Relationship Between Absorption of Radiolabeled Soluble Insulin, Subcutaneous Blood Flow, and Anthropometry

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OBJECTIVE — To evaluate the interrelationships between the rate of absorption of soluble insulin, SCBF, and anthropometry in normal subjects.

RESEARCH DESIGN AND METHODS — In 12 normal men (age range 23–30 yr, BMI 18.2–41.3 kg/m^2), simultaneous assessment of the absorption of ^{125}I-labeled soluble insulin and SCBF (^{99m}Tc clearance) was performed, on separate study days, for the anterior abdominal wall, anterior midthigh, and the upper arm sites. Each site was examined in a randomized order on two separate occasions. Absorption of ^{125}I-soluble insulin was determined by external monitoring of residual radioactivity levels at the injection site for 6 h postinjection. Residual radioactivity level-time curves, including the characteristic early phase of slow absorption of soluble insulin (the lag phase), were described using two- and three-parameter biexponential models. Anthropometric measurements included BMI, ultrasonic measurement of the subcutaneous adipose tissue layer, and caliper skin fold thickness at the anterior abdominal wall, biceps, triceps, anterior midthigh, and subscapular sites.

RESULTS — A highly significant positive relationship was observed between the rate of absorption of ^{125}I-soluble insulin and SCBF (r_S = 0.44–0.52; P < 0.01–0.001). The duration of the lag phase was inversely correlated with SCBF (r_S = −0.34–−0.51; P < 0.01–0.001). Inverse relationships also were observed for the subjects' degree of adiposity with the rate of soluble insulin absorption (r_S = −0.43–−0.71; P < 0.001) and SCBF (r_S = −0.27–−0.62; P < 0.05–0.001). Significantly shorter lag phase was observed for the abdominal site compared with thigh and arm injection sites (P < 0.05–0.01).

CONCLUSIONS — The rate of absorption of soluble insulin, including during the lag phase, is positively correlated with SCBF. Increasing adiposity prolongs the duration of the early lag phase and reduces the rate of absorption of soluble insulin and SCBF.

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RECEIVED 2 OCTOBER 1991 AND ACCEPTED IN REVISED FORM 21 MAY 1992.

SCBF, SUBCUTANEOUS BLOOD FLOW; BMI, BODY MASS INDEX; ^{99M}Tc, ^{99M}TECHNETIUM; T_{SLOW}, SLOW CLEARANCE EXPONENT; T_{FAST}, FAST CLEARANCE EXPONENT; r_S, SPEARMAN RANK CORRELATION COEFFICIENT; R^2, INDEX OF DETERMINATION; T50, TIME TO 50% ABSORPTION OF INITIAL RADIOACTIVITY; T10, TIME TO ABSORPTION OF 10% INITIAL RADIOACTIVITY; T25, TIME TO ABSORPTION OF 25% INITIAL RADIOACTIVITY; T_{MAX}, TIME TO MAXIMAL ABSORPTION; ^{133}Xe, ^{133}XENON; IDDM, INSULIN-DEPENDENT DIABETES MELLITUS.

A number of studies have reported a positive relationship between the rate of absorption of soluble insulin and SCBF (1–4). Information is not available, however, regarding the relationship with SCBF of the characteristic initial phase of slow absorption of subcutaneously administered soluble insulin, the so-called lag phase. During the lag phase, the relative rate of absorption of soluble insulin gradually increases to a maximum at ~120–180 min after injection and thereafter follows a monoexponential course (3,5–8). As a result of the lag phase, peak plasma insulin levels are attained at 90–150 min after subcutaneous administration of soluble insulin (9–11). By contrast, meal ingestion by normal subjects results in a rapid rise in plasma insulin levels, reaching a peak within 30–60 min (12–15). The delay in reaching peak insulin concentrations following subcutaneous injection of soluble insulin results in postprandial hyperglycemia and possible delayed hypoglycemia (7,9–11,13–15). In an attempt to minimize postprandial hyperglycemia caused by this initial delay in absorption, subcutaneous soluble insulin should be administered ~30 min before meal ingestion (16,17).

Despite its clinical significance in the control of postprandial glycemic excursions, the lag phase has received relatively little attention. The rates of absorption of soluble insulin previously have been represented as time to 50% absorption of initial radioactivity (T50), or fractional clearance rates of the later monoexponential absorption course, after the administration of radiolabeled preparations (1–5,7,8). By virtue of the methods utilized, these studies have not specifically addressed the lag phase.

The purpose of this study in normal subjects was *1*) to evaluate the relationship between the absorption of radiolabeled soluble insulin (including the lag phase) and SCBF for the anterior abdominal wall, anterior midthigh, and upper arm regions; and *2*) to assess the

influence on the absorption of soluble insulin and SCBF of the subjects' anthropometric measurements.

RESEARCH DESIGN AND METHODS

— Twelve normal men participated in the study (age range 23–30 yr; BMI 18.2–41.3 kg/m^2). Subjects were recruited after satisfactory clinical and laboratory screening. They did not possess a significant past medical history nor a family history of diabetes mellitus. This study was approved by the local Area Ethics Committee, and informed, written consent was obtained from each participant.

