SUPPLEMENTARY DATA

STUDY DESIGN Euglycemic clamp.

The target plasma glucose was 100 mg/dl. Subjects were admitted to the General Clinical Study Unit of the Section of Internal Medicine, Endocrinology and Metabolism, University of Perugia, between 11.00-11.30 h on the day of the study. The last administration of OHA was the day before the study. At 12:00 h subjects were served a standardized meal (688 kcal, 54% carbohydrate, 30% protein, and 16% lipids) after a s.c. dose of lispro insulin rapid-acting insulin analogue (Humalog, Lilly and Co, Indianapolis, USA). At 15.30 h, subjects were placed in bed rest and maintained a supine position till the end of the experiments. Methods for arterializing the venous blood, and infusing insulin and glucose, have been previously described (3). At 16.00 h, an i.v. infusion of regular human insulin (Humulin R; Lilly and Co, Indianapolis, USA) was initiated and continued to maintain plasma glucose at 118-135 mg/dl until 18.00 h, and at 100 mg/dl until 22.00 h (3). The study was terminated 32 hours after the s.c. injection of basal insulin, or earlier if plasma glucose increased to above 150 mg/dl in the absence of glucose infusion (end of study). At -120 min (20:00 h) from time "0 min" (22:00 h), a primed sterile, pyrogen-free constant infusion (0.222 μmol·kg^{-1}·min^{-1}) of [6,6-$^{2}H_2$]-glucose (Cambridge Isotopes Laboratories, Cambridge, MA, USA) was started and maintained throughout to determine glucose kinetics. During the clamp a variable rate of infusion of 20% glucose enriched to 2% with [6,6-$^{2}H_2$]-glucose was used to avoid non steadystate errors in measurement of glucose turnover (4).

To ensure blinding, a simple randomization was used based on computer-generated random numbers by a person who was not involved in establishing eligibility and entry of subjects.

Concealment of the randomization was ensured by having the allocation codes in a locked unreadable computer file handled by a designated investigator, who assigned subjects insulin cartridges corresponding to the week of treatment. The same independent investigator gave subjects the s.c. injection of NPH, detemir or glargine insulin in all clamp studies, by means of a 50 U insulin syringe (abdominal area, 2 cm to the left or to the right of the umbilicus).

ANALYTICAL METHODS

Plasma glucose was measured bedside (3). Plasma C-peptide, glucagon and, A1C, plasma glycerol, β-hydroxy-butyrate, lactate, alanine, free fatty acids were measured as previously described (3). Plasma insulin concentrations were quantified with a non specific human insulin radioimmunoassay (RIA, Linco Research, St. Charles, MO, USA). To determine glucose kinetics, arterialized-venous blood samples were taken every 30 min for the first 6 hours and then every 60 min during the studies. All blood samples were drawn into tubes containing EDTA and centrifuged. Plasma was stored at –80°C. Glucose enrichment was determined on its penta-acetate (penta-O-acetyl-β-D-glucopyranose) derivative by gas chromatography–mass spectrometry (GC HP 5890 II, MS HP 5972A, Hewlett-Packard Co., Palo Alto, CA, USA) in electron impact ionization mode monitoring the ions 200 and 202 for the unlabeled and D-[6,6-2H2]glucose, respectively (4).

END-POINTS OF STUDY

The primary endpoint of the study was the glucose infusion rate (GIR) [AUC 0-32h]. Secondary endpoints were glucose infusion rate (GIR) [AUC 0-16 h, AUC 16-32 h], duration of action, plasma glucose concentration, glucose kinetics, plasma C-peptide, glucagon, non-glucose substrates, infusion rates of regular insulin (total infusion of regular insulin) prior to s.c. basal insulin injection, and plasma insulin concentrations.

STATISTICAL ANALYSIS

The linear trapezoidal rule was used to calculate the area under the time concentration curve. C_{max} and T_{max} were read directly from the data for each subject. GIR data were smoothed by taking a three point moving average in order to provide reliable data for calculation of GIR C_{max} and GIR T_{max}. A mixed model for a three-period cross-over design using a two-sided test with a nominal significance level of 0.05. This model included sequence and treatment as fixed factors and patient nested within sequence as a random effect. The mean differences or mean ratios (for log-transformed data) between treatments were estimated along with their 95% confidence intervals (CI) (6). Additional pairwise comparisons among treatments were performed

using Fisher's protected testing procedure to control type I errors due to multiple comparisons. T_{max} and duration of action variables were analyzed by nonparametric analysis.
For figure construction only, when clamp studies were interrupted in advance, missing values were treated according to "last observation carried forward" principle that assumes that the variable remains constant at the last observed value. In our study this method provides a conservative estimate of missing observations.
With a sample size of 18 subjects the two-sided test at the 5% significance level of a 3x3 cross-over design had 83% power of detecting a difference of $GIR_{0\text{-}32h}$ of 30% between the treatments with the SD of the differences of 41%. Data are means ± SD unless stated otherwise. Data in figures are expressed as mean ± SE. Statistical analysis was usually performed using NCSS 2007/PASS 2008 (Kaysville, UT, USA) .

