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Pharmacokinetics and pharmacodynamics of insulin aspart in patients with type 2 diabetes: Assessment using a meal tolerance test under clinical conditions

Hitoshi Ando,*[†] Seiichiro Kurita,* Akiko Shimizu,* Ken-ichiro Kato,*

Kazuhide Ishikura,* Koumei Taji,* Masafumi Uno,* Yumie Takeshita,*

Hirofumi Misu,* Akio Fujimura,*[†] Shuichi Kaneko* and Toshinari Takamura*

*Department of Disease Control and Homeostasis, Kanazawa University Graduate School of Medical Science, Kanazawa, and [†]Division of Clinical Pharmacology, Department of Pharmacology, School of Medicine, Jichi Medical University, Shimotsuke, Japan

Short title: Pharmacokinetics of insulin aspart

Correspondence to Hitoshi Ando, MD, PhD,

Division of Clinical Pharmacology, Department of Pharmacology, School of Medicine,

Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan.

Telephone number: +81-285-58-7388   Fax number: +81-285-44-7562

E-mail: h-ando@jichi.ac.jp

SUMMARY

1. Few studies have evaluated the pharmacokinetics of rapid-acting insulin analogues in patients with type 2 diabetes, especially under clinical conditions. The aim of this study was to assess both the pharmacokinetics and pharmacodynamics of insulin aspart in type 2 diabetic patients who were being treated with the analogue alone.

2. Meal tolerance tests with and without self-injection of a customary dose of insulin aspart (0.05–0.22 U/kg) were conducted in 20 patients in a randomised crossover study.

3. The dose (per body weight) of insulin aspart was significantly correlated with both the maximum concentration ($r^2 = 0.59$, $P < 0.01$) and area under the concentration-time curve for insulin aspart ($r^2 = 0.53$, $P < 0.01$). However, the time to maximum concentration (t_{max}), which varied widely from < 60 min to ≥ 120 min, was not associated with either dosage ($r^2 = 0.02$, $P = 0.51$) or body mass index ($r^2 = 0.02$, $P = 0.57$). Injection of insulin aspart exacerbated delayed hyperinsulinaemia after meal loading, mainly in patients with t_{max} values ≥ 120 min. With regard to pharmacodynamics, insulin aspart had favourable effects on postprandial hyperglycaemia, hyperglucagonaemia, and hyperlipidaemia.

4. The t_{max} for this insulin analogue differed greatly between individuals, and delayed hyperinsulinaemia was particularly exacerbated in patients with higher t_{max} values. Identification of the factors contributing to inter-individual variation in the absorption lag time is essential for improving its efficacy and safety.

Key words: insulin aspart, rapid-acting insulin analogues, delayed hyperinsulinaemia,

glucagon, postprandial hyperlipidaemia

INTRODUCTION

The elevation of postprandial plasma glucose levels, which occurs due to the loss of first-phase insulin secretion, decreased insulin sensitivity, and consequent defective suppression of hepatic glucose output after meals, begins prior to the onset of type 2 diabetes.[1,2] Therefore, postprandial hyperglycaemia is common in patients with type 2 diabetes, even when overall glycaemic control, as assessed by measuring glycosylated haemoglobin levels, appears to be adequate.[3] Because post-challenge hyperglycaemia is known to be an independent risk factor for cardiovascular disease and all-cause mortality,[4,5] various agents that target postprandial plasma glucose, including rapid-acting insulin analogues, are now used clinically in the treatment of type 2 diabetes. Of note, mealtime dosing of a rapid-acting insulin analogue, without basal insulin or any other medications, improved both postprandial and early morning fasting plasma glucose levels in approximately one-half of 40 Japanese patients with type 2 diabetes.[6]

Insulin aspart is a rapid-acting insulin analogue in which proline 28 in the B chain has been replaced by aspartic acid.[7] Several studies have investigated the pharmacokinetics of insulin aspart and have compared the results to those for regular insulin[7-14] or other rapid-acting insulin analogues.[15-18] However, most of these studies were conducted in healthy subjects[7-10,13,18] or patients with type 1 diabetes.[12,16,17] In addition, studies in patients with type 2 diabetes used a dose of insulin aspart that was weight-adjusted, but not clinically appropriate for each patient.[11,14,15] Recently, Gagnon-Auger et al.[19] used a euglycaemic clamp to show that the pharmacokinetics of

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insulin lispro, another rapid-acting insulin analogue, differ between healthy subjects and patients with type 2 diabetes, and that the time to maximum concentration (t_{max}) is dose-dependently increased in diabetic patients. Therefore, the pharmacokinetics of insulin aspart should be assessed in patients with type 2 diabetes, especially under clinical conditions. To address the issue, we determined the pharmacokinetics of a customary dose of insulin aspart administered immediately before eating in type 2 diabetic patients receiving insulin aspart monotherapy. Another trial with a randomised crossover design was performed without insulin aspart. By comparing the concentration-time profiles of endogenous insulin, glucose, glucagon, triglycerides, and remnant-like particle (RLP)-cholesterol in these trials, we further evaluated the pharmacodynamics of insulin aspart in a clinical setting.