Subjects participated on 6 study days, each 1 wk apart, when they were administered 6 U ^{125}I-labeled soluble insulin (Actrapid HM U100; specific activity range 140–200 kBq/ml; Novo Industri, Bagsvaerd, Denmark) and 0.06 ml ^{99m}Tc as the pertechnatate ion (specific activity; nominal 0.074 MBq/ml; Amertec ^{99m}Tc Sterile Generator, Amersham International, Amersham, UK) (18). Absorption of soluble insulin and blood flow was investigated for the subcutaneous tissue of the anterior abdominal wall, anterior midthigh, and upper arm. Each site was studied in a randomized order on two separate days. The respective locations of the soluble insulin and ^{99m}Tc administrations were half-way between the anterior superior iliac spine and the umbilicus, the anterior midthigh, and at the inferior margin of the lateral body of the deltoid muscle of the upper arm.

On the night preceding each study day, subjects were premedicated with 100 mg potassium iodide to prevent thyroidal uptake of ^{125}I and ^{99m}Tc. Each study day commenced at 0800 after a 10-h overnight fast. On arrival, all participants rested in the supine position for 2 h. An intravenous cannula (Venflon) was sited into a median cubital fossa vein attached by a three-way tap, for blood sampling, to a slow-running infusion of normal saline (0.150 mM). At the end of the 2-h rest period, ^{125}I-labeled soluble insulin was administered subcutaneously (with a time of 0 min). ^{99m}Tc was administered simultaneously, 3 cm from the insulin administration site. All preparations were administered using a disposable Lo-Dose 0.5 ml syringe (Becton Dickinson, Brooklyn, New York) (needle length 12 mm, external diameter 0.4 mm). In order to standardize delivery of the preparations, injections were performed over 1 min by inserting the full length of the syringe needle at 45° into a lifted semifold. This technique was chosen to avoid accidental intramuscular injections (19). The syringe needle then was withdrawn half-way and retained for 15 sec before complete withdrawal. Injection sites then were gently wiped with cotton wool to remove material that may have refluxed to the skin surface.

Disappearance of the injected preparations from subcutaneous tissues was determined from the levels 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 and fixed 50 mm above the skin surface by means of spacer bars. Residual radioactivity at the injection site was measured continuously for the first 2 h after injection of the radioisotopes and thereafter for 5 min at half-hour intervals until the termination of the 6-h study period. Energy windows for the detector were selected to include the photopeak (full energy) emissions for ^{125}I and ^{99m}Tc at 30 and 140 KeV, respectively, thus enabling simultaneous detection of the two radioisotopes.

Subjects remained supine for the duration of the study, and smoking was not permitted. Room temperature was maintained at 22 ± 1°C, and skin surface temperature, measured by a thermocouple (2100 Tele-Thermometer, Yellow Springs, Yellow Springs, OH) placed close to the injection site, was 32 ± 2°C. All subjects' plasma glucose was monitored throughout the study period. In the event of hypoglycemic symptoms, or plasma glucose values <2.5 mM, oral carbohydrate was administered, and results for that study day were excluded from analysis.

Anthropometric measurements

BMI (weight in kg/height in m^2) was calculated on the measurement of height (without shoes) against a wall-mounted scale and the measurement of weight with subjects wearing underwear.

Measurements of skin fold thickness were performed on the right side of the body with the subject seated, using Harpenden skin-fold calipers (skin plate area 10 mm; pressure applied 10 g/mm^2 [British Indicators, St. Albans, UK]). Skin folds were picked up between the thumb and forefinger, and the caliper jaws applied at the skin fold site, ~1 cm below the forefinger. For each site, skin fold thickness was determined as a mean of three measurements, performed to the nearest 2 mm. The sites selected, as suggested by the International Biological Program (20) were: biceps—over the midpoint of the belly of the muscle; triceps—over the midpoint of the muscle belly, midway between the olecranon and the tip of the acromion; subscapular—below the tip of the inferior angle of the scapula, at a 45° angle; thigh—midpoint over anterior thigh; and abdomen—midpoint between the umbilicus and the anterior superior iliac spine, with the subject upright.

Ultrasonic measurements of the subcutaneous adipose tissue layer were performed using a high-frequency pulsed A-scan system (Cutech Systems, Cardiff, UK), with a 10-MHz lead metaniobate transducer (21). Ultrasonic measurements of the subcutaneous adipose tissue thickness were obtained, with the subject upright, at: the anterior abdominal wall—half-way between the umbilicus and the anterior superior iliac spine; thigh—anterior midthigh; arm—at the inferior margin of the lateral belly of the deltoid muscle; and at a subscap-

Table 1—*Summary of pertinent abbreviations used in the analysis of results*

^{125}I-SOLUBLE INSULIN ABSORPTION
K_2 = RATE CONSTANT REPRESENTING ABSORPTION OF SOLUBLE INSULIN FOR MODEL A
K = RATE CONSTANT REPRESENTING ABSORPTION OF SOLUBLE INSULIN FOR MODEL B
T50 = TIME TO ABSORPTION OF 50% OF INITIAL RADIOACTIVITY AT INJECTION SITE (MODEL A)
PARAMETERS REPRESENTING THE LAG PHASE
T10 = TIME TO ABSORPTION OF 10% OF INITIAL RADIOACTIVITY AT INJECTION SITE (MODEL A)
T25 = TIME TO ABSORPTION OF 25% OF INITIAL RADIOACTIVITY AT INJECTION SITE (MODEL A)
TMAX = TIME TO MAXIMAL RATE OF ABSORPTION OF SOLUBLE INSULIN = 1/K (MODEL B)
SCBF
TFAST = HALF-CLEARANCE TIME FOR FAST EXPONENT OF ^{99M}TC CLEARANCE
TSLOW = HALF-CLEARANCE TIME FOR SLOW EXPONENT OF ^{99M}TC CLEARANCE
%SLOW = RELATIVE CONTRIBUTION OF THE SLOW COMPONENT TO ^{99M}TC CLEARANCE

ular site—at the tip of the inferior angle of the scapula.

