Insulin Aspart (B28 Asp-Insulin): A Fast-Acting Analog of Human Insulin

Absorption kinetics and action profile compared with regular human insulin in healthy nondiabetic subjects

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OBJECTIVE — To study the pharmacokinetic and pharmacodynamic profile of insulin aspart (a new fast-acting human insulin analog) after subcutaneous administration in the deltoid, abdominal, and thigh sites and to compare this profile with regular human insulin (Novolin; Novo Nordisk A/S, Copenhagen).

RESEARCH DESIGN AND METHODS — A total of 20 healthy subjects were studied in a single-center six-period double-blind randomized crossover trial with 6 study days and a washout period of 1 week between each single daily dose of the trial drug. Subjects were randomized to receive a single dose of 0.2 U/kg of insulin aspart or regular insulin on each of the 6 study days in three different sites (the deltoid, the abdomen, and the thigh) during a 10-h euglycemic clamp (two drugs and three injection sites). Pharmacokinetic and pharmacodynamic measurements were derived from blood sample measurements of glucose, insulin, and C-peptide during these clamps.

RESULTS — The pharmacodynamic data from the euglycemic clamp study showed that, regardless of injection site, the maximal glucose infusion rate (GIR C_{max}) was greater and occurred at an earlier time (GIR T_{max}) after administration of insulin aspart than regular insulin (GIR C_{max}: abdomen 813 vs. 708, deltoid 861 vs. 736, and thigh 857 vs. 720 g/min, $P < 0.05$ for all; GIR T_{max}: abdomen 94 vs. 173, deltoid 111 vs. 192, and thigh 145 vs. 193 g/min, $P < 0.05$ for all). Pharmacokinetic parameters were also consistent with faster absorption and higher peak insulin concentrations after insulin aspart administration. From all sites, the peak insulin concentration (C_{max}) was higher and occurred earlier (T_{max}) after administration of insulin aspart than of regular insulin (C_{max}: abdomen 501 vs. 260, deltoid 506 vs. 252, thigh 422 vs. 220 pmol/l, $P < 0.001$ for all sites; T_{max}: abdomen 52 vs. 109, deltoid 54 vs. 98, and thigh 60 vs. 107 min, $P < 0.01$ for all sites). The absorption and glucose-lowering action of insulin aspart did not differ between sites (similar GIR C_{max}, T_{max}, and area under the curve parameters). However, the duration of the glucose-lowering effect was up to 34 min shorter ($P < 0.01$) for the abdomen injections than for the deltoid or thigh injections (lower time of 50% glucose disposal). In addition, the amount of glucose infused was significantly lower by 10–14% in the abdomen than in other sites.

CONCLUSIONS — Subcutaneous administration of insulin aspart causes a more rapid and intense maximal effect compared with regular insulin during euglycemic clamp studies in nondiabetic subjects. Abdominal administration of insulin aspart has a shorter duration of glucose-lowering effect compared with administration in the deltoid or thigh.

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The Diabetes Control and Complications Trial has conclusively demonstrated that intensive treatment of diabetes not only delays the progression of microvascular complications in type 1 diabetes but also reduces the incidence of these complications (1). This may also be true for type 2 diabetes (2,3). To achieve good glycemic control and to attempt to mimic physiological insulin secretion, treatment regimens consisting of multiple injections are often used. Basal insulin requirements are supplied as an injection of long- or intermediate-acting insulin, and meal-related glucose excursions are controlled with bolus injections of soluble insulin. Subcutaneous injection of soluble insulin in diabetic patients causes free insulin levels to rise slowly to a plateau after 3 h and then decline slowly and return to baseline ~9 h later (4). This not only results in postprandial hyperglycemia, but it also increases the risk of hypoglycemia between meals because of the long duration of the action of soluble insulin. To avoid postprandial hyperglycemia, some studies have recommended that diabetic individuals administer their injection of soluble insulin 30–60 min before a meal (5–7). This is inconvenient for the patient, and a large number of diabetic individuals inject insulin immediately before eating with consequent suboptimal glycemic control.

