Pharmacokinetics and Pharmacodynamics of NPH Insulin in Type 1 Diabetes: The Importance of Appropriate Resuspension Before Subcutaneous Injection

Paola Lucidi, Francesca Porcellati, Anna Marinelli Andreoli, Ilaria Carriero, Paola Candeloro, Patrizia Cioli, Geremia B. Bolli, and Carmine G. Fanelli

Diabetes Care 2015;38:2204–2210 | DOI: 10.2337/dc15-0801

OBJECTIVE

Crystalline NPH insulin comes in a two-phase solution with either a solvent or a rapid-acting insulin (in premixed formulations) and needs adequate mixing for complete resuspension before injection. The aim of this study was to establish pharmacokinetics (PK) and pharmacodynamics (PD) after injection of appropriately resuspended versus nonresuspended NPH insulin.

RESEARCH DESIGN AND METHODS

PK and PD were assessed after subcutaneous injection of NPH insulin 0.35 units/kg at steady state by pen either resuspended (R+, tipping of insulin pen 20 times) or nonresuspended (pen maintained in fixed position either horizontally [R- horizontal] or vertically with tip up [R- up] or tip down [R- down]). Eleven subjects with type 1 diabetes (age 31.5 ± 12 years, diabetes duration 17.5 ± 7.7 years, BMI 22.9 ± 1.5 kg/m^2, A1C 7.2 ± 0.4% [55.2 ± 4.4 mmol/mol]) were studied (euglycemic clamp) with a randomized crossover design.

RESULTS

Compared with resuspended NPH insulin (R+), nonresuspended NPH insulin resulted in profound PK/PD differences with either reduced (R- horizontal and R- up) or increased (R- down) plasma insulin concentrations [FIRI_AUC$_{(0–\text{end of study})}$ (free immunoreactive insulin area under the concentration-time curve between 0 and end of study)] and PD activity [glucose infusion rate (GIR)_AUC$_{(0–\text{end of study})}$] (all $P < 0.05$). Duration of NPH insulin action was shorter in R- up (9.4 ± 1.7 h) but longer in R- down (15.4 ± 2.3 h) compared with R+ (11.8 ± 2.6 h) ($P < 0.05$). Within-subject variability (percent coefficient of variation) among studies was as high as 23% for PK [FIRI_AUC$_{(0–\text{end of study})}$] and 62% for PD [GIR_AUC$_{(0–\text{end of study})}$].

CONCLUSIONS

Compared with resuspended NPH insulin, lack of resuspension profoundly alters PK/PD and may importantly contribute to day-to-day glycemic variability of type 1 diabetes.

Department of Medicine, Section of Internal Medicine, Endocrinology and Metabolism, Perugia University School of Medicine, Perugia, Italy

Corresponding author: Geremia B. Bolli, geremia.bolli@unipg.it.

Received 15 April 2015 and accepted 13 August 2015.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc15-0801/-/DC1.

See accompanying articles, pp. 2200, 2211, 2217, 2226, 2234, 2237, 2241, 2250, 2258, 2266, 2274, and 2282.

Because of their more physiological pharmacokinetics/pharmacodynamics (PK/PD) (1) at present, the long-acting insulin analogs glargine and detemir and the more recent basal insulin degludec (2) [along with glargine U300 soon to come (3)] have largely replaced NPH insulin in the treatment of type 1 diabetes mellitus (T1DM). However, NPH remains a popular basal insulin in type 2 diabetes mellitus (T2DM).

The PK/PD of NPH insulin (4) appears to vary considerably from day to day (5–7). The quite large variability might be related primarily to the formulation of NPH insulin per se, the lack of its appropriate resuspension before injection by patients with diabetes, or both. NPH insulin is insoluble, obtained from cocrystallization of insulin with zinc in the presence of the basic polyarginine peptide protamine at isophane ratio and neutral pH. The exact binding mode of the insulin-protamine complex is not known (8). Divalent ions like zinc and other additive ligands like phenolic derivatives and protamine, along with the type and morphology of the precipitate, play a role in the slow release of insulin from the precipitated NPH insulin crystals after subcutaneous injection (9). However, the exact mechanisms of NPH insulin dissociation are not known. Thus, variability of NPH insulin PK/PD in humans might be due to the variable mechanisms of precipitation of crystals in the subcutaneous tissue and/or variable release of insulin from the precipitate.

However, variability of NPH insulin might also be due to a lack of or insufficient resuspension before injection. In fact, NPH insulin is a two-phase solution, and the insulin of the insoluble, cloudy part has to be resuspended in the soluble, clear part by tipping the insulin vial or cartridge pen several times until a homogeneous suspension is obtained. Jehle et al. (10) reported that the majority of patients with diabetes do not optimally resuspend NPH insulin and NPH insulin–based premixed formulations, causing adverse effects on blood glucose control. However, with the exception of a pilot study in which a few subjects were examined in an incomplete crossover design (11), at present, the effects of lack of resuspension of NPH insulin before subcutaneous injection on its PK/PD in T1DM have not been systematically established. The aim of the current study was to assess the differences in PK/PD of a therapeutic dose of NPH insulin injected after appropriate resuspension compared with other occasions without resuspension in subjects with T1DM.

