Absorption Kinetics and Action Profiles of Subcutaneously Administered Insulin Analogues ($Asp^{B9}Glu^{B27}$, Asp^{B10}, Asp^{B28}) in Healthy Subjects

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Objective: The subcutaneous absorption and resulting changes in plasma insulin or analogue, glucose, C-peptide, and blood intermediary metabolite concentrations after subcutaneous bolus injection of three soluble human insulin analogues ($Asp^{B9}Glu^{B27}$, monomeric; Asp^{B28}, mixture of monomers and dimers; and Asp^{B10}, dimeric) and soluble human insulin were evaluated. Research Design and Methods: Fasting healthy male volunteers (n = 7) were studied on five occasions 1 wk apart randomly receiving 0.6 nmol · kg^{-1} s.c. ^{125}I-labeled Asp^{B10} or soluble human insulin (Novolin R, Novo, Copenhagen); 1st study and 0.6 nmol · kg^{-1} s.c. ^{125}I-labeled Asp^{B28}, $Asp^{B9}Glu^{B27}$ or soluble human insulin (2nd study). Residual radioactivity at the injection site was measured over 8 h with frequent venous sampling for plasma immunoreactive insulin or analogue, glucose, C-peptide, and blood intermediary metabolite concentrations. Results: The three analogues were absorbed 2–3 times faster than human insulin. The mean ± SE time to 50% residual radioactivity was 94 ± 6 min for Asp^{B10} compared with 184 ± 10 min for human insulin ($P < 0.001$), 83 ± 8 min for Asp^{B28} ($P < 0.005$), and 63 ± 9 min for $Asp^{B9}Glu^{B27}$ ($P < 0.001$) compared with 182 ± 21 min for human insulin. ΔPeak plasma insulin analogue levels were significantly higher after each analogue than after human insulin ($P < 0.005$). With all three analogues, the mean hypoglycemic nadir occurred earlier at 61–65 min postinjection compared with 201–210 min for the reference human insulins ($P < 0.005$). The magnitude of the hypoglycemic nadir was greater after $Asp^{B9}Glu^{B27}$ ($P < 0.05$) and Asp^{B28} ($P < 0.001$) compared with human insulin. There was a significantly faster onset and offset of responses in C-peptide and intermediary metabolite levels after the analogues than after human insulin ($P < 0.05$). Conclusions: The rapid absorption and biological actions of these analogues offer potential therapeutic advantages over the current short-acting neutral soluble insulins. *Diabetes Care* 14:1057–65, 1991

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Subcutaneously administered soluble human insulin is absorbed too slowly to mimic physiological plasma insulin profiles at meals. In nondiabetic subjects, posprandial plasma insulin concentrations rise rapidly, peaking within 30–60 min and returning to baseline by 4–5 h (1,2). In contrast, after subcutaneous injection of soluble insulin in both nondiabetic (3) and diabetic (4) subjects, plasma insulin concentrations rise more slowly to a lower peak at 90–120 min, and levels remain inappropriately high at 3–5 h (5). This slow absorption typically produces plasma insulin levels that are too low immediately postmeal and too high 3–5 h later. Consequently, both postprandial and late hypoglycemia are common problems in conventionally treated insulin-dependent diabetes (6). Although many factors influence absorption rate (7), attempts to increase the rate of subcutaneous conventional insulin absorption by techniques including massage (8,9) and the use of jet injectors (10,11) are impractical and of little clinical benefit (12). Attention has therefore turned to the development of new rapidly absorbed insulins.

Soluble insulin exists mainly as hexameric units at pharmaceutical concentrations (~10^{-3} M), whereas at physiological concentrations (~10^{-9} M), it circulates and exerts its biological effects as a monomer (13).

Dissociation into dimeric and monomeric units at the subcutaneous injection site is assumed to delay the absorption process (14–16). Advances in computer-aided molecular modeling techniques and DNA technology allowed the development of insulin analogues that remain dimeric or even monomeric at pharmaceutical concentration (17). Single amino acids in the B chain of the human insulin molecule, important for dimer formation but peripheral to the putative receptor binding site, have been targeted for mutation and substituted with amino acids favoring monomer formation. Methods by which amino acid substitutions prevent insulin self-association include the introduction of negative-charge repulsion at the monomer-monomer interface (achieved by replacing noncharged amino acids with those that carry a net negative charge at neutral pH), and the removal of metal binding sites. Examples of the former principle include the monomeric analogue $Asp^{B9}Glu^{B27}$ (Asp and Glu replacing Ser and Thr, respectively, at the B9 and B27 positions of human insulin, both substitutions introducing negative-charge repulsion; Table 1) and analogue Asp^{B28} (mixture of monomers and dimers; Asp replacing Pro at B28). An example of the second principle is the dimeric analogue Asp^{B10} (Asp replacing His at B10), in which the His-zinc binding site involved in the association of three dimers into a zinc-insulin hexamer is removed.