Analysis of results

^{125}I-soluble insulin absorption. Residual radioactivity at the injection site, expressed as counts per minute, was obtained as a mean of five 1-min periods of external monitoring. Count rates for each time point, corrected for background activity, were expressed as a percentage of the initial count rate at the time of injection. Residual radioactivity levels were calculated for 10-min intervals during the first 2 h of monitoring and subsequently for half-hour intervals up to the end of the 6-h study period. A percentage residual radioactivity at the injection site against time profile was constructed on a semilogarithmic scale, hereafter referred to as the residual radioactivity curve. Residual radioactivity curves were determined for individual subjects for each study day. Descriptive analysis of individual subjects' residual radioactivity curves was performed using three- (model A) and two- (model B) parameter diffusion, rate-limited biexponential models:

$$\text{Model A: } R(t)=R(o)(k1\exp(-k2t) - k_2\exp(-k_1t))/(k_1-k_2)$$

$$\text{Model B: } R(t) = R(o)(1+kt)\exp(-kt)$$

where $R(t)$ and $R(o)$ = residual radioactivity at injection site at times t and o min; k_1 = rate constants between two theoretical subcutaneous compartments (model A); k_2 and k = rate constants for absorption of insulin for models A and B, respectively.

The models were fitted to the observed data by an iterative function of the method of nonlinear least squares. The goodness of fit for the mathematical models to describe each individual subjects' residual radioactivity curves for each study day was judged from the value of R^2 and from the residual SDs, calculated from the sum of squared deviations between the observed and predicted data. Thereafter, the absorption of ^{125}I-soluble insulin was represented by the rate constants estimated from models A and B, respectively, and T50 min (model A). Numerical estimation of the lag phase was performed using the T10 and T25 min (model A) and Tmax (min): Tmax = $1/k$, determined from model B).

SCBF. Residual ^{99m}Tc radioactivity at the injection site, in counts per minute, was expressed as a percentage of counts at the time of injection and plotted against time on a semilogarithmic scale, hereafter referred to as the ^{99m}Tc clearance curve. The biexponential clearance curves thus obtained were analyzed using a standard sum of exponentials curve-fitting program. Tslow was calculated from the second part of the curve. Initial slope was a combination of clearance by the fast and slow exponents. Therefore, Tfast was calculated by subtraction of Tslow from the slope of the initial part of the curve. Values of Tslow and Tfast are expressed as half-clearance times in minutes. The relative contribution of the slow and fast components of ^{99m}Tc clearance was calculated as a percentage and indicated by %slow and %fast; %slow was obtained by backextrapolation of the slow exponent to the time of injection and %fast = 100 − %slow. Analysis of the relative percentage clearance of each exponent was limited to the use of %slow.

Pertinent abbreviations for parameters used in the analysis of ^{125}I-soluble insulin absorption and SCBF are summarized in Table 1.

Statistical methods

To evaluate the relationship between the rate of absorption of ^{125}I-soluble insulin and SCBF, r_S analysis was performed between individual subjects' parameters obtained from the descriptive analysis of the residual radioactivity curves and Tfast, Tslow, and %slow for ^{99m}Tc clearance. The relationship between absorption of ^{125}I-soluble insulin and the subjects' anthropometric measurements was assessed by means of r_S analysis, corrected for ties as appropriate, performed between the parameters obtained from the mathematical analysis of the residual radioactivity curves and the following measurements: BMI; ultrasonic subcutaneous adipose tissue thickness at the respective sites of soluble insulin injection; the sum of the ultrasound adipose tissue thickness for the abdominal, anterior midthigh, outer forearm, and subscapular sites (referred to as total ultrasound subcutaneous adipose tissue thickness); skin fold thickness at the sites of soluble insulin administration; and the sum of the abdominal, thigh, biceps, triceps, and subscapular skinfold thickness (referred to as total skinfold thickness).

The effect on SCBF of the subjects' anthropometric measurements was explored by means of r_S analysis between

Table 2—*The r_S values for correlation analysis between parameters of ^{125}I-soluble insulin absorption and SCBF (^{99m}Tc clearance)*

	Model A		Model B				
	K_1 (min^{-1})	K_2 (min^{-1})	K (min^{-1})	Tmax (min)	T10 (min)	T25 (min)	T50 (min)
^{99m}Tc clearance							
Tfast (min)	−0.295*	−0.457†	−0.501†	0.504†	0.341‡	0.507†	0.522†
Tslow (min)	−0.310‡	−0.459†	−0.514†	0.509†	0.357‡	0.490†	0.511†
%Slow	−0.308‡	−0.311‡	−0.401†	0.403†	0.396†	0.456†	0.437†

*$P < 0.05$.
†$P < 0.001$.
‡$P < 0.01$.

