Metabolism of Insulin Glargine After Repeated Daily Subcutaneous Injections in Subjects With Type 2 Diabetes

Paola Lucidi, md, phd[1]
Francesca Porcellati, md, phd[1]
Paolo Rossetti, md, phd[1]
Paola Candeloro, phd[1]
Anna Marinelli Andreoli, md[1]
Patrizia Cioli, rd[1]
Annke Hahn, phd[2]
Ronald Schmidt, phd[2]
Geremia B. Bolli, md[1]
Carmine G. Fanelli, md, phd[1]

OBJECTIVE—To investigate concentration of plasma insulin glargine after its subcutaneous dosing compared with concentration of its metabolites 1 (M1) and 2 (M2) in subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS—Nine subjects underwent a 32-h euglycemic glucose clamp study (0.4 units/kg glargine after 1 week of daily glargine administration). Glargine, M1, and M2 were measured by a specific liquid chromatography-tandem mass spectrometry assay.

RESULTS—Glargine was detected in only five of the nine subjects, at few time points, and at negligible concentrations. M1 was detected in all subjects and exhibited the same pattern as traditional radioimmunoassay-measured plasma insulin. M2 was not detected at all.

CONCLUSIONS—After subcutaneous injection, glargine was minimally detectable in blood, whereas its metabolite M1 accounted for most (>90%) of the plasma insulin concentration and metabolic action of the injected glargine.

Diabetes Care **35**:2647–2649, 2012

A fter subcutaneous injection in vivo, the long-acting insulin analog glargine undergoes a sequential cleavage of the COOH terminus of the B-chain–forming metabolites M1 and M2 (1,2). M1 and M2 fully retain the same metabolic properties as human insulin (HI), but in contrast to glargine, they do not differ from HI in affinity for IGF-1 receptor (IGF-1R) and mitogenesis (3). In the companion article in this issue (Bolli et al. [4]), glargine metabolism has been studied in subjects with type 1 diabetes, but there are no data on type 2 diabetes. The aim of the current study was to shed light on this question.

RESEARCH DESIGN AND METHODS—After approval by an ethics committee and informed written

consent, 18 type 2 diabetic subjects underwent a euglycemic glucose clamp study after subcutaneous injection of 0.4 units/kg glargine and 1 week of daily glargine treatment, as previously reported (5). Subjects were fasted throughout the clamp studies. Glargine metabolism was studied in nine subjects (Supplementary Table 1) from whom plasma was still available (5).

The 31-h blood samples (samples after 31 h were not available) were processed for radioimmunoassay determination of plasma concentration of insulin (which detects endogenous insulin plus injected glargine and its metabolites) (5) and for a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay specific for glargine and metabolites 1 (M1) and 2 (M2) (6) (Supplementary

Data). The lower limit of quantification for this method was 33 pmol/L for insulin glargine, M1, and M2.

The maximum plasma concentration (C_{max}), time to reach C_{max}, and predose plasma concentration were read directly from the plasma concentration-time data for each subject. The area under the concentration-time curve (AUC) between 0 and 31 h (AUC_{0-31}) was determined using the trapezoidal rule in which concentrations less than the lower limit of quantification were set at zero. Percentage of glargine and M1 was calculated according to the equation: (glargine or M1/total insulin [glargine + M1]) × 100.

Data are expressed as medians (25th and 75th percentiles). Calculations were performed using NCSS 2007 (NCSS, LLC, Kaysville, UT, www.ncss.com).

RESULTS—Plasma glucose was maintained at 101 mg/dL (25th and 75th percentiles, 99 and 103 mg/dL, respectively) as a result of variable glucose infusion (0.47 mg/kg/min [0.26 and 0.82 mg/kg/min]) for 31 h (Supplementary Fig. 1).

Glargine was detected in only five of the nine subjects (Supplementary Fig. 2). In two subjects, the last measurable concentration was at 11 h (25th and 75th percentiles, 53 and 35 pmol/L, respectively), in two other subjects at 13 h (37 pmol/L for both), and in one subject at 18 h (41 pmol/L). M1 was detected in all subjects up to 31 h, whereas M2 was not detected in any subjects at any time point. M1, but not glargine, was detected at baseline (44 pmol/L [19–131 pmol/L]), likely reflecting the metabolism of glargine injected 24 h before. In fact, 24-h postinjection values of M1 were similar to baseline values (60 pmol/L [52–118 pmol/L]). The median glargine pharmacokinetics-AUC_{0-31} was 171 pmol · h/L (0 and 314 pmol · h/L), whereas the M1 pharmacokinetics-AUC_{0-31} was 2,166 pmol · h/L (1,622 and 4,955 pmol · h/L) (Fig. 1). Glargine C_{max} was 40 pmol/L (0 and 48 pmol/L), and M1 C_{max} was 129 pmol/L (75 and 207 pmol/L). Overall, M1 represented 97% (89 and 100%, respectively) of the total amount of insulin

See accompanying commentary, p. 2426, and original article, p. 2626.

detected in blood after injection of glargine, with glargine contributing only 3% (1 and 8%). The pattern of plasma insulin concentration as measured by radioimmunoassay was similar to that of M1 as detected by LC-MS/MS (Supplementary Fig. 1).

CONCLUSIONS—In the current study in type 2 diabetic subjects on glargine treatment for 1 week, a subcutaneous injection of a therapeutic dose of insulin glargine resulted in biotransformation to M1, which constituted >90% of circulating insulin, with only a minor presence of the originally injected glargine in plasma (<10%).

To the best of our knowledge, this is the first study reporting the metabolism of insulin glargine in type 2 diabetic subjects using a specific LC-MS/MS assay (6). Overall, the results are in line with recent findings in type 1 diabetes (Bolli et al. [4]). Taken together, the results suggest that in humans, glargine is rapidly metabolized to M1, whereas the originally injected glargine is, if anything, only minimally present in plasma.