Receptor binding affinities of these analogues range from 20 to 300% that of human insulin and are closely paralleled by their in vitro biological activities as measured in the mouse free–fat cell assay (Table 1). However, in vivo biological potencies, as measured by the mouse blood glucose bioassay, are very similar to that of the human international standard (17,18).

Animal studies have shown that these analogues are absorbed two to three times faster than human insulin (17). In addition, we have already shown that, in healthy men, the monomeric analogue $Asp^{B9}Glu^{B27}$ is absorbed two to three times faster than human insulin and is associated with an earlier hypoglycemic nadir (19).

Herein, we report a study in healthy men comparing the rates of subcutaneous absorption, after bolus injection, of equimolar doses of ^{125}I-labeled soluble human insulin and ^{125}I-labeled insulin analogues ($Asp^{B9}Glu^{B27}$, Asp^{B28}, and Asp^{B10}) and the consequent changes in plasma insulin, insulin analogue, glucose, C-peptide, and blood intermediary metabolite concentrations.

TABLE 1
Biological properties of insulins

Insulin	Association state	Receptor binding affinity (%)	Biological activity	
			FFC (%)	MBGA (%)
Human	6	100	100	100
Asp^{B10}	2.2	315	207	98
Asp^{B28}	1.3	88	101	104
$Asp^{B9}Glu^{B27}$	1.1	20	31	93

Values for association state represent ratio between osmotic pressure and 6 times that of 2 Zn^{2+} human soluble insulin (hexameric at measured concn of 0.2–1 mM and with calculated mean M_r of 37,000). Receptor binding affinity (measured in human hepatoma HepG2 cell line) is expressed relative to human insulin. FFC, mouse free–fat cell assay; MBGA, mouse blood glucose assay. Data are from refs. 17 and 18.

RESEARCH DESIGN AND METHODS

The study was approved by the local ethical committee and performed in accordance with the Declaration of Helsinki. All subjects gave informed written consent.

We studied seven healthy male volunteers. None had family history of diabetes mellitus, and all had normal glucose tolerance. Their mean age was 32.6 yr (range 22–43 yr) and mean body mass index 22.7 kg · m^{-2} (range 20.7–24.8 kg · m^{-2}). None of the volunteers had a history of allergy or were receiving concomitant drugs.

Subjects were studied on five occasions (comprising 2 studies) over a 14-wk period, receiving in random order 1 wk apart 0.6 nmol · kg^{-1} (~0.1 U · kg^{-1}) soluble human ^{125}I-insulin (Novolin R, 189 MBq/L; Novo, Copenhagen) and the same dose of ^{125}I-analogue Asp^{B10} (sp act 183 MBq/L) during the first study. In the second study, subjects received in random order 0.6 nmol · kg^{-1} soluble human ^{125}I-insulin (Novolin R, sp act 180 MBq/L) and the same doses of ^{125}I-analogues $Asp^{B9}Glu^{B27}$ (sp act 139 MBq/L) and Asp^{B28} (sp act 149 MBq/L). The production and purification of the insulin analogues are identical to those of human insulin by genetic engineering (17). Because all preparations were formulated to the same concentration (0.6 mM), equal volumes of each were injected. All preparations were mono–^{125}I-labeled at the Tyr^{A14} position with the lactoperoxidase method (20).

Premedication with 500 mg potassium iodide was given the day before each study day to prevent thyroidal uptake of ^{125}I. Each study day began after an overnight fast with the siting of an intravenous cannula in an antecubital vein. The cannula was connected to a three-way tap for blood sampling and was kept patent with a slow-running infusion of isotonic saline (0.15 M). After a basal period of 1 h, the ^{125}I-labeled preparations were injected subcutaneously into the anterior abdominal wall midway between the umbilicus and the anterior superior iliac spine with a 0.5-ml disposable syringe (Lo-Dose, Becton Dickinson, Brooklyn, NY). All injections were given by the same physician, and a lifted–skinfold technique was used to avoid intramuscular injection. Three venous blood samples were taken during the basal period, and then samples were taken every 10 min during the 1st h after injection and thereafter every 15 min up to 2 h, half hourly up to 3 h, and hourly up to 8 h.

The disappearance of the injected preparations from

subcutaneous tissues was assessed from the amount of residual radioactivity at the injection site. External emission of γ-rays was measured with a 50 × 57-mm thallium-activated sodium iodide–scintillation detector with a cylindrical lead collimator fixed 50 mm above the skin surface. Residual radioactivity at the injection site was measured continuously for the 1st 2 h after injection of ^{125}I-labeled preparations and thereafter for 5-min intervals simultaneously with blood sampling. All counts were corrected for background activity, and the residual activity at a given time was expressed as a percentage of initial counts.