RESEARCH DESIGN AND METHODS

Subjects

After approval by the local ethics committee and written informed consent, 11 subjects with T1DM (Supplementary Table 1) were studied according to Declaration of Helsinki and Good Clinical Practice requirements. Subjects were free of any detectable micro- and macroangiopathic complications and of any major illness other than diabetes as indicated by medical history, physical examination, electrocardiogram, or routine laboratory tests. All were on an intensified basal-bolus insulin regimen (glargine once a day with the evening meal, rapid-acting insulin analog at mealtime).

Study Design

The study was a randomized, open-label, crossover design. During 1 week of run-in, subjects were switched from their basal insulin glargine to NPH insulin (four daily injections: breakfast, lunch, dinner, and bedtime) as previously described (12,13). Prandial insulin was continued. Subjects were subsequently studied on four different occasions at 1–3-week intervals with the euglycemic glucose clamp technique after subcutaneous injection of 0.35 units/kg NPH insulin resuspended (R+) or nonresuspended (R- horizontal, R- up, and R- down) (Protaphane FlexPen, Novo Nordisk, Bagsværd, Denmark). In all studies, the NPH insulin cartridge (pen) was maintained in a refrigerator (10°C) until 8 h before use, when it was moved to room temperature (18–20°C). In R+, the NPH pen was gently tipped 20 times over 1 min and 30 s immediately before subcutaneous injection (2 cm right or left to the umbilicus, skinfold technique). In R- horizontal, R- up, and R- down, NPH insulin was not resuspended before injection. In R- horizontal, the pen was maintained in the horizontal position. In R- up and R- down, the pen was maintained in the vertical position with the tip either up or down, respectively, for 8 h until time of injection (Fig. 1). Subjects were randomly assigned to the treatments using a Latin square, four-way, crossover design.

Clamp Study

The clamp technique has been described in detail elsewhere (4,14). In brief, subjects had their last NPH insulin injection on the evening of the day before study and the last subcutaneous injection of rapid-acting insulin analog at lunch on study day. They were admitted to the Clinical Study Unit of the Department of Medicine, Perugia University School of Medicine, between 1500 and 1600 h on study day and put on bed rest. Two venous lines were started (a superficial vein of one forearm for insulin and glucose infusion and a dorsal vein of the contralateral hand incannulated retrogradely with a G-20 butterfly needle kept at 65°C in a hot box for intermittent sampling of arterialized venous blood) and kept patent with 0.9% NaCl infusion (20 mL/h). A feedback insulin infusion was started to maintain plasma glucose between 90 and 100 mg/dL until the time of subcutaneous NPH insulin injection (2000 h). Thereafter, the rate of intravenous insulin infusion was tapered as soon as plasma glucose decreased by 5 mg/dL below baseline and eventually discontinued. Infusion of glucose was initiated after subcutaneous injection of NPH insulin to maintain plasma glucose at the target of 100 mg/dL (4,14). Studies were planned for 24 h but were ended any time plasma glucose consistently exceeded 150 mg/dL in the absence of glucose infusion.

Analytical Methods

Plasma glucose concentration was measured at the bedside using the YSI 2300 STAT glucose analyzer (YSI Inc., Yellow Springs, OH). Plasma free insulin concentration was measured by radioimmunoassay after polyethylene glycol extraction of antibodies (15) using a commercial kit specific for human insulin (Human Insulin-Specific RIA, DRG Diagnostics GmbH, Marburg, Germany).

Calculations and Statistical Analysis

Onset of action was defined as the time at which infusion of glucose was initiated in response to a decrease in plasma glucose concentration of 5 mg/dL below baseline. End of action was defined as the time at which plasma glucose concentration consistently exceeded 118 mg/dL in the absence of glucose infusion. Duration of action was calculated as the difference between end and onset of action. The end of study was at 24 h

