Identification and comparison of insulin pharmacokinetics injected with a new 4-mm needle vs 6- and 8-mm needles accounting for endogenous insulin and C-peptide secretion kinetics in non-diabetic adult males

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ABSTRACT

Aims/Introduction: Many patients with diabetes now use 5-, 6- or 8-mm needles for insulin injection. However, it is unclear whether needle length, particularly for shorter needles, affects the pharmacokinetic properties of insulin.

Materials and Methods: This was a three-way, randomized, cross-over, single-center study involving 12 healthy Japanese adult males (age 27.4 ± 4.14 years; weight 64.2 ± 5.2 kg; body fat percentage 18.2 ± 1.5%). Participants received a subcutaneous (abdomen) dose of insulin lispro (1.5 U for participants weighing 55 to <65.0 kg; 2.0 U for participants weighing 65.0 to <80.0 kg) delivered using a 32-G × 4 mm (32G × 4), 31-G × 8 mm (31G × 8) or 32-G × 6 mm (32G × 6) needle with a 3–7-day washout between doses. Pharmacokinetic parameters of exogenous insulin were identified using non-linear least squares, where the total insulin concentration was fit to the measured plasma insulin concentration using an overall combined model that accounted for C-peptide/insulin secretion in addition to the injected dose.

Results: Maximum concentration and area under the curve for 0 to infinity min for insulin were bioequivalent for the 32G × 4 needle relative to the 32G × 6 and the 31G × 8 needles. The time to the maximum insulin concentration was bioequivalent for the 32G × 4 needle relative to the 32G × 6 needle, but not the 31G × 8 needle.

Conclusions: The use of 4-mm needles is unlikely to change the pharmacokinetic properties of insulin when injected subcutaneously in adults. This trial was registered with UMIN-CTR (no. UMIN000004469). (**J Diabetes Invest** doi: 10.1111/jdi.12035, 2013)

INTRODUCTION

The goal of injections of insulin is to reliably deliver insulin into the subcutaneous space without leakage or discomfort[1]. Recent developments in insulin pen technology have overcome many of the perceived barriers associated with insulin injection. However, discomfort associated with needle insertion still has an adverse effect on quality of life.

The discomfort often associated with needle insertion is at least partly related to the diameter (gauge) and length of the needle, the wall thickness and the shape of the needle tip. Because of this, needle manufacturers have endeavored to overcome these limitations through redesigning and refining needle properties. Such improvements have had substantial benefits on quality of life and patients often prefer the newer needles[2–7]. However, needle length is subject to some limitations, as it governs the depth of injection and must be long enough to reach the subcutaneous tissue, without penetrating the underlying muscle.

Many of the currently available needles are 5, 6 or 8 mm long. In Japan, 5- or 6-mm needles are more frequently used than 8-mm needles. Some patients use longer needles (mainly 8 mm) for no specific reason other than their long-held habit or their physicians' preference.

Although subcutaneous tissue thickness varies between injection sites, it is possible that 8-mm needles inserted perpendicular to the skin could be inserted into muscle tissue[8]. Intramuscular injection is associated with increased pain relative to subcutaneous injection and can increase the rate of uptake of insulin, because of differences in tissue vascularity[9–11].

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The use of shorter needles might overcome these problems. Shorter needles have reduced the likelihood of insertion into muscle and are often better tolerated. However, issues such as what is the minimal length of an insulin pen needle or whether shorter needles could be used in patients from demographically different backgrounds, have not been fully evaluated. A recent study showed that injection-site skin thickness does not markedly differ among demographically diverse adults with diabetes[8]. Conversely, subcutaneous thickness shows a much wider range. The results of that study suggested that a 4–5-mm pen needle could enter the subcutaneous tissue with minimal risk of intramuscular injection, and can be safely and effectively used by most diabetic adults.

A 4-mm-long 32-G needle was recently introduced. A clinical trial carried out in USA patients with type 1 or 2 diabetes showed equivalent glycemic control, but reduced pain and greater preference for the 32-G × 4 mm (32G × 4) needle relative to the two other needles[12].

Although glucose control is an essential outcome of such studies, it is also important to evaluate possible implications on insulin pharmacokinetic properties and confirm that needle length does not adversely influence the properties of the injected insulin. Studies in other patient populations are also necessary, considering possible differences in subcutaneous fat depth. Therefore, the aim of the present study was to compare the new 32-G × 4 mm (32G × 4) needle with two other available needles with lengths of 6 mm (32G) and 8 mm (31G) in terms of insulin pharmacokinetics in healthy Japanese adult males.

MATERIALS AND METHODS
Objective
The primary objective of the present three-way, randomized, cross-over, single-center study was to compare the blood insulin pharmacokinetic properties of three different insulin pen needles.

Participants
Healthy, non-diabetic adult males aged 20–40 years were eligible for the present study. Further inclusion criteria included weight 55–80 kg, body mass index 18.5–25.0 kg/m², body fat percentage 15–20%, systolic blood pressure 90–140 mmHg, diastolic blood pressure <90 mmHg, pulse rate 40–100 b.p.m., glycated hemoglobin <5.2% (Japan Diabetes Society value; National Glycohemoglobin Standardization Program value <5.6%)[13,14], fasting blood glucose <110 mg/dL and blood glucose <140 mg/dL at 2 h after a 75-g glucose load. The protocol was described to the participants and written informed consent was obtained. The present study was approved by the institutional review board at Kyushu Clinical Pharmacology Research Clinic (Fukuoka, Japan).