When given by subcutaneous injection, monomeric or dimeric insulin is absorbed more rapidly than regular human insulin (8,9). With insulin aspart, the amino acid proline in the β-chain has been replaced by the amino acid aspartic acid. In human insulin lispro (another fast-acting insulin),

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Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; C_{max}, maximal concentration; ECG, electrocardiogram; GIR, glucose infusion rate; MRT, mean residence time; $T_{AUC\ 1/2}$, time of 50% glucose disposal; T_{max}, time to maximal concentration.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

the natural amino acid sequence of the β-chain at positions 28 and 29 is inverted (8). The effect of these changes is to reduce the tendency of the insulin molecule to self-associate into hexamers. Extensive preclinical testing has shown that the chemical and biological properties of insulin aspart are similar to those of human regular insulin in terms of potency and binding characteristics to the insulin/IGF-1 receptor (10,11). In clinical studies, both insulin aspart and regular human insulin appear to be equally potent in terms of glucose-lowering effect (12). However, the time of onset of action of insulin aspart is faster and the duration of action is shorter than that of human insulin. Thus, when injected before a meal, insulin aspart may provide better control of postprandial blood glucose than regular human insulin, even if it is given immediately before a meal. Additionally, the more rapid decline in plasma insulin levels after insulin aspart administration may decrease the risk of hypoglycemia between meals. The site of subcutaneous insulin administration also plays an important part in glycemic control. A subcutaneous injection of regular insulin is absorbed most rapidly from the abdomen, less rapidly from the deltoid region, and most slowly from the thighs and the gluteal region (13). Subcutaneous injection of regular insulin in the abdomen has also been shown to produce a substantially greater reduction of plasma glucose than an injection of regular insulin in the thigh (13). This study was designed to compare the pharmacokinetic profiles of and the pharmacodynamic responses to single doses of insulin aspart and regular human insulin (Novolin; Novo Nordisk, Copenhagen) when injected subcutaneously into three different sites (abdomen, thigh, and deltoid) in healthy nondiabetic subjects during a euglycemic clamp.

RESEARCH DESIGN AND METHODS

— The experimental protocol for this study was approved by the Human Subjects Committee of the University of California at San Diego. After explaining the protocol, written informed consent was obtained from all subjects. Before study entry, 20 healthy male subjects who were nonsmokers and had no family history of diabetes were screened with a medical history, physical examination, complete blood count, serum chemistry analysis, hepatitis B and human immunodeficiency virus screenings, a urine screening for drugs of abuse, and an electrocardiogram (ECG). The mean age of the subjects was 31.1 ± 6.51 years, and the mean BMI was 23.6 ± 2.2 kg/m^2. All participants were in good health and had fasting plasma glucose values of <104 mg/dl.

Study design

By using the euglycemic clamp technique (14), we compared insulin aspart and regular human insulin (Novolin) given subcutaneously (0.2 U/kg body wt) at three different sites (deltoid, thigh, and abdomen on the nondominant side). Thus, all subjects were randomized (block factorial design) to six interventions (two drugs and three sites). There was a washout period of at least 1 week and not more than 2 weeks between each clamp session. Before the commencement of each study, the subjects were asked not to exercise strenuously for 4 days and not to consume alcohol for 1 day. All subjects were admitted to the Special Diagnostic and Treatment Unit at 8:00 P.M. on the day before each study and fasted overnight except for water. On the day of the study, a urine analysis for drugs of abuse and an alcohol breath test were performed. Throughout the clamp study, cardiac rhythm was monitored by using continuous telemetry, and pre- and post-study ECGs were performed.

The euglycemic clamp study was performed after inserting two catheters (an antecubital venous catheter for infusing 20% dextrose and a retrograde venous catheter in the hand for measuring plasma glucose). Basal plasma glucose measurements were obtained every 30 min for 90 min, and then crystalline human insulin (Novolin) or Novolin Aspart insulin (Novo Nordisk A/S, Copenhagen, Denmark) was injected subcutaneously into the study site. During the entire clamp study period of 10 h, the plasma glucose concentration was clamped at euglycemia (i.e., the mean of basal plasma glucose measurements) by a variable infusion of exogenous 20% dextrose based on the 10- to 15-min plasma glucose measurement. The average corrected glucose infusion rate (GIR) was calculated for each 10-min period in the first hour of the clamp and then for each 15-min period for the remainder of the study. Blood for serum insulin and C-peptide levels was drawn every 30 min in the basal period, every 10 min during the first hour of the clamp, and then every 30 min throughout the entire study.