During each study period, subjects remained fasted and supine, and smoking was not permitted. Room temperature was maintained constant at 22°C.

Blood samples were aliquoted into fluoride for plasma glucose estimation (glucose analyzer, Chemlab, Hornchurch, UK) and lithium-heparin for determination of immunoreactive C-peptide and insulin or analogue levels. For measurement of lactate, 3-hydroxybutyrate, glycerol, and alanine, 0.5-ml aliquots were taken into 2 ml 3% (wt/vol) perchloric acid. All samples were centrifuged at 4°C and 2500 × *g* within 5 min of sampling, and the plasma was stored at −20°C until assay. A highly sensitive radioimmunoassay with ethanol separation (Novo) was used for measurement of plasma insulin or analogue concentraitons (21). ^{125}I-labeled pork (Tyr^{A19}) insulin was used as the tracer and anti-pork insulin guinea pig serum (antibody M8170) as the antibody. There appeared to be complete cross-reactivity of this antibody with human insulin and the three analogues. As an extra precaution, concentrations of the analogues were read off standard curves constructed from freeze-dried preparations of the respective analogue. The coefficients of variation (C.V.) of the insulin and insulin analogue assays for the middle part of the linear range were 4.2% (human insulin), 4.2% (Asp^{B28}), 4% (Asp^{B10}), and 7.9% ($Asp^{B9}Glu^{B27}$). Plasma C-peptide was measured by a highly sensitive radioimmunoassay with ethanol precipitation (Novo) with sensitivity 0.02 nM and intra-assay C.V. 3% (22). Intermediary metabolites were assayed by automated fluorometric methods (23) with intra-assay C.V. <5%, except in the case of very low levels of 3-hydroxybutyrate, where it was higher.

Results are expressed as means ± SE. ΔPeak and ΔNadir represent the maximum increase and decrease, respectively, from basal of the measured parameter and were calculated from values obtained by subtracting basal (min 0) values from all subsequent values. The time taken to reach ΔPeak and ΔNadir is represented by *t*Peak and *t*Nadir, respectively. The time taken for values to return to basal (*t*Basal) was obtained by calculation of cutoff points of curves with baseline by linear interpolation. Areas under the curve (AUC) were calculated by the trapezoidal method; $iAUC_t$ represents the cumulative incremental AUC for the specified time period *t*. Each analogue was compared with its respective control human insulin by two-tailed Student's paired *t* test (i.e., analogue Asp^{B10} with human insulin day 1; analogues $Asp^{B9}Glu^{B27}$ and Asp^{B28} with human insulin day 2). When more than two groups were compared, e.g., in the case of the second study, statistical differences between the treatments were first demonstrated by repeated-measures analysis of variance before paired *t* testing. $P < 0.05$ was considered significant.

RESULTS

All preparations were well tolerated by all subjects with no local or systemic adverse effects recorded.

Disappearance of the three insulin analogues from the subcutis, as depicted by the residual radioactivity at the site of injection, was faster than that of the reference human insulin from as early as 10 min postinjection ($P < 0.01$; Fig. 1*A*). Absorption of analogues was two to three times faster than that of human insulin when times to 50% residual radioactivity (T_{50}) were compared ($P < 0.005$; Table 2). Approximately 80% of the analogues were absorbed during the first 3 h compared with only 50% of human insulin. From 3 to 8 h, the percentage of human insulin absorbed was two to three times higher than that of the analogues ($P < 0.005$; Table 2).

Basal insulin levels were similar on the analogue and control study days (Table 2). Injection of each of the three analogues resulted in a rapid rise from basal of plasma insulin analogue concentrations, reaching peaks between 30 and 40 min postinjection that were significantly higher than those after human insulin ($P < 0.005$), followed by a rapid return toward basal (Fig. 1*B*; Table 2). In contrast, injection of human insulin produced plasma insulin levels that plateaued between 30 and 240 min postinjection and then slowly returned toward basal. The $iAUC_{0-8\,h}$ of plasma insulin analogue levels after Asp^{B28} was similar to that after human insulin, whereas those after Asp^{B10} and $Asp^{B9}Glu^{B27}$ were significantly different at 50 and 380%, respectively, relative to human insulin at 100% ($P < 0.001$; Table 3).

