


The association between anti-insulin aspart antibodies and the pharmacokinetic and pharmacodynamic characteristics of fast-acting insulin aspart in children and adolescents with type 1 diabetes

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Abstract

Background: Fast-acting insulin aspart (faster aspart) is a novel formulation of insulin aspart (IAsp) ensuring ultrafast absorption and effect.

Aim: To compare the pharmacokinetics between faster aspart and IAsp, based on free or total IAsp measurement, and investigate the association between anti-IAsp antibodies and faster aspart and IAsp pharmacological properties in children and adolescents with type 1 diabetes (T1D).

Methods: In a randomized, two-period crossover trial, 12 children, 16 adolescents, and 15 adults (6-11, 12-17, and 18-64 years) received 0.2 U/kg double-blind single-dose subcutaneous faster aspart or IAsp followed by a standardized liquid meal test.

Results: Across age groups, the pharmacokinetic profile was left-shifted including greater early exposure for faster aspart vs IAsp irrespective of free or total IAsp assay. Onset of appearance occurred 2.4 to 5.0 minutes (free) or 1.8 to 3.0 minutes (total) earlier for faster aspart vs IAsp ($P < .05$). Treatment ratios (faster aspart/IAsp) for 0 to 30 minutes IAsp exposure were 1.60 to 2.11 and 1.62 to 1.96, respectively (children, free: $P = .062$; otherwise $P < .05$). The ratio of free/total IAsp for overall exposure ($AUC_{IAsp,0-t}$) was negatively associated with anti-IAsp antibody level across age. Pooling with a previous similar trial showed no clear association between anti-IAsp antibodies and meal test 1- or 2-hour postprandial glucose increment independent of age and insulin treatment ($R^2 \leq .070$; $P \geq .17$).

Conclusions: In children and adolescents with T1D, faster aspart provides ultrafast pharmacokinetics irrespective of free or total IAsp assay. Elevated anti-IAsp antibodies are associated with higher total IAsp concentration, but do not impact faster aspart and IAsp glucose-lowering effect.

KEYWORDS

adolescents, children, insulin antibodies, pharmacodynamics, pharmacokinetics

1 | INTRODUCTION

The introduction of recombinant human insulin and insulin analogs has reduced the risk of developing anti-insulin antibodies. Still, the prevalence of diabetes patients of all ages with detectable anti-insulin antibodies remains considerable.¹⁻⁴ It has been proposed that anti-insulin antibodies may serve a buffering effect, thereby modifying the insulin pharmacokinetic profile and/or may lead to diminished insulin action through neutralizing effects.^{1,5-7} Furthermore, anti-insulin antibodies interfere with assays of exogenous insulin concentration.⁸ While total insulin (ie, bound and unbound) can be assayed without previous steps, measurement of the free, unbound fraction requires that anti-insulin antibodies are precipitated prior to analysis using polyethylene glycol (PEG).⁹ The pharmacokinetics of exogenous insulin in children and adolescents with type 1 diabetes (T1D) have been determined by measuring either free¹⁰ or total insulin.¹¹⁻¹³

Fast-acting insulin aspart (faster aspart) is a novel formulation of insulin aspart (IAsp) in which two additional excipients, L-arginine and niacinamide, are included to ensure formulation stability with ultrafast initial absorption and effect.¹⁴ Based on free IAsp measurement in adults, faster aspart is associated with accelerated pharmacokinetics compared with IAsp, which translate into earlier onset of action, 74% larger initial 30-minute glucose-lowering effect and reduced postprandial glucose (PPG) levels for faster aspart.¹⁵⁻¹⁹ In children and adolescents, based on free IAsp, faster aspart has also shown accelerated pharmacokinetics and the potential to reduce PPG relative to IAsp.²⁰ However, assessment of faster aspart pharmacokinetics based on total IAsp has been a request from some regulatory bodies. It was recently shown in adults that faster aspart pharmacokinetics were accelerated relative to IAsp irrespective of free or total IAsp assay.²¹ Moreover, a higher level of anti-IAsp antibodies was associated with an overall greater concentration of total IAsp but had no influence on faster aspart and IAsp glucose-lowering effect.²¹ The level of anti-insulin antibodies is often higher in children and adolescents vs adults, which may be due to increased immunological potential during childhood and higher level of insulin autoantibodies before development of T1D at a younger age.²

The aims of the present analysis in children, adolescents, and adults with T1D were to compare the pharmacokinetic properties between faster aspart and IAsp both when assessed as free and total IAsp, to investigate the association between anti-IAsp antibody level and measured serum concentrations of IAsp using both assays and, finally, to elucidate if there is any effect of anti-IAsp antibody level on faster aspart and IAsp glucose-lowering effect.

2 | METHODS

2.1 | Trial design

This was a single-center (Kinder- und Jugendkrankenhaus AUF DER BULT, Hannover, Germany), randomized, double-blind, two-period crossover trial in children, adolescents, and adults with T1D. Before

initiation of the trial, the appropriate health authority and ethics committee reviewed and approved the protocol. The trial was performed according to the Declaration of Helsinki, Good Clinical Practice and regulatory guidance on pediatric clinical trials.²²⁻²⁵ Adults, adolescents, and parents or legally accepted representatives of children and adolescents were fully informed orally and in writing, and gave written informed consent before initiation of any trial-related activities.

