SUPPLEMENTARY DATA

Pharmacokinetics and Pharmacodynamics of Three Different Formulations of Insulin Aspart: A Randomized, Double-Blind, Crossover Study in Men with Type 1 Diabetes

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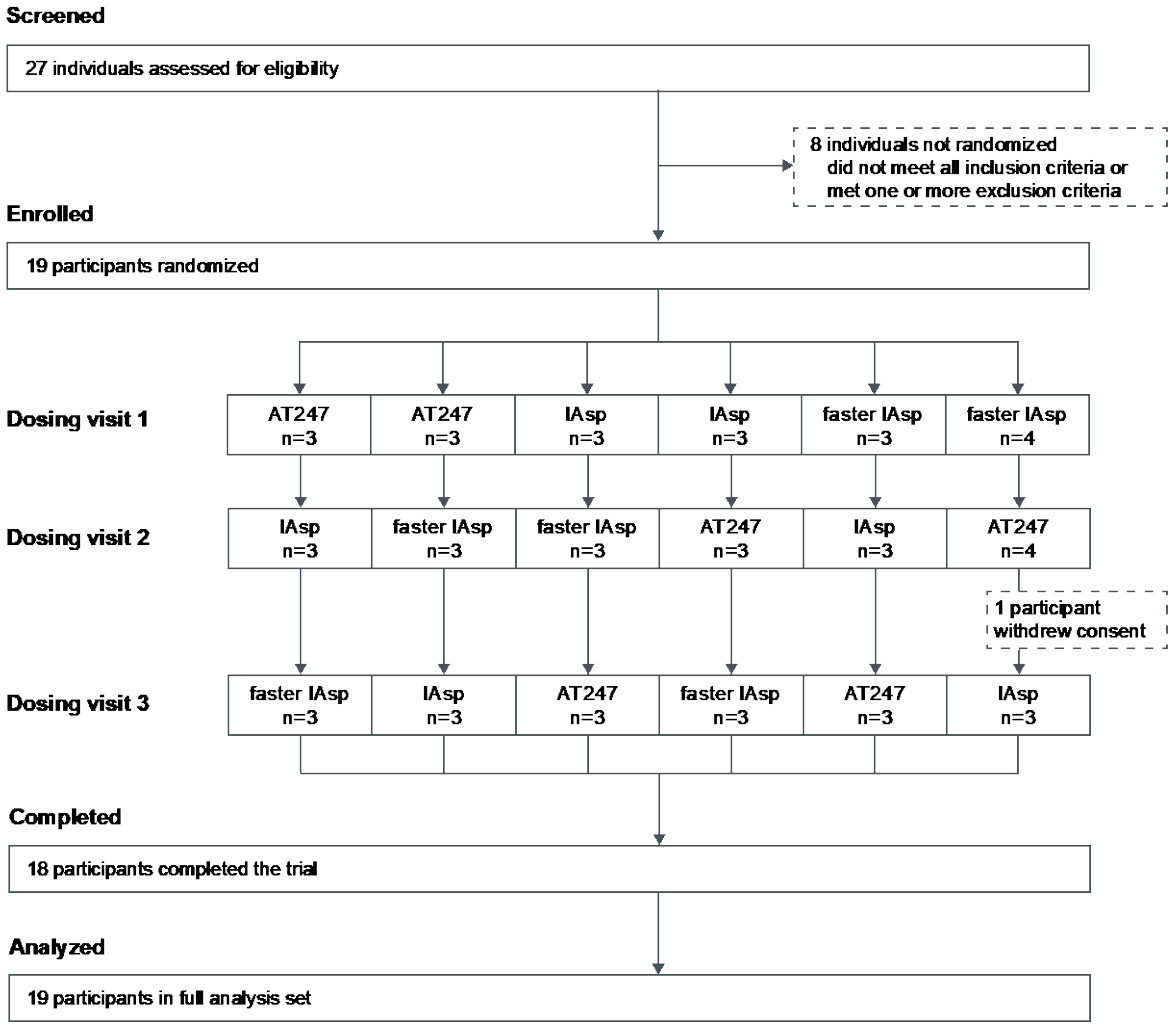
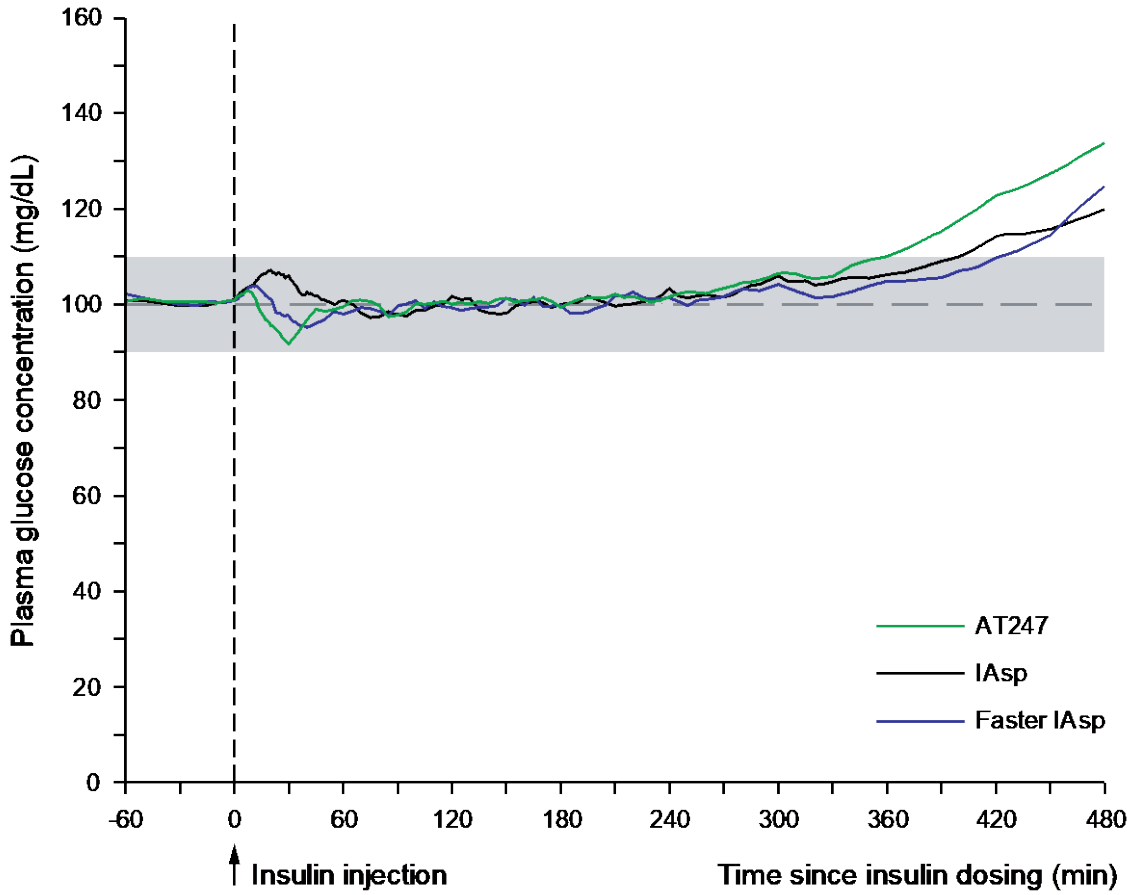
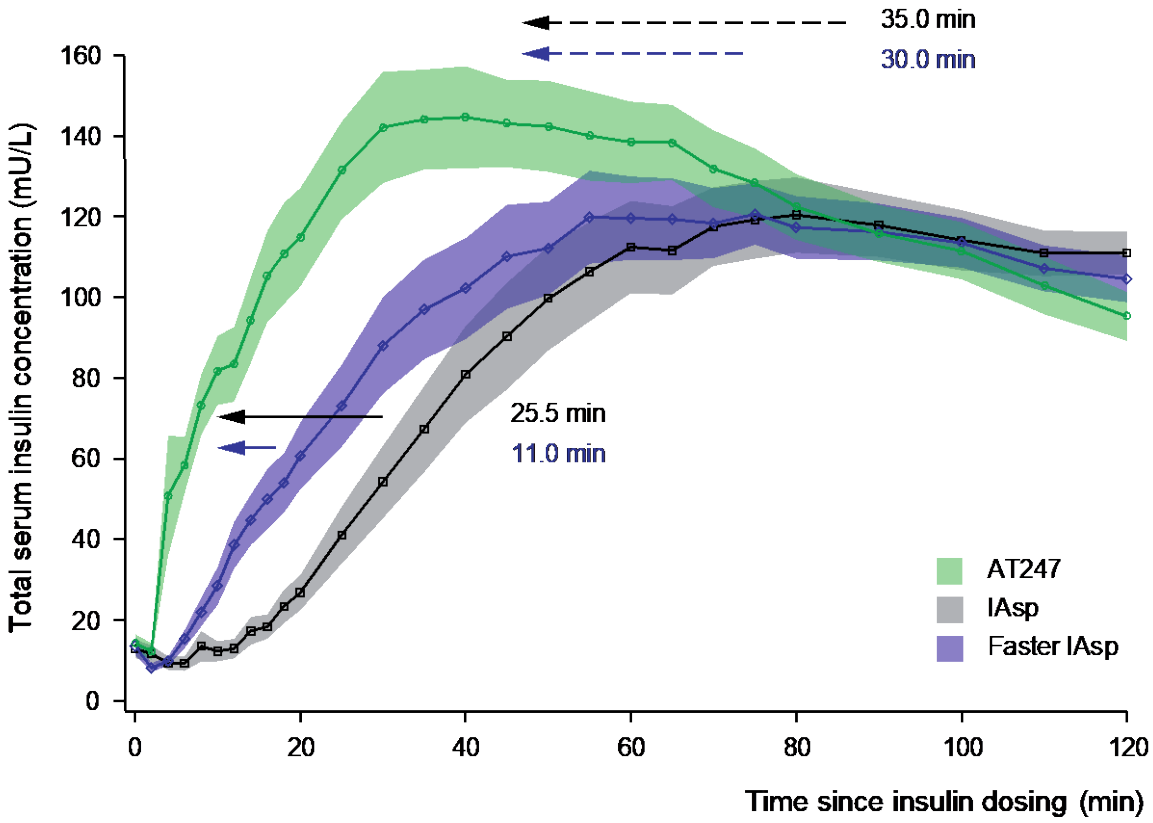