Basal levels of plasma glucose were similar on the analogue and control study days (Table 2). The three analogues resulted in a rapid fall in plasma glucose followed by a fast return toward basal, with the hypoglycemic nadir in each case occurring significantly earlier at ~60 min postinjection compared with human insulin ($P < 0.005$; Fig. 1*C*; Table 2). After human insulin, there was a less well-defined nadir between 3 and 4 h followed by a slow return toward fasting levels. The magnitude of the early hypoglycemic fall (as measured by ΔNadir and $iAUC_{0-3\,h}$) was significantly greater after injection of the two monomeric analogues ($P < 0.05$–0.001).

Basal C-peptide levels were similar on the analogue and control study days. Endogenous insulin release was largely suppressed after injection of insulin or analogues as shown by the rapid fall in C-peptide levels to low levels that were 12–25% those of fasting basal values

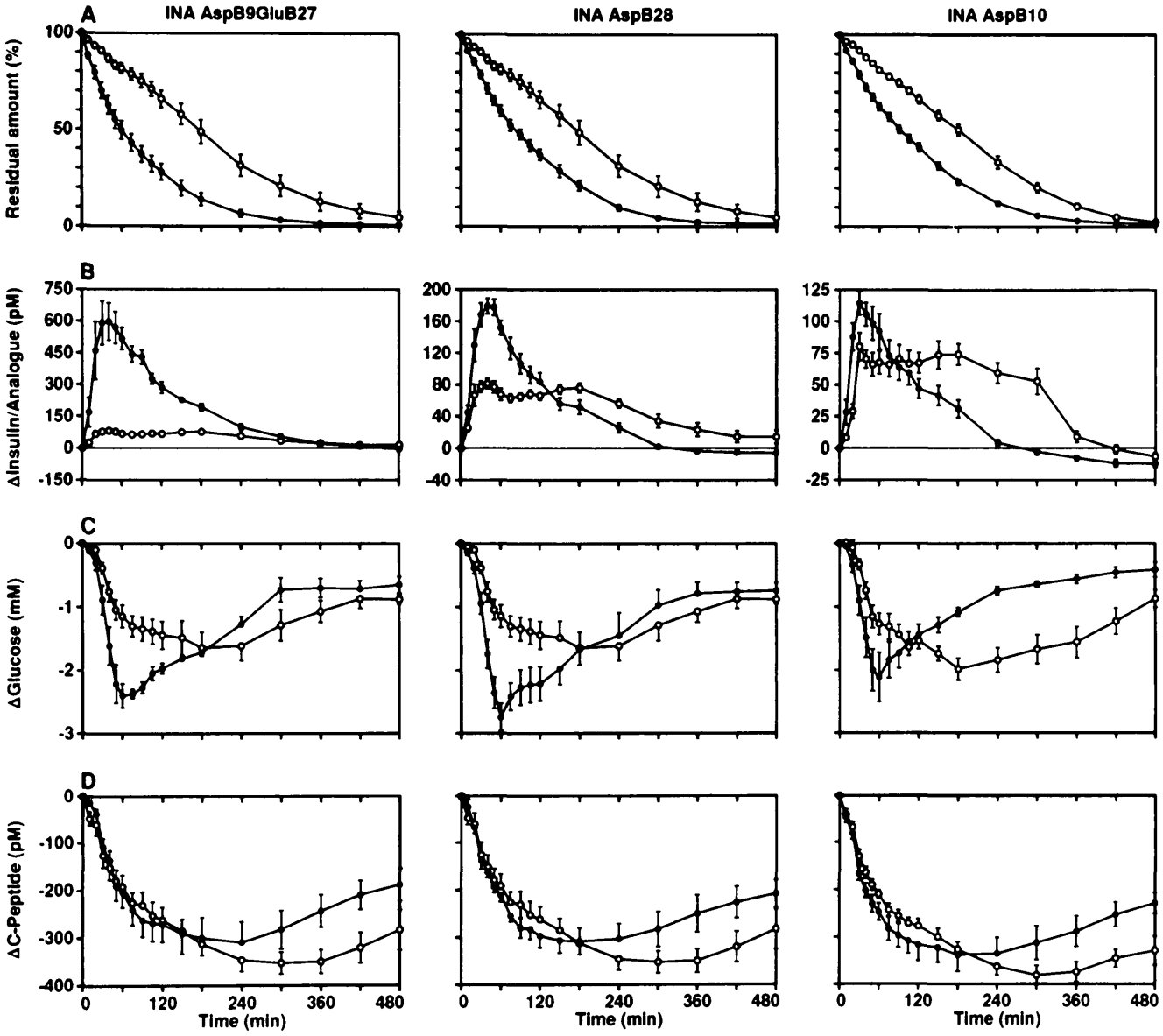

FIG. 1. Mean ± SE residual radioactivity at injection site (*A*) and change from basal of plasma immunoreactive insulin and insulin analogue (*B*), glucose (*C*), and C-peptide (*D*) concentrations after injection of 0.6 nmol · kg^{-1} s.c. soluble human insulin (○) or insulin analogue (INA; ●) into healthy subjects (n = 7) at min 0.