In order to investigate the relation between level of anti-IAsp antibodies and faster aspart and IAsp glucose-lowering effect, data from the current trial were integrated with data from a previous pediatric clinical pharmacology trial with faster aspart and IAsp having an overall similar design and conducted in the same hospital with standardized settings.²⁰ The previous pediatric clinical pharmacology trial did not measure total IAsp and could therefore not contribute to the pharmacokinetic investigations of free vs total IAsp presented in the current manuscript. Both the current trial and the previous trial were registered at ClinicalTrials.gov: NCT03407599 and NCT02035371. Trial procedures were consistent in both protocols.

2.2 | Trial participants

Eligible subjects were males and females 6 to 11 years (children), 12 to 17 years (adolescents), or 18 to 64 years (adults) of age, with a diagnose of T1D at least 12 months prior to screening, treated with multiple daily injections of insulin or continuous subcutaneous insulin infusion for at least 12 months with a total daily insulin dose below 1.2 (I)U/kg and a total daily bolus insulin dose of at least 0.3 (I)U/kg and below 0.7 (I)U/kg. Participants were also required to have body mass index (BMI) within the 3rd and 97th BMI percentiles for children and adolescents²⁶ or no higher than 28.0 kg/m² for adults and HbA_{1c} no higher than 10.0% (86 mmol/mol). Individuals with clinically significant concomitant diseases, clinically significant abnormal values in laboratory screening tests or unusual eating patterns or special dietary requirements were excluded from participation. Smokers, individuals treated with drug(s) that might cause interference with glucose metabolism and pregnant or breast-feeding females were also not included in the trial.

2.3 | Trial procedures

The trial comprised four visits: a screening visit, two dosing visits conducted 3 to 22 days apart, and a follow-up visit. At the dosing visits, subjects were dosed with faster aspart (100 U/mL; Novo Nordisk, Bagsværd, Denmark) or IAsp (NovoRapid; 100 U/mL; Novo Nordisk) following a randomized sequence. Trial products were injected subcutaneously using a blinded PDS290 pen-injector prefilled pen (Novo Nordisk).

Procedures at each of the two dosing visits, including dosing visit exclusion criteria, were as previously described.²⁰ In brief, after subjects arrived at the hospital in the evening, glucose was maintained at a stable level overnight by means of a previously used procedure of

variable intravenous regular human insulin infusion (Actrapid, Novo Nordisk) in combination with infusion of glucose (if PG was below 300 mg/dL [16.6 mmol/L]) or infusion of 0.9% saline (if PG was at least 300 mg/dL [16.6 mmol/L]), based on body weight.²⁷ In the morning, the insulin and glucose infusions were terminated after it had been verified that two PG measurements 30 minutes apart were both between 100 and 160 mg/dL (5.6–8.9 mmol/L). After 15 minutes, a 0.2 U/kg blinded single dose of faster aspart or IAsp was then subcutaneously injected into a lifted skin fold of the lower abdominal wall above the inguinal area. Within 2 minutes postdose, subjects consumed a standardized liquid meal (Ensure Original Therapeutic Nutrition; Abbott Nutrition, Green Oaks, Illinois; containing 105 kcal per 100 mL, 62 energy percent [E%] carbohydrate, 16 E% protein, and 22 E% fat) within 8 minutes. The volume of the liquid meal was adjusted based on body weight with a maximum of 532 mL (Table S1).

Sampling of blood for pharmacokinetic, PG, and β -hydroxybutyrate measurement was performed at predefined time points until 12 hours postdose as listed in Table S2. Treatment to alleviate hypoglycemia was initiated in case PG decreased to below 56 mg/dL (3.1 mmol/L) or in case of a higher PG level if deemed necessary by the Investigator for safety reasons. Regular human insulin (Actrapid) was administered intravenously in case PG was consistently elevated above 342 mg/dL (19 mmol/L) and/or β -hydroxybutyrate was above 18.7 mg/dL (1.8 mmol/L) (there were none of these cases in this trial).

Until 2 hours postdose, subjects stayed in a semisupine position, and between 2 and 12 hours postdose, subjects stayed in a supine or semisupine position. They refrained from consumption of water until 2 hours postdose to the extent possible and were not allowed to eat until 6 hours postdose. From 6 hours postdose, subjects were offered meals and snacks. Only regular human insulin was allowed as short-acting insulin between 6 and 12 hours after dosing. Subjects stayed at the hospital until 12 hours after dosing.

2.4 | Assessments

Measurement of free and total serum IAsp concentrations was performed by means of a validated IAsp-specific enzyme-linked immunosorbent assay with a lower limit of quantification (LLOQ) of 10 pmol/L. Prior to measuring free IAsp, precipitation of serum samples was performed using PEG thereby obtaining a supernatant fraction containing free IAsp. The baseline level of total anti-IAsp antibodies (ie, antibodies specific to IAsp and those cross reacting with human insulin) was determined by means of a validated subtraction radioimmunoassay using ¹²⁵I-labeled IAsp as previously described.²¹

PG concentration for pharmacodynamic evaluation and assessment of safety and β -hydroxybutyrate concentration for assessment of safety were determined using a blood glucose and ketone monitoring system (FreeStyle Precision Neo, Abbott).