Analytical techniques

Blood drawn for glucose was immediately separated by an Eppendorf microcentrifuge (Brinkman, Westbury, NY), and plasma glucose was determined via the glucose oxidase method (model 2300 STAT; Yellow Springs Instrument, Yellow Springs, OH). Blood for serum insulin and C-peptide measurements was collected in untreated tubes and was allowed to clot at room temperature for 60 min before the supernatant was removed. All specimens were stored at −20°C until assayed. Serum insulin or insulin aspart was assayed by using a standard commercial radioimmunoassay kit validated for both human soluble insulin and insulin aspart at Medi-Lab (Copenhagen, Denmark). C-peptide was measured by using a standard commercial enzyme-linked immunosorbent assay kit at Medi-Lab.

Statistical methods

The primary effectiveness end points were the following parameters derived from the GIR profiles during the time interval from 0 to 600 min: *1*) maximal glucose infusion rate (GIR C_{max}) during the interval from drug administration (0 min) to last meas-

Table 1—*Pharmacodynamic parameters (GIR)*

	Abdomen			Deltoid			Thigh		
	Regular	Aspart	*P* value	Regular	Aspart	*P* value	Regular	Aspart	*P* value
C_{max} (mg/min)	708 ± 20	813 ± 23	<0.01	736 ± 24	861 ± 28	<0.05	720 ± 23	857 ± 33	<0.01
T_{max} (min)	173 ± 62	94 ± 46	<0.001	192 ± 51	111 ± 59	<0.001	193 ± 60	145 ± 122	<0.05
AUC (g)	192 ± 47	168 ± 48	<0.01	205 ± 56	189 ± 56	<0.01	206 ± 54	200 ± 58	NS

Data are means ± SD.

ured time point (600 min), *2*) time of maximal glucose infusion rate (GIR T_{max}) during the interval from 0 to 600 min, *3*) the area under the curve (AUC) during the interval from 0 to 600 min (GIR AUC), and *4*) time of 50% glucose disposal ($T_{AUC\ 1/2}$). This is the median of the GIR profile. Secondary effectiveness end points were the following parameters derived from the serum insulin profiles: *1*) mean residence time (MRT), *2*) maximal concentration (C_{max}) during the interval from 0 to 600 min, *3*) time to maximal concentration (T_{max}), and *4*) AUC during the interval from 0 to 600 min (AUC 0–600). MRT was included because a difference in MRT is equal to a difference in mean absorption time. The null hypothesis that the three injection sites were equal was tested against the alternative that at least two of the three sites differed. Also, the primary and secondary effectiveness parameters for the two insulins were compared regarding injection site. Mean differences between the three injection sites and between treatments for each of the three sites were estimated, and 95% CIs were constructed. GIR AUC (0–600 min), GIR C_{max}, $T_{AUC\ 1/2}$, AUC insulin, C_{max}, and MRT were log transformed before analysis of variance (ANOVA) with injection site, treatment, and period as fixed effects and subject as a random effect. T_{max} was compared by nonparametric methods. ANOVA was carried out with drug, sites, and drug-by-site interaction as fixed factors and subject as random block. No significant drug-by-site interaction was observed. Therefore, we can interpret the results separately by drug and site.

RESULTS — Two subjects dropped out of the study after receiving three and four doses, one because of personal reasons and the other because of a positive urine drug screen. A total of 18 subjects completed the entire study.

Primary effectiveness end points: pharmacodynamics

The results of the pharmacodynamic parameters are summarized in Table 1 and are shown in Fig. 1. The GIR C_{max} during the interval from drug administration (0 min) to last measured time point (600 min) was significantly higher for insulin aspart (at all injection sites) than for regular insulin (abdomen 813 vs. 708, deltoid 861 vs. 736, thigh 857 vs. 720 g/min, $P < 0.05$ for all). Between injection sites, the GIR C_{max} was highest after the deltoid injection (followed by the thigh) and lowest after the