(Fig. 1*D*; Table 2). The maximum C-peptide suppression was similar but occurred significantly earlier after each of the three analogues ($P < 0.02$–0.005). This was followed by a significantly faster return toward basal levels, as indicated by C-peptide concentrations that were significantly higher between 3 and 8 h for analogues Asp^{B28} and Asp^{B10} ($P < 0.05$; Table 2) and between 4 and 8 h for analogue Asp^{B9}Glu^{B27} ($P < 0.05$). Total C-peptide suppression between 0 and 8 h was similar on the analogue and human insulin treatment days (Table 3).

Blood lactate levels tended to be higher after injection of each of the three analogues compared with human insulin, but statistical significance was reached only for the Asp^{B10} and Asp^{B28} analogues (Fig. 2*A*; Table 4).

Injection of insulin or analogue resulted in an initial suppression of plasma 3-hydroxybutyrate to nearly undetectable levels followed by a subsequent posthypoglycemic rise above fasting levels (Fig. 2*B*). The maximum 3-hydroxybutyrate suppression was similar but occurred significantly earlier after injection of each of the three analogues compared with human insulin ($P < 0.05$; Table 4). In the case of analogues Asp^{B9}Glu^{B27} and Asp^{B28}, there was a significantly faster return to basal levels ($P < 0.005$). The pattern of response of glycerol levels (Fig. 2C) was similar to that of 3-hydroxybutyrate, with the two monomeric analogues producing a significantly earlier maximum suppression followed by a faster return to basal fasting levels ($P < 0.05$; Table 4).

TABLE 2
Summary of results

Parameter	Human insulin (day 2)	Analogue			Human insulin (day 1)
		$Asp^{B9}Glu^{B27}$	Asp^{B28}	Asp^{B10}	
Disappearance					
T_{50} (min)	182 ± 21	63 ± 9*	83 ± 8†	94 ± 6*	184 ± 11
$Fraction_{0-3\,h}$ (%)	51 ± 6	86 ± 3*	79 ± 3†	77 ± 2*	50 ± 3
$Fraction_{3-8\,h}$ (%)	44 ± 4	13 ± 3*	20 ± 3†	22 ± 2*	48 ± 3
Insulin/analogue					
Basal (pM)	38 ± 4	51 ± 5	38 ± 4	41 ± 7	41 ± 5
ΔPeak (pM)	95 ± 9	644 ± 94†	189 ± 11†	131 ± 10†	93 ± 10
*t*Peak (min)	80 ± 26	41 ± 6	39 ± 5	34 ± 6	119 ± 37
$iAUC_{(3-8\,h)}$ (pM · min · 10^3)	10.4 ± 2.0	19.8 ± 2.7‡	2.5 ± 0.6†	−0.5 ± 0.6*	9.2 ± 1.5
Glucose					
Basal (mM)	5.3 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	5.6 ± 0.1	5.6 ± 0.2
ΔNadir (mM)	−1.9 ± 0.2	−2.7 ± 0.1‡	−2.9 ± 0.2*	−2.4 ± 0.4	−2.1 ± 0.2
*t*Nadir (min)	201 ± 34	62 ± 5†	65 ± 5†	61 ± 6*	210 ± 20
$iAUC_{(0-3\,h)}$ (mM · min)	−200 ± 30	−306 ± 14§	−328 ± 30*	−238 ± 25	−222 ± 22
C-peptide					
Basal (pM)	434 ± 22	376 ± 48	398 ± 33	452 ± 49	447 ± 24
ΔNadir (pM)	−363 ± 21	−311 ± 44	−321 ± 29	−340 ± 36	−396 ± 22
*t*Nadir (min)	369 ± 24	219 ± 14†	236 ± 21§	174 ± 15§	317 ± 28
$iAUC_{(3-8\,h)}$ (pM · min · 10^3)	−100.1 ± 7.5	−76.4 ± 1.1	−79.2 ± 1.0‡	−88.4 ± 9.2‡	−107.5 ± 5.3

Values are means ± SE for measured parameters (relating to disappearance of radioactivity at injection site and plasma concentrations of immunoreactive insulin or analogue, glucose, and C-peptide) after injection of 0.6 nmol · kg^{-1} s.c. soluble human insulin or insulin analogues $Asp^{B9}Glu^{B27}$, Asp^{B28}, or Asp^{B10} into healthy male volunteers (n = 7). T_{50}, 50% residual radioactivity; iAUC, incremental area under curve.
*P < 0.001, †P < 0.005, ‡P < 0.05, §P < 0.02, vs. respective human insulin by paired t test.

