

Plasma Exposure to Insulin Glargine and Its Metabolites M1 and M2 After Subcutaneous Injection of Therapeutic and Supratherapeutic Doses of Glargine in Subjects With Type 1 Diabetes

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OBJECTIVE—In vivo, after subcutaneous injection, insulin glargine (21^A-Gly-31^B-Arg-32^B-Arg-human insulin) is enzymatically processed into 21^A-Gly-human insulin (metabolite 1 [M1]). 21^A-Gly-des-30^B-Thr-human insulin (metabolite 2 [M2]) is also found. In vitro, glargine exhibits slightly higher affinity, whereas M1 and M2 exhibit lower affinity for IGF-1 receptor, as well as mitogenic properties, versus human insulin. The aim of the study was to quantitate plasma concentrations of glargine, M1, and M2 after subcutaneous injection of glargine in male type 1 diabetic subjects.

RESEARCH DESIGN AND METHODS—Glargine, M1, and M2 were determined in blood samples obtained from 12, 11, and 11 type 1 diabetic subjects who received single subcutaneous doses of 0.3, 0.6, or 1.2 units · kg⁻¹ glargine in a euglycemic clamp study. Glargine, M1, and M2 were extracted using immunoaffinity columns and quantified by a specific liquid chromatography-tandem mass spectrometry assay. Lower limit of quantification was 0.2 ng · mL⁻¹ (33 pmol · L⁻¹) per analyte.

RESULTS—Plasma M1 concentration increased with increasing dose; geometric mean (percent coefficient of variation) M1-area under the curve between time of dosing and 30 h after dosing (AUC_{0-30h}) was 1,261 (66), 2,867 (35), and 4,693 (22) pmol · h · L⁻¹ at doses of 0.3, 0.6, and 1.2 units · kg⁻¹, respectively, and correlated with metabolic effect assessed as pharmacodynamics-AUC_{0-30h} of the glucose infusion rate following glargine administration ($r = 0.74$; $P < 0.01$). Glargine and M2 were detectable in only one-third of subjects and at a few time points.

CONCLUSIONS—After subcutaneous injection of glargine in male subjects with type 1 diabetes, exposure to glargine is marginal, if any, even at supratherapeutic doses. Glargine is rapidly and nearly completely processed to M1 (21^A-Gly-human insulin), which mediates the metabolic effect of injected glargine.

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Insulin glargine design followed the physiology of human insulin formation in β -cells in which 31^B-Arg-32^B-Arg-human insulin is a final intermediate of the processing from proinsulin to

human insulin (1–4). Although unmodified 31^B-Arg-32^B-Arg-human insulin failed subcutaneously despite being fully active intravenously (5), substitution of 21^A-Asp for Gly rendered the molecule

both chemically stable (6) and fully active subcutaneously without substantial alterations in receptor affinities (7–10). Soluble at acidic pH, glargine precipitates amorphously upon subcutaneous injection and becomes subject to enzymatic maturation into 21^A-Gly-human insulin upon slow release from the depot (11). As a result, glargine exhibits a nearly flat action profile and duration beyond 24 h after multiple dosing in subjects with type 1 and type 2 diabetes (12,13). Glargine is preferred to human NPH insulin because it protects from the risk of hypoglycemia, primarily nocturnal (14).

In vitro studies have indicated that glargine has greater binding affinity for the IGF-1 receptor (IGF-1R) and greater potency on DNA synthesis (so-called mitogenic effects) compared with human insulin, at least in malignant cell lines expressing primarily IGF-1R, not insulin receptors (IR) (8). However, the in vitro data are not directly applicable in vivo in humans; the natural precursor 31^B-Arg-32^B-Arg-human insulin shows even greater IGF-1R affinity than glargine (8,9). In addition, it is presently proposed that the mitogenic potential of insulin analogs is mediated primarily via IR, not IGF-1R (15,16). Nevertheless, the safety of glargine in humans has been questioned (16) based on in vitro experiments, even though glargine does not promote tumor growth in vivo in animals (17), in contrast to the insulin analog X10 (10^B-Asp-human insulin) (16), which presents with greater affinity for both IR and IGF-1R. Some controversial registry studies have suggested a possible greater cancer risk in humans using glargine versus non-glargine insulin (18,19).