RESULTS

Glycemic control and insulin doses in the week prior to studies

After randomization, glycemic control (mean blood glucose from self-monitoring data) over days 1-7 of the one-week treatment with NPH (128±14 mg/dl), detemir (130±15 mg/dl) and glargine (129±13mg/dl mg/dl) was no different (p=0.512). Total daily insulin doses over 3 days prior to studies were greater with detemir (0.65±0.38) vs NPH (0.62±0.36, p=0.006) and glargine (0.59±0.34 U/Kg, p=0.001) due to greater doses of rapid-acting analog needed with detemir (0.31±0.26 vs NPH 0.28±0.23, p=0.004, and glargine 0.26±0.23 U/kg, p=0.001) with no difference between NPH and glargine (p=0.109).

Plasma Glucose concentration and rates of i.v. insulin infusion prior to s.c. insulin injection

Plasma glucose concentration decreased similarly during i.v. insulin infusion on the three occasions, and at time-point 0 was similar with NPH, detemir and glargine (98±4.3, 96±6.4 mg/dl and 97±3.7 mg/dl, respectively, p= 0.680). Over the last two hours of feed-back insulin infusion, the insulin dose given to maintain euglycemia was greater with detemir (0.04±0.07 U/kg) as compared to NPH and glargine (0.02±0.08, and 0.01±0.05 U/Kg, respectively, although the difference was not significant (p=0.071) (Appendix Table 3). After the s.c. injection of basal insulin at time 0 h (22:00 h), the rate of i.v. insulin infusion decreased similarly among treatments and was ended within one hour.

SUPPLEMENTARY DATA

Table 1. Demographic and clinical characteristics of the subjects.

Number	18
M/F	12/6
Age (years)	60 ± 7 (49-73)
Weight (Kg)	79 ± 9.5 (58-93)
BMI (Kg/m^2)	29.1 ± 3.2 (23.8-35)
Known DM duration (years)	12.8 ± 7.5 (2-34)
A1C (%)	7.5 ± 0.6 (6.8-8.5)
C-peptide (ng/ml)	1.54 ± 0.91 (0.11-3.54)
Treatment	all on insulin ± OHA[§]
Insulin dose (U/kg/day)*	total 0.64 ± 0.42 (0.12-1.54)
	basal 0.34 ± 0.17 (0.12-0.82)

[§] All 18 subjects on basal insulin, 13 on basal-bolus;

9 subjects were also on metformin (5 on basal-bolus,

3 basal insulin only, 1 basal insulin only + repaglinide;

1 subject on basal insulin only + repaglinide)

* prandial: rapid-acting insulin analogue; basal: NPH insulin

Data are Mean±SD and ranges (min-max)

Supplementary Table 2. Results of PD/PK study (0-32h) of insulins NPH, detemir and glargine in T2DM.