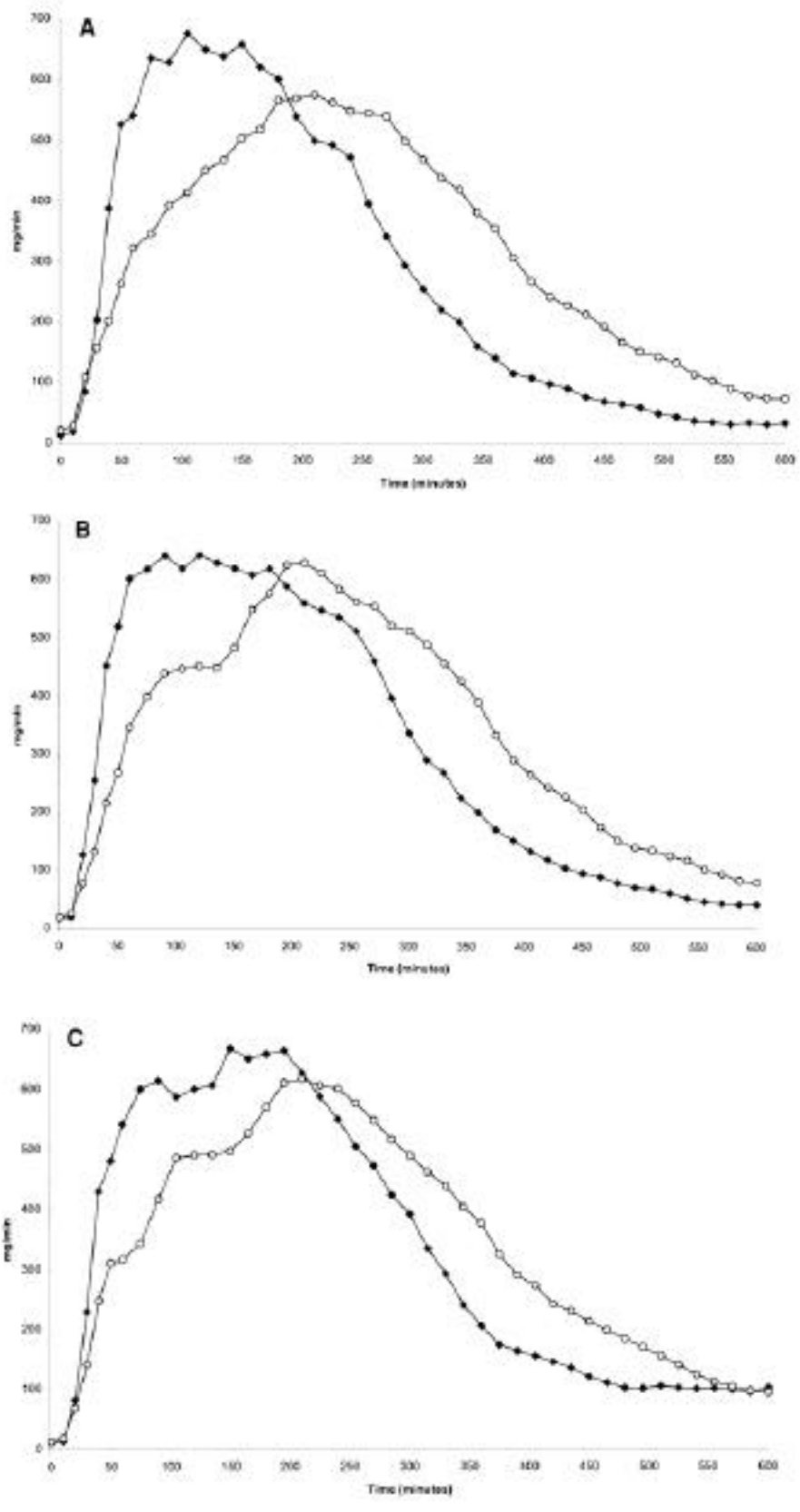

Figure 1—*The mean GIR response in nondiabetic subjects after subcutaneous injection of human insulin aspart (◆) and human regular insulin (Novolin) (○) at various sites.* A*: Abdomen injection.* B*: Deltoid injection.* C*: Thigh injection.*

Table 2—*Pharmacokinetic parameters (insulin concentration)*

	Abdomen			Deltoid			Thigh		
	Regular	Aspart	*P* value	Regular	Aspart	*P* value	Regular	Aspart	*P* value
C_{max} (pmol/l)	260 ± 99	501 ± 111	<0.001	252 ± 99	506 ± 202	<0.001	221 ± 64	422 ± 171	<0.001
T_{max} (min)	109 ± 68	52 ± 20	<0.01	98 ± 77	54 ± 26	<0.01	107 ± 59	60 ± 37	<0.001
AUC (pmol · min^{-1} · l^{-1})	65,373 ± 102	77,975 ± 130	<0.01	65,423 ± 116	81,633 ± 211	<0.01	62,478 ± 58	75,916 ± 122	NS

Data are means ± SD. *P* values were calculated for the ANOVA models.

abdominal injection for both insulin aspart and regular insulin. However, this was not statistically significant.