However, in vivo, after subcutaneous injection, glargine undergoes an enzymatic removal of the basic arginine pair at positions 30^B and 31^B to yield 21^A-Gly-human insulin (metabolite 1 [M1]), analogous to prohormone activation (4), with some further loss of threonine to

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See accompanying commentary, p. 2426, and brief report, p. 2647.

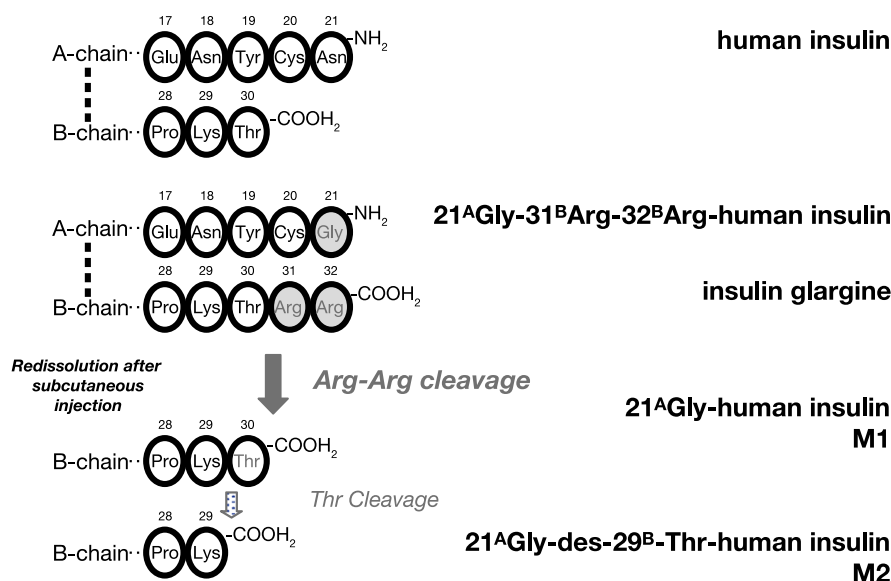


Figure 1—Insulin glargine maturation and metabolism after subcutaneous injection. Enzymatic removal of the COOH-terminal basic arginine pair yields 21^A-Gly-human insulin metabolite M1, the principal active moiety of glargine. Subsequent cleavage of 30^B-threonine yields M2.

21^A-Gly-des-30^B-Thr-human insulin (metabolite 2 [M2]) (Fig. 1) (9,11). Both M1 and M2 exhibit lower affinity for IGF-1R and lower mitogenic potential in vitro compared with glargine, and even with human insulin, while fully retaining its metabolic properties (9,10). Thus, it is understood that most, if not all, of the glargine injected subcutaneously in humans is rapidly transformed to M1 and partly further to M2, resulting in minimal, if any, plasma exposure to parent glargine. Yet, because of technical constraints, the in vivo quantification of glargine metabolism to M1 and M2 in humans has so far been limited (11,20) and of uncertain interpretation (21).

Recently, a new bioanalytical method has been developed for specific measurement of glargine and its metabolites M1 and M2 in human plasma. Therefore, the current study was undertaken to characterize the in vivo metabolism of glargine after subcutaneous injection of therapeutic as well as suprathreshold doses in type 1 diabetic subjects and to correlate the glucodynamic effects of injected glargine with plasma concentration of glargine and/or its M1 and M2 metabolites.