Additional assessments of safety comprised adverse events (AEs), hypoglycemic episodes, injection site reactions, safety laboratory

assessments, physical examination, and vital signs. Within each treatment period, AEs were defined as treatment emergent if their onset occurred between the time of dosing and 7 days later. Hypoglycemic episodes were classified based on the definitions from the American Diabetes Association (ADA)²⁸ and the International Society for Pediatric and Adolescent Diabetes (ISPAD)²⁹ and furthermore categorized as “confirmed” if documented by PG below 56 mg/dL (3.1 mmol/L) with or without symptoms consistent with hypoglycemia. Hypoglycemic episodes were defined as treatment emergent if their onset occurred from time of dosing of trial product until the next dosing of any insulin, although no later than 16 hours after dosing of trial product.

2.5 | Trial endpoints

All pharmacokinetic endpoints used to evaluate onset of exposure, early exposure, offset of exposure, and overall exposure were defined and calculated as described previously.¹⁵ During blinded review of serum IAsp profiles, it was seen that the predose concentrations were higher than the LLOQ and remained higher than the LLOQ during the full sampling period for selected total IAsp profiles. Therefore, these profiles were baseline-corrected using a baseline value calculated as the mean of all predose concentrations. Following baseline correction, all predose concentrations and negative concentrations were set to zero.

The following pharmacodynamic endpoints were used to assess early glucose-lowering effect during the standardized meal test: PPG increment from the time of dosing until 1 hour (Δ PPG_{1h}) and 2 hours (Δ PPG_{2h}) after dosing. If subjects received glucose to alleviate hypoglycemia, pharmacodynamic endpoints were calculated after the last observation before glucose intervention had been carried forward. No glucose intervention was needed during the first hour after dosing, and only three glucose interventions were needed between 1 and 2 hours postdose (in one adult for faster aspart and one child and one adult for IAsp). Between 2 and 6 hours postdose, glucose intervention was needed in six subjects for faster aspart (three children and three adults) and in seven subjects for IAsp (three children, one adolescent, and three adults) but this did not influence the presented pharmacodynamic endpoints covering the 0 to 2 hour postdose period.

2.6 | Statistical analysis

A minimum of 12 children, 15 adolescents, and 15 adults were required to complete the trial. These numbers were set with no formal sample size calculation according to guidelines on the conduct of clinical pharmacology trials in the pediatric population.^{22,25} With this sample size, the power of detecting a treatment difference in one of the key pharmacokinetic endpoints, $\log(\text{AUC}_{\text{IAsp},0-30\text{min}})$, was 76% in children and 99% in adolescents, assuming within-subject SDs of 0.50 and 0.43 and treatment differences (faster aspart-IAsp) of 0.57 and 0.68 for children and adolescents, respectively.²⁰

SAS version 9.4 (SAS Institute, Cary, North Carolina) was used to conduct all statistical analyses at a 5% significance level including all randomized subjects who received at least one dose of trial product. Pharmacokinetic endpoints were compared between faster aspart and IAsp in a linear mixed model with age group, treatment, age group-by-treatment interaction, and period as fixed effects and subject as random effect. The variance of the random subject effect and the residual variance depended on age group. AUCs and maximum concentration ($C_{max, IAsp}$) were log-transformed before analysis. Within each age group, least square means for each treatment along with treatment ratio and 95% confidence interval (CI) (for log-transformed endpoints) and treatment difference and 95% CI (for other endpoints) were estimated.

The association between anti-IAsp antibody level and the free and total IAsp exposure was evaluated by presenting a plot of the relationship between anti-IAsp antibody level and the ratio of $AUC_{IAsp,0-t}$ for free vs total IAsp. The plot only included subjects with available data for anti-IAsp antibody level and $AUC_{IAsp,0-t}$ for both free and total IAsp (for faster aspart and/or IAsp).

The potential effect of anti-IAsp antibody level on the early glucose-lowering effect of faster aspart and IAsp was evaluated by presenting plots of the relationship between anti-IAsp antibody level and ΔPPG_{1h} and ΔPPG_{2h} , respectively, for both faster aspart and IAsp. This was done in a pooled analysis of the current trial and a previous pediatric clinical pharmacology trial with faster aspart and IAsp.²⁰

3 | RESULTS

3.1 | Subject disposition and baseline characteristics

A total of 55 subjects were screened, 46 randomized (13 children, 16 adolescents, and 17 adults) and 43 exposed to trial product. Three randomized subjects were withdrawn before first dosing (a child experiencing hypoglycemia within 24 hours of planned dosing, an adult who withdrew consent, and an adult with difficulties in gaining venous access). All 43 exposed subjects completed the trial (12 children, 16 adolescents, and 15 adults). Baseline characteristics for the 43 exposed subjects in the current trial are presented in Table 1.

Baseline characteristics for subjects in the pooled analysis of the current trial and a previous pediatric clinical pharmacology trial are summarized in Table S3. Subjects in the pooled analysis had baseline characteristics comparable to subjects in the current trial alone.