The GIR T_{max} during the interval from 0 to 600 min was significantly less for insulin aspart at all injection sites than for regular insulin (abdomen 94 vs. 173, deltoid 111 vs. 192, thigh 145 vs. 193 min, $P < 0.05$ for all). Thus, the peak glucose-lowering effect of insulin aspart occurred much faster with insulin aspart than with regular insulin. When comparing drugs for every injection site, the GIR T_{max} was shortest for abdominal administration followed by the deltoid, and GIR T_{max} was longest after injection in the thigh. Although this was not statistically significant, this suggests a more rapid glucose-lowering effect after abdominal injections than after deltoid or thigh injections.

The GIR AUC that expressed the cumulative glucose infused was slightly smaller after administration of insulin aspart compared with regular insulin at all injection sites (abdomen 168 vs. 192, deltoid 189 vs. 205, thigh 200 vs. 206 g). These differences of 3–12% were statistically significant only after abdominal and deltoid injections and suggest that insulin aspart may have a slightly smaller total glucose-lowering effect than regular insulin. When comparing between sites, GIR AUC was significantly lower ($P < 0.01$) after abdominal injection compared with deltoid and thigh injections of insulin aspart.

$T_{AUC\ 1/2}$ was significantly shorter for insulin aspart at all injection sites compared with regular insulin (abdomen 69 min shorter, deltoid 62 min shorter, thigh 48 min shorter). For insulin aspart, $T_{AUC\ 1/2}$ was shortest after abdominal injection (followed by the deltoid) and longest after administration in the thigh.

Secondary effectiveness end points: pharmacokinetics

The results of the pharmacokinetic parameters are summarized in Table 2 and are shown in Fig. 2. Comparison of treatment with insulin aspart and regular insulin showed the following results.

C_{max} during the interval from 0 to 600 min was higher after administration of insulin aspart than regular insulin (abdomen 501 vs. 260, deltoid 506 vs. 252, thigh 422 vs. 221 pmol/l, $P < 0.001$ for all sites). This was the case for any injection site, and the differences were highly statistically significant and more pronounced after the values were adjusted for endogenous insulin (data not shown). Between sites, the C_{max} was significantly lower for insulin aspart at the abdomen than at the thigh.

T_{max} was significantly lower after insulin aspart than after regular insulin injection, and again this was regardless of injection site (abdomen 52 vs. 109, deltoid 54 vs. 98, thigh 60 vs. 107 min, $P < 0.01$ for all sites). Insulin aspart was absorbed significantly faster than regular insulin, and there was no intersite difference in insulin aspart absorption.

The AUC for insulin concentration was also greater after administration of insulin aspart than after regular insulin for all injection sites (abdomen 77,975 vs. 65,373, deltoid 81,633 vs. 65,423, thigh 75,916 vs. 62,478 pmol · min^{-1} · l^{-1}, $P < 0.01$ for all). Again, there was no intersite difference in insulin aspart absorption.

The MRT for each site was lower for insulin aspart than for regular insulin (abdomen 150 vs. 213, deltoid 160 vs. 220, thigh 172 vs. 222 min, $P < 0.001$ for all). Between sites, the MRT was significantly lower after abdominal administration than after thigh administration.