Supplementary Figure 1. Participant disposition and study design (AT247, novel formulation of insulin aspart; IAsp, NovoRapid®; faster IAsp, Fiasp®). Dosing visits were separated by a 3- to 15-day washout period.



Supplementary Figure 2. Mean plasma glucose concentration during the euglycemic clamps after subcutaneous injection of 0.3 units/kg of a novel insulin aspart formulation (AT247), IAsp, or faster IAsp in men with type 1 diabetes. The gray-shaded area shows the plasma glucose clamp target range of 100 mg/dL (5.5 mmol/L) ± 10%. Number of participants: 19 for AT247 and faster IAsp; 18 for IAsp.



Supplementary Figure 3. Mean total insulin (sum of human insulin and insulin aspart) concentration-time profile after subcutaneous administration of 0.3 units/kg of a novel insulin aspart formulation (AT247), IAsp, or faster IAsp for 2 h postdose. Variability bands show the SEM. Arrows represent differences in time to 50% of maximum total insulin concentration ($t_{Early50\%Cmax}$ treatment difference [95% CI] AT247–IAsp: -25.5 min [-31; -19], *P*=0.0004; AT247–faster IAsp: -11.0 min [-13; -6], *P*=0.0004) and in time to maximum total insulin concentration (t_{max} treatment difference [95% CI] AT247–IAsp: -35.0 min [-80; -20], *P*=0.0004; AT247–faster IAsp: -30 min [-50; -10], *P*=0.0028). Number of participants: 19 for AT247 and faster IAsp; 18 for IAsp.

Supplementary Table 1. Glucose clamp quality for a novel formulation of insulin aspart (AT247) and currently marketed insulin aspart formulations (IAsp and faster IAsp)

	AT247* *n* = 19	IAsp* *n* = 18	Faster IAsp* *n* = 19
Precision (%)†	4.5 ± 1.2	5.9 ± 2.6	4.4 ± 1.4
Control deviation (mg/dL)‡	0.40 ± 1.49	2.80 ± 4.04	0.62 ± 1.65

* Data are presented as arithmetic mean ± SD.

† Coefficient of variation of the plasma glucose measurements.

‡ Mean difference between the plasma glucose measurements and the target glucose level.