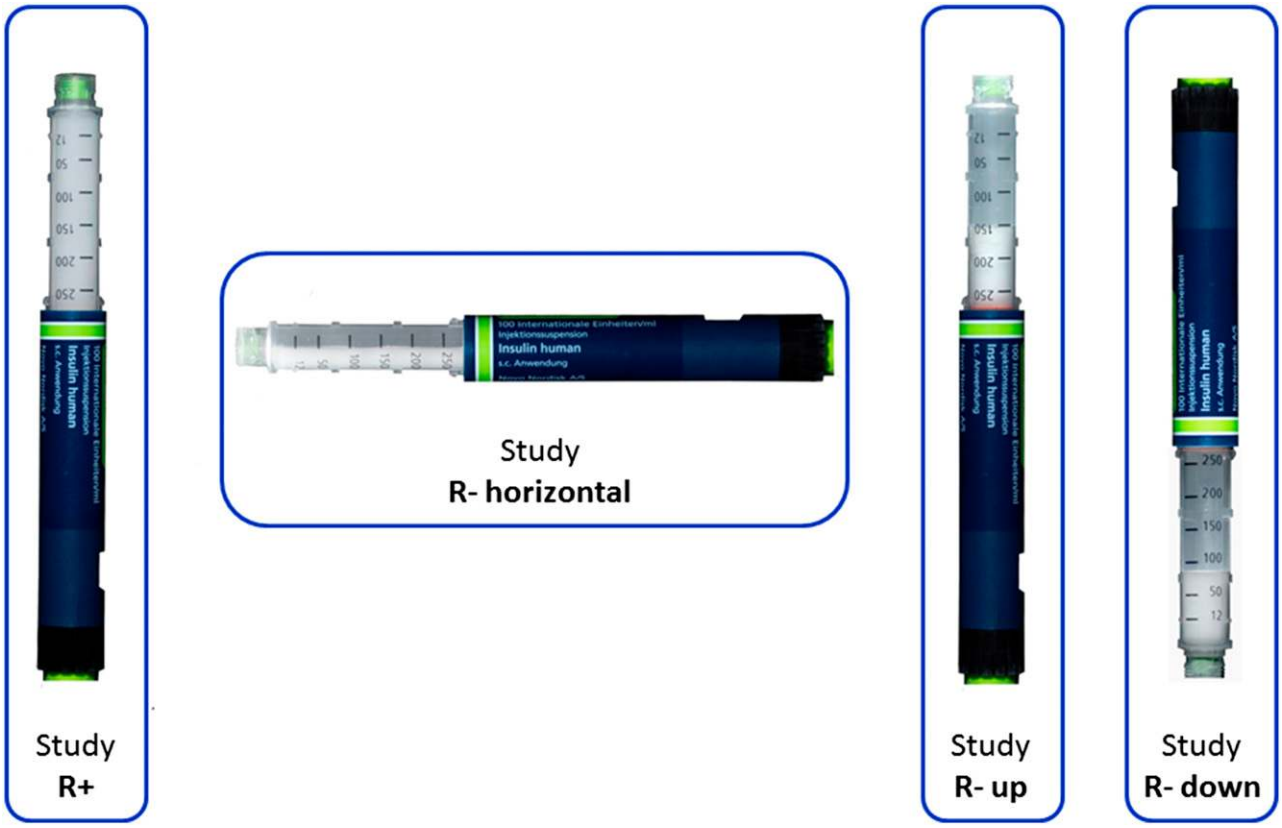

Figure 1—How the NPH insulin pen appears immediately before its use for subcutaneous injection. In the R+ study, NPH insulin was appropriately resuspended before injection. In the R- horizontal, R- up, and R- down studies, NPH insulin was injected without prior resuspension (R- horizontal, pen maintained horizontally; R- up and R- down, pen maintained vertically with R- up tip up and R- down tip down).

or any time plasma glucose consistently exceeded 150 mg/dL in the absence of glucose infusion. The linear trapezoidal rule was used to calculate the area under the concentration-time curve between 0 and 24 h ($AUC_{0–24\,h}$) or 0 and end of study if terminated before 24 h ($AUC_{0–end\ of\ study}$) for plasma free insulin concentration and glucose infusion rate (GIR). Incremental AUC for plasma glucose was calculated by subtracting the basal value from the sample points before integrating AUC (16). Maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) for the same variables were read directly from the plasma concentration-time data for each subject. The determination of C_{max} and T_{max} for GIR were derived from a smoothed three-point running average GIR curve for each subject to provide reliable data for calculation.

The primary analysis of the PK/PD parameters was performed using ANOVA with subject, treatment, sequence group, and period effects on natural log–transformed data. Within-subject variability was assessed as intraindividual coefficient of variation (CV) values calculated using the root mean square approach described by Bland (17).

The primary end point of the study was duration of action. Secondary end points were plasma insulin concentration and GIR ($AUC_{0–end\ of\ study}$). A sample size of 11 subjects was chosen to achieve 91% power to detect a mean of paired differences of 2.0 h with an SD of differences of 2.0 h and with a significance level (α) of 0.05 using a two-sided paired *z* test. All tests of statistical hypothesis were carried out at the 5% level of significance, and comparisons were two-sided. Data in the text are expressed as mean ± SD and median with 25th and 75th percentiles as appropriate; in figures, data are expressed as mean ± SE. Statistics were performed using NCSS PASS software (Kaysville, UT; www.ncss.com).

RESULTS

Plasma Insulin and Glucose Concentrations and GIRs

After subcutaneous NPH insulin injection, plasma insulin concentration initially increased for 4–5 h and subsequently decreased in all four studies but to a different extent (Fig. 2). Compared with resuspended NPH insulin (R+), injection of nonresuspended NPH insulin resulted in either lower (R- horizontal and R- up) or higher (R- down) plasma insulin concentration (Fig. 2), $AUC_{(0–end\ of\ study)}$, and C_{max} (Table 1). In contrast, T_{max} was no different among the four studies.

