SUPPLEMENTARY DATA

Glargine, M1 and M2 specific assay

A total of 300 µl of plasma sample was mixed with 50 µl of a working internal standard solution (50 ng.ml-1 of 15N72- glargine, 25 ng.ml-1 of 15N64-M1 and 25 ng.ml-1 of 15N63-M2) and 300 µl of PBS buffer pH 7.8. Subsequently, glargine and its metabolites were extracted using an immunoaffinity purification protocol (1) and determined with a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system.

A total of 60 µL of the extract (300 µL in total) was injected into the liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. Lower limit of quantification was 0.2 ng/mL for insulin glargine, M1 and M2. The LC-MS/MS system consisted of an API 5000 triple quadrupole mass spectrometer (AB Sciex, Darmstadt, Germany) equipped with a Turbo-V-source operating in positive mode and connected to an Ultimate 3000 HPLC system (Dionex, Idstein, Germany). An inlet valve was used to truncate non-relevant signals (10-port, VICI Valco Instruments, Houston, TX, USA). For the chromatography of insulin glargine, M1 and M2, a reversed phase column was used at 40 °C. A linear gradient was employed at a flow rate of 0.6 ml/min using water/formic acid (100:0.5, v/v) as mobile phase A and acetonitrile/formic acid (100:0.5, v/v) as mobile phase B. Total run time was 8.25 min. Retention time of insulin glargine, M1 and M2 were 2.07, 2.13 and 2.13 min, respectively. Retention time of the internal standards $^{15}N_{72}$-insulin glargine, $^{15}N_{64}$-M1 and $^{15}N_{63}$-M2 were 2.07, 2.13 and 2.13 min, respectively. The mass spectrometer was operated in the positive ion mode with an electrospray voltage of +5500 V at 600 °C. Nebulizer gas (GS1) was set to 80 psi, heater gas (GS2) to 90 psi and curtain gas to 50 psi. Collision gas was set to 7 instrument units. Position of the electrospray needle was 5 mm (horizontal) and 5 mm (vertical). Multiple reaction monitoring (MRM) was used for quantification. The mass transitions used were *m/z* 867.0 → *m/z* 136.1 for insulin glargine, *m/z* 959.7 → *m/z* 136.1 for M1, *m/z* 942.9 → *m/z* 136.1 for M2, *m/z* 877.4 → *m/z* 137.2 for $^{15}N_{72}$-insulin glargine, *m/z* 970.0 → *m/z* 137.2 for $^{15}N_{64}$-M1 and *m/z* 953.0 → *m/z* 137.2 for $^{15}N_{63}$-M2. All quadrupoles were working at unit resolution. Quantitation was performed with Analyst Software V1.4.2 (AB Sciex, Darmstadt, Germany) using the internal standard method. Ratios of analyte peak area and internal standard peak area (y-axis) were plotted against concentration (x-axis), and calibration curves were calculated by least square regression with 1/concentration^2 weighting.

There was no cross reactivity of the LC-MS/MS assay against human insulin, insulin lispro (Liprolog®), liraglutide (Victoza®), exenatide (Byetta®), insulin glulisine (Apidra®), detemir (Levemir®) or insulin aspart (Novorapid®).