Supplementary Table 2. Primary analysis results for area under the curve endpoints

	Treatment ratio* (95% CI) *P* AT247-to-IAsp	Treatment difference† (95% CI) *P* AT247–IAsp	Treatment ratio* (95% CI) *P* AT247-to-faster IAsp	Treatment difference† (95% CI) *P* AT247–faster IAsp
Exposure (mU•h/L)				
$AUC_{Asp,0\text{-}16min}$	nc	11.97 (6.72; 17.06) 0.0004	nc	8.38 (4.68; 12.27) 0.0003
$AUC_{Asp,0\text{-}30min}$	6.91 (5.09; 9.38) <0.0001	nc	2.25 (1.67; 3.04) <0.0001	nc
$AUC_{Asp,0\text{-}60min}$	2.52 (2.13; 2.97) <0.0001	nc	1.56 (1.33; 1.83) <0.0001	nc
$AUC_{Asp,0\text{-}90min}$	1.72 (1.50; 1.97) <0.0001	nc	1.31 (1.15; 1.50) 0.0003	nc
$AUC_{Asp,0\text{-}2h}$	1.43 (1.26; 1.62) <0.0001	nc	1.19 (1.05; 1.35) 0.0068	nc
$AUC_{Asp,0\text{-}8h}$	1.03 (0.94; 1.12) 0.5612	nc	0.96 (0.89; 1.05) 0.3929	nc
Glucose-lowering effect (mg/kg)				
$AUC_{GIR,0\text{-}16min}$	nc	0.00 (0.00; 3.67) 0.012	nc	0.00 (0.00; 3.67) 0.0124
$AUC_{GIR,0\text{-}30min}$	nc	36.79 (11.64; 45.09) 0.0004	nc	18.94 (5.43; 38.06) 0.0007
$AUC_{GIR,0\text{-}60min}$	nc	143.86 (82.98; 189.76) 0.0004	nc	81.43 (59.76; 139.01) 0.0009
$AUC_{GIR,0\text{-}90min}$	nc	197.47 (129.58; 259.86) 0.0004	nc	94.22 (30.53; 205.50) 0.0128
$AUC_{GIR,0\text{-}2h}$	1.81 (1.49; 2.20) <0.0001	nc	1.27 (1.05; 1.53) 0.015	nc
$AUC_{GIR,0\text{-}8h}$	nc	44.38 (-171.81; 323.37) 0.4268	nc	-75.23 (-164.96; 196.00) 0.7133

Number of participants: 19 for AT247 and faster IAsp; 18 for IAsp. nc: not calculable/calculated.

* Mean treatment ratios based on log-transformed data analyzed by means of a mixed effects model and results back-transformed to the original scale, *P* calculated using the student t-test.

† Median treatment difference based on untransformed data, *P* calculated using the Wilcoxon rank sum test.

Supplementary Table 3. Exposure and glucose-lowering effect for a novel formulation of insulin aspart (AT247) versus currently marketed insulin aspart formulations (IAsp and faster IAsp)

	AT247* *n* = 19	IAsp* *n* = 18	Faster IAsp* *n* = 19	Treatment ratio† (95% CI) AT247-to-IAsp	Treatment ratio† (95% CI) AT247-to-faster IAsp
Exposure (mU•h/L)					
$AUC_{Asp,0\text{-}16min}$	14.3 ± 8.0	1.2 ± 1.2	5.2 ± 3.9	12.0 (9.3; 19.0)	2.7 (2.1; 3.8)
$AUC_{Asp,0\text{-}30min}$	41.6 ± 20.4	8.1 ± 6.8	20.4 ± 13.3	5.3 (4.3; 7.5)	2.0 (1.7; 2.6)
$AUC_{Asp,0\text{-}60min}$	111.1 ± 45.0	50.4 ± 31.9	73.8 ± 38.6	2.3 (1.9; 2.9)	1.5 (1.3; 1.8)
$AUC_{Asp,0\text{-}90min}$	174.3 ± 61.7	108.1 ± 51.3	133.8 ± 54.7	1.7 (1.4; 2.0)	1.3 (1.1; 1.5)
$AUC_{Asp,0\text{-}2h}$	226.8 ± 72.1	163.9 ± 61.7	189.4 ± 64.0	1.4 (1.2; 1.7)	1.2 (1.1; 1.4)
$AUC_{Asp,0\text{-}8h}$	415.4 ± 87.7	403.9 ± 71.0	426.6 ± 74.8	1.1 (1.0; 1.1)	1.0 (0.9; 1.1)
Glucose-lowering effect (mg/kg)					
$AUC_{GIR,0\text{-}16min}$	1.6 ± 2.5	0.0 ± 0.0	0.0 ± 0.0	nc	nc
$AUC_{GIR,0\text{-}30min}$	33.9 ± 24.8	2.9 ± 7.6	11.7 ± 13.0	11.9 (nc)	2.9 (2.1; 5.0)
$AUC_{GIR,0\text{-}60min}$	220.0 ± 113.1	81.2 ± 97.7	132.1 ± 94.3	2.8 (2.0; 5.5)	1.7 (1.3; 2.3)
$AUC_{GIR,0\text{-}90min}$	423.8 ± 193.0	240.7 ± 221.3	317.2 ± 164.0	1.8 (1.4; 2.7)	1.3 (1.1; 1.6)
$AUC_{GIR,0\text{-}2h}$	655.9 ± 289.1	440.0 ± 318.0	517.6 ± 236.7	1.5 (1.3; 2.0)	1.3 (1.1; 1.5)
$AUC_{GIR,0\text{-}8h}$	1741.7 ± 610.7	1719.1 ± 662.4	1751.6 ± 544.9	1.0 (0.9; 1.1)	1.0 (0.9; 1.1)