	NPH (A)	Detemir (B)	Glargine (C)	Difference /Ratio	95% confidence intervals	*P* values
$GIR_{AUC\ 0\text{-}32\ h}$ [mg/kg] †	1170±703	1081±785	1538±688	89 (A-B)	-226, 403	0.568
P value‖		**0.014**		-368 (A-C)	-683, -53	**0.023**
				-457 (B-C)	-771, -141	**0.006**
GIR C_{max} [$mg \cdot kg^{-1} \cdot min^{-1}$]‡	1.25±1.58	1.26±0.55	1.42±0.54	-0.01 (A-B)	-0.2 ;0.21	0.931
P value‖		0.251		-0.16 (A-C)	-0.4 ; 0.37	0.123
				-0.15 (B-C)	-5.5 ; 0.36	0.144
GIR T_{max} [hours]‡	18 (6- 23)	11 (8-15)	15 (2-16)	3.8 (A-B)	-2 ; 10	0.214
P value‖		0.211		4.3 (A-C)	-2.5 ; 9.5	0.144
				-0.3 (B-C)	-5.2 ; 4.5	0.861
$FFA_{AUC\ 0\text{-}32\ h}$ [mM/l] †	13.8±2.4	16.3±3.3	13.0±2.9	85% (A/B)	77 ; 94	**0.003**
P value‖		**0.000**		107% (A/C)	97 ; 116	0.157
				126% (B/C)	115 ; 139	**0.000**
$B\text{-}OH_{AUC\ 0\text{-}32\ h}$ [mM/l] †	14±6.2	19±16	13±9.7	80% (A/B)	61; 104	0.095
P value‖		**0.039**		113% (A/C)	86 ; 147	0.370
				141% (B/C)	108 ; 184	**0.013**
$PG_{AVG\ 0\text{-}32\ h}$ [ml/dl] †	108±15	108±14	102±2	99% (A/B)	95; 104	0.802
P value‖		**0.049**		105% (A/C)	100; 109	**0.044**
				105% (B/C)	101; 110	**0.025**
$C\text{-}P_{AUC\ 0\text{-}32\ h}$ [ng/ml] †	31±22	33±27	28±20	96% (A/B)	88; 104	0.319
P value‖		**0.002**		112% (A/C)	103;122	**0.012**
				117% (B/C)	103;122	**0.000**
$IRG_{AUC\ 0\text{-}32\ h}$ [pg/ml] †	1690±643	1731±569	1577±497	97% (A/B)	90; 104	0.338
P value‖		**0.031**		106% (A/C)	99 ; 114	0.084
				110% (B/C)	102 ; 117	**0.009**
$EGP_{AVG\ 0\text{-}32\ h}$ (µmol/Kg/min] †	5.2±2.5	5.8±2.7	4.5±2.2	-0.53 (A-B)	-1.58; 0.51	0.308
P value‖		**0.039**		0.73 (A-C)	-0.29; 1.8	0.152
				1.28 (B-C)	0.24; 2.34	**0.017**
$GU_{AVG\ 0\text{-}32\ h}$ [µmol/Kg/min] †	7.8±1.6	8.2±1.3	7.9±1.3	-0.39 (A-B)	-0.99; 0.15	0.194
P value‖		0.397		-0.10 (A-C)	-0.70; 0.5	0.747
				0.29 (B-C)	-0.31; 0.89	0.329
MDA(Minimal Duration of Action) (h) ‡	32 (30-32)	31(27-32)	32 (32-32)	0.4 (A-B)	-1.75; 2.7	0.431
P value‖		**0.013**		0 (A-C)	-2.75; 0	0.499
				-1.4 (B-C)	-4; -0.5	**0.002**
DA (Duration of Action) (h) ‡	32 (32-32)	32(30-32)	32 (32-32)	0 (A-B)	0; 1.25	0.241
P value‖		**0.040**		0 (A-C)	-1.5; 0.25	0.813
				-0.75 (B-C)	-2.5; 0	**0.008**

†Arithmetic mean±SD. ‡Median (25th- to 75th-percentile). ‖ *P* value across all comparisons.

Supplementary data Table 3(I). Results of PD/PK study (0-16h; 16-32h) of NPH, detemir and glargine in T2DM.

	NPH (A)	Detemir (B)	Glargine (C)	Difference /Ratio	95% confidence intervals	*P* values
$GIR_{AUC\ 0\text{-}16\ h}$ [mg/kg] †	461±382	593±437	693±365	-132 (A-B)	-360; 95	0.242
P value□		0.128		-232 (A-C)	-485; 20	0.069
				-100 (B-C)	-327; 127	0.376
$GIR_{AUC\ 16\text{-}32\ h}$ [mg/kg] †	709±441	488±386	845±483	221 (A-B)	62; 381	**0.008**
P value□		**0.000**		-136 (A-C)	-294; 24	0.093
				-357 (B-C)	-516; -197	**0.000**
$FFA_{AUC\ 0\text{-}16\ h}$ [mM/l] †	6.4±1.7	6.8±1.7	5.5±1.3	93% (A/B)	83; 105	0.287
P value□		**0.005**		115% (A/C)	101; 129	**0.024**
				122% (B/C)	108; 137	**0.002**
$FFA_{AUC\ 16\text{-}32\ h}$ [mM/l] †	7.4±1.0	9.5±1.8	7.4±1.9	79% (A/B)	70; 88	**0.000**
P value□		**0.000**		102% (A/C)	108; 113	0.643
				129% (B/C)	116; 144	**0.000**
$B\text{-}OH_{AUC\ 0\text{-}16\ h}$ [mM/l] †	4.6±2.62	4.7±3.6	3.2±2.0	102% (A/B)	85; 122	0.824
P value□		**0.000**		146% (A/C)	122; 175	**0.000**
				143% (B/C)	119; 171	**0.000**
$B\text{-}OH_{AUC\ 16\text{-}32\ h}$ [mM/l] †	9.2±4	14.7±13	9.8±8	73% (A/B)	53; 102	0.061
P value□		0.067		105% (A/C)	76; 145	0.775
				142% (B/C)	99; 204	0.055
$PG_{AVG\ 0\text{-}16\ h}$ [ml/dl] †	106±10	105±10	102±3	101% (A/B)	98; 104	0.467
P value□		0.069		104% (A/C)	99; 108	0.067
				103% (B/C)	99; 106	0.108
$PG_{AVG\ 16\text{-}32\ h}$ [ml/dl] †	110±20	112±18	103±3	98% (A/B)	92; 104	0.465
P value□		**0.050**		105% (A/C)	99 ; 112	0.091
				108% (B/C)	101; 114	**0.018**
$PG_{0\text{-}16}\ C_{max}$ [ml/dl] †	119±18	116±18	111±7	102% (A/B)	97; 108	0.364
P value□		**0.050**		107% (A/C)	101; 113	**0.016**
				104% (B/C)	99; 110	0.117
$C\text{-}P_{AUC\ 0\text{-}16\ h}$ [ng/ml] †	16±11	16±12	14±11	102% (A/B)	93; 112	0.677
P value□		**0.011**		115% (A/C)	105; 127	**0.005**
				113% (B/C)	103; 124	**0.025**
$C\text{-}P_{AUC\ 16\text{-}32\ h}$ [ng/ml] †	15±10	17±14	14±9	90% (A/B)	81; 100	**0.043**
P value□		**0.003**		109% (A/C)	98; 120	0.111
				121% (B/C)	109; 133	**0. 000**