These findings in type 2 diabetic subjects are relevant for the interpretation in vivo of the in vitro data reporting greater affinity of glargine for IGF-1R and greater

mitogenic potency compared with HI in some malignant cell lines (7). The present studies indicate that in vivo, glargine concentration in plasma is negligible and well below that shown in vitro to demonstrate greater affinity for IGF-1R (3,8) and greater mitogenesis (9). Because glargine is rapidly and nearly totally metabolized to M1, which has effects on mitogenesis no different than HI (3,7), it is unlikely that there is an increased risk of cancer in humans following administration of insulin glargine, at least via IGF-1R stimulation. Therefore, the findings of in vitro studies on mitogenesis using glargine as such (10) are of limited informative value for interpretation of the results of glargine administration in vivo.

We acknowledge that the current study has limitations (i.e., small sample size and a dose of insulin glargine just above the therapeutic range of the subjects studied). Nevertheless, the results are homogeneous in all subjects, consistent with those in type 1 diabetes (Bolli et al. [4]) and with recent findings of no stimulation of IGF-1R bioactivity by plasma of type 2 diabetic subjects who were given glargine doses greater than those described in the current study (11).

In conclusion, after subcutaneous injection of a therapeutic dose in glargine-treated

type 2 diabetic subjects, glargine is only transiently, and at minimal concentration, detectable in plasma, whereas its metabolite M1 accounts for most (>90%) of the plasma insulin concentration. Thus, in vivo, glargine does not exert its long-acting metabolic effects directly as glargine but predominantly via its main metabolite M1.

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/antiapoptotic 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.
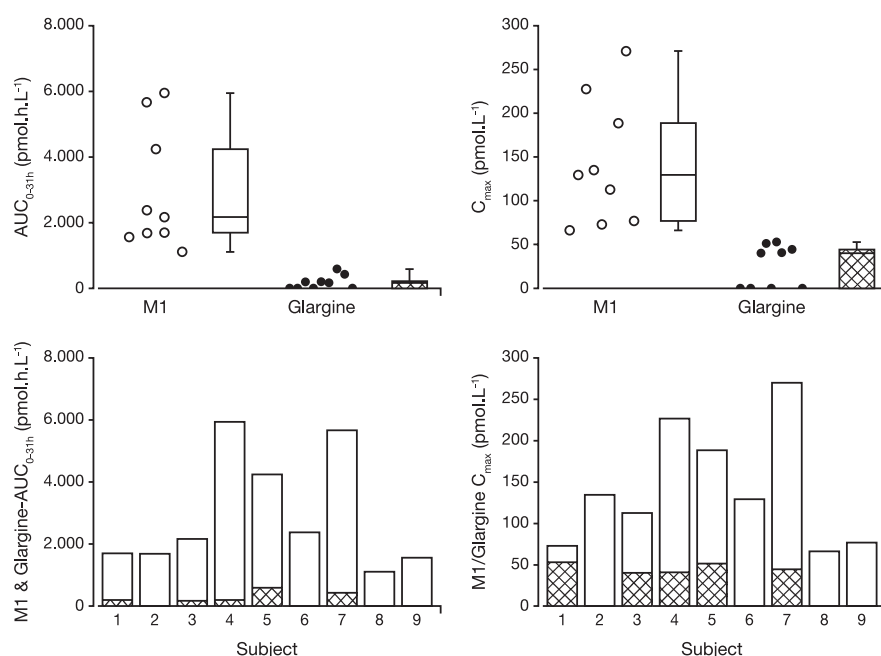
Figure 1—Top panel: AUC_{0-31h} of M1 and glargine in the nine individual subjects (M1, open circles; glargine, filled circles) and median and 10th, 25th, 75th, and 90th percentiles AUC_{0-31h} in the group of M1 (open box) and glargine (hatched box) (top left panel) and C_{max} of M1 (open circles) and glargine (filled circles) in individual subjects and median and 10th, 25th, 75th, and 90th percentiles C_{max} in the group of M1 (open box) and glargine (hatched box) (top right panel). Bottom panel: individual values of AUC_{0-31h} of M1 and glargine (LC-MS/MS) (bottom left panel) and C_{max} of M1 and glargine (M1 open columns; glargine, hatched columns) in the nine subjects.

References

1. 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

2. 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

3. 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

4. Bolli GB, Hahn AD, Schmidt R, et al. 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. Diabetes Care 2012;35:2626–2630

5. 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

6. 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

7. 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

8. Ciaraldi TP, Sasaoka T. Review on the in vitro interaction of insulin glargine with the insulin/insulin-like growth factor system: potential implications for metabolic and mitogenic activities. Horm Metab Res 2011;43:1–10

9. Shukla A, Grisouard J, Ehemann V, Hermani A, Enzmann H, Mayer D. Analysis of signaling pathways related to cell proliferation stimulated by insulin analogs in human mammary epithelial cell lines. Endocr Relat Cancer 2009;16:429–441

10. Schmidt N, Welte S, Sommereld M, Muller G, Tschank G, Tennagels N. Analysis of signalling pathways stimulated by insulin, [Asp^{B10}]insulin and insulin glargine and its metabolites in the human mammary epithelial cell lines MCF7 and MCF10. Diabetologia 2011;54 (Suppl. 1):S235

11. Varewijck AJ, Janssen JA, Vähätalo M, Hofland LJ, Lamberts SW, Yki-Järvinen H. Addition of insulin glargine or NPH insulin to metformin monotherapy in poorly controlled type 2 diabetic patients decreases IGF-I bioactivity similarly. Diabetologia 2012;55:1186–1194