3.2 | Faster aspart vs IAsp pharmacokinetics assessed as free or total IAsp

Not surprisingly, free IAsp concentrations were lower than total IAsp concentrations independent of trial product (Figures 1 and S1). Notably, however, in all three age groups, a comparable shift to the left of

TABLE 1 Baseline characteristics

	Children (N = 12)	Adolescents (N = 16)	Adults (N = 15)
Age, y	10.0 (1.3)	14.9 (1.7)	19.7 (1.5)
Sex			
Female, N (%)	7 (58.3)	10 (62.5)	6 (40.0)
Male, N (%)	5 (41.7)	6 (37.5)	9 (60.0)
Race			
White, N (%)	12 (100.0)	16 (100.0)	15 (100.0)
Body weight, kg	41.5 (9.4)	62.8 (8.9)	74.8 (9.9)
BMI, kg/m ²	18.7 (2.4)	21.6 (2.0)	23.5 (2.3)
Duration of diabetes, y	4.9 (2.2)	8.0 (4.2)	11.4 (5.4)
HbA _{1c} , mmol/mol	55.5 (8.2)	56.7 (8.7)	57.6 (13.8)
%	7.2 (0.7)	7.3 (0.8)	7.4 (1.3)
Anti-IAsp antibodies, %B/T	30.3 (17.5)	36.0 (18.3)	18.9 (11.6)

Note: Data are mean (SD) unless otherwise stated.

Abbreviations: %B/T, percent bound/total; BMI, body mass index; HbA_{1c}, glycosylated hemoglobin; IAsp, insulin aspart; N, number of subjects.

the serum IAsp concentration-time profile was observed with faster aspart vs IAsp irrespective of whether free or total IAsp was measured.

In general, independent of age group, earlier onset of appearance and shorter $t_{Early\ 50\% C_{max}}$ were seen for faster aspart vs IAsp for free as well as for total IAsp (Table 2). Except for $t_{Early\ 50\% C_{max}}$ based on total IAsp in children ($P = .069$), onset of appearance and $t_{Early\ 50\% C_{max}}$ differed statistically significantly between faster aspart and IAsp for all pairwise comparisons. Furthermore, for both onset of appearance, $t_{Early\ 50\% C_{max}}$ and t_{max} , all pairwise differences between faster aspart and IAsp were of comparable magnitude for free and total IAsp in all three age groups (Tables 2 and S4).

Independent of age group, greater early exposure within 30 minutes postdose was generally observed for faster aspart vs IAsp for both free and total IAsp measurement (Table 3). However, the greater $AUC_{IAsp,0-30min}$ with faster aspart vs IAsp in children based on free IAsp did not reach statistical significance ($P = .062$). With respect to early exposure, the pairwise differences between faster aspart and IAsp were of comparable magnitude irrespective of pharmacokinetic assay method or age group (Tables 3 and S5).

Regarding offset of exposure, point estimates for the difference of faster aspart-IAsp ($t_{Late\ 50\% C_{max}}$) were generally negative and point estimates for the ratio of faster aspart/IAsp ($AUC_{IAsp,2-t}$) were generally below 1, indicating a left-shift of the late-phase pharmacokinetic profile for faster aspart vs IAsp independent of pharmacokinetic assay method (Tables 4 and S6). However, only few comparisons were statistically significant and results were not entirely consistent across age group and assay method, presumably because the number of subjects per age group was relatively low.

FIGURE 1 Mean 2-hour serum IAsp concentration-time profiles after 0.2 U/kg s.c. faster aspart or IAsp administration in (A and B) children, (C and D) adolescents, and (E and F) adults with T1D when assaying free or total IAsp. N = 12 for children, N = 16 for adolescents, and N = 15 for adults. Variability bands show the SE of the mean. IAsp, insulin aspart