†Arithmetic mean±SD. □*P* value across all comparisons from ANOVA.

Supplementary data Table 3(II). Results of PD/PK study (0-16h; 16-32h) of NPH, detemir and glargine in T2DM.

	NPH (A)	Detemir (B)	Glargine (C)	Difference /Ratio	95% confidence intervals	*P* values
IRG*$_{AUC\ 0\text{-}16\ h}$ [pg/ml] †	853±321	857±280	781±233	99% (A/B)	91; 106	0.693
P value□		**0.037**		107% (A/C)	99; 116	0.075
				109% (B/C)	101; 118	**0. 032**
IRG*$_{AUC\ 16\text{-}32\ h}$ [pg/ml] †	836±333	874±297	796±272	95% (A/B)	86; 104	0.274
P value□		0.101		105% (A/C)	96; 116	0.278
				111% (B/C)	99; 1119	0. 072
EGP$_{AVG\ 0\text{-}16\ h}$ [µmol/Kg/min] †	6.2±2.8	6.2±3.1	5.4±2.5	-0.01 (A-B)	-1.39; 1.42	0.987
P value□		0.369		0.85 (A-C)	-0.6; 2.2	0.226
				0.86 (B-C)	-0.54; 2.3	0.221
EGP$_{AVG\ 16\text{-}32\ h}$ [µmol/Kg/min] †	3.5±2.9	4.7±2.3	3.1±2.9	-1.2 (A-B)	-2.4; -0.38	**0.038**
P value□		**0.003**		0.5 (A-C)	-0.58; 1.6	0.348
				1.68 (B-C)	0.58; 2.8	**0.000**
GU$_{AVG\ 0\text{-}16\ h}$ [µmol/Kg/min] †	8.3±1.8	8.7±1.6	8.5±1.6	-0.4 (A-B)	-1.1; 0.4	0.331
P value□		0.617		-0.16 (A-C)	-0.9; 0.6	0.652
				0.18 (B-C)	-0.5; 0.9	0.597
GU$_{AVG\ 16\text{-}32\ h}$ [µmol/Kg/min] †	6.8±1.6	7. 2±1.1	6.9±1.2	-0.37 (A-B)	-0.96; 0.2	0.211
P value□		0.410		-0.07 (A-C)	-0.7; 0.6	0.808
				0.3 (B-C)	-0.28; 0.89	0.302
IIR§$_{AUC\text{-}2\text{-}0\ h}$ [U/Kg] †	0.02±0.08	0.04±0.17	0.01±0.05	-0.02 (A-B)	-0.01; 1.01	0.064
P value□		0.071		0.0 (A-C)	-0.14; 0.19	0.771
				0.02 (B-C)	-0.01; 0.37	0.061

†Arithmetic mean±SD. *P* value across all comparisons from ANOVA. IRG*: Immunoreactive Glucagon.

IIR§: Insulin Infusion Rate during feedback

SUPPLEMENTARY DATA **FIGURE 1**