RESEARCH DESIGN AND METHODS

Subjects

Male subjects (aged 21–56 years) with type 1 diabetes on stable basal and

prandial insulin regimen (<1.2 units \cdot kg⁻¹), fasting serum C-peptide (<0.3 nmol \cdot L⁻¹), and glycated hemoglobin (HbA_{1c} ≤ 7.5 mmol \cdot mol⁻¹, [9.0%]) for at least 2 months participated in the study. The study was performed in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines for Good Clinical Practice and adhered to the principles of the Declaration of Helsinki and with all the laws, regulations, and guidelines of Germany, where the study was conducted at the Profil Institute (Neuss). The study protocol and its amendments received local institution review board approval.

Study design

Within a single-center, double-blind euglycemic clamp study, subjects were randomized to receive single doses of 0.3, or 0.6, or 1.2 units \cdot kg⁻¹ glargine. Each subject was studied with only one glargine dose. Subjects were admitted to the study center before the clamp experiment for baseline evaluation, and those taking glargine or detemir had their last injection of these insulins that evening. All subjects used human isophane insulin as basal insulin at their usual dose on the next day with the last injection no later than noon.

Euglycemic clamp

For the euglycemic clamp, subjects were connected to a Biostator (MTB Medizin-

technik, Amstetten, Germany) in the morning, in the fasting state, ~ 4 – 6 h before administration of the glargine test dose, as previously described (22). In short, a variable manual intravenous infusion of insulin glulisine or 20% glucose was initiated to obtain a clamp target blood glucose level of 5.5 mmol \cdot L⁻¹ (100 mg \cdot dL⁻¹) $\pm 30\%$ that was to be maintained without glucose infusion for at least 1 h before subcutaneous periumbilical injection of glargine at approximately 9:00 A.M., and the glulisine infusion was stopped immediately before injection. After glargine injection, an intravenous variable-rate glucose infusion was initiated to maintain blood glucose at 5.5 mmol \cdot L⁻¹ (100 mg \cdot dL⁻¹) for 30 h. Subjects fasted for the duration of the clamp.

Analytical methods

Blood samples for determination of the plasma concentration of glargine and its metabolites M1 and M2 were collected before dosing and at 3, 6, 12, 18, 24, and 30 h postdose. A total of 300 μ L of plasma from each sample was mixed with 50 μ L of a working internal standard solution (50 μ g \cdot L⁻¹ ¹⁵N₇₂-glargine, 25 μ g \cdot L⁻¹ ¹⁵N₆₄-M1, and 25 μ g \cdot L⁻¹ ¹⁵N₆₃-M2) and 300 μ L of PBS buffer (pH 7.8). Subsequently, glargine and its metabolites were extracted using an immunoaffinity purification protocol that was based on a protocol from Thevis et al. (23) and determined with a liquid chromatography-tandem mass spectrometry system (Supplementary Data). The lower limit of quantification (LLOQ) for this method was 33 pmol \cdot L⁻¹ for glargine, M1, and M2.

Statistical analyses and calculations

All subjects were included in the pharmacokinetics (PK) and pharmacodynamics (PD) population. PK and PD data were summarized by dose using descriptive statistics. Data in the text are expressed as geometric mean with coefficient of variation.

Glargine and its metabolite concentrations were determined for a 30-h interval and were integrated for areas under concentration-time curves (PK-AUC_{0–30h} [pmol \cdot h \cdot L⁻¹]) using the trapezoidal rule. The PD effect was determined as the glucose infusion rate and integrated for the area under the curve between time of dosing and 30 h after dosing (PD-AUC_{0–30h} [mg \cdot kg⁻¹]) using the linear rectangle method.

RESULTS—A total of 34 normal weight male subjects with type 1 diabetes without

substantial intersubject differences for diabetes complications and concomitant medications were included in the study to receive glargine at doses of 0.3 ($n = 12$), 0.6 ($n = 11$), or 1.2 units \cdot kg⁻¹ ($n = 11$). Their clinical characteristics are shown in Supplementary Table 1.