Glycerol and alanine were not measured in the Asp^{B10} study. There was no difference in the pattern of response in blood alanine levels, which gradually declined during the study period after injection of human insulin or analogues $Asp^{B9}Glu^{B27}$ and Asp^{B28} (Fig. 2*D*; Table 4).

TABLE 3
Receptor binding affinity and plasma insulin or analogue levels

Insulin	Receptor binding affinity (%)	$iAUC_{0-8\,h}$		
		Insulin or analogue		C-peptide (pM · min · 10^3)
		%	pM · min · 10^3	
Asp^{B10}	315	50	10.5 ± 1.6*	−134 ± 13
Human	100	100	21.3 ± 1.8	−143 ± 8
Asp^{B28}	88	97	20.7 ± 4.5	−120 ± 13
$Asp^{B9}Glu^{B27}$	20	380	81.0 ± 6.5*	−115 ± 16

Values are means ± SE and, those for human insulin are mean of 2 human insulin study days. Receptor binding affinities of preparations (defined in Table 1) and cumulative incremental areas under curve (iAUCs) between 0 and 8 h for immunoreactive insulin or analogue and C-peptide plasma concentrations after injection of 0.6 nmol · kg^{-1} s.c. of each preparation into healthy male volunteers (n = 7).
*P < 0.001 vs. human insulin by paired t test.

CONCLUSIONS

This study demonstrated the rapid subcutaneous absorption of insulin analogues Asp^{B28} and Asp^{B10} in humans and extended our earlier findings with the monomeric analogue $Asp^{B9}Glu^{B27}$ (19). Assuming that elimination of insulin and analogues from the plasma is first order, the plasma appearance curves predicted from the disappearance data agree with their measured appearance when corrected for C-peptide suppression (P. Hougaard, Novo Research Inst., Bagsvaerd, Denmark, unpublished observations). Thus, significant degradation of insulin or analogues at the injection site is unlikely, and the disappearance curves provide a reliable measure of absorption. More-detailed analysis of the disappearance data than presented here revealed that the monomeric analogue does not exhibit a lag phase of absorption and that the known increasing rate of subcutaneous absorption of soluble insulin can be explained by the progressive breakdown of hexameric aggregates into dimeric and monomeric units at the subcutaneous site (S.K. et al., this issue, p. 1057–1065).

The rapid absorption of the three analogues was accompanied by a rapid rise in plasma insulin analogue levels followed by a rapid return toward basal levels (Fig. 1*B*). The resulting plasma insulin analogue profiles are qualitatively very similar to the physiological insulin