Study Design
The present study was carried out at the LTA Clinical Pharmacology Center, Sumida Hospital (Tokyo, Japan). The following needles that are commonly used in Japan were used in the present study: Becton Dickinson Company 32G × 4 and 31-G × 8 mm (31G × 8), and Novo Nordisk 32G × 6. Huma-Pen Luxura HD (Eli Lilly Japan K.K., Kobe, Japan) pens were used to administer insulin lispro (Humalog; Eli Lilly Japan K.K.). A single pen was used for each patient, and all pens used were from the same manufacturing batch. This pen meets JIS T 3226-1:2005 and ISO 11608-1:2000 for accuracy of injection devices.

Each participant attended the institute four times, with one screening visit and three test visits. At the screening visit, biochemical blood and urine tests were carried out, informed consent was obtained, and the participants were randomized to one of six groups using a computer-generated randomization list. The order of needles to be used differed between each group to minimize possible bias (Figure 1).

The three test visits were carried out at 3–7-day intervals to allow washout, and to reduce the physical and mental burden on the participants. After a 12-h fast, the participants arrived at the clinic, and height, weight, body temperature, blood pressure and heart rate were measured, and a physical examination was carried out. At approximately 09.00 hours, the participants received a dose of insulin (1.5 U for participants weighing 55 to <65.0 kg; 2.0 U for participants weighing 65.0 to <80.0 kg)
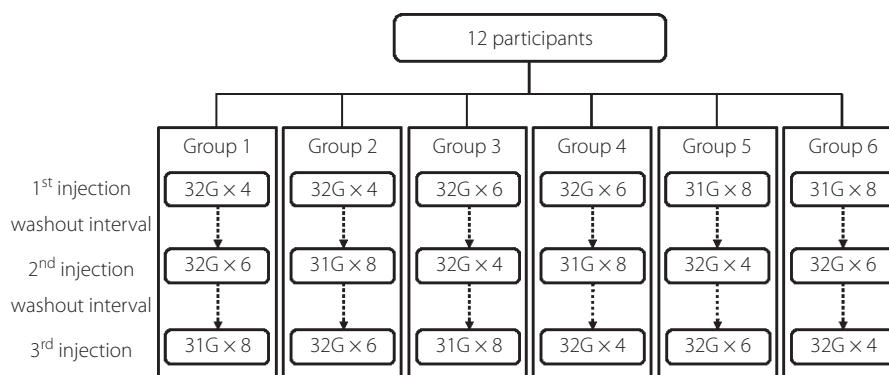
Figure 1 | A total of 12 participants were randomized to six groups. The order of needle use differed between each group to minimize possible bias.

with the insulin pen fitted with the allocated needle. The dose was decided based on the doses used in previous studies [15,16]. Needles were to be replaced if they were blocked, bent or damaged on inspection.

All injections were administered into the abdomen, approximately 5 cm left of the navel. After cleaning the participant's abdomen using a disposable alcohol gauze swab, the insulin was injected without lifting a skin fold for the 32G × 4 needle, and with a lifted skin fold for the 31G × 8 and the 32G × 6 needles to reduce the risk of intramuscular injection and standardize injection depth. All needles were inserted perpendicularly to the skin surface.

Blood samples (through an indwelling venous catheter placed at the antebrachial region) were taken at 15 min and just before injection, and at 10, 20, 30, 40, 50, 60, 90, 120 and 180 min after injection. Blood pressure and heart rate were measured during this time and another physical examination was carried out after obtaining the last blood sample. The duration of blood sampling was selected based on the Guidelines for Bioequivalence Studies of Generic Products (2006), which recommends that sampling should be continued until the area under the curve (AUC) for time T (AUC_T) exceeds 80% of the AUC to infinity[17]. Considering that the reported time[16] to the maximum insulin concentration (T_{max}) for insulin lispro is approximately 37 min, and the half-life is 53 min for a 0.025-U/kg dose, the AUC_T should be determined for 196 min; thus, we considered that the AUC for 0 to infinity min ($AUC_{0-\infty}$) would be appropriate.

All tests were carried out in a single-blind manner, with the participants blinded to which needle was being used at each test. The investigator was aware of which needle was being used. All procedures were carried out by physicians, nurses and medical technicians who were well familiarized with the procedures. All injections were carried out by a single physician with extensive experience of using insulin pens and needles.

Biochemical Assays

Serum immunoreactive insulin (IRI) and C-peptide concentrations were measured in all blood samples using validated chemiluminescence assays. Biochemical tests were carried out by the Mitsubishi Chemical Medience Corporation (Tokyo, Japan).