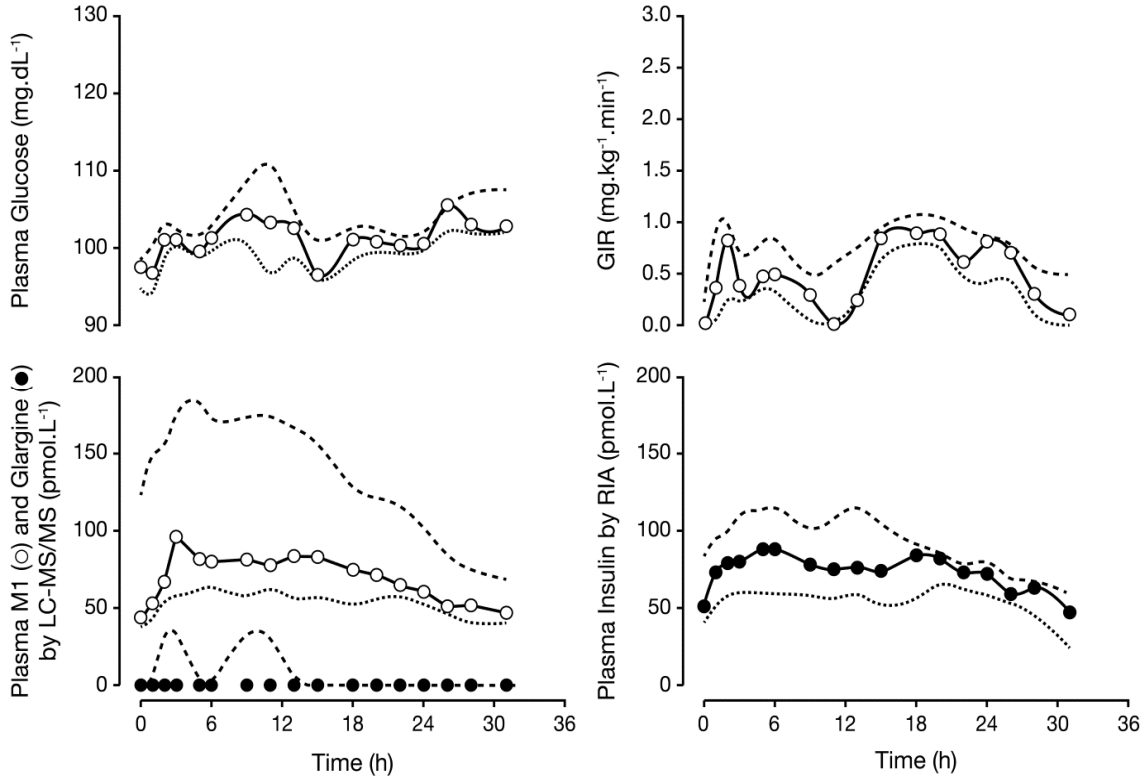
SUPPLEMENTARY DATA

Supplementary Table 1. *Clinical characteristics of the nine subjects with type 2 diabetes studied*

M/F	6/3
Age (y)	61.9 ± 8.7
Weight (kg)	72.5 ± 8.0
Body mass index (kg/m^2)	29.3 ± 3.0
Known DM duration (y)	12.7 ± 10
A1C (%)	7.4 ± 0.8
Fasting C-peptide (ng/ml)	1.4 ± 1.1
Treatments	all on insulin ± OHA[§]
Insulin dose (U/kg/day)*	total 0.69 ± 0.47 basal 0.35 ± 0.21

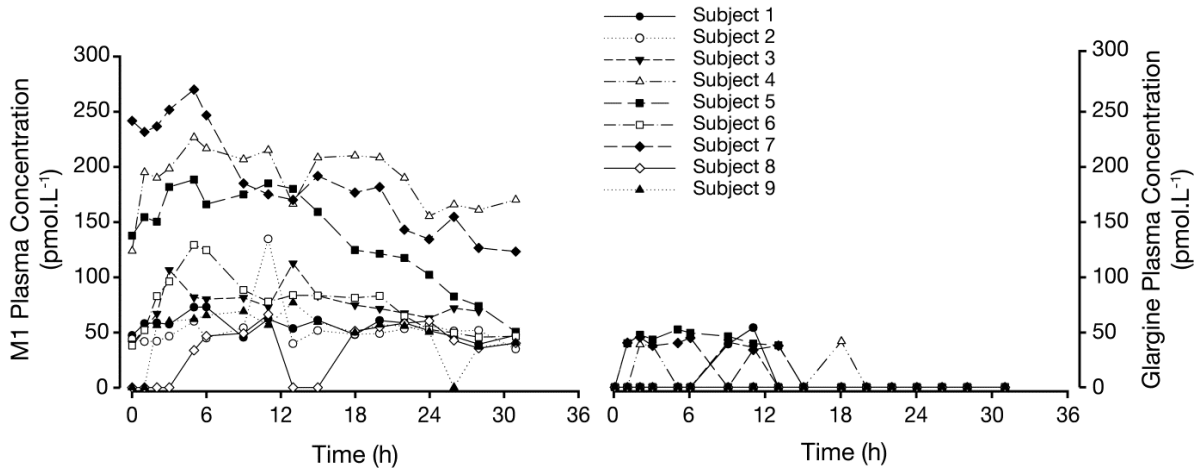
Data are mean ± SD. [§] All nine subjects on basal insulin (neutral protamine Hagedorn [NPH]), seven on basal-bolus; five subjects were also on metformin (three on basal-bolus, two basal insulin only) and one basal insulin only + repaglinide. *Prandial: rapid-acting insulin analogue; basal: NPH insulin. OHA, oral hypoglycemic agent.

Supplementary Figure 1. Median plasma glucose (upper left panel), rates of glucose infusion (GIR) (upper right panel), plasma concentrations of M1 (open circles) and glargine (full circles) measured by LC-MS/MS (lower left panel), and plasma concentration of insulin measured by RIA (lower right panel) in the nine subjects with type 2 diabetes given s.c. injection of glargine 0.4 U/Kg. Continuous and broken lines below and above the median represent 25th and 75th percentile distribution.



SUPPLEMENTARY DATA

Supplementary Figure 2. Individual plasma M1 (left panel) and glargine (right panel) concentrations in the 9 subjects with T2DM measured by LC-MS/MS.



Reference

1. Thevis M, Thomas A, Delahaut P, Bosseloir A, Schanzer 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.