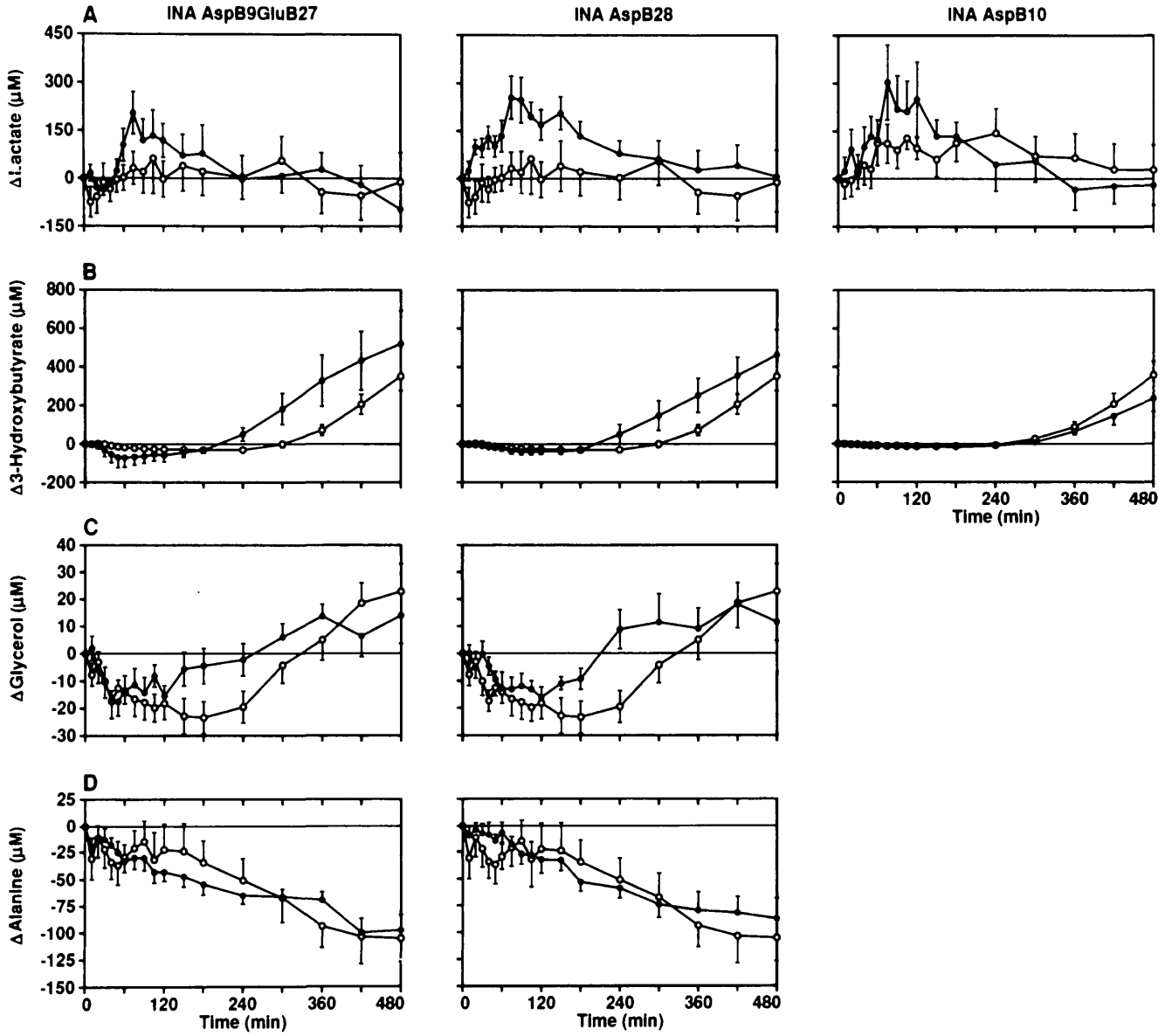

FIG. 2. Mean ± SE change from basal of blood lactate (*A*), 3-hydroxybutyrate (*B*), glycerol (*C*), and alanine (*D*) concentrations after injection of 0.6 nmol · kg^{-1} s.c. soluble human insulin (○) or insulin analogue (INA; ●) into healthy subjects (n = 7) at min 0. Glycerol and alanine were not measured for Asp^{B10} study.

response to a meal in nondiabetic subjects (1). The faster delivery of the analogues was accompanied by a more-rapid onset and offset of action as seen by changes in glucose, C-peptide, and intermediary metabolites. The recovery from insulin-induced hypoglycemia involves both the dissipation of insulin and activation of glucose counterregulatory systems (24,25). It is therefore possible that the rapid offset of action of the analogues may in part have resulted from a greater counterregulatory response secondary to the more pronounced degree of hypoglycemia. However, the manner in which the plasma glucose changes closely mirror the plasma insulin analogue changes (Fig. 1, *C* and *B*) is very striking and suggests that the recovery in glucose levels is aided by the rapidly declining plasma insulin analogue concentrations. Furthermore, the percentage of human insulin absorbed between 3 and 8 h is at least twofold greater than that of insulin analogues, and in comparison with analogue Asp^{B28} (the only analogue for which quantitative comparison between plasma insulin and insulin analogue concentrations is possible; see below), this results in plasma insulin levels that are fourfold greater than corresponding insulin analogue levels ($P < 0.005$; Table 2). These findings also agree with the observations of Heineman et al. (26), who used the euglycemic clamp technique to compare subcutaneously administered human insulin and analogues Asp^{B9}Glu^{B27} and Asp^{B10} in healthy volunteers. After injection of 72 nmol (~12 U) of each analogue, glucose infusion rates were higher with the analogues from as early as 20–30

TABLE 4
Summary of intermediary metabolite results

Parameter	Human insulin (day 2)	Analogue			Human insulin (day 1)
		$Asp^{B9}Glu^{B27}$	Asp^{B28}	Asp^{B10}	
Lactate					
Basal (μM)	768 ± 74	594 ± 42	542 ± 60*	539 ± 56	501 ± 55
ΔPeak (μM)	230 ± 88	292 ± 65	336 ± 230	421 ± 89†	251 ± 56
$iAUC_{0-1\ h}$ (μM · min · 10^3)	1.4 ± 9.1	13.7 ± 7.5	28.7 ± 6.6*	27.8 ± 11.2	12.8 ± 8.1
3-Hydroxybutyrate					
Basal (μM)	38 ± 12	98 ± 61	51 ± 16	25 ± 6	24 ± 5
ΔNadir (μM)	−33 ± 11	−81 ± 51	−44 ± 15	−20 ± 6	−15 ± 3
tNadir (min)	206 ± 32	84 ± 8*	115 ± 17†	81 ± 10†	118 ± 10
tBasal (min)	315 ± 24	215 ± 24‡	239 ± 23‡	237 ± 17	281 ± 27
Glycerol					
Basal (μM)	88 ± 9	88 ± 12	83 ± 12		
ΔNadir (μM)	−30 ± 5	−27 ± 5	−23 ± 5		
tNadir (min)	182 ± 35	107 ± 27†	82 ± 12†		
tBasal (min)	350 ± 25	231 ± 36†	219 ± 38†		
Alanine					
Basal (μM)	305 ± 27	253 ± 15	275 ± 27		
$iAUC_{0-8\ h}$ (μM · min · 10^3)	−27.7 ± 9.3	−28.7 ± 3.0	−26.1 ± 4.8		