Glucose metabolism

Blood glucose concentrations were <6.5 mmol \cdot L⁻¹ (118 mg \cdot dL⁻¹) for 17, 30, and 30 h following 0.3, 0.6, and 1.2 units \cdot kg⁻¹ glargine, respectively (Fig. 2), and PD-AUC_{0-30h} increased from 562 (61) to 2,726 (37) and 6,260 (29) mg \cdot kg⁻¹, verifying a positive correlation between injected dose of glargine and glucose metabolism.

Plasma concentrations of glargine, M1, and M2

M1 was the predominant metabolite of glargine found in plasma (Fig. 2), and its exposure increased with increasing dose

of injected glargine. M1-maximum plasma concentration was 78 (percent coefficient of variation 52), 136 (37), and 206 (26) pmol \cdot L⁻¹, and M1-AUC_{0-30h} was 1,261 (66), 2,867 (35), and 4,693 (22) pmol \cdot h \cdot L⁻¹ for doses of 0.3, 0.6, and 1.2 units \cdot kg⁻¹ of injected glargine, respectively. The PK profile of M1 showed maximum plasma concentrations \sim 12 h after subcutaneous injection of glargine and by 30 h was still elevated over baseline at doses >0.3 units \cdot kg⁻¹ (Fig. 2). The metabolic activity (PD-AUC_{0-30h}) observed after the single doses of injected glargine correlated with the PK-AUC_{0-30h} of M1 plasma concentrations ($r = 0.74$; $P < 0.001$) (Fig. 3).

Glargine or M2 was not detectable in the plasma of most subjects at any dose of glargine and at any time point of the study. When detectable, glargine appeared early; however, plasma concentrations did not increase with increasing dose of injected glargine. M2 first appeared

after 12 h and was associated with the presence of a high M1 concentration. Individual values are given in Supplementary Fig. 1.

Two subjects given 0.6 units \cdot kg⁻¹ presented with M1 values more than three times the interquartile spread (Supplementary Fig. 1) that did not correlate with glucodynamic efficacy. Therefore, their M1 as well as glargine and M2 values were excluded from statistical and graphical PK analysis. Both subjects presented with uncommon nonneutralizing insulin-antibody profiles at baseline that may have caused the observed high insulin concentrations.

CONCLUSIONS—This investigation was undertaken to specifically address the question of insulin glargine metabolism in vivo, after subcutaneous injection of therapeutic and suprathreshold doses in subjects with type 1 diabetes. The results indicate there is virtually no parent