Plasma glucose concentration was maintained at the target in all four studies in the initial 4 h but subsequently increased by various rates in the different studies. Compared with resuspended NPH insulin (R+), plasma glucose increased earlier to >110 mg/dL in R- horizontal and R- up (Fig. 2), although the difference in T_{max} was statistically significant only in R- up (Table 1). In contrast, in R- down, T_{max} occurred later than in the other studies (Table 1). Compared with R+, hyperglycemia (calculated as incremental AUC) was more pronounced in R- horizontal and R- up, but reduced in R- down, the latter difference being statistically significant (Table 1).

Onset of action of NPH insulin was earlier in R- down versus R+; end of action tended

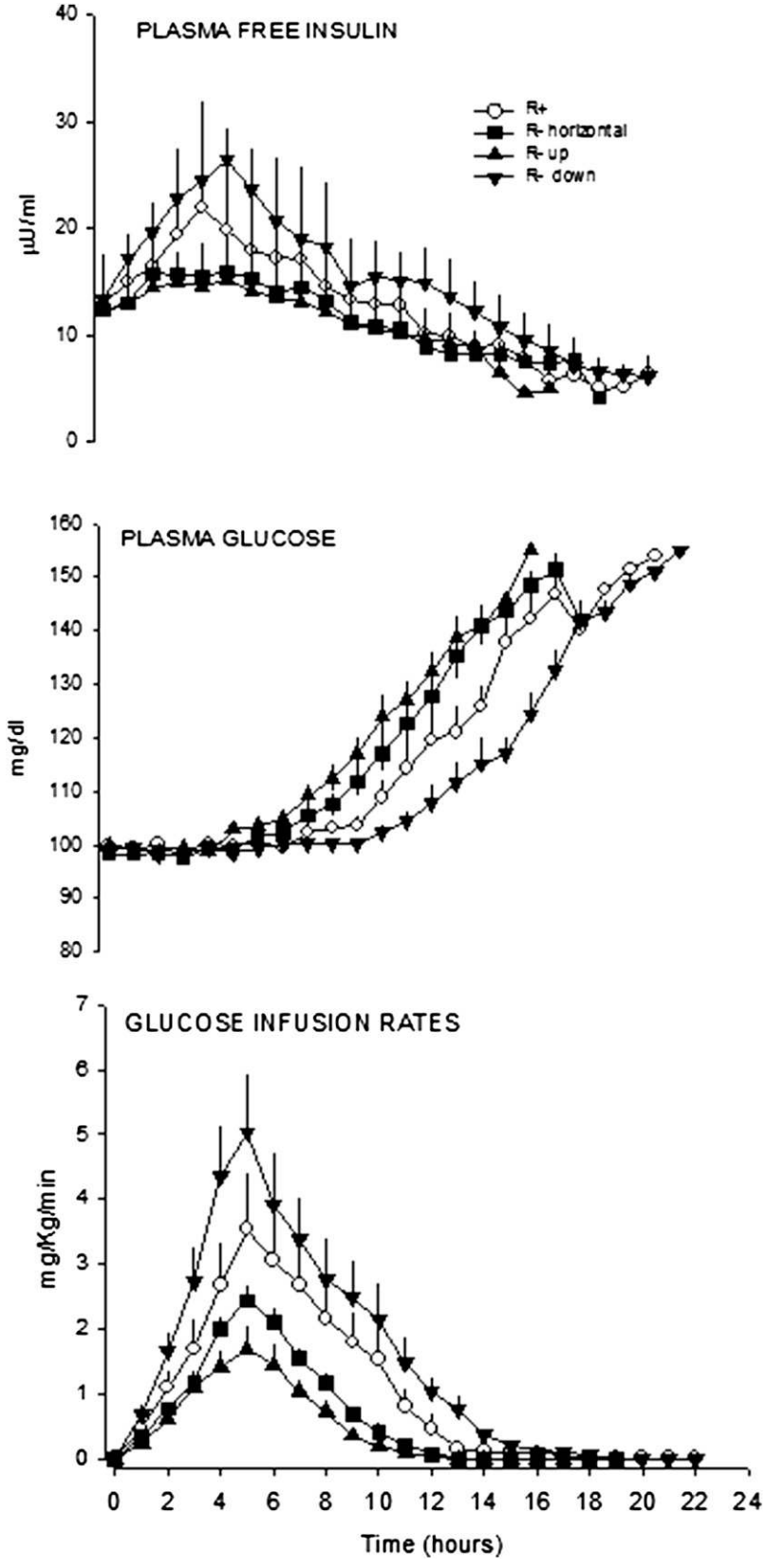

Figure 2—Plasma free insulin and glucose concentrations and GIRs to maintain euglycemia in the four studies after subcutaneous injection of 0.35 units/kg NPH insulin in 11 subjects with T1DM. In the R+ study, NPH insulin was properly resuspended before injection. In the R- horizontal, R- up, and R- down studies, NPH insulin was injected without prior resuspension (R- horizontal, pen maintained horizontally; R- up and R- down, pen maintained vertically with R- up tip up and R- down tip down).

to be earlier in R- horizontal and R- up, but the difference was not statistically significant. In contrast, in R- down, end of action occurred later than in the other studies (Table 1). Duration of action was shorter in R- horizontal and R- up than in R+ (the difference statistically significant only in R- up), whereas it was longer in R- down than in the other studies (Table 1).