METHODS

Patients

This study was conducted between February and June 2007. A total of 20 Japanese patients with type 2 diabetes were recruited from the outpatient clinic at Kanazawa University Hospital (Kanazawa, Japan) based on the following inclusion criteria: 1) treatment with insulin aspart monotherapy, i.e., dosing of pure insulin aspart at each mealtime without combined administration of any oral antidiabetic agent or other insulin preparation[6] for at least 3 months; 2) relatively good and stable glycaemic control, as defined by two consecutive haemoglobin A_{1c} measurements of $< 7.4\%$ and a difference between measurements of $< 0.5\%$; and 3) all chronic diseases other than type 2 diabetes, such as hypertension and dyslipidaemia, being stable. For insulin aspart monotherapy, the breakfast, lunch, and dinner doses of insulin aspart were adjusted in an attempt to achieve plasma glucose levels of 4.4–6.7 mmol/l before lunch, dinner, and bedtime, respectively. Patients with anaemia, acute diseases, existing malignancy, or severe renal or hepatic dysfunction, as well as women of childbearing potential, were excluded. Five healthy, non-diabetic Japanese individuals were recruited as controls. The characteristics of the subjects are shown in Table 1. Eight patients with diabetes had been treated with one or more antihypertensive medications: angiotensin receptor blocker ($n = 5$), calcium channel blocker ($n = 5$), diuretic ($n = 2$), beta-blocker ($n = 1$), or angiotensin-converting enzyme inhibitor ($n = 1$). Six patients received continuous treatment with a lipid-lowering agent: statin ($n = 5$) or fibrate ($n = 1$).

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This study was approved by the ethics committee of Kanazawa University (Kanazawa, Japan) and was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent before participating in the study.

Study design

In the morning following an overnight fast, participants consumed a test meal developed by a working group of the Japan Diabetes Society (460 kcal; protein, 18.0 g; fat, 18.0 g; carbohydrate, 56.5 g; E460F18, Q. P. Corp., Tokyo, Japan), along with 100 ml of water, within 15 min.[20] The participants were asked to remain seated and blood samples were obtained immediately before and at 30, 60, 120, and 180 min after ingestion of the test meal. In diabetic patients, tests with and without subcutaneous administration of insulin aspart (NovoRapid; Novo Nordisk Pharma Ltd., Tokyo) just before meal loading were conducted according to a randomised crossover schedule with an interval ranging from 7 to 56 days (mean, 20 days). Insulin aspart was administered in the abdomen at a customary dose for breakfast time (range, 3–12 U; mean, 7.0 U). The insulin injection site and technique were checked by at least one doctor. All participants completed the study, and the medications taken by the diabetic patients did not change throughout the study period.

Assays

Serum concentrations of total insulin (i.e., endogenous insulin plus exogenous insulin aspart) were measured using an E-test TOSOH II IRI immunoassay kit (Tosoh

Corp., Shunan, Japan), because the cross-reactivity of the insulin monoclonal antibody with insulin aspart is reported to be approximately 100%.[21] In addition, endogenous insulin concentrations were assayed using another enzyme immunoassay kit (LUMIPULSE Presto Insulin; Fujirebio Inc., Tokyo) because we found that the reagent does not cross-react with insulin aspart (Supplemental Fig. 1). The circulating insulin aspart concentration was calculated by subtracting the endogenous insulin concentration from the total insulin concentration. Plasma glucagon and serum RLP-C concentrations were determined using a Daiichi II radioimmunoassay kit (TFB Inc., Tokyo) and a JIMRO II assay kit (Otsuka Pharmaceutical Co., Tokyo), respectively. The other variables were measured by standard methods. The inter-assay coefficients of variation were all $\leq$ 15%. Haemoglobin A_{1c} (National Glycohemoglobin Standardization Program) values were estimated using the following formula:[22]

Haemoglobin A_{1c} (National Glycohemoglobin Standardization Program) (%) = haemoglobin A_{1c} (Japan Diabetes Society) (%) + 0.4%

Data analysis

Metabolic parameter profiles during meal tolerance tests were characterised by recording the values for maximum concentration (C_{max}), t_{max}, and the area under the concentration-time curve between 0 and 180 min after meal loading (AUC_{0-180}). C_{max} and t_{max} were determined directly from the observed data. AUC_{0-180} was calculated according to the trapezoidal rule.

Raw or log-transformed data were analysed using a Student's t-test or by an

analysis of variance (ANOVA) or Kruskal-Wallis test followed by Bonferroni *post-hoc* tests. Associations between variables were assessed using Spearman's rank correlation coefficient. The values are presented as means ± standard deviation (SD) unless otherwise indicated. Two-sided *P* values of < 0.05 were considered statistically significant. All calculations were performed using SPSS for Windows version 11 (SPSS Japan, Tokyo). Graphic representations and curve fitting were performed using Microsoft Excel version 2003.

RESULTS

In non-diabetic healthy subjects, the concentration of endogenous insulin rapidly and transiently increased after ingestion of the test meal, with a mean t_{max} value of 36 min (Fig. 1 and Table 2). The t_{max} value for endogenous insulin was significantly higher in patients with type 2 diabetes (mean, 84 min; Table 2), and the serum insulin concentration at 180 min after meal loading was higher in diabetic patients than in control subjects (121 ± 87 vs. 39 ± 18 pmol/L; $P < 0.05$; Fig. 1). Thus, diabetic patients exhibited delayed mild hyperinsulinaemia after meal loading.

The serum concentration of insulin aspart increased after subcutaneous administration of a therapeutic dose (0.11 ± 0.04 U/kg), with a mean t_{max} of 63 min. The values of C_{max} and AUC_{0-180} for insulin aspart were 391 ± 282 pmol/L and 42.8 ± 31.9 nmol·min/L, respectively. Consequently, the t_{max} for total insulin decreased slightly, and total insulin concentrations (C_{max} and AUC_{0-180}) in the trial involving insulin aspart were twice as high as those in the trial not involving insulin aspart (Table 2). Moreover, insulin aspart administration reduced the AUC_{0-180} for endogenous insulin by 29% ($P < 0.01$).