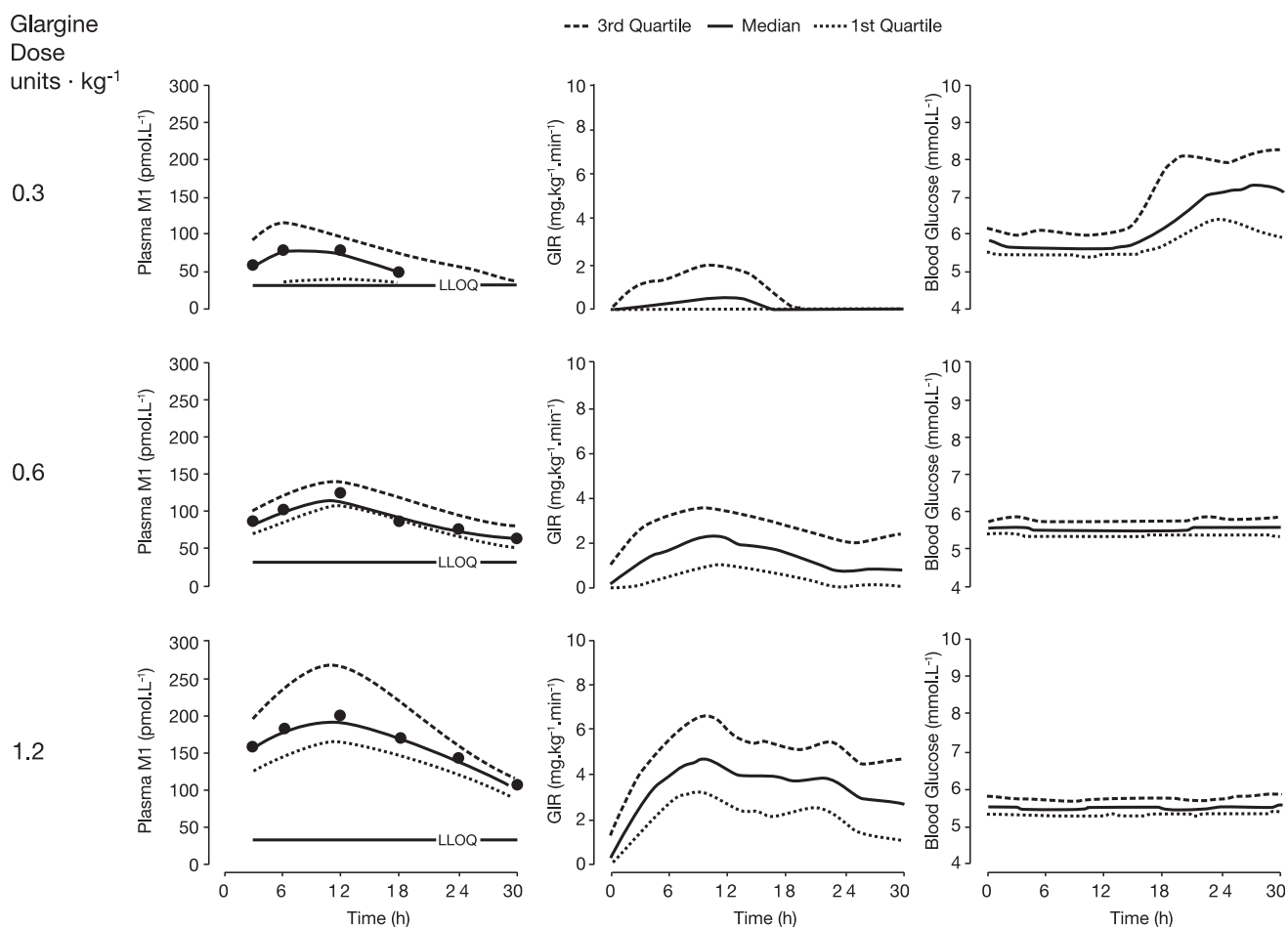


Figure 2—Plasma concentration of metabolite M1 (left panel), glucose infusion rate (GIR) to maintain euglycemia (middle panel), and blood glucose concentration (right panel) in the clamp studies after subcutaneous injection of 0.3, 0.6, and 1.2 units \cdot kg⁻¹ glargine (upper, middle, and lower panels) in subjects with type 1 diabetes. Medians (solid lines) and 25th–75th percentiles are given (dotted and dashed lines). LLOQ: 33 pmol \cdot L⁻¹ (solid line) for M1. Plasma glargine and M2 concentrations are not presented since its concentrations nearly always fall below LLOQ (see RESULTS).

