. To be as meaningful as possible, the comparison should be done in experimental conditions as close to the real life of persons with diabetes.

Establishing PK of Long-Acting Insulins

In the physiology of glucose homeostasis, plasma insulin concentrations over time (PK) closely predict insulin action, i.e., PD (4). An example of this is the PK of NPH and glargine after subcutaneous injection of same dose (5). In turn, PD closely predicts the clinical outcomes and the advantages of glargine as compared with NPH insulin (6,7). However, this is true only if plasma insulin concentration derives exclusively from absorption of the subcutaneously injected insulin, a condition occurring in subjects with T1D with absent endogenous insulin secretion (5). In contrast, in normal volunteers (1) or subjects with T2D (8), the endogenous insulin secretion largely contributes to plasma insulin concentration in addition to the subcutaneous absorption of the injected insulin, therefore interfering with PK and PD. Previous attempts to suppress the confounder endogenous insulin secretion (by infusing somatostatin or exogenous insulin or by clamping at a target blood glucose concentration lower than fasting levels) have not successfully addressed the issue. Even the attempt of Linnebjerg et al. (1) to subtract the calculated endogenous insulin based on mathematically

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modeled C-peptide from the total plasma insulin does not completely eliminate the interference by endogenous insulin on PK and/or PD. In fact, the PK reported by Linnebjerg et al. (1) with a peak of glargine in plasma at ~12 h differs from those observed in subjects with T1D where the increase occurs approximately between 3 and 6 h after first injection (5) and at steady state (9). Therefore, subjects with T1D, not healthy volunteers, are generally considered more suitable to determine the time-action profile of long-acting insulins (2). However, if Linnebjerg et al. had measured the main metabolite of glargine after the subcutaneous injection, M1 (21A-Gly-insulin), by the liquid chromatography–tandem mass spectrometry system (no cross-reactivity to human or analog insulin) (10–12), then they might have produced accurate and reliable glargine PK data that was meaningful to T1D patients, even in a study in normal subjects. Parenthetically, such an approach would have also resulted in an additional value of the study, i.e., to prove similarity between LY IGlar and IGlar in terms of metabolism of LY IGlar as compared with IGlar after subcutaneous injection.

PK is not so useful when the injected long-acting insulin is acylated. Acylated insulins circulate largely bound to albumin, and their concentrations cannot be determined in plasma with the current methods as "free insulin" but only as total insulin (bound + free), which does not predict metabolic activity (9,13).

Establishing PD of Long-Acting Insulins

Establishing PD requires performing and interpreting experiments using the glucose clamp technique. In the pioneering view of Reubin Andres (14,15), who initiated the euglycemic clamp with intravenous insulin more than 50 years ago, the dynamics of the rate of glucose infusion to maintain euglycemia reflect the glucose-lowering effects of infused insulin, thus providing an accurate estimate of insulin sensitivity. Subsequently, the "hyperglycemic" (to study pancreatic islet cell function) and "hypoglycemic" (to study glucose counterregulation) variants of the "euglycemic" clamp with intravenous insulin were also introduced, both with automated (16) and manual (17) techniques. At that time, the PD of subcutaneously injected insulin was still derived from absorption kinetics of radiolabeled insulin injected subcutaneously (18). After 1990, the principle of the glucose clamp was adopted to establish the PD of subcutaneously injected insulin, initially for rapid-acting insulins and later for long-acting insulins. As the glucose clamp was, and is, "a physiological principle" rather than a univocal, universally accepted methodology, different researchers have done different studies varying insulin doses, type of subjects studied, study designs, and clamp methodology (automated vs. manual) at their own discretion. It is no surprise that conflicting and controversial results have been produced (6,7). However, in research absolute contradictions do not exist, the discrepancies being explained by different premises in different studies. Establishing PD of a subcutaneously injected long-acting insulin requires assessing its onset of action, duration of action, peak (if any), and total metabolic activity (quantified by the total amount of glucose infused to maintain euglycemia).