As expected, the dose (per body weight) of insulin aspart was significantly correlated with both C_{max} (Fig. 2a) and AUC_{0-180} (Fig. 2b). However, the dosage did not affect the t_{max} for insulin aspart (Fig. 2c). In contrast, the t_{max} for insulin lispro has been reported to increase dose-dependently.[19] In addition, body mass index did not correlate with the t_{max} for insulin aspart (Fig. 2d). These results suggest that the t_{max} for insulin aspart in clinical practice is more greatly affected by factors other than dosage or level

of obesity.

The total insulin concentration at 180 min after meal loading tended to remain higher in the trial involving insulin aspart than in the one not involving insulin aspart (170 ± 128 vs. 121 ± 87 pmol/L; $P = 0.11$). Moreover, the t_{max} for insulin aspart was significantly correlated with the total insulin concentration at 180 min (Fig. 2e). Collectively, these results indicate that the clinical use of insulin aspart exacerbates delayed hyperinsulinaemia in a subset of patients with type 2 diabetes, notably those with higher t_{max} values.

Concentration-time profiles for circulating glucose, glucagon, triglycerides, and RLP-cholesterol after ingestion of the test meal are shown in Fig. 3. As shown in Table 2, the administration of insulin aspart significantly attenuated postprandial hyperglycaemia (C_{max} and AUC_{0-180} for glucose) ($P < 0.01$). The plasma glucose level at 180 min after meal loading was lower than that before meal loading (6.1 ± 1.7 vs. 7.7 ± 1.0 mmol/L; $P < 0.01$). Although the plasma glucose level fell to less than 3.9 mmol/L in two patients, symptoms of hypoglycaemia were not observed in any patient. In addition, insulin aspart significantly increased the t_{max} for glucagon (Table 2). Compared with the respective values in healthy subjects, postprandial concentrations of triglycerides and RLP-cholesterol (C_{max} and AUC_{0-180}) were significantly higher and tended to be higher, respectively, in diabetic patients (Table 2). However, the abnormal postprandial increases in lipid levels were not statistically significant in the trial involving insulin aspart.

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DISCUSSION

Previous studies have demonstrated that insulin aspart has a faster onset and shorter duration of action than regular human insulin.[7-14] In healthy subjects, the mean t_{max} for insulin aspart has been reported to be 31–33 min (at a dose of 0.025 U/kg),[10,18] 39 min (0.05 U/kg),[10] 52 min (0.1 U/kg),[9] and 52–70 min (0.2 U/kg),[7,8] suggesting that the t_{max} for insulin aspart increases in a dose-dependent manner. In the present study, however, a dose-dependent increase in t_{max} was not observed for doses of insulin aspart in the range 0.05 to 0.22 U/kg (Fig. 2c). The value of t_{max} differed widely between individuals and was 120 min or higher in 20% of patients (4 of 20). It is known that increasing adiposity (i.e., obesity) delays insulin absorption.[23] In addition, the absorption and hypoglycaemic action of insulin analogues were reported to be delayed in obese patients with type 2 diabetes.[19] However, no correlation between t_{max} for insulin aspart and body mass index was observed in this study (Fig. 2d). Because body mass index does not accurately reflect body fat percentage or subcutaneous fat thickness, it remains to be determined whether or not these factors are associated with t_{max} for insulin aspart. Regardless, this study demonstrated for the first time that inter-individual differences in the absorption of insulin aspart exist in patients with type 2 diabetes under clinical conditions, irrespective of differences in insulin dosage and body mass index.

Multiple factors, including injection site, skin temperature, physical activity, smoking, and age, can affect the absorption of exogenous insulin.[7,24,25] Injection depth, which depends on the injection technique, also affects insulin absorption.[26] Additionally,

repeated injection of exogenous insulin or one of its analogues (including insulin aspart) at the same site may induce lipoatrophy, resulting in erratic absorption.[27] In the present study, all patients self-injected insulin aspart into their customary abdominal region using their own devices. In addition, lifestyle habits such as morning exercise and smoking were not restricted. Therefore, various factors may have influenced the value of t_{max} for insulin aspart. On the other hand, these factors appear to have had minimal effects on the values of C_{max} and AUC_{0-180}, which were clearly dependent on the dosage of insulin aspart (Figs 2a and 2b). Interestingly, the reported t_{max} for insulin aspart at a dose of 0.15 U/kg differed between patients with type 1 diabetes (40 min)[12] and those with type 2 diabetes (70 min),[14] whereas the values of both C_{max} (493 and 507 pmol/L, respectively) and AUC (71.0 and 73.7 nmol·min/L, respectively) were comparable between the two patient groups. These observations indicate that factors contributing to variation in the value of t_{max} for insulin aspart need to be identified. However, because multiple factors, rather than a single factor, generally affect insulin absorption in a patient,[24] and because these factors may influence each other,[24] it might be difficult to identify the most important factor. Therefore, at least at present, it is important to remember that the value of t_{max} for insulin aspart differs between individuals and that delayed hyperinsulinaemia is pronounced in some patients.

Delayed hyperinsulinaemia after meals, with and without resulting hypoglycaemia, is likely to increase hunger and hence increase food intake.[28,29] Moreover, it may stimulate the secretion of counter-regulatory hormones to restore euglycaemia.[28] As a consequence, delayed hyperinsulinaemia may result in weight gain and worsen

glycaemic control. In the present study, treatment with insulin aspart exacerbated

delayed hyperinsulinaemia, especially in patients with t_{max} values $\geq$120 min. Therefore,

it is possible that these patients are susceptible to the adverse effects of insulin aspart.