Values are means ± SE for measured parameters (relating to blood concentrations of lactate, 3-hydroxybutyrate, glycerol, and alanine) after injection of 0.6 nmol · kg^{-1} s.c. soluble human insulin or insulin analogues $Asp^{B9}Glu^{B27}$, Asp^{B28}, and Asp^{B10} into healthy male volunteers (n = 7). Glycerol and alanine were not measured for Asp^{B10} study. iAUC, Incremental areas under curve.
*$P < 0.02$, †$P < 0.05$, ‡$P < 0.005$, vs. respective human insulin by paired t test.

min postinjection, whereas, an earlier and faster decrease in glucose infusion rate was also observed with the analogues between 5 and 6 h after administration. Therefore, it might be predicted that the risk of late hypoglycemia would be less with such analogues.

Because endogenous insulin release was largely suppressed during our study, the $iAUC_{0-8\ h}$ for plasma insulin and analogue levels reflected the appearance in the circulation of the exogenously adminstered preparations. The $iAUC_{0-8\ h}$ for the insulin analogue plasma levels spanned an eightfold range (Table 3), and yet their effects on plasma glucose and C-peptide levels were very similar. In euglycemic-hyperinsulinemic clamp studies in pigs (27), a similar relationship between plasma insulin analogue levels and biological action has been observed after equimolar infusions of analogues and human insulin. In these pig studies, the low-affinity analogue ($Asp^{B27}Glu^{B27}$) reached two to three times higher and the high-affinity analogue (Asp^{B10}) significantly lower steady-state plasma concentrations than did human insulin. The calculated metabolic clearance rate (MCR) of $Asp^{B9}Glu^{B27}$ (7 ml · kg^{-1} · min^{-1}) was lower and that of Asp^{B10} (26 ml · kg^{-1} · min^{-1}) was higher than that of human insulin (20 ml · kg^{-1} · min^{-1}). Another study in miniature pigs showed similar rankings in MCRs for the low- and high-affinity analogues and human insulin (28). Therefore in our study, although the shape of the insulin analogue concentration curves (rapid rise to peak followed by fast return toward basal) largely reflects their rapid absorption, their differing AUCs were consistent with their expected differing metabolic clearance rates (as predicted by the pig clamp studies). The nearly full in vivo potency of the low-affinity analogue $Asp^{B9}Glu^{B27}$ may therefore be explained by high circulating levels, resulting from its low MCR, counteracting its low receptor binding affinity. Conversely, low levels of Asp^{B10} result from its high MCR and offset its high receptor binding affinity. It also follows that direct quantitative comparison between circulating analogue and human insulin levels are not possible except for the relatively normal affinity analogue (Asp^{B28}), whose MCR is similar to human insulin (unpublished observations).

Receptor binding and internalization of the bound ligand–receptor complex is a major pathway of insulin degradation (29,30). The relationship described above between receptor binding affinity and MCR in pigs (27,28) and the observation that high-affinity insulin analogues are internalized more rapidly than low-affinity analogues by human hepatoma (HepG2) cells (18) suggest that the insulin analogues are also largely cleared by a receptor-mediated mechanism. Moreover, the similar inverse relationship shown between the receptor binding affinities of the preparations in this study and their iAUC for insulin analogue levels provides strong indirect in vivo evidence that receptor-mediated degradation is also a major pathway of insulin and analogue clearance in humans.

In conclusion, this study has shown that each analogue is absorbed some two to three times faster than human insulin in healthy male volunteers. Despite their widely different in vitro characteristics, all three ana-

logues have similar biological actions to human insulin, as evidenced by their effects on glucose, C-peptide, and intermediary metabolites. However, the time course of these effects differs from that of human insulin and is consistent with the faster absorption profiles of the analogues. The rapid onset and offset of action of these insulin analogues make them promising candidates for the provision of meal-related insulin requirements in diabetes. We have already shown in insulin-dependent diabetic subjects that these insulin analogues achieve more physiological plasma insulin profiles and reduce postprandial glucose excursions compared with soluble human insulin (31,32).

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Preliminary analysis of part of these data appears in ref. 33.

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