Pharmacokinetic Modeling and Parameter Identification

To determine the pharmacokinetics of the injected insulin, the contribution of endogenous insulin must be considered, because the participants were not diabetic. We used a combined model that involves kinetic analysis of both endogenous plasma insulin and C-peptide, as well as of exogenous insulin (Figure 2). System modeling and parameter identification were carried out using Matlab version 7.13 (Mathworks, Natick, MA, USA). Endogenous insulin and C-peptide components of the model
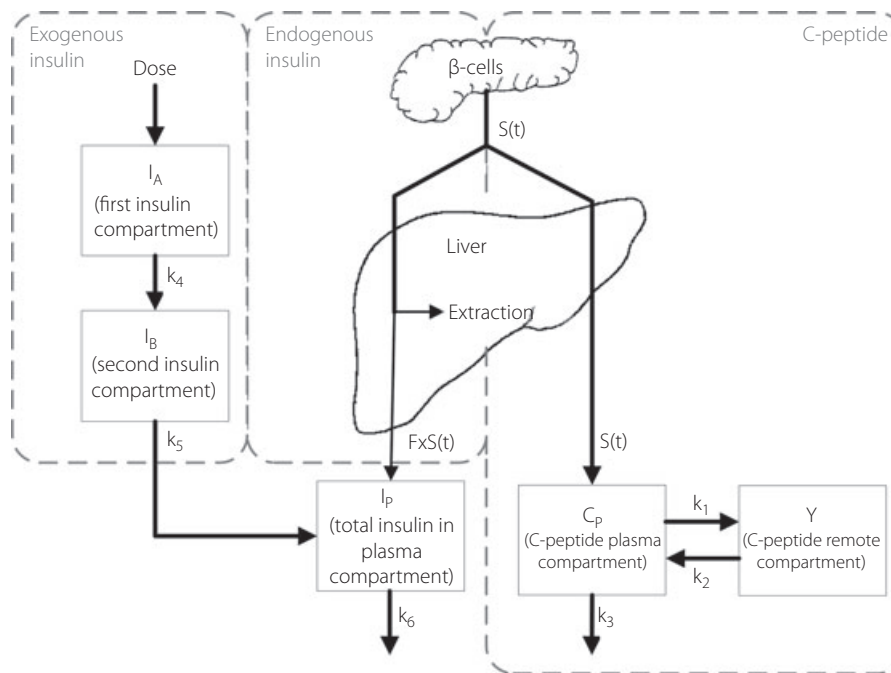
Figure 2 | Schematic diagram of the extended combined model that accounts for the kinetics of endogenous insulin and C-peptide, in addition to exogenous insulin. Insulin and C-peptide are assumed to be secreted by β-cells in an equimolar fashion (i.e. S[t]). A fraction, F, of the secreted insulin survives hepatic transit, appears in the systemic circulation and is distributed within the single plasma compartment. All of the C-peptide survives hepatic portal transport and is assumed to follow two-compartment distribution kinetics. The injected insulin goes through two compartments before being distributed into the plasma compartment along with the endogenous insulin.

were taken from previous studies that have accurately described the insulin and C-peptide secretion, and their appearance in plasma under changing conditions[17–19].

$S(t)$ denotes equimolar insulin–C-peptide release from β-cells. All of the C-peptide survives hepatic portal transport and follows two-compartment distribution kinetics (Equations 1 and 2). $C(t)$ and $Y(t)$ are C-peptide concentrations in the plasma and the remote compartments, respectively. A fraction of the secreted insulin, $FS(t)$, survives hepatic portal transport and enters the plasma compartment (Equation 5), whereas the remainder is extracted by the liver. The injected insulin passes through two compartments before entering the plasma compartment, along with endogenous insulin, to yield the total plasma insulin concentration that was measured experimentally.

Mass transfer equations:

$$S(t) = \frac{dC(t)}{dt} + (k_1 + k_3)C(t) - k_2 Y(t) \tag{1}$$

$$\frac{dY(t)}{dt} = k_1 C(t) - k_2 Y(t) \tag{2}$$

$$\frac{dI_A(t)}{dt} = -k_4 I_A(t), \quad \text{where } I_A(0) = Dose \tag{3}$$

$$\frac{dI_B(t)}{dt} = k_4 I_A(t) - k_5 I_B(t) \tag{4}$$

$$\frac{dI_P(t)}{dt} = FS(t) + k_5 I_B(t) - k_6 I_P(t) \tag{5}$$

Plasma insulin concentration:

$$\frac{I_P}{V_I} \tag{6}$$

The units on the left hand side of Equations 1–5 are in nmol/min. After dividing plasma insulin in nmol by the plasma insulin distribution volume in L in Equation 6, a conversion factor of 166.667 yielded the insulin concentration in units of μU/mL.

The rates k_1, k_2 and k_3, which describe the secretion of C-peptide to its appearance in plasma and the C-peptide distribution volume in the plasma, V_{CP}, were previously determined in a large population, and were mathematically related to the participants' sex, age and body surface area[20] with a high degree of correlation[17]. The same mathematical relationships were used to predetermine k_1, k_2, k_3 and V_{CP} for all of the participants.