Tfast, Tslow, and %slow for ^{99m}Tc clearance and the anthropometric measurements described above. Results are expressed as mean ± SE, unless otherwise stated. For illustrative purposes, subjects were divided into nonobese (BMI <25 kg/m^2, range 18.2–24.1 kg/m^2, $n = 6$) and obese (BMI >27.5 kg/m^2, range 27.8–41.3 kg/m^2, $n = 6$) (22,23). Comparisons within and between the two groups of subjects were conducted by means of Wilcoxon signed rank and Mann-Whitney tests, respectively. Statistical significance was defined as $P < 0.05$.

RESULTS— Descriptive analysis of individual subjects' residual radioactivity curves revealed R^2 values greater than 0.992 for both models. Residual plots constructed between the observed and predicted data revealed no systematic deviations. R^2 values and residual SDs revealed no significant differences between curve-fitting by models A and B.

Relationship between ^{125}I-soluble insulin absorption and SCBF

The values of r_S for the relationship between parameters of ^{125}I-soluble insulin absorption and ^{99m}Tc clearance are presented in Table 2.

Significant negative correlations were observed for k_2 and k with Tfast ($P < 0.001$). Similarly, significant negative correlations also were observed for the rate constants k_1, k_2, and k with Tslow ($P < 0.01$–0.001). Strong negative associations were observed for k_1, k_2, and k with %slow ($P < 0.01$–0.001). Highly significant positive correlations were observed for T50 with Tfast, Tslow, and %slow ($P < 0.001$). The rate of absorption of soluble insulin is, therefore, positively correlated with SCBF. The relationships of k_2 and k with Tfast and Tslow are illustrated in Fig. 1.

Examination of the relationship between parameters representing the lag phase and ^{99m}Tc clearance revealed significant positive correlations for Tmax, T10, and T25 with Tfast, Tslow and %slow ($P < 0.01$–0.001). Therefore, the duration of the lag phase increased with declining SCBF. The relationships of T10 and T25, with Tfast and Tslow, are illustrated in Fig. 2.

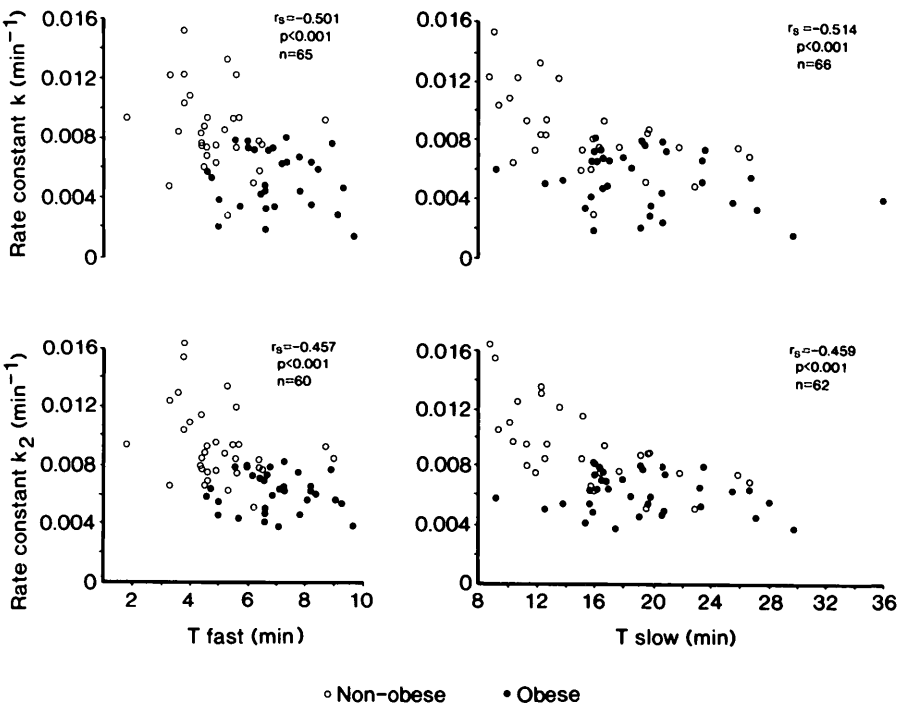

Figure 1—*Relationships for the rate constants k_2 (model A) and k (model B) for ^{125}I-soluble insulin absorption with the half-clearance times Tfast and Tslow of ^{99m}Tc clearance.*