Our results demonstrate that treatment with insulin aspart affects not only glucose

concentrations but also glucagon concentrations in the postprandial state. Postprandial

hyperglucagonaemia and the consequent decreased suppression of hepatic glucose

output are thought to contribute to postprandial hyperglycaemia.[30,31] Specifically,

suppression of plasma glucagon concentrations after glucose ingestion is weak in

subjects with impaired glucose tolerance[31] and type 2 diabetes[32] as compared with

normal subjects. In the present study, the loading of a test meal, which contains

glucagon secretion-stimulating amino acids,[33] appeared to increase glucagon

concentrations, even in non-diabetic subjects (Fig. 3b). Although postprandial

hyperglucagonaemia may be attributable to the reduced action of insulin on pancreatic α

cells,[30,33] insulin aspart significantly affected the value of only t_{max}, and not the values of

C_{max} and AUC_{0-180} for glucagon in the present study. Therefore, whether the effect of

insulin aspart on glucagon secretion contributes to its hypoglycaemic action remains

unclear and needs to be determined in future studies.

Similar to postprandial hyperglycaemia, postprandial hyperlipidaemia is a potential

risk factor for the development of cardiovascular disease.[34] Notably, cholesterol-rich

remnant-like lipoproteins, whose levels are increased in hypertriglyceridaemia, are

thought to be atherogenic factors.[34] It has been demonstrated that both insulin aspart[35]

and insulin lispro[36] have more favourable effects than human insulin on postprandial

hypertriglyceridaemia. Our results confirm the beneficial effect of insulin aspart on postprandial hypertriglyceridaemia and further show its impact on postprandial RLP-cholesterol accumulation.

In summary, the rapid action and favourable pharmacodynamic properties of insulin aspart were preserved in patients with type 2 diabetes, even under clinical conditions. However, the t_{max} for this insulin analogue differed greatly among individuals. Moreover, delayed hyperinsulinaemia was exacerbated, particularly in patients with higher t_{max} values. Therefore, to improve the efficacy and safety of insulin aspart in the treatment of patients with type 2 diabetes, the factors responsible for inter-individual variation in the absorption lag time need to be identified.

ACKNOWLEDGEMENTS

REFERENCES

1. Pratley RE, Weyer C. The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. *Diabetologia* 2001;**44**:929-45.

2. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J. Clin. Invest.* 1999;**104**:787-94.

3. Bonora E, Corrao G, Bagnardi V, *et al.* Prevalence and correlates of post-prandial hyperglycaemia in a large sample of patients with type 2 diabetes mellitus. *Diabetologia* 2006;**49**:846-54.

4. DECODE Study Group. Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-hour diagnostic criteria. *Arch. Intern. Med.* 2001;**161**:397-405.

5. Sorkin JD, Muller DC, Fleg JL, Andres R. The relation of fasting and 2-h postchallenge plasma glucose concentrations to mortality: data from the Baltimore Longitudinal Study of Aging with a critical review of the literature. *Diabetes Care* 2005;**28**:2626-32.

6. Takamura T, Sakurai M, Nakamura M, *et al.* Factors associated with improvement of fasting plasma glucose level by mealtime dosing of a rapid-acting insulin analog in type 2 diabetes. *Diabetes Res. Clin. Pract.* 2007;**75**:278-84.

7. Mudaliar SR, Lindberg FA, Joyce M, *et al.* 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. *Diabetes Care* 1999;**22**:1501-6.

8. Heinemann L, Weyer C, Rauhaus M, Heinrichs S, Heise T. Variability of the metabolic effect of soluble insulin and the rapid-acting insulin analog insulin aspart. *Diabetes Care* 1998;**21**:1910-4.

9. Home PD, Barriocanal L, Lindholm A. Comparative pharmacokinetics and pharmacodynamics of the novel rapid-acting insulin analogue, insulin aspart, in healthy volunteers. *Eur. J. Clin. Pharmacol.* 1999;**55**:199-203.

10. Kaku K, Matsuda M, Urae A, Irie S. Pharmacokinetics and pharmacodynamics of insulin aspart, a rapid-acting analog of human insulin, in healthy Japanese volunteers. *Diabetes Res. Clin. Pract.* 2000;**49**:119-26.

11. Krones R, Schutte C, Heise T. The rapid-acting properties of insulin aspart are preserved in elderly people with type 2 diabetes. *Diabetes Obes. Metab.* 2009;**11**:41-4.

12. Lindholm A, McEwen J, Riis AP. Improved postprandial glycemic control with insulin aspart. A randomized double-blind cross-over trial in type 1 diabetes. *Diabetes Care* 1999;**22**:801-5.

13. Osterberg O, Erichsen L, Ingwersen SH, Plum A, Poulsen HE, Vicini P. Pharmacokinetic and pharmacodynamic properties of insulin aspart and human insulin. *J Pharmacokinet. Pharmacodyn.* 2003;**30**:221-35.

14. Perriello G, Pampanelli S, Porcellati F, *et al.* Insulin aspart improves meal time glycaemic control in patients with Type 2 diabetes: a randomized, stratified, double-blind and cross-over trial. *Diabet. Med.* 2005;**22**:606-11.

15. Bolli GB, Luzio S, Marzotti S, *et al.* Comparative pharmacodynamic and pharmacokinetic characteristics of subcutaneous insulin glulisine and insulin aspart

prior to a standard meal in obese subjects with type 2 diabetes. *Diabetes Obes. Metab.* 2011;**13**:251-7.

16. Homko C, Deluzio A, Jimenez C, Kolaczynski JW, Boden G. Comparison of insulin aspart and lispro: pharmacokinetic and metabolic effects. *Diabetes Care* 2003;**26**:2027-31.

17. Plank J, Wutte A, Brunner G, *et al.* A direct comparison of insulin aspart and insulin lispro in patients with type 1 diabetes. *Diabetes Care* 2002;**25**:2053-7.