The plasma C-peptide concentrations were interpolated minute to minute between the measured samples. A 31-min symmetric (–15, +15) min Savitzky–Golay finite impulse response derivative filter[21], equivalent to a moving linear fit

(first-order polynomial), was used to determine the rate of change of plasma C-peptide, $\frac{dC(t)}{dt}$, from 0 to 165 min. An ordinary differential equation (ODE) solver was used to integrate Equation 2 to deconvolute plasma C-peptide concentrations in nmol/L and estimate insulin–C-peptide secretion ($S[t]$) via Equation 1 per needle injection experiment in all of the participants. To determine C-peptide secretion in units of nmol/min, the C-peptide concentration was multiplied by V_{CP} to yield C-peptide in nmol before deconvolution. The mean secretion rates and the mean measured C-peptide concentrations, from which they were derived, are shown in Figure 3.

After determining insulin secretion, $S(t)$, for each of the 36 experiments, the parameters k_4, k_5 and k_6 that define the pharmacokinetics of the injected insulin as well as F, the fraction of secreted insulin that survived hepatic transport and V_{IP}, the distribution volume of insulin in plasma, were determined by non-linear least squares on the set of ODEs defined by Equations 3, 4, 5 and 6. The system was assumed to be at steady state immediately before insulin injection. The parameters were determined by minimizing the sum-squared error between the measured total insulin concentration and the modeled plasma insulin concentration, in which the parameters were constrained to be positive.

After parameter determination in each experiment, the model was simulated with fit parameters in which insulin secretion was set to 0 to yield the estimated exogenous plasma insulin curve attributable to the injected insulin. Conversely, the plasma insulin curve attributable to endogenous secretion was obtained by setting the dose to 0. The sum of both profiles yields the total fit plasma insulin curve.

Calculation of T_{max}, Maximum Concentration and $AUC_{0-\infty}$

The primary end-point was to test for bioequivalence of insulin injected with the 32G × 4 needle relative to the 32G × 6 and the 31G × 8 needles by three pharmacokinetic metrics; T_{max} (the time after the injection corresponding to the maximal exogenous plasma insulin concentration), maximum concentration (C_{max}; the corresponding insulin concentration) and $AUC_{0-\infty}$ (the area under the curve from time 0 to infinity). The solution to Equations 3, 4, 5 and 6, with no endogenous insulin secretion, is given by Equation 7, which calculates the exogenous plasma insulin concentration attributable to the injected dose:

$$I_P^{Dose} = \frac{Dose}{V_{IP}}(A \cdot \exp(-k_4 t) + B \cdot \exp(-k_5 t) + C \cdot \exp(-k_6 t)) \tag{7}$$

where:

$$A = \frac{k_4 k_5}{(k_6 - k_4)(k_5 - k_4)} \tag{8}$$
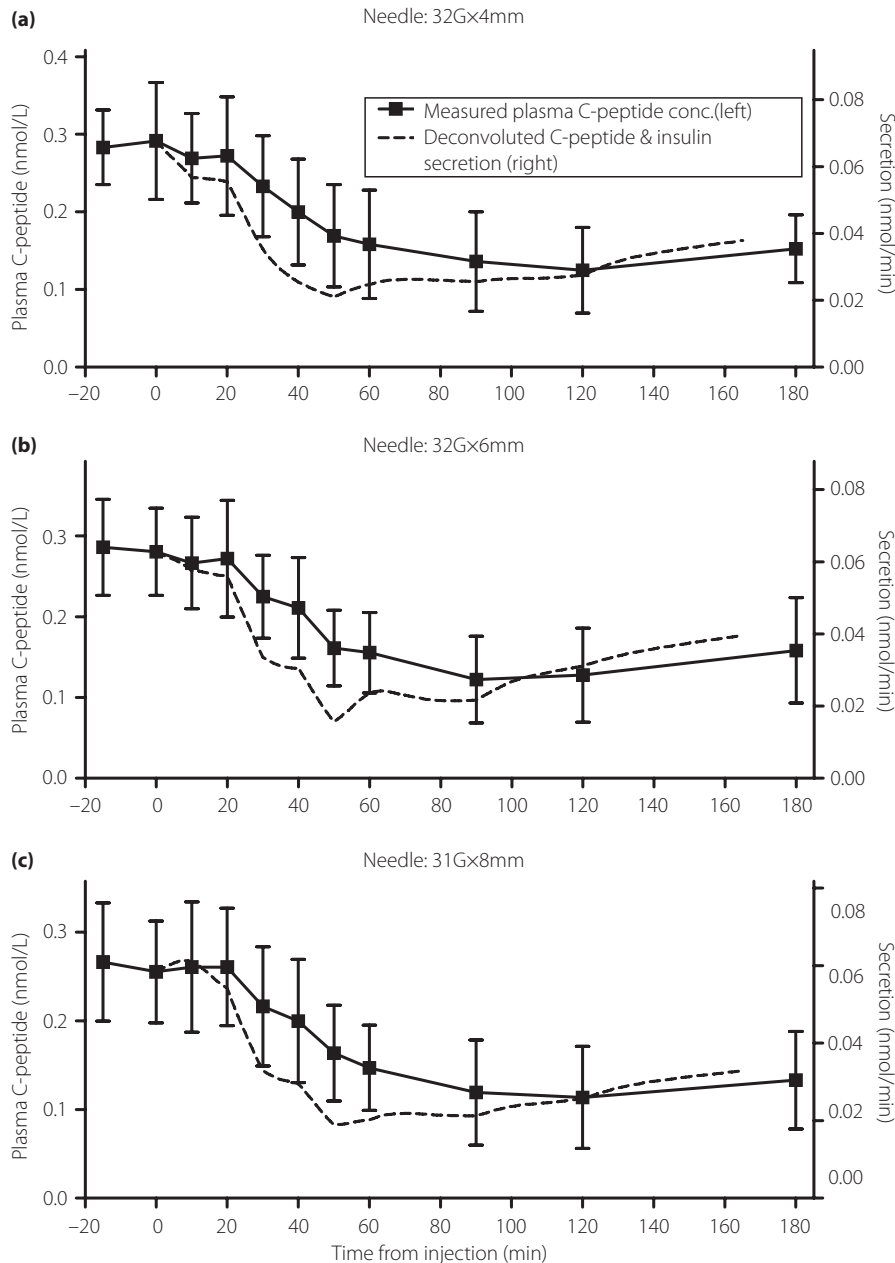
Figure 3 | Plots of the measured plasma C-peptide concentrations (squares; mean and standard deviation) and interpolated values (solid line, left axis) along with C-peptide and insulin secretion rates (dashed line, right axis; mean values). (a) 32-G × 4 mm (32G × 4) needle. (b) 32-G × 6 mm (32G × 6) needle. (c) 31-G × 8 mm (31G × 8) needle. The left and right axis scales are normalized so that the C-peptide concentration and secretion overlap at 0 min.