The choice of the subjects to be studied is critical. To reliably assess PD, subjects with T1D and virtually undetectable endogenous insulin secretion should be studied. In this population, NPH injected subcutaneously wanes considerably earlier than glargine after subcutaneous injection, and consequently plasma glucose increases earlier to greater values (5). In contrast, in normal volunteers, despite infusion of either somatostatin (19) or intravenous insulin (20) to suppress endogenous insulin secretion, the PK/PD of NPH and glargine appear superimposable, especially in the last 12 h of a 24-h study, and euglycemia is observed (Fig. 1). This paradoxical result is driven by the confounder endogenous insulin secretion, which clearly is not corrected by somatostatin or exogenous insulin infusion (19,20). In normal volunteers, this also occurs when a glargine insulin synthetized in China is compared with NPH (21). The confounder endogenous insulin secretion may be so important that the three basal insulins, NPH, glargine, and detemir, may all appear similar when each one is examined against the two others in normal subjects (22). Taken together, these results suggest that endogenous insulin secretion of normal volunteers might mask potentially existing PK/PD differences between LY IGlar and IGlar. Clearly, ad hoc studies in T1D subjects are needed to finally answer the question. As expected, the PK/PD of LY IGlar and IGlar in T1D (23) differ from those found in normal volunteers in the study by Linnebjerg et al. (1).

The dose of insulin, the condition of steady state, and time of insulin dosing are also critical points. How PD of a subcutaneously injected insulin performs at doses higher than those needed in real life of people may be interesting from pharmacological point of view in the study by Linnebjerg et al. (1), in which 0.5 units/kg glargine was given. However, this is not so useful for people with diabetes, at least those with T1D who generally need <0.3 units/kg/day basal insulin. The dose of basal insulin to be studied in a clamp should be as close as possible to that used by the subjects with T1D and T2D in their real life. T1D and T2D subjects should be studied after several days of use of long-acting insulin, not after the first dose, as was done in the study by Linnebjerg et al. (1), which does not result in steady-state conditions (24) and may lead to incorrect interpretations (25). Therefore, single-dose studies or studies that do not establish steady state are not appropriate for evaluating long-acting insulins (2). In addition, subjects should be studied with insulin dosing in the evening, as this is the most popular time of basal injection among T1D and T2D subjects. However, if a morning dosing is studied, then the results should be interpreted considering that the morning dosing may generate PD different from evening dosing (26).

Last, but not least (and this comment does not apply to the study of Linnebjerg et al. [1] who studied normal volunteers), the metabolic condition of the subjects with diabetes (both T1D and T2D) in the 12 h prior to the glucose clamp is critical. The principle of the euglycemic clamp of Reubin Andres predicates that the dynamics of the glucose infused "in study" are a specific mirror of the activity of insulin infused (or injected) after or at "zero" time. However, sometimes in clamp studies in subjects with T1D or T2D there is a glucose infusion occuring before and at "zero" time, i.e., before the subcutaneous injection of the insulin to be tested. This is likely an artifact due to inappropriately elevated