18. von Mach MA, Brinkmann C, Hansen T, Weilemann LS, Beyer J. Differences in pharmacokinetics and pharmacodynamics of insulin lispro and aspart in healthy volunteers. *Exp. Clin. Endocrinol. Diabetes* 2002;**110**:416-9.

19. Gagnon-Auger M, du Souich P, Baillargeon JP, *et al.* Dose-dependent delay of the hypoglycemic effect of short-acting insulin analogs in obese subjects with type 2 diabetes: a pharmacokinetic and pharmacodynamic study. *Diabetes Care* 2010;**33**:2502-7.

20. Shimabukuro M, Higa N, Chinen I, Yamakawa K, Takasu N. Effects of a single administration of acarbose on postprandial glucose excursion and endothelial dysfunction in type 2 diabetic patients: a randomized crossover study. *J. Clin. Endocrinol. Metab.* 2006;**91**:837-42.

21. Moriyama M, Hayashi N, Ohyabu C, Mukai M, Kawano S, Kumagai S. Performance evaluation and cross-reactivity from insulin analogs with the ARCHITECT insulin assay. *Clin. Chem.* 2006;**52**:1423-6.

22. Seino Y, Nanjo K, Tajima N, *et al.* Report of the Committee on the classification and

diagnostic criteria of diabetes mellitus. *Diabetol. Int.* 2010;**1**:2-20.

23. Vora JP, Burch A, Peters JR, Owens DR. Relationship between absorption of radiolabeled soluble insulin, subcutaneous blood flow, and anthropometry. *Diabetes Care* 1992;**15**:1484-93.

24. Heinemann L. Variability of insulin absorption and insulin action. *Diabetes Technol. Ther.* 2002;**4**:673-82.

25. Sindelka G, Heinemann L, Berger M, Frenck W, Chantelau E. Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous insulin absorption in healthy subjects. *Diabetologia* 1994;**37**:377-80.

26. Hofman PL, Derraik JG, Pinto TE, *et al.* Defining the ideal injection techniques when using 5-mm needles in children and adults. *Diabetes Care* 2010;**33**:1940-4.

27. Holstein A, Stege H, Kovacs P. Lipoatrophy associated with the use of insulin analogues: a new case associated with the use of insulin glargine and review of the literature. *Expert Opin. Drug Saf.* 2010;**9**:225-31.

28. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA* 2002;**287**:2414-23.

29. Rodin J, Wack J, Ferrannini E, DeFronzo RA. Effect of insulin and glucose on feeding behavior. *Metabolism* 1985;**34**:826-31.

30. Meier JJ, Kjems LL, Veldhuis JD, Lefebvre P, Butler PC. Postprandial suppression of glucagon secretion depends on intact pulsatile insulin secretion: further evidence for the intraislet insulin hypothesis. *Diabetes* 2006;**55**:1051-6.

31. Mitrakou A, Kelley D, Mokan M, *et al.* Role of reduced suppression of glucose

production and diminished early insulin release in impaired glucose tolerance. *N. Engl. J. Med.* 1992;**326**:22-9.

32. Ohneda A, Watanabe K, Horigome K, Sakai T, Kai Y, Oikawa S. Abnormal response of pancreatic glucagon to glycemic changes in diabetes mellitus. *J. Clin. Endocrinol. Metab.* 1978;**46**:504-10.

33. Tsuchiyama N, Takamura T, Ando H, *et al.* Possible role of alpha-cell insulin resistance in exaggerated glucagon responses to arginine in type 2 diabetes. *Diabetes Care* 2007;**30**:2583-7.

34. Stalenhoef AF, de Graaf J. Association of fasting and nonfasting serum triglycerides with cardiovascular disease and the role of remnant-like lipoproteins and small dense LDL. *Curr. Opin. Lipidol.* 2008;**19**:355-61.

35. Schmoelzer I, de Campo A, Pressl H, *et al.* Biphasic insulin aspart compared to biphasic human insulin reduces postprandial hyperlipidemia in patients with Type 2 diabetes. *Exp. Clin. Endocrinol. Diabetes* 2005;**113**:176-81.

36. Ceriello A, Del Prato S, Bue-Valleskey J, *et al.* Premeal insulin lispro plus bedtime NPH or twice-daily NPH in patients with type 2 diabetes: acute postprandial and chronic effects on glycemic control and cardiovascular risk factors. *J. Diabetes Complications* 2007;**21**:20-7.

Table 1 Characteristics of the subjects

	Non-diabetic subjects	Patients with type 2 diabetes
n	5	20
Age (years)	35 ± 7	60 ± 11 **
Men	3 (60)	14 (70)
Body mass index (kg/m^2)	20.7 ± 2.2	24.0 ± 2.5 *
Fasting plasma glucose (mmol/l)	5.3 ± 0.3	7.6 ± 1.2 **
Fasting insulin (pmol/l)	34 (29.5)	40 (50.2)
Hemoglobin A$_{1c}$ (%)	5.3 ± 0.1	6.7 ± 0.5 **

Data are *n*, *n* (%), means ± SD, or geometric mean (geometric CV%).

*$P < 0.05$, **$P < 0.01$ *vs*. non-diabetic subjects.