$$B = \frac{k_4 k_5}{(k_4 - k_5)(k_6 - k_5)} \qquad (9)$$

$$C = \frac{k_4 k_5}{(k_4 - k_6)(k_5 - k_6)} \qquad (10)$$

Equation 7 was simulated with the parameters V_{IP} k_4, k_5 and k_6 for each experiment. T_{max} and C_{max} were derived from the

peak of the simulated plasma insulin curve in response to the dose. $AUC_{0-\infty}$ was calculated by integrating Equation 7 from 0 to infinity, yielding:

$$AUC_{0-\infty} = \frac{Dose}{V_{IP}} \left(\frac{A}{k_4} + \frac{B}{k_5} + \frac{C}{k_6} \right) \qquad (11)$$

Normalized C_{max} and normalized $AUC_{0-\infty}$ were calculated by dividing C_{max} and $AUC_{0-\infty}$ by the dose.

Statistical Analyses

Based on previous reports[16,22,23] and considering the possible risks/ethical concerns of injecting insulin in healthy individuals, we enrolled 12 participants, with two participants allocated to each needle order group. Bioequivalence analyses were carried out as previously described[22]. For C_{max} and $AUC_{0-\infty}$, bioequivalence of the 32G × 4 needle relative to either the 32G × 6 needle or the 31G × 8 needle was accepted if the mean difference (ratio) of the log-converted C_{max} and $AUC_{0-\infty}$ was within the range log(0.80)–log(1.25). For T_{max}, bioequivalence of the 32G × 4 needle relative to either the 32G × 6 needle or the 31G × 8 needle was accepted if the mean difference (ratio) of T_{max} between pairs of needles was within ±20%.

To identify the pharmacokinetic factors influencing C_{max}, T_{max} and $AUC_{0-\infty}$ for exogenous insulin resulting from the dose, analysis of variance was carried out with adjustment for needle type and order of needles as dependent variables. Values of $P < 0.05$ were considered statistically significant. Statistical analyses were carried out using Prism version 5.04 (GraphPad Software, San Diego, CA, USA).

RESULTS

All 12 participants completed all three tests, and all of their data were used in the analyses. No participants withdrew from the study and no needle replacements were required for blocked or damaged needles. The characteristics of the 12 participants are presented in Table 1. The pre-estimated C-peptide rate constants and C-peptide distribution volume in the plasma of the C-peptide secretion submodel used to derive insulin secretion are also shown in Table 1.

The fit parameters from the insulin submodel for each needle were averaged across the 12 participants and are shown in Table 2. In this model, parameters k_4 and k_5 are interchangeable; although the two rates are identifiable, their order is not. In Table 2, the smaller of the two parameters is reported as k_4,

Table 1 | Participant characteristics and calculated C-peptide secretion parameters

	Mean ± SD	Median (range)
Age (years)	27.4 ± 4.14	26 (23–36)
Height (cm)	172.4 ± 5.1	171.4 (165.3–179.6)
Weight (kg)	64.2 ± 5.2	66 (55.8–70.7)
BMI (kg/m²)	21.6 ± 1.3	21.2 (19.9–23.7)
Body fat percentage (%)	18.2 ± 1.5	18.6 (15.7–19.9)
C-peptide secretion parameters		
V_{CP} (L)	3.99 ± 0.1	4.03 (3.82-4.1)
k_1 (per min)	0.0522 ± 0.0006	0.052 (0.0515–0.0534)
k_2 (per min)	0.0496 ± 0.0003	0.0496 (0.0490–0.0499)
k_3 (per min)	0.0593 ± 0.0007	0.0595 (0.0579–0.06)

BMI, body mass index; k_1, k_2 and k_3, rate constants used in the C-peptide secretion submodel; SD, standard deviation; V_{CP}, distribution volume of C-peptide in plasma.