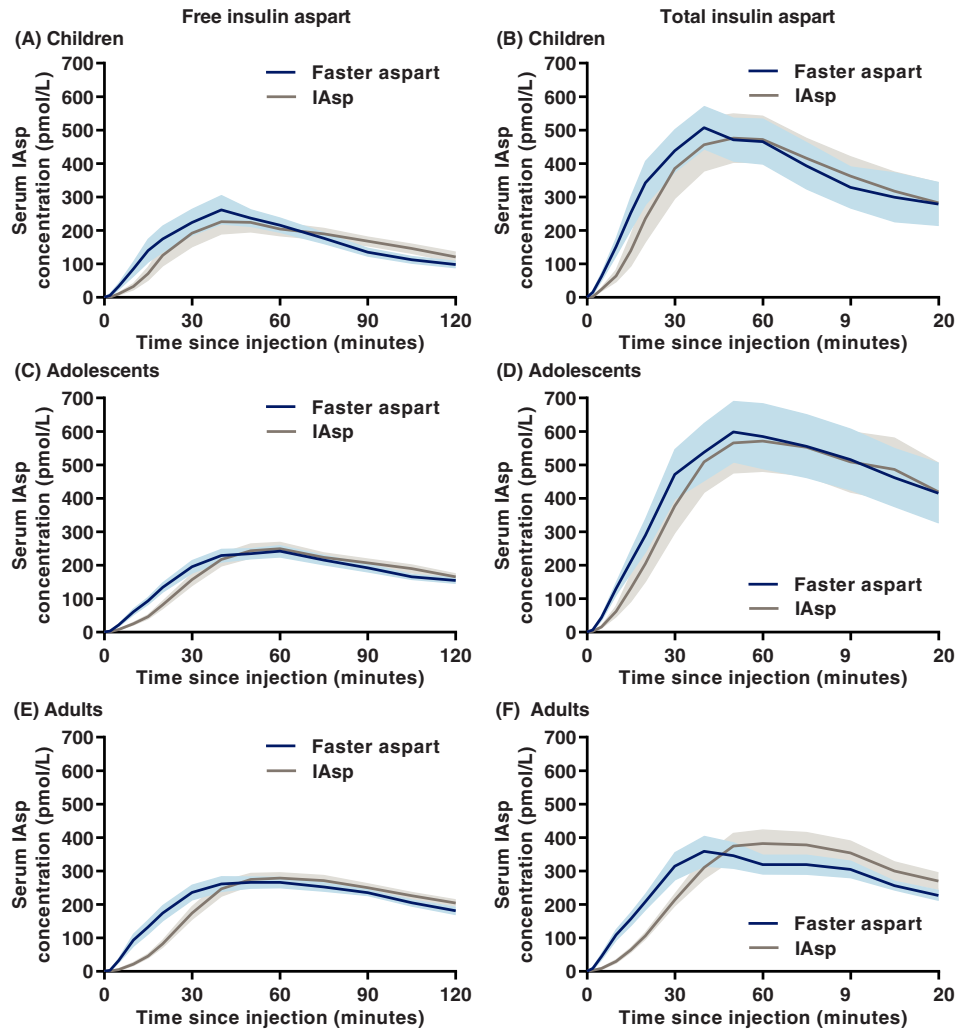


TABLE 2 Onset of exposure for faster aspart vs IAsp after 0.2 U/kg s.c. administration in children, adolescents, and adults with T1D when assaying free or total IAsp

	Free IAsp		Total IAsp	
	Treatment difference Faster aspart - IAsp [95% CI]	P-value ^a	Treatment difference Faster aspart - IAsp [95% CI]	P-value ^a
Onset of appearance (min)				
Children	-5.0 [-8.2; -1.7]	.006	-2.9 [-4.4; -1.5]	.001
Adolescents	-2.4 [-3.7; -1.0]	.002	-1.8 [-3.2; -0.5]	.013
Adults	-4.6 [-6.7; -2.4]	<.001	-3.0 [-4.9; -1.1]	.004
$t_{\text{Early } 50\% C_{\text{max}}}$ (min)				
Children	-6.4 [-12.5; -0.3]	.042	-6.3 [-13.1; 0.6]	.069
Adolescents	-6.6 [-9.5; -3.7]	<.001	-6.3 [-10.1; -2.5]	.003
Adults	-10.3 [-13.8; -6.9]	<.001	-8.3 [-12.7; -4.0]	.001
t_{max} (min)				
Children	-11.8 [-28.8; 5.1]	.152	-16.7 [-33.9; 0.6]	.057
Adolescents	-1.2 [-14.0; 11.7]	.846	2.3 [-13.2; 17.8]	.763
Adults	-10.9 [-22.9; 1.1]	.072	-8.5 [-17.2; 0.2]	.055

Note: N = 12 for children, N = 16 for adolescents, and N = 15 for adults.

Abbreviations: CI, confidence interval; IAsp, insulin aspart; $t_{\text{Early } 50\% C_{\text{max}}}$, time to 50% of maximum IAsp concentration in the early part of the pharmacokinetic profile; t_{max} , time to maximum concentration.

^aFor treatment comparison of faster aspart vs IAsp.

TABLE 3 Early exposure for faster aspart vs IAsp after 0.2 U/kg s.c. administration in children, adolescents, and adults with T1D when assaying free or total IAsp

	Free IAsp		Total IAsp	
	Treatment ratio Faster aspart/IAsp [95% CI]	P-value ^a	Treatment ratio Faster aspart/IAsp [95% CI]	P-value ^a
AUC_{IAsp,0-15min} (pmol-h/L)				
Children	3.62 [1.44; 9.09]	.011	2.86 [1.45; 5.64]	.006
Adolescents	2.28 [1.67; 3.12]	<.001	2.38 [1.66; 3.43]	<.001
Adults	3.94 [2.34; 6.61]	<.001	3.29 [2.14; 5.07]	<.001
AUC_{IAsp,0-30min} (pmol-h/L)				
Children	1.78 [0.97; 3.29]	.062	1.75 [1.02; 3.03]	.045
Adolescents	1.60 [1.30; 1.98]	<.001	1.62 [1.26; 2.07]	<.001
Adults	2.11 [1.56; 2.85]	<.001	1.96 [1.50; 2.56]	<.001
AUC_{IAsp,0-1h} (pmol-h/L)				
Children	1.22 [0.89; 1.68]	.191	1.21 [0.89; 1.65]	.193
Adolescents	1.15 [0.97; 1.37]	.099	1.20 [0.99; 1.47]	.065
Adults	1.27 [1.09; 1.48]	.005	1.24 [1.05; 1.47]	.018

Note: N = 12 for children, N = 16 for adolescents, and N = 15 for adults.

Abbreviations: AUC, area under the curve; CI, confidence interval; IAsp, insulin aspart.

^aFor treatment comparison of faster aspart vs IAsp.