CONCLUSIONS — The results of this study demonstrate the significantly different pharmacokinetic and pharmacodynamic properties of insulin aspart compared with regular human insulin when injected subcutaneously. The substitution of aspartate for proline in insulin aspart reduces the tendency of monomers to self-associate, and this property greatly enhances the rate of insulin absorption. By using the euglycemic clamp technique, pharmacodynamic data from our study indicate that, regardless of injection site, the peak glucose-lowering effect after injection of insulin aspart, as measured by the GIR C_{max}, was significantly greater and occurred much earlier than after the administration of regular insulin. In this study, healthy nondiabetic subjects with intact islet cell function were studied. Thus, endogenous insulin secretion may magnify the dynamics of the injected insulin and overestimate the onset of action, peak activity, and duration of action. With the administration of exogenous insulin and induction of its glucose-lowering effect, the intact counterregulatory hormone response in these subjects may possibly lead to an underestimation of the true insulin effect. However, in this study, C-peptide levels were measured (data not shown), and the differences between insulin aspart and human insulin remained significant even after C-peptide levels were used to adjust for endogenous insulin production.

The more rapid and intense glucose-lowering response with insulin aspart (similar to that seen with insulin lispro [15]) mimics the normal physiological response to endogenous insulin and may help provide better control of the rapid rise in postprandial glucose levels. Moreover, the $T_{AUC\ 1/2}$ was also lower for insulin aspart, which suggests a more rapid taper of its glucose-lowering effect. This rapid tapering may be beneficial in reducing the occurrence of late postprandial hypoglycemia that is known to occur with regular insulin.

In keeping with its rapid metabolic effect, insulin aspart was absorbed more rapidly from all injection sites and reached a higher serum peak concentration (higher C_{max} and lower T_{max} insulin). In addition, despite equivalent dose administration, more insulin aspart was absorbed from all sites (greater AUC insulin). Similar results were reported by Braak et al. (15) in a study

of 12 healthy nondiabetic volunteers who were injected with 0.2 U/kg of either insulin lispro or regular insulin and were followed with a euglycemic clamp for 12 h, as in our study. These differences were even more pronounced when values were adjusted for endogenous insulin (data not shown). However, even though a greater amount of insulin aspart was absorbed, its total glucose-lowering effect (as measured by AUC for glucose) was slightly lower (~3–12%). This suggests that, although insulin aspart is absorbed faster and has a more rapid and intense glucose-lowering effect, its total glucose-lowering effect may be slightly less than that of regular insulin, especially when administered at abdominal or deltoid sites. Braak et al. (15) also reported that regular insulin induced a greater overall glucose demand than insulin lispro, especially after deltoid and femoral injections.

Insulin absorption is known to be affected by injection site. Differences in absorption rates reflect physiological differences in the subcutis and blood flow in the three injection sites (16). Deltoid and femoral sites are associated with prolonged insulin absorption due to reduced blood flow (16). Subcutaneous injection of regular insulin in the abdomen is not only absorbed more rapidly (13,16–19) but also has been shown to produce a substantially greater reduction of plasma glucose than an injection of regular insulin in the thigh (13). In contrast with previous studies, in our study, the amount of glucose infused indicates that the onset of the glucose-lowering action of insulin aspart did not significantly differ between the injection sites as reflected by similar GIR T_{max}. This finding is supported by Braak et al. (15), who could not demonstrate any significant difference in the onset of the glucose-lowering effect between sites after insulin lispro injection. However, the duration of the glucose-lowering action was up to 34 min shorter after abdominal injection than after deltoid or thigh injections ($P < 0.001$) as assessed by GIR $T_{AUC\ 1/2}$. The total amount of glucose infused was also significantly lower (10–14%) in the abdomen than in other sites. Insulin lispro injected into the abdomen induces a 4–7% lower glucose demand ($P = 0.066$) (15). Thus, insulin aspart injected into the abdomen, although absorbed as quickly as from the deltoid and thigh regions, would have a more rapid taper of its glucose-lowering effect and potentially have less propensity to cause late postmeal hypoglycemia. The pharma-