**Table 2** | Insulin pharmacokinetic parameters and metrics

	32G × 4	32G × 6	31G × 8
Identified parameters			
k_4 (per min)	0.0275 ± 0.0105	0.0252 ± 0.007	0.0236 ± 0.0069
k_5 (per min)	0.0378 ± 0.0164	0.037 ± 0.0135	0.0365 ± 0.0221
k_6 (per min)	0.2737 ± 0.0334	0.2567 ± 0.0587	0.2559 ± 0.0456
V_{IP} (L)	4.35 ± 0.37	4.46 ± 0.43	4.39 ± 0.33
F	0.39 ± 0.11	0.34 ± 0.11	0.37 ± 0.10
PK metrics			
T_{max} (min)	39.39 ± 9.26	39.76 ± 6.09	43.01 ± 8.03
C_{max} (µU/mL)	16.99 ± 4.17	16.95 ± 3.22	17.19 ± 4.08
$AUC_{0-\infty}$ (min µU/mL)	1420 ± 255	1506 ± 259	1539 ± 279
C_{max}/dose (µU/mL)	9.65 ± 2.79	9.69 ± 2.41	9.70 ± 2.45
$AUC_{0-\infty}$/dose (min µU/mL)	795 ± 104	845 ± 115	860 ± 101

Values are means ± standard deviation. The identified exogenous parameters were determined by fitting the overall model to the total measured insulin by the non-linear least squares method. The parameters for C-peptide and insulin secretion compartments were calculated using mathematical relationships as a function of participant characteristics. Although the two-rate constants k_4 and k_5 are identifiable, they are interchangeable. In this table, k_4 is reported as the smaller of the two and k_5 is reported as the larger of the two. The maximum insulin concentration (T_{max}), maximum concentration (C_{max}) and area under the curve for 0 to infinity min ($AUC_{0-\infty}$) are derived from the simulated exogenous plasma insulin curve resulting from the identified parameters when insulin secretion was set to 0. PK, pharmacokinetic.

and the larger value is reported as k_5. T_{max}, C_{max} and $AUC_{0-\infty}$ values are also reported in Table 2.

For each of the three needles, the fit (total) plasma insulin curves, the estimated plasma insulin curve resulting from the injection and the estimated plasma insulin attributable to endogenous secretion were averaged across the 12 participants, and are plotted in Figure 4. The means and standard deviations of the insulin concentrations measured at each time are also plotted.