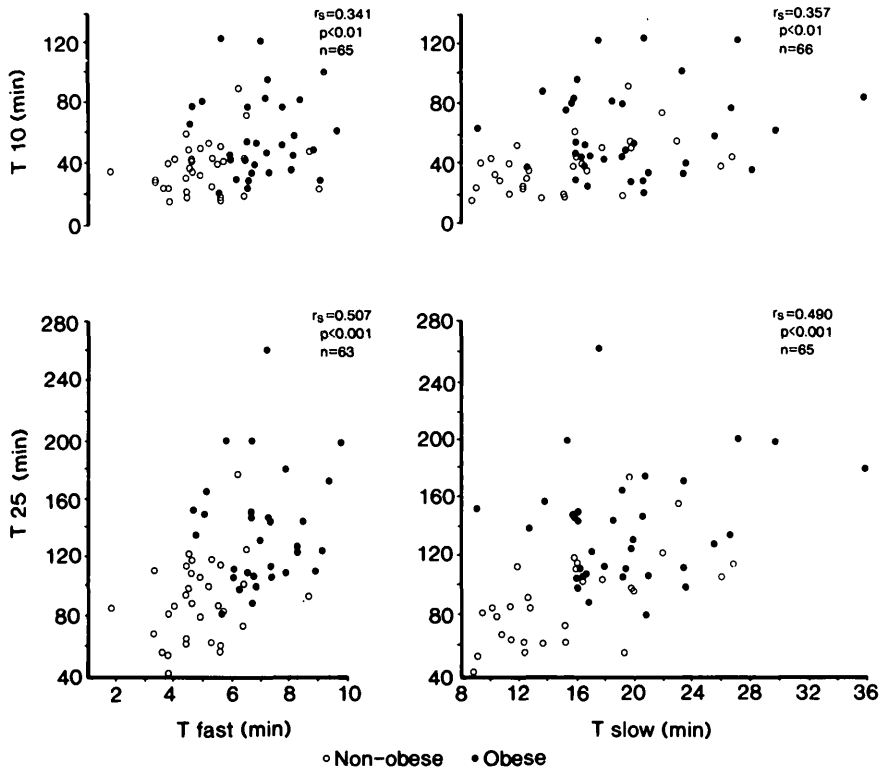

Figure 2—*Relationships for T10 and T25 for ^{125}I-soluble insulin absorption with the half-clearance times Tfast and Tslow for ^{99m}Tc clearance.*

Relationship between ^{125}I-soluble insulin absorption and anthropometric measurements

The values of r_S for correlation analysis between parameters of ^{125}I-soluble insulin absorption and the subjects' anthropometric measures are summarized in Table 3.

Mean (range) for the anthropometric measures were: BMI, 27.1 kg/m^2 (18.2–41.3); ultrasound subcutaneous adipose tissue thickness at the sites of soluble insulin administration, 7.8 mm (2.4–22.3); total ultrasound adipose tissue thickness, 29.3 mm (13.3–49.4); skinfold thickness at the site of soluble insulin injection, 15.4 mm (4.8–50.0); and total skinfold thickness, 81.0 mm (34.6–205.3).

Correlation analysis revealed inverse relationships for k_1, k_2, and k with BMI ($P < 0.01$–0.001). A strong positive correlation was observed between T50 and BMI ($P < 0.001$). Significant relationships were not observed between the parameters of ^{125}I-soluble insulin absorption and ultrasound adipose tissue thickness at the injection site. However, highly significant negative correlations were noted for k_1, k_2, and k with total ultrasound adipose tissue thickness ($P < 0.01$–0.001). Rate constants k_2 and k were inversely correlated with skin fold thickness at the injection site ($P < 0.001$). Similarly, significant negative correlations were observed for k_2 and k with total skin fold thickness ($P < 0.001$). T50 demonstrated a strong positive correlation with total skin fold thickness ($P < 0.001$). The rate of absorption of soluble insulin declines with increasing subcutaneous adiposity, including skin fold thickness at the injection site. The relationships for k_2 and k with BMI and total skin fold thickness are illustrated in Fig. 3.

Concerning the numerical pa-

Table 3—*The r_S values for the relationship between parameters of ^{125}I-soluble insulin absorption and subjects' anthropometric measures*

	Model A		Model B				
	K_1 (min^{-1})	K_2 (min^{-1})	K (min^{-1})	Tmax (min)	T10 (min)	T25 (min)	T50 (min)
BMI (kg/m^2)	−0.350*	−0.615†	−0.693†	0.701†	0.527†	0.681†	0.705†
Total ultrasound adipose tissue thickness (mm)	−0.317*	−0.434†	−0.543†	0.553†	0.436†	0.547†	0.554†
Skin fold thickness at injection site (mm)	−0.169	−0.506†	−0.526†	−0.528†	0.387†	0.503†	0.520†
Total skin fold thickness (mm)	−0.270‡	−0.512†	−0.606†	0.614†	0.471†	0.618†	0.625†

*$P < 0.01$.
†$P < 0.001$.
‡$P < 0.05$.

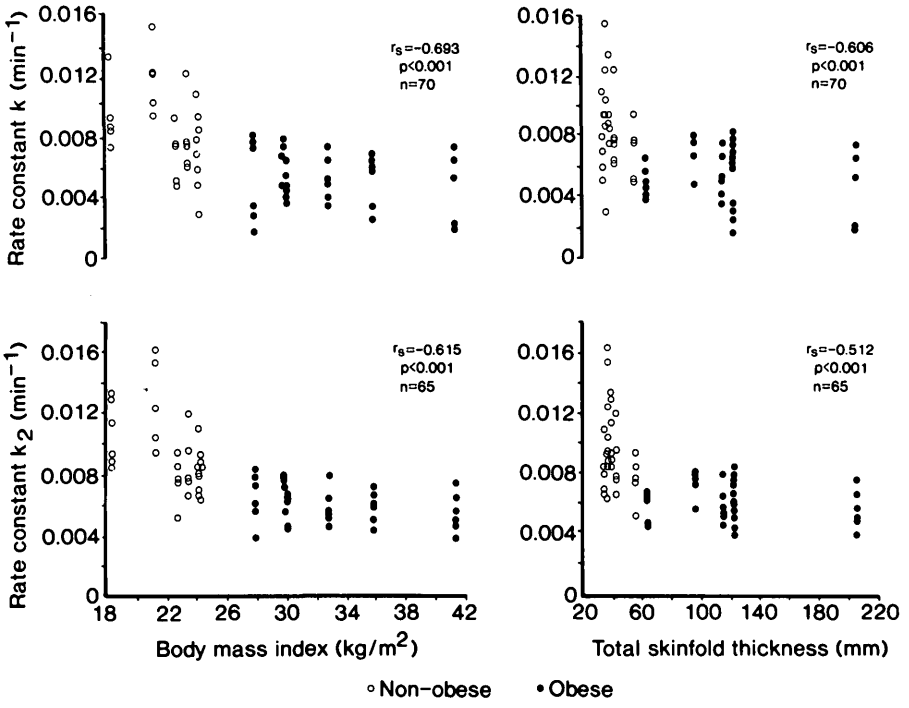

Figure 3—*Relationships for the rate constants k_2 (model A) and k (model B) for ^{125}I-soluble insulin absorption with BMI and total skin fold thickness.*