TABLE 4 Offset of exposure for faster aspart vs IAsp after 0.2 U/kg s.c. administration in children, adolescents, and adults with T1D when assaying free or total IAsp

	Free IAsp		Total IAsp	
	Treatment difference Faster aspart - IAsp [95% CI]	P-value ^a	Treatment difference Faster aspart - IAsp [95% CI]	P-value ^a
t_{Late 50% C_{max}} (min)				
Children	-13.8 [-44.6; 16.9]	.344	-0.5 [-34.1; 33.1]	.975
Adolescents	-14.4 [-40.1; 11.3]	.251	-12.3 [-39.0; 14.5]	.342
Adults	-8.1 [-14.5; -1.6]	.018	-15.5 [-30.1; -0.9]	.039
	Free IAsp		Total IAsp	
	Treatment ratio Faster aspart/IAsp [95% CI]	P-value ^a	Treatment ratio Faster aspart/IAsp [95% CI]	P-value ^a
AUC_{IAsp,2-t} (pmol-h/L)				
Children	0.97 [0.68; 1.38]	.852	1.02 [0.85; 1.21]	.826
Adolescents	0.83 [0.71; 0.97]	.022	0.93 [0.80; 1.08]	.299
Adults	0.90 [0.78; 1.04]	.154	0.86 [0.77; 0.96]	.010

Note: N = 12 for children, N = 16 for adolescents, and N = 15 for adults.

Abbreviations: AUC, area under the curve; CI, confidence interval; IAsp, insulin aspart; t_{Late 50% C_{max}}, time to 50% of maximum IAsp concentration in the late part of the pharmacokinetic profile.

^aFor treatment comparison of faster aspart vs IAsp.

In all three age groups, total exposure (AUC_{IAsp,0-t}; primary endpoint) and maximum concentration (C_{max,IAsp}) were generally comparable between faster aspart and IAsp both for free and total IAsp (Table S7). Only exceptions were minor treatment differences for AUC_{IAsp,0-t} in adolescents based on free IAsp (estimated ratio faster aspart/IAsp [95% CI] 0.93 [0.87;1.00], P = .045) and in adults based on total IAsp (0.95 [0.90;1.00], P = .049). All point estimates for the treatment ratio of faster aspart vs IAsp were between 0.93 and 1.03 across age groups and assay method (Table S7).

3.3 | The association between anti-IAsp antibody level and free vs total IAsp pharmacokinetics

The association between anti-IAsp antibody level and the ratio of AUC_{IAsp,0-t} for free IAsp vs AUC_{IAsp,0-t} for total IAsp is presented in Figure 2 for all three age groups together. A clear negative association was observed between the level of anti-IAsp antibodies and the ratio of free vs total IAsp for AUC_{IAsp,0-t} irrespective of insulin product (faster aspart or IAsp). Thus, for both insulin products there was a

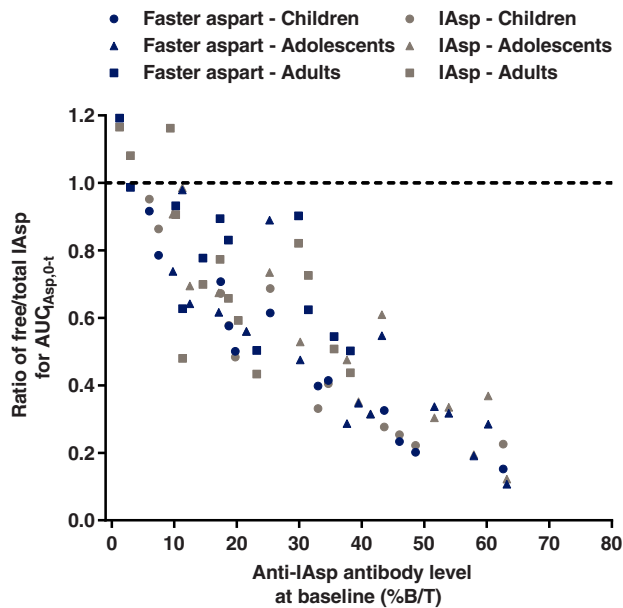


FIGURE 2 Relationship between individual anti-IAsp antibody level and the ratio of free vs total IAsp exposure after 0.2 U/kg s.c. faster aspart or IAsp administration in children, adolescents, and adults with T1D. $N = 12$ for children, $N = 16$ for adolescents, and $N = 14$ for adults (baseline anti-IAsp antibody measurement was missing in one adult subject). The horizontal dotted line reflects equal IAsp exposure for free and total IAsp. %B/T, percent bound/total; AUC, area under the curve; IAsp, insulin aspart

clear positive association between the level of anti-IAsp antibodies and $AUC_{IAsp,0-t}$ for total IAsp, while no such apparent association was observed between the level of anti-IAsp antibodies and $AUC_{IAsp,0-t}$ for free IAsp (Figure S2).

3.4 | The association between anti-IAsp antibody level and PPG increment for faster aspart and IAsp

A pooled analysis of the current trial and a previous pediatric clinical pharmacology trial was conducted to investigate any impact of anti-IAsp antibody level on the early glucose-lowering effect of faster aspart and IAsp. Independent of age group or trial product, there was no clear association between anti-IAsp antibody level and 1-hour or 2-hour PPG increment in a meal test (Figure 3).