The GIR required to maintain euglycemia was lower in R- horizontal and R- up than in R+ (with only the difference in R- up being statistically significant), whereas it was higher in R- down than in the other studies. GIR C_{max} was lower in R- horizontal and R- up (again, the difference statistically significant only in R- up), whereas it was higher in R- down than in the other studies. GIR T_{max} did not differ among the four studies (Table 1).

Subjects in Study

All subjects in all four studies ended before the planned conclusion of 24 h because plasma glucose increased to >150 mg/dL (threshold for definition of end of study). Consequently, there were fewer subjects in R- horizontal and R- up than in R+ and, particularly, than in R- down after the 11th hour (Fig. 3).

Intraindividual CVs of Plasma Insulin Concentrations and GIRs

The intraindividual CVs, a measure of PK/PD variability, were calculated in all four studies, comparing R+ with R- horizontal, R- up, and R- down. In the latter three studies, the greatest variability for free immunoreactive insulin (FIRI) C_{max} and GIR C_{max} occurred between R+ and R- down. This was also the case for FIRI_$AUC_{(0–end\ of\ study)}$. The GIR_$AUC_{(0–end\ of\ study)}$ had the highest CV in all comparisons, the more elevated values being between R+ and R- horizontal (Table 2).

CONCLUSIONS

The current study demonstrates that a therapeutic dose of NPH insulin (0.35 units/kg) commonly used in patients with T1DM results in quite different PK/PD depending on whether NPH insulin is appropriately resuspended before subcutaneous injection. Compared with resuspended NPH insulin, nonresuspended NPH insulin may result in either potentiated or reduced insulin PK/PD depending on the position of the pen before injection (held horizontally or vertically with tip either up or down). At one extreme, when the pen is held

Table 1—PK/PD parameters

Parameter	Study				*P* value
	R+	R- horizontal	R- up	R- down	
PK					
FIRI_AUC$_{(0–\text{end of study})}$ (μU/mL · h^{-1}) (*$P < 0.05$ vs. R+, R- horizontal, R- up; §$P < 0.05$ vs. R+)	248 ± 88	206 ± 41§	188 ± 35§	323 ± 52*	**0.001**
FIRI C_{max} (μU/mL) (*C_{max} $P < 0.05$ vs. R- horizontal, R- up)	24 ± 9.3*	19 ± 3.5	17 ± 2.9	27 ± 3.1*	**0.001**
FIRI T_{max} (h)	4.7 ± 0.6	3.6 ± 2.2	4.6 ± 1.6	4.7 ± 0.6	0.143
PD					
AUC plasma glucose (mg/dL · h^{-1}) (*$P < 0.05$ vs. R- up)	171 ± 61	205 ± 55	208 ± 53	164 ± 44*	**0.049**
Plasma glucose T_{max} (h) (*$P < 0.05$ vs. R+, R- horizontal, R- up; §$P < 0.05$ vs. R+)	17.3 ± 2.6	16.5 ± 1.8	15.4 ± 2.2§	19.7 ± 2.2*	**0.001**
Onset of action† (h) (*$P < 0.05$ vs. R+)	2.2 ± 1.2	1.7 ± 1.2	1.7 ± 1	1 ± 0*	**0.008**
End of action† (h) (*$P < 0.05$ vs. R+, R- horizontal, R- up)	13.9 ± 2.3	11.9 ± 2.1	11.1 ± 2.1	16.4 ± 2.3*	**0.001**
Duration of action† (h) (*$P < 0.05$ vs. R+, R- horizontal, R- up; §$P < 0.05$ vs. R+)	11.8 ± 2.6	10.1 ± 1.8	9.4 ± 1.7§	15.4 ± 2.3*	**0.001**
GIR					
C_{max} (mg/kg/min) (*$P < 0.05$ vs. R- horizontal, R- up; §$P < 0.05$ vs. R+)	3.8 ± 2.8	2.6 ± 1.7	1.7 ± 1.2§	5.1 ± 3.0*	**0.001**
T_{max} (h)	6.2 ± 1.4	6.3 ± 1.2	5.4 ± 0.9	5.1 ± 0.7	0.206
$AUC_{0–\text{end of study}}$ (mg/kg) (*$P < 0.05$ vs. R+, R- horizontal, R- up; §$P < 0.05$ vs. R+)	1,342 ± 1,129	774 ± 637	481 ± 322§	1,957 ± 1,253*	**0.001**

Data are mean ± SD. Boldface indicates significance at $P < 0.05$. †See RESEARCH DESIGN AND METHODS for definition.

vertically tip down with no resuspension before injection (R- down), the injected NPH insulin is predominantly the cloudy part (Fig. 1), which is rich in insulin crystals. In this case, plasma insulin concentration, insulin effect (GIR), and duration of action are potentiated compared with the other extreme of nonresuspended NPH insulin, with the predominant injected part being clear (theoretically no or very few insulin crystals [R- horizontal and R- up]). Within-subject variability of PK/PD of NPH insulin is elevated both when all four suspension conditions are considered and when the resuspended NPH insulin is compared with one occasion of nonresuspension (Table 2). In contrast to these characteristics of the insoluble NPH insulin, the modern long-acting insulin analogs (1,2) are soluble and, therefore, do not need resuspension before injection. For this reason, they are expected to be less variable than NPH insulin.