rameters of the lag phase, significant positive correlations were observed for Tmax, T10, and T25 with BMI, total ultrasound adipose tissue thickness, skin fold thickness at the injection site, and total skin fold thickness ($P < 0.001$). Thus, prolongation of the lag phase was observed with increasing degree of adiposity, including skin fold thickness at specific injection sites. The relationships for T10 and T25 with BMI and total skin fold thickness are illustrated in Fig. 4.

Regional differences in lag phase and the rate of absorption of ^{125}I-soluble insulin

Parameters representing the rate of absorption of ^{125}I-soluble insulin, including during the lag phase, for the abdominal, thigh, and arm injection sites for nonobese and obese subjects are presented in Table 4.

Comparisons of the rate of absorption of soluble insulin for the three injection regions in nonobese subjects revealed larger rate constants (k_2 and k) and shorter T50 for the abdomen than both the thigh and arm injection sites ($P < 0.05–0.001$). These parameters however, were, similar for the latter two sites. For the lag phase, shorter T_{10}, T_{25}, and T_{max} values were noted for the abdominal than the thigh and arm sites ($P < 0.05–0.001$), with no significant differences between the latter two sites.

Absorption of soluble insulin in the obese subjects was similar for the abdominal and arm sites. These two sites demonstrated faster rates of absorption, with higher rate constants and shorter T50 than the thigh ($P < 0.05–0.001$). In parallel, T10, T25, and Tmax values were similar for the abdominal and arm regions but lower for these two sites than the thigh ($P < 0.01–0.001$).

To further evaluate the effect of subjects' anthropometry on the rate of absorption of soluble insulin, comparisons also were conducted between the nonobese and obese subjects for each respective site. In keeping with the inverse relationship between the rate of absorption of soluble insulin and the subjects' degree of adiposity described previously, nonobese subjects demonstrated faster absorption with shorter lag phases for the abdomen and thigh injection sites compared with their obese counterparts ($P < 0.01–0.001$). Although a higher rate of absorption of soluble insulin and shorter lag phase also was observed for the arm site of nonobese compared with obese subjects ($P < 0.05–0.01$), the differences were less marked than for the other two sites examined.

Relationship between SCBF and anthropometric measurements

The values of r_S for the relationship between SCBF and the subjects' anthropometry are presented in Table 5.

Highly significant positive correlations were observed for Tfast with BMI, total ultrasound adipose tissue thickness, and total skin fold thickness ($P < 0.001$). A positive correlation also was noted between Tfast and skinfold thickness at the site of insulin injection ($P < 0.01$). Tslow also demonstrated a positive relationship with BMI ($P < 0.01$). A weaker correlation was observed for Tslow with total ultrasound adipose tissue thickness and total skin fold thickness ($P < 0.05$). Tslow and skin fold thickness at the injection site were not related. None of the anthropometric measures demonstrated significant relationship with %slow. No significant correlations were observed between the parameters of ^{99m}Tc clearance and ultrasound thickness of adipose tissue at the injection site.

CONCLUSIONS— The relationship between the absorption of soluble insulin and SCBF was investigated in resting normal subjects, using mathematical modeling of the entire ^{125}I-soluble insulin residual radioactivity curve and the