Table 2　Profiles of the metabolic parameters during the meal tolerance test

| | Non-diabetic subjects | Diabetic patients | |
		Without insulin aspart	With insulin aspart
Serum total insulin			
C_{max} (pmol/l)	246 (36.4)	245 (57.1)	505 (57.3) †**
t_{max} (min)	30, (30, 30)	60, (60, 120) ††	60 (53, 60)
AUC_{0-180} (nmol·min/l)	22.4 (47.8)	29.5 (58.0)	57.8 (62.3) ††**
Serum endogenous insulin			
C_{max} (pmol/l)	246 (36.4)	245 (57.1)	183 (61.4)
t_{max} (min)	30, (30, 30)	60, (60, 120) ††	60, (60, 60) †
AUC_{0-180} (nmol·min/l)	22.4 (47.8)	29.5 (58.0)	21.0 (65.6)
Plasma glucose			
C_{max} (mmol/l)	6.7 (22.6)	13.7 (14.5) ††	10.9 (13.1) ††**
t_{max} (min)	30, (30, 60)	60, (60, 120)	60, (30, 60) *
AUC_{0-180} (mmol·min/l)	988 (13.7)	2086 (14.9) ††	1549 (15.5) ††**
Plasma glucagon			
C_{max} (ng/l)	130 (27.0)	139 (24.7)	129 (27.2)
t_{max} (min)	60, (30, 60)	30, (30, 60)	90, (53, 120) *
AUC_{0-180} (µg·min/l)	20.5 (26.9)	20.9 (25.1)	21.1 (25.0)
Serum triglycerides			
C_{max} (mmol/l)	1.05 (29.4)	1.64 (30.0) †	1.55 (28.5) †
t_{max} (min)	120, (120, 120)	120, (120, 180)	120, (60, 135)
AUC_{0-180} (mmol·min/l)	162 (37.1)	256 (28.9) ††	235 (28.0) †
Serum RLP-cholesterol			
C_{max} (mmol/l)	0.121 (26.8)	0.172 (29.6)	0.149 (31.5)
t_{max} (min)	120, (90, 120)	120, (83, 180)	120, (60, 180)
AUC_{0-180} (mmol·min/l)	18.0 (38.7)	25.9 (29.9)	22.4 (28.0)

C_{max}, maximum concentration; t_{max}, time to maximum concentration; AUC_{0-180}, area under the concentration-time curve between 0 and 180 min after meal loading; RLP, remnant-like particle.

Data are geometric mean (geometric CV%) or median (lower and upper quartiles).

†$P < 0.05$, ††$P < 0.01$ *vs*. non-diabetic subjects.

*$P < 0.05$, **$P < 0.01$ *vs*. diabetic patients in the trial without insulin aspart.

ANOVA or Kruskal-Wallis test with Bonferroni *post-hoc* test; three comparisons.

FIGURE LEGENDS

Figure 1

Concentration-time profiles for insulin aspart and endogenous insulin after ingestion of a test meal. After an overnight fast, 20 patients with type 2 diabetes (white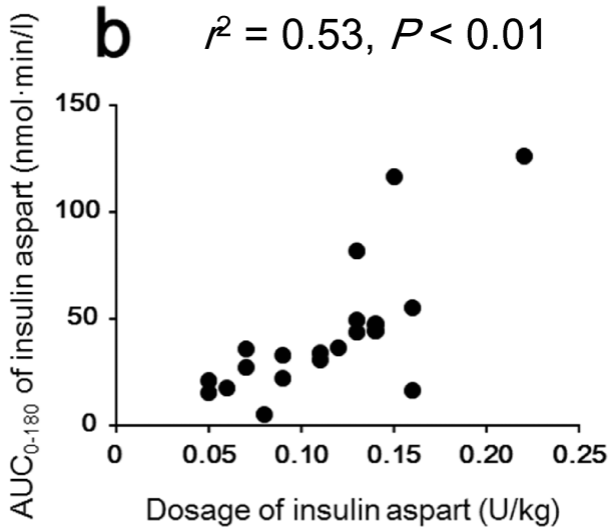 circles) and five non-diabetic subjects (white triangles) underwent a meal tolerance test without administration of insulin aspart (dashed lines). The same diabetic patients underwent another test in which insulin aspart was administered subcutaneously just before meal loading (solid lines). The mean dosage of insulin aspart was 7.0 U (range, 3–12 U). Data represent the means ± SE. White squares, insulin aspart plus endogenous insulin; black squares, insulin aspart; black circles, endogenous insulin. *$P < 0.05$ *vs*. all the other concentration-time profiles (repeated measures ANOVA with Bonferroni *post-hoc* tests; ten comparisons).

Figure 2

Relationships between the pharmacokinetic parameters of insulin aspart and drug dose (**a**, **b**, **c**), body mass index (**d**), and total insulin concentration at 180 min after dosing (**e**).

Figure 3

Concentration-time profiles for circulating glucose (**a**), glucagon (**b**), triglycerides (**c**), and RLP-cholesterol (**d**) after ingestion of a test meal. After an overnight fast, 20 patients with type 2 diabetes (white circles) and five non-diabetic subjects (white