nc: not calculable

* Data are presented as arithmetic mean ± SD.

† Mean treatment ratio (calculated using the Fieller method).

Supplementary Table 4. Per-protocol and post hoc analysis results for onset of action

Onset of action	AT247* *n* = 19	IAsp* *n* = 18	Faster IAsp* *n* = 19	Treatment ratio† (95% CI) *P* AT247-to-IAsp	Treatment ratio† (95% CI) *P* AT247-to-faster IAsp
Per-protocol definition	17.0 min (13.0; 24.0)	37.0 min (35.0; 63.0)	23.0 min (22.0; 35.0)	-23.0 min (-37; -15) 0.0004	-9.0 min (-11; -3) 0.0006
Post hoc definition	14.0 min (13.0; 16.0)	28.5 min (23.0; 36.0)	19.0 min (17.0; 24.0)	-14.0 min (-22; -10) 0.0006	-5.0 min (-9; -3) 0.0003

Per-protocol definition: time after insulin injection until plasma glucose concentration has decreased at least 5 mg/dL (0.3 mmol/L) from the baseline.
Post hoc definition: time after insulin injection until plasma glucose concentration has decreased at least 5 mg/dL (0.3 mmol/L) from highest plasma glucose level measured postdose.
* Data are presented as median (25th percentile; 75th percentile).
† Median treatment difference (treatment comparison calculated using the Wilcoxon rank sum test using untransformed parameters).

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CONSORT checklist of information to include when reporting a randomized crossover trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomized crossover trial in the title	1
	1b	Specify a crossover design and report all information according to the CONSORT for abstracts checklist (structured summary of trial design, methods, results, and conclusions)	1
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	2
	2b	Specific objectives or hypotheses	2
Methods			
Trial design	3a	Rationale for a crossover design. Description of the design features including allocation ratio, especially the number and duration of periods, duration of washout period, and consideration of carry over effect	2
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n.a.
Participants	4a	Eligibility criteria for participants	2
	4b	Settings and locations where the data were collected	2
Interventions	5	The interventions with sufficient details to allow replication, including how and when they were actually administered	2 - 3
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	3
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n.a.
Sample size	7a	How sample size was determined, accounting for within participant variability	3

	7b	When applicable, explanation of any interim analyses and stopping guidelines	n.a.
Randomization:			
Sequence generation	8a	Method used to generate the random allocation sequence	2
	8b	Type of randomization; details of any restriction (such as blocking and block size)	2
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	2
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to the sequence of interventions	2
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	2
	11b	If relevant, description of the similarity of interventions	2
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes which are appropriate for crossover design	3
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	3
Results			
Participant flow (a diagram is strongly recommended)	13a	The numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome, separately for each sequence and period	Supplementary Figure 1
	13b	No of participants excluded at each stage, with reasons, separately for each sequence and period	Supplement Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	3
	14b	Why the trial ended or was stopped	n.a.
Baseline data	15	A table showing baseline demographic and clinical characteristics by sequence and period	3 - 4
Numbers analyzed	16	Number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	3; Supplementary Figure 1

Outcomes and estimation	17a	For each primary and secondary outcome, results including estimated effect size and its precision (such as 95% confidence interval)	4 - 6; Table 1; Supplementary Table 2 - 4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	n.a.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Supplementary Table 3 and 4
Harms	19	Describe all important harms or unintended effects in a way that accounts for the design	6
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses. Consider potential carry over effects.	6 - 7
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	7
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	6 - 7
Other information			
Registration	23	Registration number and name of trial registry	1 and 2
Protocol	24	Where the full trial protocol can be accessed, if available	n.a.
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	7

* Dwan K, Li T, Altman DG, Elbourne D. CONSORT 2010 statement: extension to randomised crossover trials. BMJ 2019; 366:l4378