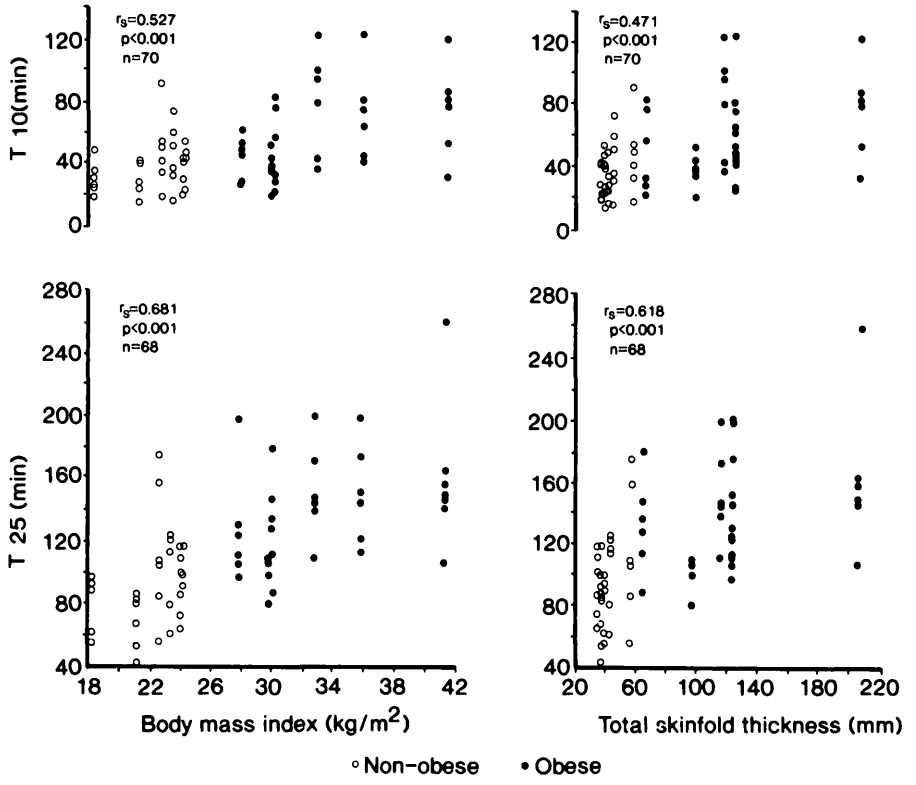

Figure 4—*Relationships for T10 and T25 for ^{125}I-soluble insulin absorption with BMI and total skin fold thickness.*

clearance of the lipophobic radioisotope ^{99m}Tc, respectively. Previous studies have reported a positive relationship between the rate of absorption of radiolabeled soluble insulin, represented by T50 or fractional clearance rates of the monoexponential part of the residual radioactivity curves, and the clearance from contralateral injection sites of the lipophilic radioisotope ^{133}Xe (1–4). Representation of the absorption of soluble insulin by these methods has excluded specific examination of the characteristic initial phase of slow relative rate of absorption of soluble insulin (the lag phase) and its relationship with SCBF. The three- and two-parameter diffusion rate-limited biexponential functions of models A and B provided appropriate descriptive analyses of the residual radioactivity curves, in accordance with previous modeling of similar curves and plasma insulin profiles after subcutaneous soluble insulin administration (6, 24–27). Therefore, parameters that characterize individual subjects' residual radioactivity curves, including the lag phase, could be estimated from the models used.

Measurement of SCBF, in relationship to the absorption of soluble insulin, has been performed by the clearance of ^{133}Xe (1–4). The lipophilic properties of this radioisotope result in slow clearance from the subcutaneous adipose tissue and allow diffusion into arterioles, venules, and arteriovenous anastomoses, thereby measuring a component of total blood flow in addition to flow through nutritive capillaries (28, 29). Interpretation of the ^{133}Xe clearance requires knowledge of the tracers' tissue: blood partition coefficient for that particular injection site (30). Earlier estimates of the coefficient, obtained in vitro or in experimental animals, were grossly inaccurate when applied to human subcutaneous adipose tissue (31). The partition coefficient also varies between lean and obese subjects and for different sites within an individual (31). Further uncertainties regarding ^{133}Xe clearance are introduced by the subjects' hematocrit and the prevailing nutritional and neurohumoral status (28,32–34). These properties of ^{133}Xe compromise its use in comparison with human subcutaneous adipose tissue blood flow within and between subjects, and its use should ideally be reserved for fat-free areas (35). The hydrophilic properties of ^{99m}Tc overcome the uncertainties relating to lipophilic tracers, allowing for rapid clearances through nutritive capillaries and not larger vessels (36), thus assessing blood flow at the putative site of insulin absorption.

This study confirms the presence of a positive relationship between the rate of absorption of soluble insulin from the abdomen, thigh, and arm injection sites and SCBF, determined simultaneously within the same region. Therefore, significant correlations were observed for the rate constants representing soluble insulin absorption (k_2 [model A] and k [model B]), and T50 with Tfast, Tslow, and %slow for ^{99m}Tc clearance. Surprisingly, no data exist on the relationship between the lag phase of soluble insulin absorption and SCBF. Of greater significance, therefore, this study reveals a negative relationship between the lag phase and SCBF. Therefore, Tmax, T10, and T25 demonstrate positive correlations with Tfast, Tslow and %slow for ^{99m}Tc clearance.

Current evidence suggests that after subcutaneous administration of soluble insulin, the injected depot undergoes substantial dilution and removal of zinc from its predominant configuration of a two-zinc-hexameric unit (37–39). Thereafter, the hexameric insulin dissociates, diffuses to the capillaries, and is absorbed in a predominantly dimeric or

Table 4—*Rates of absorption of ^{125}I-soluble insulin and parameters of the lag phase for the abdominal, thigh, and arm injection sites for nonobese and obese normal subjects*

	Model A		Model B				
	K_1 (min^{-1})	K_2 (min^{-1})	K (min^{-1})	Tmax (min)	T10 (min)	T25 (min)	T50 (min)
Nonobese							
Abdomen	0.018	0.010	0.011	98	29	72	145
	(0.002)	(0.001)	(0.001)	(8)	(3)	(7)	(13)
Thigh	0.009	0.008	0.008	130	53	117	211
	(0.001)	(0.001)	(0.001)	(11)	(6)	(10)	(18)
Arm	0.010	0.009	0.009	113	44	99	181
	(0.001)	(0.001)	(0.001)	(7)	(5)	(7)	(11)
Obese							
Abdomen	0.011	0.006	0.007	160	62	140	253
	(0.002)	(0.001)	(0.001)	(12)	(8)	(13)	(16)
Thigh	0.010	0.004	0.006	190	80	170	319
	(0.001)	(0.001)	(0.001)	(11)	(10)	(10)	(21)
Arm	0.007	0.007	0.007	140	53	122	223
	(0.001)	(0.001)	(0.001)	(7)	(8)	(7)	(12)