The insulin pharmacokinetic metrics (i.e. T_{max}, C_{max} and $AUC_{0-\infty}$) are summarized for each needle in Table 2. The comparisons of T_{max} between the 32G × 4 needle (39.39 ± 9.26 min) and either the 32G × 6 needle (39.76 ± 6.09 min) or the 31G × 8 needle (43.01 ± 8.03 min) were not statistically significant ($P = 0.9098$ and $P = 0.3177$, respectively). Similarly, the comparisons of C_{max} between the 32G × 4 needle (16.99 ± 4.17 µU/mL) and either the 32G × 6 needle (16.95 ± 3.22 µU/mL) or the 31G × 8 needle (17.19 ± 4.08 µU/mL) were not significant ($P = 0.9823$ and $P = 0.9059$, respectively). Last, the comparisons of $AUC_{0-\infty}$ between the 32G × 4 needle (1420 ± 255 min µU/mL) and either the 32G × 6 needle (1506 ± 259 min µU/mL) or the 31G × 8 needle (1539 ± 279 min µU/mL) were not statistically significant ($P = 0.4212$ and $P = 0.2843$, respectively). As shown in Table 3, C_{max} and
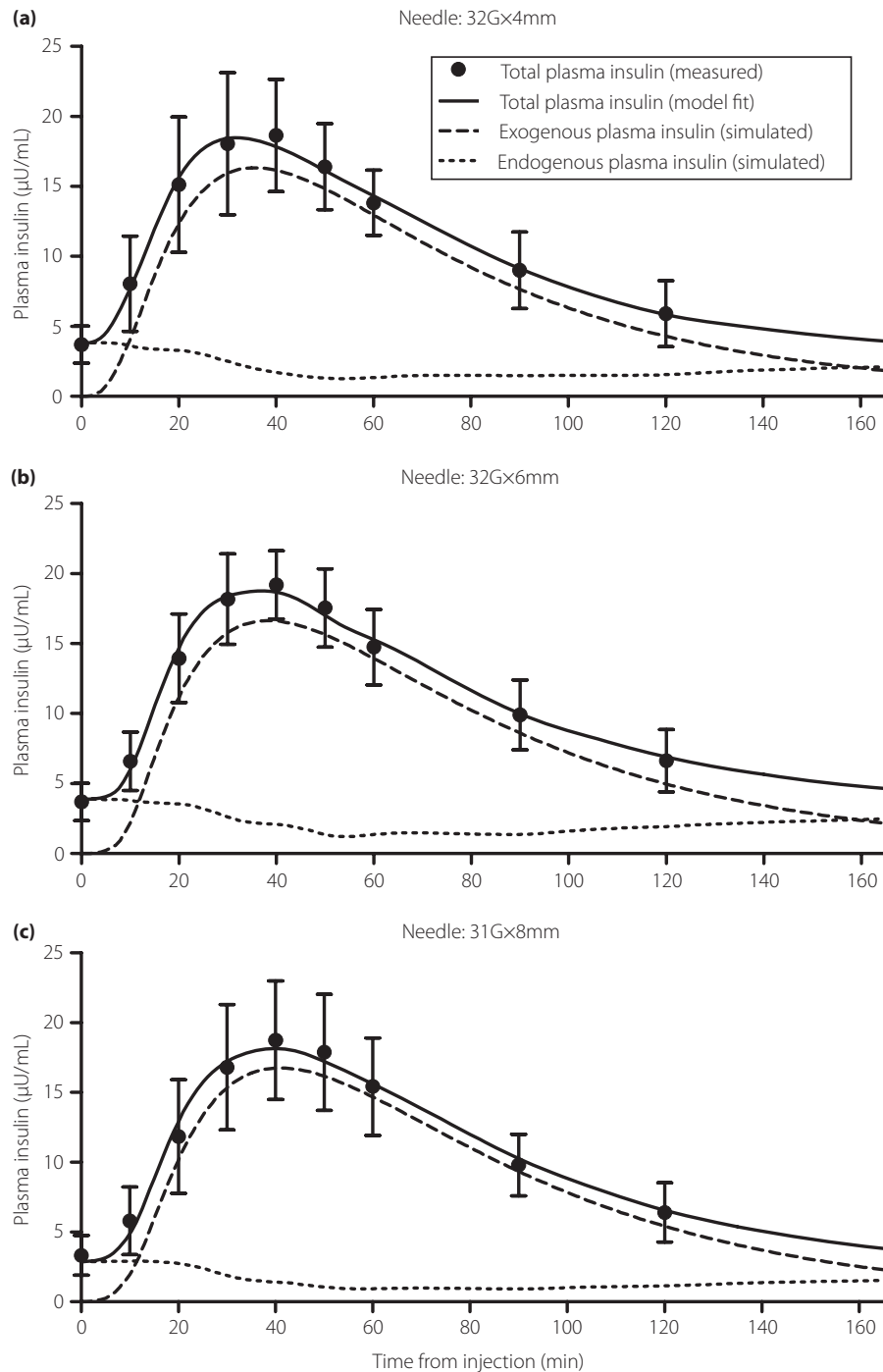
Figure 4 | Measured plasma insulin concentrations (circles; mean and standard deviation) and the model fits of total insulin (solid line). The simulated exogenous plasma insulin curves (dashed line; mean values) and of simulated endogenous plasma insulin curves (dotted line; mean values). The simulated exogenous and endogenous plasma insulin curves were generated by simulating the model using the identified parameters with insulin secretion set to 0 and the injection dose set to 0, respectively. The sum of the exogenous and endogenous curves yield the model fit of total insulin. (a) 32-G × 4 mm (32G × 4) needle. (b) 32-G × 6 mm (32G × 6) needle. (c) 31-G × 8 mm (31G × 8) needle.

$AUC_{0-\infty}$ for the 32G × 4 needle were bioequivalent to both the 32G × 6 needle and the 31G × 8 needle, as the 90% confidence intervals for both comparisons were within the prespec-ified limits. Interestingly, T_{max} tended to be longer for the 31G × 8 needle relative to the 32G × 4 and 32G × 6 needles. Consequently, the 32G × 4 needle was bioequivalent to the

Table 3 | Bioequivalence analysis of 32-G × 4 mm vs 32-G × 6 mm and 31-G × 8 mm needles for insulin pharmacokinetics

	Ratio*	90% CI	Lower/upper limits†
T_{max} (min)			
32G × 4 vs 32G × 6	−0.3667	−5.8597, 5.1264	−7.9517, 7.9517
32G × 4 vs 31G × 8	−3.6167	−9.6907, 2.4574	−8.6017, 8.6017
C_{max} (μU/mL)			
32G × 4 vs 32G × 6	−0.0109	−0.1713, 0.1496	−0.2230, 0.2231
32G × 4 vs 31G × 8	−0.0164	−0.1893, 0.1565	−0.2230, 0.2231
$AUC_{0-\infty}$ (min μU/mL)			
32G × 4 vs 32G × 6	−0.0608	−0.1827, 0.0611	−0.2230, 0.2231
32G × 4 vs 31G × 8	−0.0810	−0.2076, 0.0457	−0.2230, 0.2231

CI, confidence interval; T_{max}, time to the maximum concentration; C_{max}, maximum concentration; $AUC_{0-\infty}$, area under the curve from 0 to infinity min.
*Ratio of the mean values.
†±20% for time to the maximum concentration (T_{max}), log (0.80), log (1.25) for maximum concentration (C_{max}) and area under the curve from 0 to infinity min ($AUC_{0-\infty}$).