The present studies examined three extreme situations of nonresuspension (pen held horizontally or vertically with tip either up or down) and not the more common condition where patients inject NPH insulin after only partial, incomplete resuspension. Still, the results emphasize how critical resuspension is to obtain the expected PK/PD of NPH insulin. The wide fluctuations in plasma glucose control reported in subjects with T1DM who do not adequately resuspend NPH insulin before injection (10) are likely explained by the PK/PD results of the current study. A large part of the reported variability of NPH insulin (5–7,10,18) is possibly explained by a lack of or insufficient resuspension before injection, as shown in the current study; thus, the variability of NPH insulin might be substantially decreased in the everyday life of patients with diabetes by simply adhering to the long-well-known recommendation of resuspending NPH insulin before injection, no matter the manufacturer (19). In fact, the major insulin companies all recommend injecting NPH insulin at room temperature after full resuspension obtained with at least 10–20 times of tipping the pen to allow full movement of the small glass ball present in the Novo Nordisk FlexPen and Lilly KwikPen or the three small metal balls of the Sanofi Lantus SoloSTAR pen. However, in the current study, appropriate resuspension of NPH insulin required up to 1 min and 30 s, which is quite long for preparation of an insulin

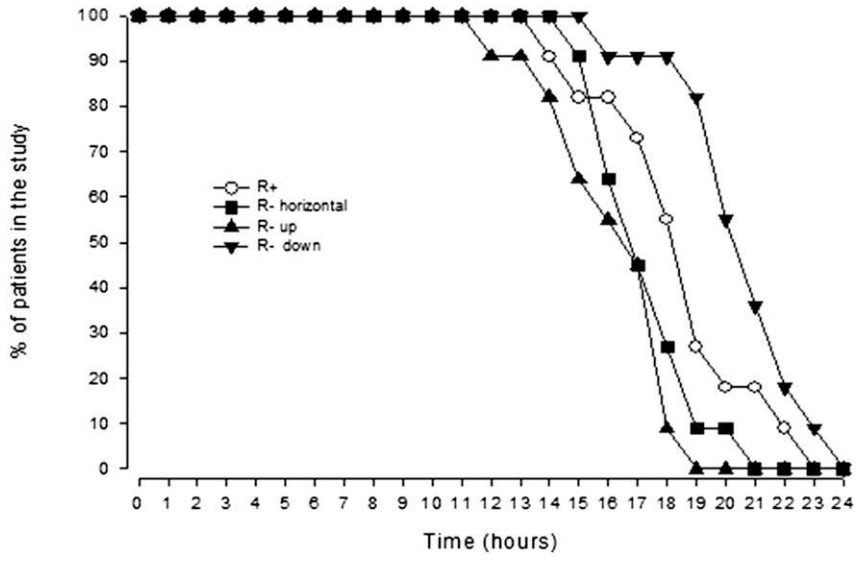

Figure 3—Time course of subjects in the four studies. Studies were terminated at the end of NPH action (plasma glucose >150 mg/dL) or at 24 h. As compared to study R+, in studies R- either fewer (R- horizontal, R- up) or more subjects (R- down) remained in the study.

Table 2—Intraindividual CVs of plasma insulin concentrations and GIRs in the four studies

	$FIRI\_AUC_{(0–end\ of\ study)}$	$GIR\_AUC_{(0–end\ of\ study)}$
Studies R+, R- horizontal, R- up, R- down	16 (14–19)	45 (38–53)
Studies R+, R- horizontal	14 (8–21)	62 (51–73)
Studies R+, R- up	20 (11–29)	54 (45–64)
Studies R+, R- down	23 (13–34)	48 (40–56)
	FIRI T_{max}	GIR T_{max}
Studies R+, R- horizontal, R- up, R- down	63 (52–75)	9 (8–10)
Studies R+, R- horizontal	39 (33–46)	7 (6–8)
Studies R+, R- up	68 (56–81)	10 (8–11)
Studies R+, R- down	47 (40–55)	9 (8–11)
	FIRI C_{max}	FIRI T_{max}
Studies R+, R- horizontal, R- up, R- down	18 (16–21)	28 (24–33)
Studies R+, R- horizontal	20 (17–23)	29 (16–44)
Studies R+, R- up	20 (17–23)	30 (16–44)
Studies R+, R- down	28 (24–33)	31 (17–47)

Data are mean (95% CI).

injection and a key step that some patients might totally or partially skip.