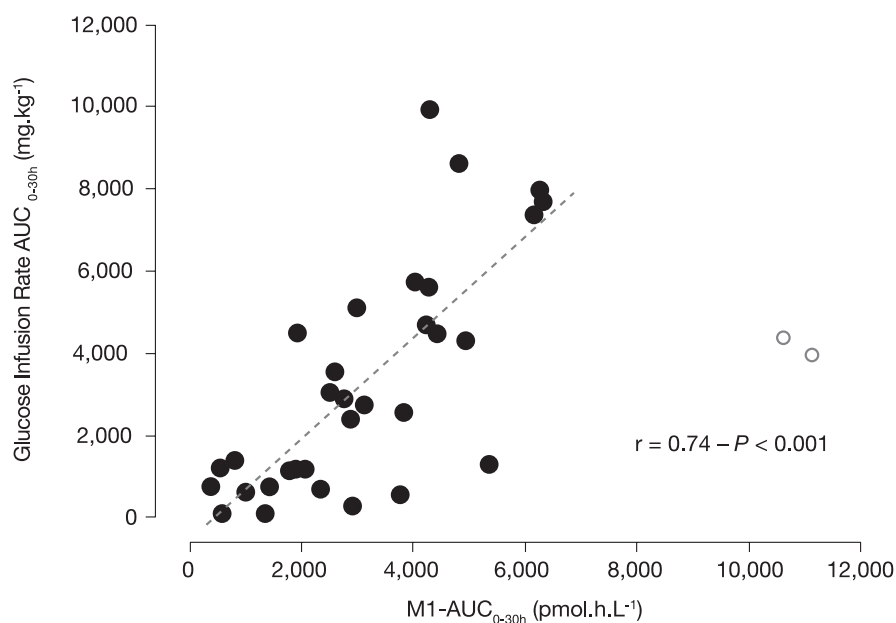


Figure 3—Linear regression of exposure to metabolite M1 (closed circles) and glucose infusion as AUC_{0-30h} . Open circles indicate two far outside values, excluded from linear regression analysis.

glargine circulating in plasma regardless of the dose given. In contrast, there is a rapid increase in plasma 21^A-Gly-human insulin (M1) concentration that is proportional to the dose of glargine injected. Because M2 is also virtually not present in plasma, it is concluded that in vivo in humans glargine is rapidly processed to M1, and M1, not glargine itself, mediates the glucodynamic effects. This conclusion is also supported by the correlation between plasma concentration of M1 and the metabolic effects of injected glargine on glucose metabolism.

Moreover, the same metabolic pattern has been observed in steady state in preschool children with type 1 diabetes (24) and also in type 2 diabetes (25), showing that glargine is rapidly metabolized to 21^A-Gly-human insulin regardless of type of diabetes or age.

This study has not examined the effects of glargine metabolites further to glucose metabolism, but it is reasonable to assume that M1 conveys the entire array of well-known insulin-mediated effects, such as those on lipid, protein, and endothelium metabolism.

The processing of glargine after subcutaneous injection as presented in this study has been previously reported in humans with a less specific and sensitive bioanalytical method (11). Other determinations used a radioimmunoassay that did not discriminate between parent glargine, M1, and M2, or a radioimmunoassay

sensitive for human insulin and so detected almost exclusively M1 (21). The present report, based on a new, specific methodology in a dose-response study in subjects with type 1 diabetes, confirms and more precisely quantifies glargine metabolism to yield almost completely M1 in humans compared with previous observations. It is reasonable to assume that exposure data using the unspecific radioimmunoassay method also reflect in effect M1 exposure, as indicated by a study in type 2 diabetes (25). The late appearance of M2 points to unspecific degradation once M1 is formed. Corroborating evidence is given by an in vitro study using mass spectrometry, which found that, within 1 h at 37°C, insulin glargine in human serum is quantitatively degraded into M1, which was used to quantify glargine by radioimmunoassay in the presence of human insulin (22).

Even the assumption that parent glargine concentrations quantified below LLOQ are not zero but rather at the limit of 33 pmol · L⁻¹, this would be a quite low concentration of glargine, which, according to in vitro findings, is not capable of promoting greater binding to IGF-1R in vivo or of promoting greater mitogenesis in cancer cell lines (26) compared with human insulin. Thus, in the absence of glargine, and because M1 and M2 exhibit even lower binding to IGF-1R and less mitogenetic potential (10), the hypothesis that after subcutaneous injection

glargine could promote mitogenesis in humans more than human insulin does not find scientific support.