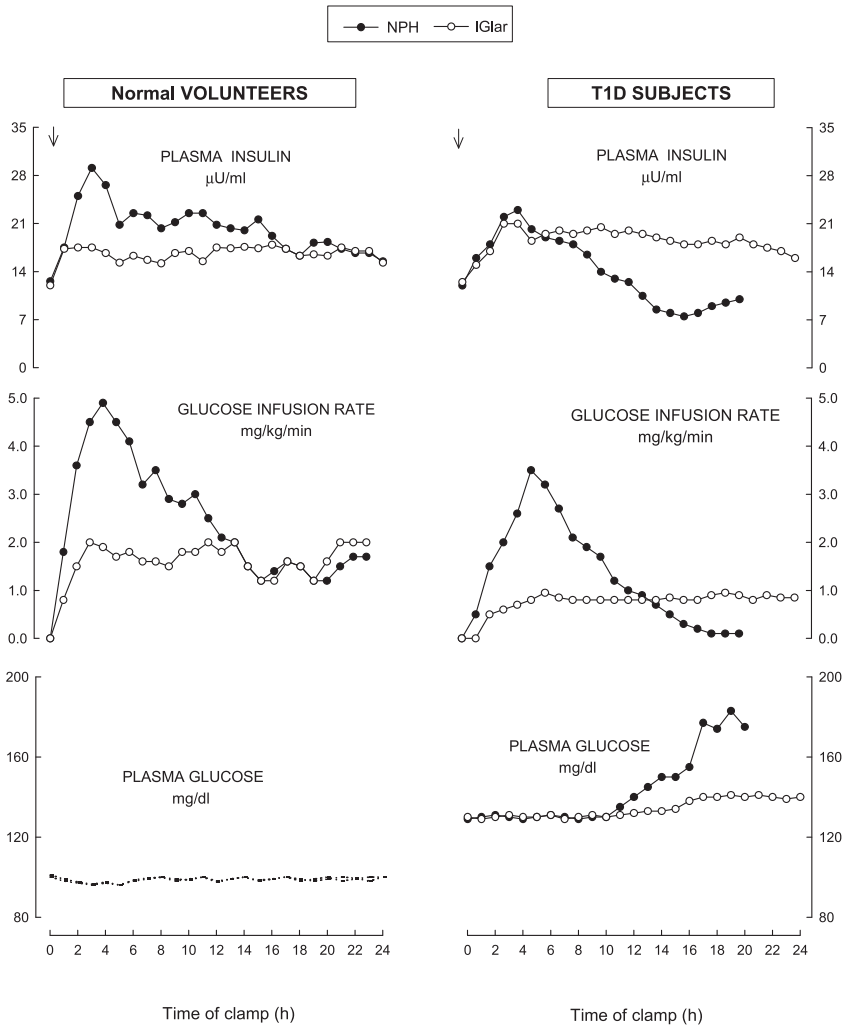

Figure 1—PK and PD of insulin NPH and insulin IGlar in normal volunteers at a dose of 0.4 units/kg (left panel) (20) and in T1D subjects at a dose of 0.3 units/kg (right panel) (5). In both studies, glucose was infused to maintain plasma glucose at the target euglycemia in normal volunteers and at 130 mg/dL in T1D subjects. In the second 12 h of the studies (time 12–24 h) in T1D subjects treated with NPH, plasma insulin concentration fell below that of IGlar and the insulin deficiency resulted in lower glucose infusion rate and earlier and greater hyperglycemia as compared with IGlar (right panel). In contrast, in normal volunteers, in the same time interval of 12–24 h, plasma insulin concentrations, glucose infusion rate, and plasma glucose concentrations with NPH and IGlar did not differ (left panel). Thus, the ongoing endogenous insulin secretion of normal volunteers masked the different PK/PD of NPH and IGlar seen in T1D. Figure made after data were extrapolated from the original figures of Heinemann et al. (20) and Lepore et al. (5).

intravenous insulin infusions in the hours before the clamp to correct hyperglycemia (13,27,28). Such a glucose infusion is carried over for a nonquantifiable number of hours after the subcutaneous injection of insulin, thus confounding the interpretation of the glucose infused in the study.

Conclusions

The similarity between PK/PD of LY IGlar and IGlar reported by Linnebjerg et al. (1) in normal volunteers cannot be immediately extrapolated to the T1D and T2D populations, primarily because of the confounder endogenous insulin secretion in the population studied. Additional studies in T1D and T2D subjects at steady state, at a clinically relevant dose given in the evening, and with assessment of glargine metabolism (plasma M1) (11,12) are needed to convincingly document the similarity of these products in the population that is going to use them under everyday real-life conditions.

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