The two conditions of nonresuspended NPH insulin in the current study (R- up and R- down) should establish the PK/PD of either the clear (R- up) or the cloudy (R- down) part of the injectant. In fact, the cloudy part, which is rich in insulin crystals, exhibits the greatest insulin activity (R- down). Of note, however, the clear part, which should contain no or very few insulin crystals, has a definite insulin activity (Fig. 2) and, thus, may indicate an important presence of insulin. However, it should be observed that in R- up, the clear part was likely enriched with insulin crystals from the cloudy part just before injection when the pen had to be inverted from the initial tip-up position to the tip-down position.

The variability in PK/PD of resuspended or nonresuspended NPH insulin, reported as CVs in the present study, is not directly comparable with that of another study where NPH insulin was resuspended before injection (7). In that study, the variability of NPH insulin was higher than glargine and detemir in subjects with T1DM. However, the study examined parallel groups of subjects, not the same subjects receiving different basal insulins in a crossover comparison as in the present study. In addition, the clamp technique used (automatic Biostator) (7) is not comparable with that of the current study (manual clamp) (4,14). One limitation of the present study is that calculating within-subject variability for resuspended NPH insulin was not possible because the subjects were studied only once in the R+ condition. Only within-subject variability of resuspended and nonresuspended NPH insulin was calculated (Table 2). As expected, the highest variability occurred between the two extreme situations of injection of the clear and cloudy parts of NPH insulin (R- up and R- down).

As mentioned, NPH insulin is used less and less in T1DM but continues to be popular in the treatment of T2DM either as basal insulin or as part of premixed formulations (20,21). Although PK/PD of NPH insulin in T2DM treatment (22) differs from that in T1DM treatment (4), the results observed with NPH insulin in the current study in T1DM could be largely applicable to T2DM. The high variability of NPH insulin observed in the present study is expected to occur also with the premixed formulations where NPH insulin is combined in variable percentages with rapid-acting insulin. Such a variability of the NPH insulin component might contribute to the elevated risk for hypoglycemia of NPH insulin–based premixed formulations (23) in addition to its PK/PD (20).

The current study has some limitations in addition to those already mentioned. The number of subjects studied was small. However, the fact that differences are quite evident with so few subjects speaks in favor of the importance of the resuspension process as a main factor in determining PK/PD of injected NPH insulin. The study examined NPH insulin from only one manufacturer (Novo Nordisk), but it is assumed that NPH insulin from other manufacturers would result in similar characteristics (19) and action profiles at least in subjects without diabetes (24). In the present study, the effect of NPH insulin was examined for 24 h after administration of a 0.35 units/kg dose, mimicking the subjects' prior daily basal insulin need (Supplementary Table 1). Although NPH insulin should have been studied in the clamp according to the more common clinical use of two (or more) daily administrations, we speculate that the effects of resuspension versus nonresuspension of NPH insulin given in two (or more) doses compared with the one dose used in the current study would be qualitatively similar. Finally, this study examined NPH insulin injected with pens rather than syringes from NPH insulin vials. Although we would anticipate similar findings between pen and syringe injections, some differences might exist depending on the vial size, effects of needle aspiration, presence/absence of rolling balls, and so forth.

In conclusion, nonresuspended NPH insulin before injection may result in an approximately twofold difference in glucodynamic effect (either higher or lower) compared with resuspended NPH insulin, depending on the position of the pen. For real-life insulin-treated patients with T1DM, and likely T2DM, this may translate to a risk of either hyperglycemia or hypoglycemia. Additional studies are required to establish the comparative within-subject variability of properly resuspended NPH insulin with that of modern long-acting insulin analogs, such as glargine, detemir, degludec, and the new glargine U300 soon to come (2), to finally answer the long-discussed question of whether soluble basal insulins are less variable than insoluble NPH insulin.

Acknowledgments. The authors thank Mauro Lepore, Sanofi Italy, for long-term intellectual contribution to the research in this topic.

Duality of Interest. P.L. has received travel grants for scientific meetings from Sanofi, Medtronic, and Theras Group. F.P. has received honoraria for speaker fees and/or travel grants from Sanofi, Eli Lilly & Co., Bristol-Myers Squibb, and Merck & Co. G.B.B. received honoraria from Sanofi and Eli Lilly & Co. for scientific advising and consulting. C.G.F. has served on scientific advisory panels for Sanofi and received speaker fees and/or travel grants from Bristol-Myers Squibb, Merck & Co., and The

Menarini Group. No other potential conflicts of interest relevant to this article were reported.

This is an independent research study not supported by pharmaceutical companies.

Author Contributions. P.L. enrolled patients, performed clamps, analyzed data, and reviewed and edited the manuscript. F.P. enrolled patients, performed clamps, and reviewed and edited the manuscript. A.M.A., I.C., and P.Ci. performed clamps and reviewed and edited the manuscript. P.Ca. performed clamps and laboratory assays and reviewed and edited the manuscript. G.B.B. provided the study concept and design, supervised the protocol development and research, and wrote the manuscript. C.G.F. performed clamps, analyzed data, performed the statistical analysis, and reviewed and edited the manuscript. P.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in poster form at the 74th Scientific Sessions of the American Diabetes Association, San Francisco, CA, 13–17 June 2014.