32G × 6 needle, but not the 31G × 8 needle. The type of needle and the needle order did not affect T_{max}, C_{max} or $AUC_{0-\infty}$ ($P = 0.1309$, $P = 0.8025$ and $P = 0.8318$, respectively; two-way analysis of variance).

Because we expected the possibility of mild hypoglycemia in the present study, we were determined to treat only severe hypoglycemia (<50 mg/dL) or mild hypoglycemia (<70 mg/dL) with any symptoms consistent with hypoglycemia, such as sickness or general fatigue. Therefore, mild hypoglycemia without symptoms was not included as an adverse event in the present study. None of the participants developed severe hypoglycemia, and none of the participants who showed temporary mild hypoglycemia reported any symptoms.

DISCUSSION

In the present study of healthy Japanese adult males, we found that C_{max} and $AUC_{0-\infty}$ for insulin lispro injected using a 32G × 4 needle were bioequivalent to those for the 32G × 6 needle and 31G × 8 needle, whereas T_{max} was bioequivalent between the 32G × 4 needle and the 32G × 6 needle.

Our findings expand on those of a cross-over study that compared the pharmacodynamic characteristics of the 32G × 4 needle with two other needles (31G × 5 mm and 31G × 8 mm) in two 3-week treatment periods[12]. The percent absolute change in serum fructosamine, a marker of glycemic control over 2–3 weeks, was bioequivalent for the 32G × 4 needle relative to both comparators. In a study in obese subjects, a 31G × 5-mm needle was associated with comparable metabolic control to a 31G × 8-mm needle[24]. However, it was reported that approximately 5% of injections with a 5-mm needle in children were intramuscular[25].

In the present study, T_{max} tended to be longer for the 31G × 8 needle than the shorter needles, which meant that

bioequivalence for the 32G × 4 and the 31G × 8 needles was not shown. However, T_{max} was not significantly different between the 32G × 4 and 31G × 8 needles ($P = 0.3177$, *t*-test). Considering that C_{max} and $AUC_{0-\infty}$ were comparable for all three needles with bioequivalence for both parameters relative to the 32G × 4 needle, we suspect this finding is related to the timing of blood samples (every 10 min), which might mask the true timing of the peak insulin concentration, and the small sample size, which was too small given the large standard deviation of T_{max}. More frequent blood sampling and more subjects might be necessary in future studies to confirm whether the differences in T_{max} are due to the method used, or whether the depth of injection in the abdomen affects the duration of insulin exposure.

Regarding the impact of injection depth on insulin uptake, one study compared radioactivity levels at the injection sites where radiolabeled regular insulin was injected subcutaneously to a depth of 3 mm beneath the skin surface or to 2 mm above the muscle fascia in the thigh and abdomen, under ultrasound guidance[26]. The authors found no significant differences in insulin uptake between the deep and superficial injections at either site[26].

The results of the present study should be considered in light of some limitations. First, the insulin assay was non-specific for insulin lispro, measuring both insulin lispro and endogenous insulin. To overcome this, our combined model accounted for C-peptide and insulin secretion dynamics, which we obtained from previous publications. We added exogenous insulin submodel compartments to the endogenous insulin and C-peptide combined model. This allowed us to estimate exogenous insulin and endogenous insulin plasma concentrations by fitting the total measured insulin to the overall combined model. Studies in patients with type 1 diabetes would help to confirm these findings. Second, bioequivalence of the 32G × 6 and 32G × 8 needles was not evaluated. However, this was not a prespecified analysis. Based on the descriptive data, it is likely that these needles would provide the same pharmacokinetics in clinical settings. Finally, the present study was carried out in Japanese adult males, with injections into the abdomen. Nevertheless, the results can be generalizable to other ethnicities, age-groups, or even obese people, as even the shortest needle penetrates through the skin. The results of a recent study on skin/subcutaneous tissue thickness[8] concluded that skin thickness varies minimally between patient groups of differing demographics (e.g. age, body mass index, sex and race), and rarely exceeds 3 mm. However, differences in subcutaneous tissue thickness, subcutaneous vascularity and adiposity between common injection sites (e.g. thigh, buttocks and abdomen) might influence the resulting insulin pharmacokinetic profiles, and the risk of intramuscular injection, highlighting the importance of good injection technique. Studies in children and other populations are required to confirm and expand on the present results.

Pharmacokinetic studies in obese, particularly abdominally obese, patients will be needed to confirm these results. Future

studies could also include intramuscular injection as an additional control.

In conclusion, the new 32G × 4 needle was bioequivalent to two widely used needles, namely the 32G × 6 and 31G × 8 needles in terms of the peak insulin concentration (C_{max}) and total insulin exposure ($AUC_{0-\infty}$). The 32G × 4 needle was also bioequivalent to the 32G × 6 needle, but not the 31G × 8 needle, for the time to reach peak insulin concentration (T_{max}), which was longer in the latter needle than in the 32G × 4 and 32G × 6 needles. Taken together, we believe the use of a 4-mm needle does not adversely affect the pharmacokinetic properties of insulin compared with longer needles, when injected subcutaneously in adults.

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