triangles) underwent a meal tolerance test without administration of insulin aspart (dashed lines). The same diabetic patients underwent another test in which insulin aspart was administered subcutaneously just before meal loading (black circles and solid lines). Data represent the means ± SE. *$P < 0.05$, **$P < 0.01$ (repeated measures ANOVA with Bonferroni *post-hoc* test; three comparisons).
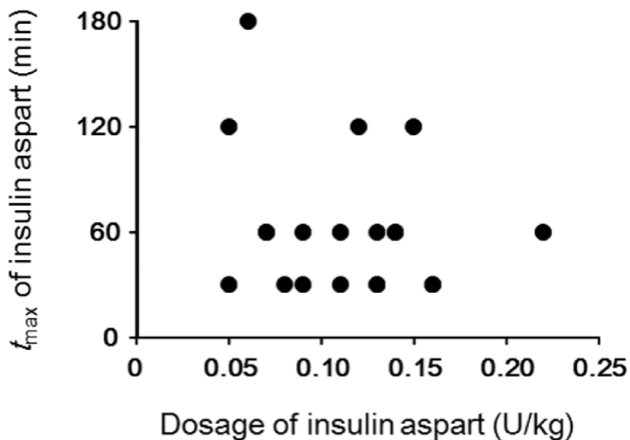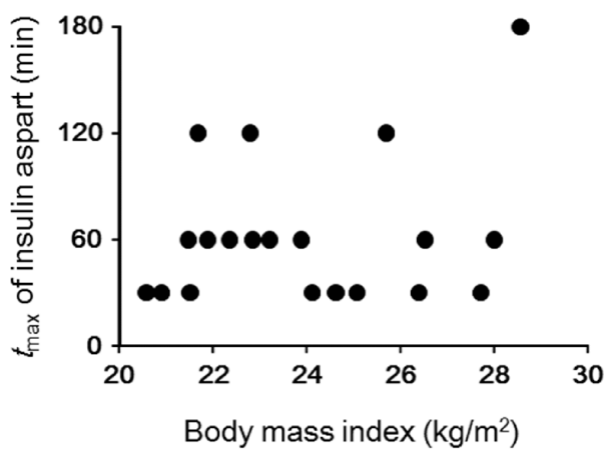
Trial with insulin aspart (Diabetic patients)
- □ Insulin aspart + endogenous
- ■ Insulin aspart
- ● Endogenous

Trial without insulin aspart
- △ Non-diabetic patients
- ○ Diabetic patients

a $r^2 = 0.59$, $P < 0.01$

C_{max} of insulin aspart (pmol/l) vs Dosage of insulin aspart (U/kg)

b $r^2 = 0.53$, $P < 0.01$

AUC_{0-180} of insulin aspart (nmol·min/l) vs Dosage of insulin aspart (U/kg)

c $r^2 = 0.02$, $P = 0.51$

t_{max} of insulin aspart (min) vs Dosage of insulin aspart (U/kg)

d $r^2 = 0.02$, $P = 0.57$

t_{max} of insulin aspart (min)

Body mass index (kg/m^2)