References

1. Bolli GB, Di Marchi RD, Park GD, Pramming S, Koivisto VA. Insulin analogues and their potential in the management of diabetes mellitus. Diabetologia 1999;42:1151–1167
2. Gough SC, Harris S, Woo V, Davies M. Insulin degludec: overview of a novel ultra long-acting basal insulin. Diabetes Obes Metab 2013;15:301–309
3. Bolli GB, DeVries JH. New long-acting insulin analogs: from clamp studies to clinical practice. Diabetes Care 2015;38:541–543
4. Lepore M, Pampanelli S, Fanelli C, et al. Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro. Diabetes 2000;49:2142–2148
5. Galloway JA, Spradlin CT, Nelson RL, Wentworth SM, Davidson JA, Swarner JL. Factors influencing the absorption, serum insulin concentration, and blood glucose responses after injections of regular insulin and various insulin mixtures. Diabetes Care 1981;4:366–376
6. Heinemann L. Variability of insulin absorption and insulin action. Diabetes Technol Ther 2002;4:673–682
7. Heise T, Nosek L, Rønn BB, et al. Lower within-subject variability of insulin detemir in comparison to NPH insulin and insulin glargine in people with type 1 diabetes. Diabetes 2004;53:1614–1620
8. Norrman M, Hubálek F, Schluckebier G. Structural characterization of insulin NPH formulations. Eur J Pharm Sci 2007;30:414–423
9. Brange J. *Galenics of Insulin*. Berlin, Heidelberg, Springer-Verlag, 1987
10. Jehle PM, Micheler C, Jehle DR, Breitig D, Boehm BO. Inadequate suspension of neutral protamine Hagendorn (NPH) insulin in pens. Lancet 1999;354:1604–1607
11. Lepore M, Bartocci L, Fanelli CG, et al. Effect of adequate vs inadequate resuspension of NPH insulin prior to s.c. injection on pharmacokinetics and –dynamics in T1DM (Abstract). Diabetes 2001;50(Suppl. 2):A70
12. Lalli C, Ciofetta M, Del Sindaco P, et al. Long-term intensive treatment of type 1 diabetes with the short-acting insulin analog lispro in variable combination with NPH insulin at mealtime. Diabetes Care 1999;22:468–477
13. Rossetti P, Pampanelli S, Fanelli C, et al. Intensive replacement of basal insulin in patients with type 1 diabetes mellitus given rapid-acting insulin analog at mealtime: a 3-month comparison between administration of NPH four times daily and glargine insulin at dinner or bedtime. Diabetes Care 2003;26:1490–1496
14. Porcellati F, Rossetti P, Busciantella NR, et al. Comparison of pharmacokinetics and dynamics of the long-acting insulin analogs glargine and detemir at steady state in type 1 diabetes: a double-blind, randomized, crossover study. Diabetes Care 2007;30:2447–2452
15. Kuzuya H, Blix PM, Horwitz DL, Steiner DF, Rubenstein AH. Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes 1977;26:22–29
16. Le Floch JP, Escuyer P, Baudin E, Baudon D, Perlemuter L. Blood glucose area under the curve. Methodological aspects. Diabetes Care 1990;13:172–175
17. Bland M. How should I calculate a within-subject coefficient of variation? [article online], 2006. Available from http://www-users.york.ac.uk/~mb55/meas/cv.htm. Accessed 5 March 2015
18. Binder C, Lauritzen T, Faber O, Pramming S. Insulin pharmacokinetics. Diabetes Care 1984;7:188–199
19. Kaiser P, Maxeiner S, Weise A, et al. Assessment of the mixing efficiency of neutral protamine Hagedorn cartridges. J Diabetes Sci Technol 2010;4:652–657
20. Luzio S, Dunseath G, Peter R, Pauvaday V, Owens DR. Comparison of the pharmacokinetics and pharmacodynamics of biphasic insulin aspart and insulin glargine in people with type 2 diabetes. Diabetologia 2006;49:1163–1168
21. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2015;38:140–149
22. Lucidi P, Porcellati F, Rossetti P, et al. Pharmacokinetics and pharmacodynamics of therapeutic doses of basal insulins NPH, glargine, and detemir after 1 week of daily administration at bedtime in type 2 diabetic subjects: a randomized cross-over study. Diabetes Care 2011;34:1312–1314
23. Rys P, Wojciechowski P, Rogoz-Sitek A, et al. Systematic review and meta-analysis of randomized clinical trials comparing efficacy and safety outcomes of insulin glargine with NPH insulin, premixed insulin preparations or with insulin detemir in type 2 diabetes mellitus. Acta Diabetol 2015;52:649–662
24. Starke AAR, Heinemann L, Hohmann A, Berger M. The action profiles of human NPH insulin preparations. Diabet Med 1989;6:239–244