Values in parentheses are SE.

monomeric form (5,38–41). Dilution of the injected depot and dissociation of the hexameric insulin unit would account for the initial delay in the absorption of soluble insulin. Such a delay would, therefore, not be anticipated with monomeric insulin analogues, as has been demonstrated (42). Our results indicate an inverse relationship between the duration of the lag phase and SCBF, although the rate of absorption of soluble insulin during this period should be predominantly limited by the physical processes thought to occur. However, the rate of linear diffusion is proportional to the concentration gradient. Alteration of this gradient, by increasing blood flow, would increase the rate of dilution of the injected depot and thereby enhance the dissociation of the two-zinc-hexamer of insulin. Predominance of the physical processes occurring during the lag phase is emphasized by only modest enhancement observed in the rate of absorption of soluble insulin when administered after admixture with potent vasodilators, such as aprotinin (43), prostaglandin E_1 (44), and phenoxybenzamine (45).

Conflicting results have been reported for the influence on the rate of absorption of soluble insulin of the subjects' anthropometry. The absorption of radiolabeled soluble insulin was not affected by the ultrasound thickness of subcutaneous adipose tissue at the injection site (46), but declined with increasing sum of skinfold thickness measured at three sites (47). In IDDM patients, a positive correlation was observed between the size of the subcutaneous depot during basal rate continuous subcutaneous insulin infusion and skinfold thickness at the infusion site (48). Our results demonstrate a clear inverse relationship between the rate of absorption of soluble insulin and the subjects' degree of adiposity. Anthropometric measurements used provide reliable indicators of body fat mass, and correlate closely with estimates obtained by complex techniques of body densitometry and total body water and potassium determinations (49–51). Thus, the rate constants representing soluble insulin absorption demonstrated significant negative correlations not only with indicators of body fat mass, such as the BMI and total skinfold thickness, but also skinfold thickness at the specific injection sites. Concerning the lag phase, Tmax, T10, and T25 demonstrated significant positive correlations with BMI, total ultrasound adipose thickness, and total and skin fold thickness at the injection site. Increasing adiposity and skin fold thickness at injection sites thus result in lengthening of the lag phase.

The relationship between the rate of absorption of soluble insulin and the subjects' anthropometry may, in part, be explained by the previously described negative correlation between anterior abdominal wall adipose tissue blood flow and the subjects' adiposity (30,34). A similar inverse relationship between SCBF for the abdominal, thigh, and arm injection sites and the subjects' degree of adiposity were observed in this study.

Table 5—*The r_S values for correlation analysis between SCBF (^{99m}Tc clearance) and subjects anthropometric measures*

	^{99m}Tc clearance		
	Tfast	Tslow	%Slow
BMI (kg/m^2)	0.618*	0.300†	0.171
Total ultrasound thickness (mm)	0.501*	0.271‡	0.091
Skin fold thickness at injection site (mm)	0.338†	0.155	0.097
Total skin fold thickness (mm)	0.561*	0.278‡	0.169

*$P < 0.001$
†$P < 0.01$.
‡$P < 0.05$.

The inverse relationship between SCBF and the subjects' degree of adiposity may result from an increase in adipocyte size with increasing adiposity, which in turn leads to a reduction in SCBF and capillary density (33,52).

Our data corroborates the previously reported faster absorption of soluble insulin from abdominal sites compared with other regions (5,9,10,43). Of greater clinical significance in terms of control of postprandial hyperglycemia, the shortest lag phase was observed following abdominal injection for both nonobese and obese subjects. Estimation of the lag phase, particularly to Tmax, indicates the subcutaneous tissue of the abdominal wall as the preferred site for administration of soluble insulin. However, the Tmax values for even this site emphasize the currently recommended practice of injection of soluble insulin 30–45 min before meal ingestion (16, 17). Furthermore, the lag-phase parameters for the thigh and arm sites highlight the inadequacy of these sites for delivery of preprandial boluses of soluble insulin. Numerical estimation of the lag-phase parameters demonstrates the limitations of currently available soluble insulin preparations in controlling postprandial glycemic excursions. This goal may be better attained in the near future with rapid insulin delivery following subcutaneous administration of novel insulin analogues (38,39,42).

This study demonstrates a positive relationship between the absorption of soluble insulin and SCBF. The rate of absorption of soluble insulin rises, with a lag phase of shorter duration, as SCBF increases. Increasing BMI and subcutaneous adiposity result in a reduction of SCBF and the rate of absorption of soluble insulin, with prolongation of the lag phase. The shortest duration lag phase is observed following abdominal injection of soluble insulin.

Acknowledgments— This study was presented in part at the British Diabetic Association, April 1991 and published in abstract form (*Diabetic Med* 8:32, 1991).

We are grateful to Novo Research Institute (Bagsvaerd, Denmark) for the generous supplies of radiolabeled soluble insulin, Mr. S. Luzio for technical assistance, and Mr. A.C. Shaw for the illustrations. Statistical analyses were conducted under the guidance of Dr. R. Newcombe (Department of Medical Statistics, University of Wales College of Medicine) and Dr. A. Volund (Novo Research Institute). Ms. M. Evans and Mrs. E. Walters provided expert assistance in the preparation of this manuscript.

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