e $r^2 = 0.30$, $P < 0.05$

Total insulin at 180 min (pmol/l)
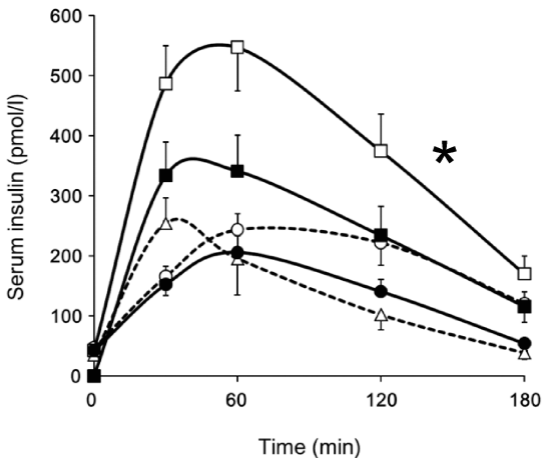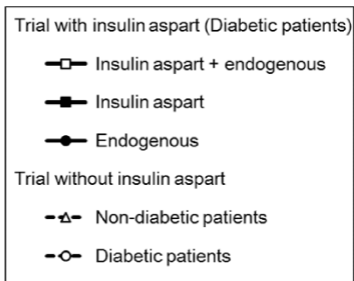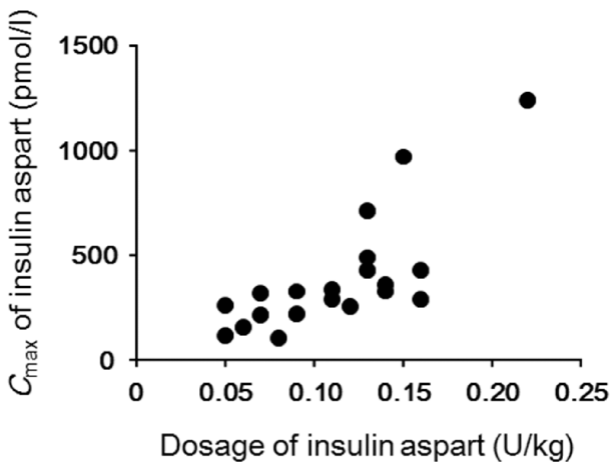
t_{max} of insulin aspart (min)
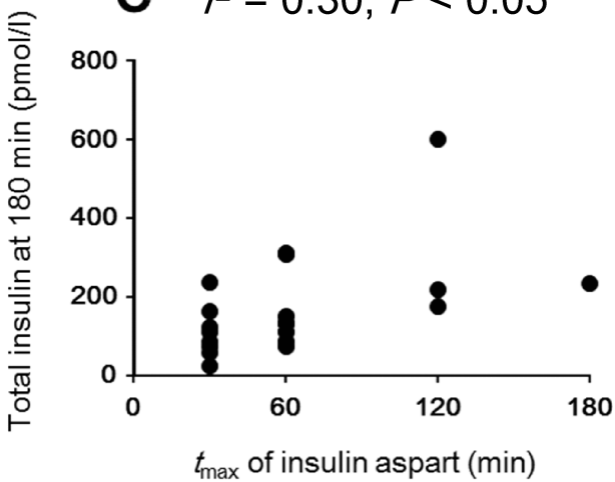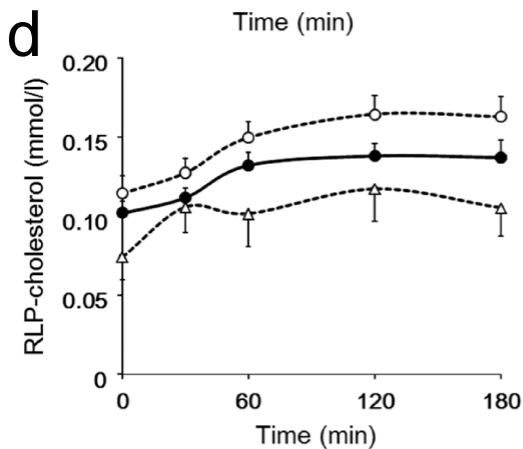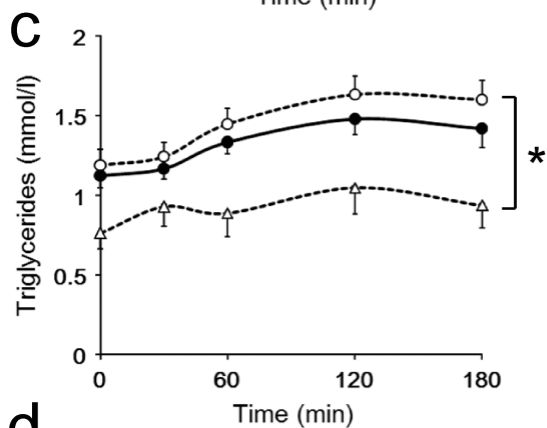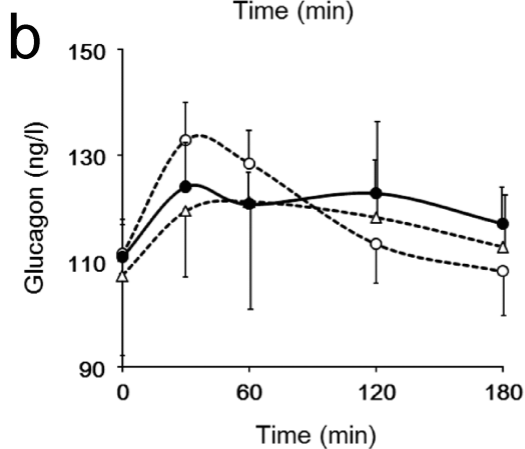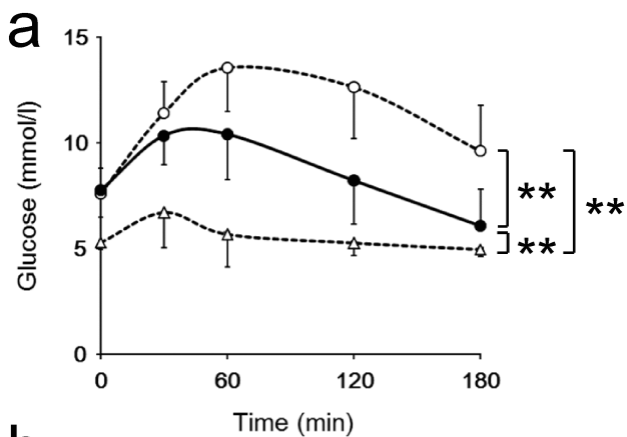
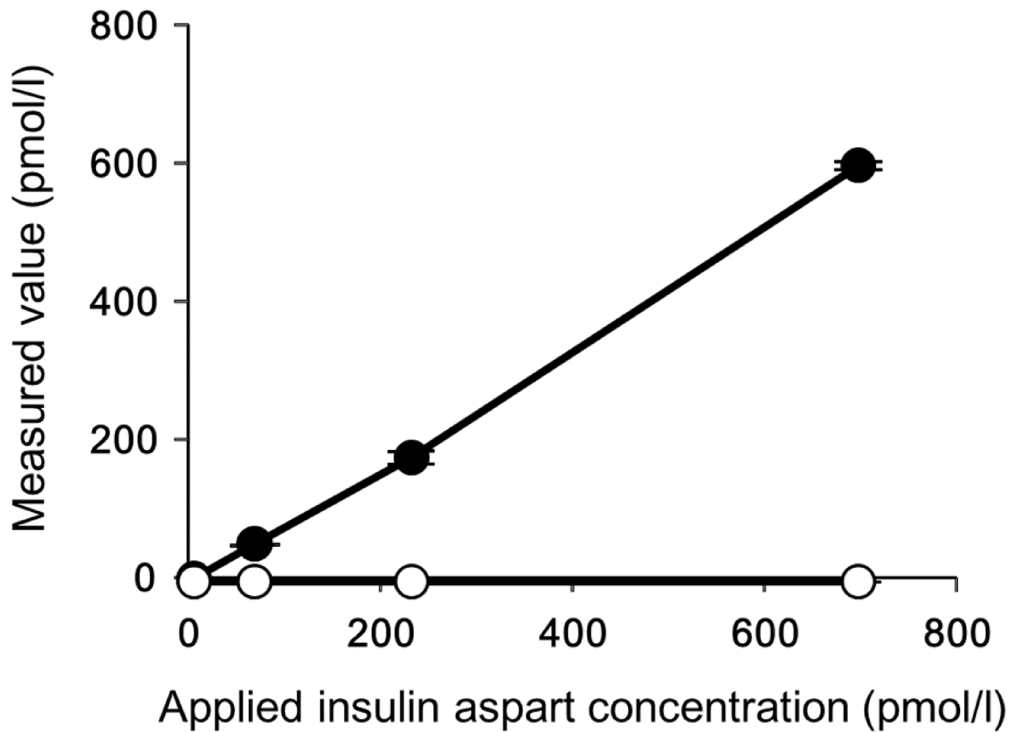## Supplemental Figure

***In vitro* measurements of insulin aspart concentrations.**
Concentrations of serial dilutions of insulin aspart in purified
water were measured using an E-test TOSOH II IRI kit (black
circles) and a LUMIPULSE Presto Insulin kit (white circles).
Data represent the means ± SD of three measurements.