3.5 | Safety

Faster aspart and IAsp were safe and well tolerated across the three age groups. No serious AEs were observed, no withdrawals of subjects occurred due to AEs and the majority of AEs were mild (12 of 13 events). According to the ADA/ISPAD definition, 25 treatment emergent hypoglycemic episodes were reported: 8 in children (3 for faster aspart and 5 for IAsp), 1 in adolescents (IAsp), and 16 in adults (6 for faster aspart and 10 for IAsp). Six confirmed hypoglycemic

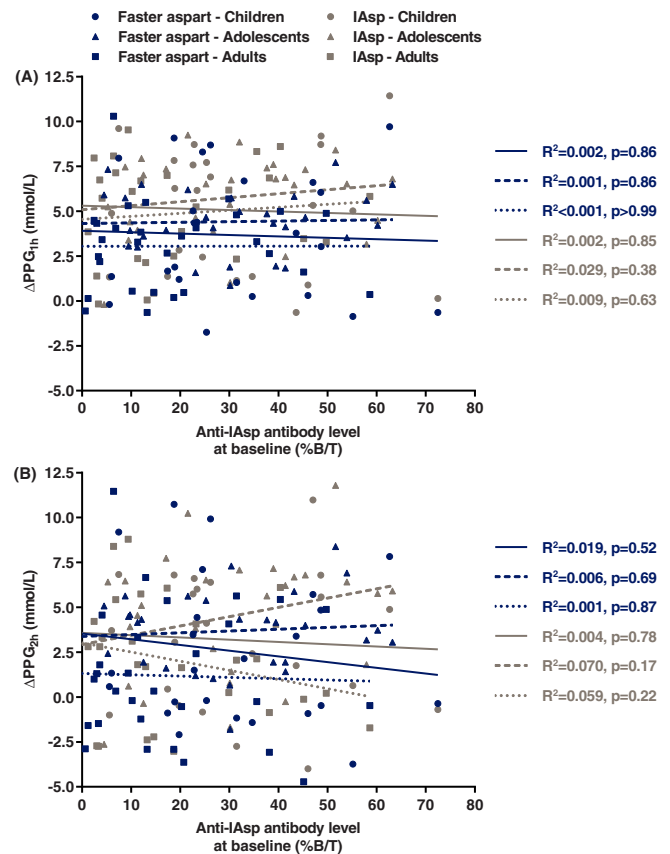


FIGURE 3 Relationship between individual anti-IAsp antibody level and, A, 1-hour PPG increment (ΔPPG_{1h}) or, B, 2-hour PPG increment (ΔPPG_{2h}) in a meal test following 0.2 U/kg s.c. faster aspart or IAsp administration in children, adolescents, and adults with T1D. Results are based on 24 children, 29 adolescents, and 29 adults in a pooled analysis of two pediatric clinical pharmacology trials. Lines are the estimated regression lines in children (full), adolescents (dashed), and adults (dotted) for faster aspart (blue) and IAsp (gray), respectively. The coefficients of determination (R^2) as well as the P -values for the testing of a slope of zero (ie, horizontal regression line) are included. %B/T, percent bound/total; IAsp, insulin aspart; PPG, postprandial glucose

episodes were reported (one for IAsp in a child and two for faster aspart and three for IAsp in adults). No severe hypoglycemic episodes were reported. A total of two injection site reactions occurred: one in a child after IAsp dosing and one in an adolescent after faster aspart dosing. Both injection site reactions were mild. No clinically relevant observations were done with respect to laboratory safety assessments, physical examination, or vital signs.

4 | DISCUSSION

In the current investigation of children, adolescents, and adults with T1D, the main observations were that the pharmacokinetic profile was similarly left-shifted for faster aspart vs IAsp irrespective of assaying free or total IAsp, that overall higher serum concentrations

were observed for total vs free IAsp, that a lower ratio of free-to-total IAsp for $AUC_{IAsp,0-t}$ was correlated with a higher level of anti-IAsp antibodies, and, finally, that no association occurred between anti-IAsp antibody level and faster aspart or IAsp glucose-lowering effect. Faster aspart and IAsp were both well tolerated by the subjects with no new safety findings.

The current findings in children, adolescents, and adults of earlier onset and greater initial exposure for faster aspart vs IAsp irrespective of assaying free or total IAsp are in accordance with a previous study in adults only.²¹ The present trial also further extends the previous findings in children and adolescents of accelerated pharmacokinetic properties of faster aspart vs IAsp, which were based on free IAsp measurement, to also cover total IAsp, although with a generally higher concentration of total IAsp.²⁰ Faster aspart and IAsp contain the same active drug substance, and the same assay, with or without PEG precipitation, was used to determine serum concentrations for both insulins, which may explain why the comparison of pharmacokinetics between the two insulin products provide similar results for free and total IAsp. It is, however, not possible to conclude based on the present results that the pharmacokinetics of other insulins can be compared based on free or total insulin with similar outcome. Consequently, it is always important when reporting the pharmacokinetics of an exogenous insulin to be transparent on whether free or total insulin is measured.

The present investigation of the association between anti-IAsp antibody level and the ratio of free-to-total IAsp for $AUC_{IAsp,0-t}$ suggests that anti-IAsp antibody level explains to some extent the higher concentration seen for total vs free IAsp across children, adolescents, and adults with T1D. This is in accordance with a study with faster aspart and IAsp in adults²¹ and also with several previous observations with other insulin products.^{7,8,30} It has been proposed that the effects of insulin, as well as other hormones existing in the bloodstream in a bound as well as a free pool, are reflected by the concentration of their free pool rather than their total concentration.^{31,32} If this is indeed the case, then it must be kept in mind that investigations of the pharmacokinetic-pharmacodynamic relationship for such hormones should always be based on free hormone concentrations.