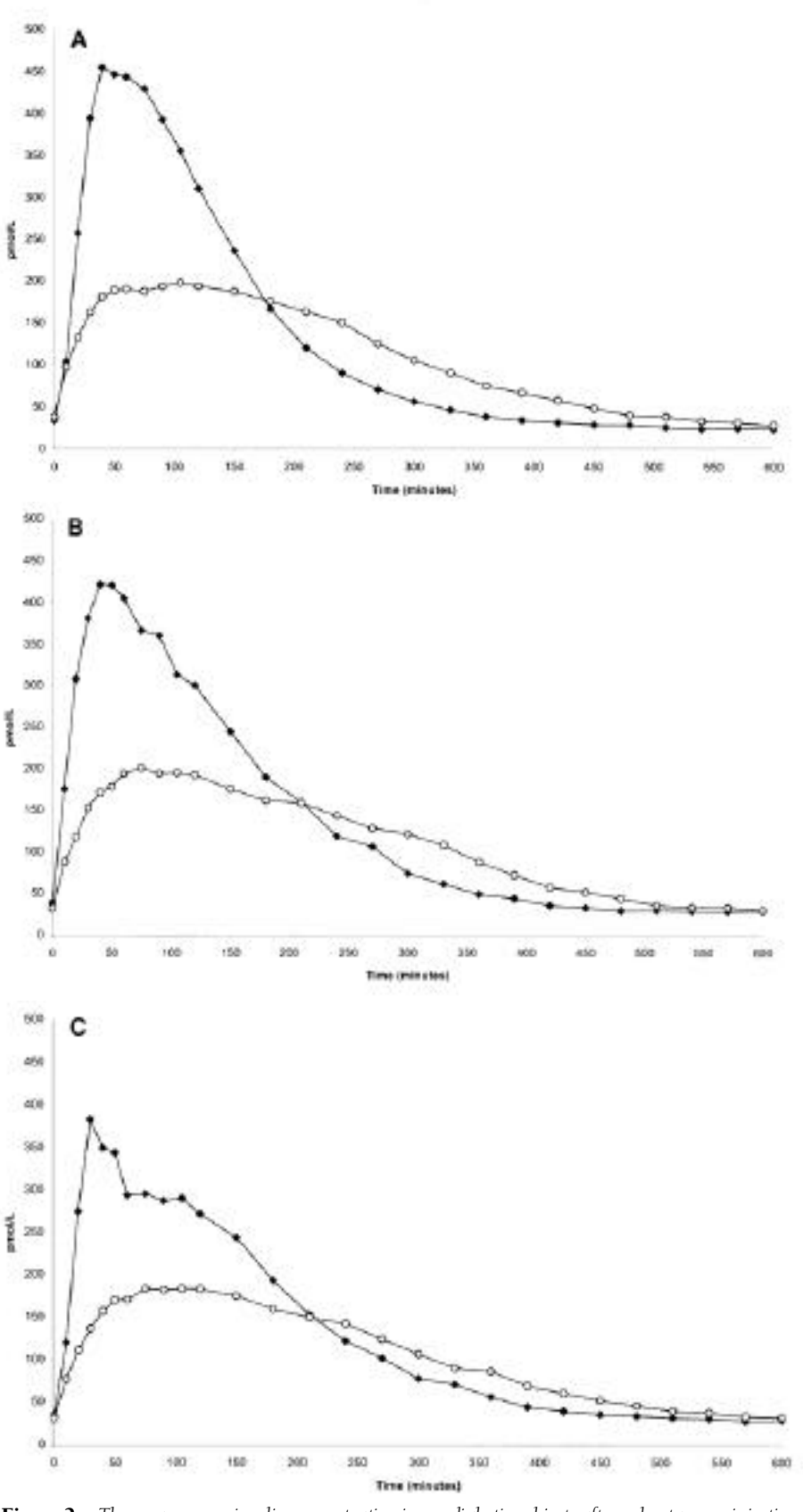

Figure 2—*The mean serum insulin concentration in nondiabetic subjects after subcutaneous injections (0.2 U/kg) of human insulin aspart (◆) and human regular insulin (Novolin) (○) at various sites.* A: *Abdomen injection.* B: *Deltoid injection.* C: *Thigh injection.*

cokinetic and pharmacodynamic parameters for insulin aspart injections into the deltoid or thigh regions were similar.

In conclusion, insulin aspart is absorbed significantly faster, reaches a higher serum peak concentration, and evokes a more rapid glucose-lowering response than regular human insulin in healthy nondiabetic subjects. It is well tolerated, and its duration of action is shortest after abdominal subcutaneous injection. These properties make insulin aspart an attractive option for use at meal times to ameliorate postmeal hyperglycemia in diabetic subjects. The rapid taper of its metabolic effect may be beneficial in reducing the occurrence of late postmeal hypoglycemia.

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