One limitation of the current study is that glargine metabolism has been assessed after first, not multiple, daily injections of insulin glargine (steady state). In the latter condition, insulin glargine increases in plasma more, and its PD effects are more pronounced and of longer duration compared with those following first injection (27). In theory, one might expect at steady state greater glargine, M1, and M2 concentrations in plasma compared with those of the current study. However, the small increase in plasma insulin observed at steady-state glargine administration (27) is still far below the concentrations observed with the 1.2 units · kg⁻¹ dose in this study and therefore is likely also attributed more to the increase in M1 than to glargine itself.

The current study has examined male subjects with type 1 diabetes without obesity, and although findings in subjects with type 2 diabetes corroborate the proposed glargine metabolism (Lucidi et al. [25]), additional studies are needed in obese, older type 2 diabetic subjects to verify in these subjects given a high dose of glargine that the metabolism of glargine follows a pattern quantitatively similar to that demonstrated in type 1 diabetes.

In conclusion, the current study confirms that after subcutaneous injection, insulin glargine, even at a supratherapeutic dose, is rapidly and near completely processed to 21^A-Gly-human insulin, the prime mediator of the metabolic effects of injected glargine.

ADDENDUM—While this article was in proof, an elegant in vitro study was published that showed that glargine displays higher potency than human insulin for stimulation of insulin/IGF-1 hybrid receptors with greater proliferative/apoptotic effects in MCF-7 cells. In contrast, M1 and M2 display lower potency than human insulin.

Pierre-Eugene C, Pagesy P, Nguyen TT, Neuillé M, Tschank G, Tennagels N, Hampe C, Issad T. Effect of insulin analogues on insulin/IGF1 hybrid receptors: increased activation by glargine but not by its metabolites M1 and M2. *PLoS One* 2012;7:e41992.

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G.B.B. jointly initiated the investigation, contributed to the discussion, wrote the manuscript, and reviewed and edited the manuscript. A.D.H. jointly initiated the investigation, established the bioanalytical method, researched the data, and contributed to the discussion. R.S. validated the bioanalytical method, analyzed the samples, and reviewed and edited the manuscript. T.E. contributed the pharmacokinetic data and reviewed and edited the manuscript. R.D. supervised the clinical part, researched the data, and reviewed and edited the manuscript. T.H. headed the clinical part and reviewed and edited the manuscript. R.H.A.B. jointly initiated the investigation, pooled the information, contributed to the discussion, wrote the manuscript, and reviewed and edited the manuscript. R.H.A.B. 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.