Some previous trials have suggested that high anti-insulin antibody titers are associated with delayed increase in insulin concentration initially after dosing as well as longer time to maximum concentration and a prolonged half-life.^{5,6} Still, the majority of trials have not shown any impact of anti-insulin antibodies on the glucose-lowering effect of different insulins including faster aspart and IAsp.^{7,21,33,34} Furthermore, trials with IAsp have shown no correlation between anti-IAsp antibody level and various efficacy parameters, suggesting that there is no clinically relevant influence of anti-IAsp antibodies on the efficacy of IAsp.³⁵ In accordance, based on the current pooled analysis, postprandial increase in glucose during a meal test was not affected by anti-IAsp antibody level in children, adolescents, and adults. In several phase 3 trials comparing faster aspart and IAsp, including one in children and adolescents, faster aspart provided better control of PPG, overall glycemic control to at least the same

extent as IAsp and with similar or reduced overall hypoglycemia risk.^{17-19,36} Taken together, the clinical significance of anti-IAsp antibody development for the efficacy of faster aspart is assessed to be limited in patients with diabetes including children and adolescents. Thus, faster aspart may be able to fulfill the unmet need that exists not only in adults but also in children and adolescents with diabetes for an ultrafast mealtime insulin with absorption characteristics, which better resemble endogenous insulin secretion in healthy individuals.³⁷

Children and adolescents are characterized by having generally higher and more variable anti-insulin antibody levels compared with adults (Figure 2).² Thus, it was a strength of the current trial that inclusion of children and adolescents ensured a population with a broad spectrum of anti-IAsp antibody levels for determining the association between level of anti-IAsp antibodies and pharmacological properties of faster aspart and IAsp. A limitation was that the initial pharmacodynamic characteristics of faster aspart and IAsp were determined by a meal test and not by the glucose clamp method, which is considered the golden standard to assess pharmacodynamics of exogenous insulin. A meal test was chosen in both trials in order to minimize the stress on the children and adolescents with respect to invasive procedures and blood volume drawn. Another limitation was that the number of subjects per age group was relatively low. This is particularly relevant to remember when interpreting the statistical analysis of treatment differences in pharmacokinetic characteristics in each age group. Except for two endpoints for total IAsp (t_{max} in adolescents and $AUC_{IAsp,2-t}$ in children), the point estimates for the comparison of faster aspart vs IAsp were in favor of faster aspart. Moreover, the vast majority of differences turned out to be statistically significant despite the relatively low number of subjects.

In conclusion, across children, adolescents, and adults with T1D, faster aspart was associated with earlier onset and larger initial exposure compared with IAsp independent of measuring free or total IAsp. Total serum IAsp concentration was generally higher than free serum IAsp concentration, and across all anti-IAsp antibody levels the ratio between free and total IAsp was negatively related to anti-IAsp antibody level. It was, however, reassuring that the anti-IAsp antibody level had no impact on faster aspart or IAsp glucose-lowering effect. Thus, the present trial in children, adolescents, and adults representing a broad spectrum of anti-IAsp antibody levels supports previous conclusions on the pharmacological characteristics of faster aspart and IAsp determined as free or total IAsp and the association with anti-IAsp antibody level based only on adults with low to moderate anti-IAsp antibody levels.²¹

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CONFLICT OF INTERESTS

T. B. has received speaker honoraria from AstraZeneca, DexCom, Medtronic, Roche Diabetes, Sanofi and Ypsomed. L. Q. B. and N. R. are employees of Novo Nordisk. M. D. B. and H. H. are employees and shareholders of Novo Nordisk. T. D. has received speaker honoraria and research support and has consulted for Abbott, Bayer, BMS, AstraZeneca, Boehringer Ingelheim, DexCom, Eli Lilly, Medtronic, Novo Nordisk, Sanofi, and Roche. He is a shareholder of DreaMed Ltd. T. v. d. B. has received speaker honoraria from Medtronic, Sanofi, and Ypsomed.

AUTHOR CONTRIBUTIONS

Torben Biester and Thomas Danne contributed with trial design, data acquisition, and data interpretation. Thekla von dem Berge contributed with data acquisition and data interpretation. Line Quist Bendtsen contributed with trial design, trial management, and data interpretation. Mette Dahl Bendtsen contributed with data analysis and data interpretation. Naveen Rathor contributed with data interpretation. Hanne Haahr contributed with trial conception, trial design, and data interpretation. All authors have contributed with manuscript writing, critical manuscript revision, and final manuscript approval.

DATA ACCESSIBILITY

Will individual participant data be available (including data dictionaries)? Individual participant data will be shared in data sets in a deidentified/anonymized format.

What data in particular will be shared? Data sets from Novo Nordisk sponsored clinical research completed after 2001 for product indications approved in both the EU and United States.

What other documents will be available? Trial protocol and redacted Clinical Trial Report (CTR) will be available according to Novo Nordisk data sharing commitments.

When will data be available (start and end dates)? The data will be available permanently after research completion and approval of product and product use in both EU and United States. No end date.

With whom will data be shared? With bona fide researchers submitting a research proposal requesting access to data.

For what types of analyses? For use as approved by the Independent Review Board (IRB) according to the IRB Charter (see novonordisk-trials.com).

By what mechanism will data be made available? Access request proposal form and the access criteria can be found at novonordisk-trials.com. The data will be made available on a specialized SAS data platform.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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