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References

1. Grau U. Insulin-Arg²: A new retarding principle based on a natural pro-insulin derived processing intermediate. *Diabetes Res Clin Pract* 1985;1(Suppl.1):S204
2. Kemmler W, Peterson JD, Steiner DF. Studies on the conversion of proinsulin to insulin. I. Conversion in vitro with trypsin and carboxypeptidase B. *J Biol Chem* 1971;246:6786–6791
3. Seipke G, Geisen K, Neubauer H-P, Pittius C, Roskamp R, Schwabe D. New insulin preparations with prolonged action profiles: A21-modified arginine insulins. *Diabetologia* 1992;35(Suppl.1):A4
4. Steiner DF. Adventures with insulin in the islets of Langerhans. *J Biol Chem* 2011;286:17399–17421
5. Monti LD, Poma R, Caumo A, et al. Intravenous infusion of diarginylinsulin, an insulin analogue: effects on glucose turnover and lipid levels in insulin-treated type II diabetic patients. *Metabolism* 1992;41:540–544
6. Markussen J, Diers I, Hougaard P, et al. Soluble, prolonged-acting insulin derivatives. III. Degree of protraction, crystallizability and chemical stability of insulins substituted in positions A21, B13, B23, B27 and B30. *Protein Eng* 1988;2:157–166
7. Zeuzem S, Stahl E, Jungmann E, Zoltbrocki M, Schöffing K, Caspary WF. In vitro activity of biosynthetic human diarginylinsulin. *Diabetologia* 1990;33:65–71
8. Kurtzhals P, Schäffer L, Sørensen A, et al. Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use. *Diabetes* 2000;49:999–1005
9. Kohn WD, Micanovic R, Myers SL, et al. pI-shifted insulin analogs with extended in vivo time action and favorable receptor selectivity. *Peptides* 2007;28:935–948
10. Sommerfeld MR, Müller G, Tschank G, et al. In vitro metabolic and mitogenic signaling of insulin glargine and its metabolites. *PLoS ONE* 2010;5:e9540
11. Kuerzel GU, Shukla U, Scholtz HE, et al. Biotransformation of insulin glargine after subcutaneous injection in healthy subjects. *Curr Med Res Opin* 2003;19:34–40
12. 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
13. 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
14. Home PD, Fritsche A, Schinzel S, Massi-Benedetti M. Meta-analysis of individual patient data to assess the risk of hypoglycaemia in people with type 2 diabetes using NPH insulin or insulin glargine. *Diabetes Obes Metab* 2010;12:772–779
15. Bonnesen C, Nelander GM, Hansen BF, et al. Synchronization in G0/G1 enhances the mitogenic response of cells overexpressing the human insulin receptor A isoform to insulin. *Cell Biol Toxicol* 2010;26:293–307
16. Smith U, Gale EA. Does diabetes therapy influence the risk of cancer? *Diabetologia* 2009;52:1699–1708
17. Stammberger I, Bube A, Durchfeld-Meyer B, Donaubaue H, Troschau G. Evaluation of the carcinogenic potential of insulin glargine (LANTUS) in rats and mice. *Int J Toxicol* 2002;21:171–179
18. Hemkens LG, Grouven U, Bender R, et al. Risk of malignancies in patients with diabetes treated with human insulin or insulin analogues: a cohort study. *Diabetologia* 2009;52:1732–1744
19. Jonasson JM, Ljung R, Talbäck M, Haglund B, Gudbjörnsdóttir S, Steineck G. Insulin glargine use and short-term incidence of malignancies—a population-based follow-up study in Sweden. *Diabetologia* 2009;52:1745–1754
20. Agin A, Jeandidier N, Gasser F, Grucker D, Sapin R. Glargine blood biotransformation: in vitro appraisal with human insulin immunoassay. *Diabetes Metab* 2007;33:205–212
21. Hansen BF, Kurtzhals P, Jensen AB, Dejgaard A, Russell-Jones D. Insulin X10 revisited: a super-mitogenic insulin analogue. *Diabetologia* 2011;54:2226–2231
22. Heise T, Nosek L, Ronn 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
23. Thevis M, Thomas A, Delahaut P, Bosseloir A, Schänzer W. Qualitative determination of synthetic analogues of insulin in human plasma by immunoaffinity purification and liquid chromatography-tandem mass spectrometry for doping control purposes. *Anal Chem* 2005;77:3579–3585
24. Danne T, Philotheou A, Goldman D, Guo T, Ping L, Johnston P. The Preschool Study: Hypoglycemia assessed by continuous glucose monitoring in 125 children under age 6 with Type 1 diabetes treated with multiple daily insulin injections. *Pediatr Diabetes* 2011;12(Suppl. 15):122
25. Lucidi P, Porcellati F, Rossetti P, et al. Metabolism of insulin glargine after repeated daily subcutaneous injections in subjects with type 2 diabetes. *Diabetes Care* 2012;35:2647–2649
26. Sandow J. Growth effects of insulin and insulin analogues. *Arch Physiol Biochem* 2009;115:72–85
27. Porcellati F, Rossetti P, Ricci NB, et al. Pharmacokinetics and pharmacodynamics of the long-acting insulin analog glargine after 1 week of use compared with its first administration in subjects with type 1 diabetes. *Diabetes Care* 2007;30:1261–1263