scores in reading and mathematics between the extreme categories of smoking represented 0·12 and 0·18 of the standard deviation of the population. The equivalent figures for the scale of qualifications at age 23 are 0·22 for men and 0·31 for women (which is not explained by including the additional category of smoking in this analysis).

Of course these are observational data and an unequivocal indication of a causal relation cannot be claimed. The analysis did not include, for example, a measure of the mother's intellectual development, which might be expected to be related both to her smoking habits (but perhaps less so in 1958 than now) and to the achievements of her child. Nevertheless, the evidence seems strong enough to justify warnings against smoking in pregnancy because of the possible implications for the long term physical and intellectual development of the child, in addition to the other dangers.

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Recombinant DNA derived monomeric insulin analogue: comparison with soluble human insulin in normal subjects

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Abstract

***Objective*—To compare the rate of absorption from subcutaneous tissue and the resulting hypoglycaemic effect of iodine-125 labelled soluble human insulin and a monomeric insulin analogue derived by recombinant DNA technology.**

***Design*—Single blind randomised comparison of equimolar doses of ^{125}I labelled soluble human insulin and insulin analogue.**

***Setting*—Study in normal people at a diabetes research unit and a university department of medical physics.**

***Subjects*—Seven healthy male volunteers aged 20-39 not receiving any other drugs.**

***Interventions*—After an overnight fast and a basal period of one hour two doses (0·05 and 0·1 U/kg) of ^{125}I labelled soluble human insulin and insulin analogue were injected subcutaneously into the anterior abdominal wall on four separate days.**

***End point*—To find a fast acting insulin for meal related requirements in insulin dependent diabetics.**

***Measurements and main results*—Residual radioactivity at the injection site was measured continuously for the first two hours after injection of the ^{125}I labelled preparations and thereafter for five minutes simultaneously with blood sampling. Frequent venous blood samples were obtained over six hours for determination of plasma immunoreactive insulin, insulin analogue, glucose, and glucagon values. Time to 50% of initial radioactivity at the injection site for the insulin analogue compared with soluble insulin was 61 *v* 135 minutes ($p<0·05$) with 0·05 U/kg and 67 *v* 145 minutes ($p<0·001$) with 0·1 U/kg. Concentrations in plasma increased faster after the insulin analogue compared with soluble insulin, resulting in higher plasma concentrations between 10 and 150 minutes ($0·001<p<0·05$) after 0·05 U/kg and between 40 and 360 minutes ($0·001<p<0·05$) after 0·1 U/kg. The hypoglycaemic response to insulin analogue was a plasma glucose nadir at 60 minutes with both doses compared with 90 and 120 minutes with soluble insulin at 0·5 and 0·1 U/kg respectively. The response of glucagon substantiated the earlier and more dramatic hypoglycaemic effect with the insulin analogue.**

***Conclusions*—The much faster absorption from subcutaneous tissue of the disubstituted monomeric insulin analogue compared with soluble insulin suggests that the analogue may be a potential candidate for rapid insulin delivery after subcutaneous bolus injection.**

Introduction

In normal people physiological endogenous insulin secretion is related to the availability of substrate and the prevailing glycaemic value.[1-4] Eating a meal results in a rapid rise in plasma insulin concentration, reaching a peak within 30-60 minutes.[3 5 6] By contrast, the subcutaneous injection of soluble insulin produces peak plasma concentrations at 90-120 minutes,[7-9] necessitating injection around 30 minutes before a meal in an attempt to minimise postprandial hyperglycaemia.[10 11]

The initial delay in the absorption of commercially available soluble insulin from the subcutaneous tissue has been attributed to the rate of dissociation from

hexameric units into monomeric insulin molecules.[12-16] Recently substitution of amino acid residues at the monomer-monomer interface of the insulin dimer has become possible by utilising recombinant DNA technology, resulting in monomeric insulin analogues.[17 18] One such insulin analogue is the result of substitution of the B9 serine and B27 threonine residues of human insulin with aspartate and glutamate respectively. Preliminary studies in animals have shown substantially faster absorption of the insulin analogue from subcutaneous tissue compared with soluble human insulin.[19]

We report a study in normal people comparing the rate of absorption from subcutaneous tissue and the hypoglycaemic response of equimolar doses of iodine-125 (^{125}I) labelled soluble human insulin and ^{125}I labelled insulin analogue.

Subjects and methods

We studied seven healthy male volunteers aged 20-39. None had a family history of diabetes mellitus and all had normal glucose tolerance and were within 10% of ideal body weight. None of the volunteers had a history of allergy or were receiving concomitant drugs. The study was approved by the local ethical committee and was performed in accordance with the Declaration of Helsinki. All subjects gave informed written consent.

Subjects were studied on four occasions at weekly intervals receiving, in random order, 0·05 U/kg and 0·1 U/kg ^{125}I labelled soluble human insulin (Actrapid HM U100, specific activity 185 MBq/l; Novo Industri A/S, Copenhagen) and equimolar doses of ^{125}I labelled insulin analogue (specific activity 198 MBq/l).[20]

Premedication with 100 mg potassium iodine was given the evening before each study day to prevent thyroidal uptake of ^{125}I. Each study day began after an overnight fast with the siting of an intravenous cannula in an antecubital vein. The cannula was connected to a three way tap for blood sampling and was kept patent by a slow running infusion of isotonic saline (0·15 mol/l). After a basal period of one hour the ^{125}I labelled preparations were injected subcutaneously into the anterior abdominal wall midway between the umbilicus and the anterior superior iliac spine, a 0·5 ml disposable insulin syringe being used (Lo-Dose; Becton Dickinson, Brooklyn, New York). Venous blood samples were obtained half hourly during the basal period, every 10 minutes during the first hour after injection, and thereafter every 15 minutes up to two hours, half hourly up to four hours, and hourly up to six hours. Blood samples were aliquoted into fluoride for plasma glucose estimation (glucose analyser; Chemlab Instruments, Hornchurch, Essex) and into lithium-heparin for determination of immunoreactive insulin[21] and glucagon concentrations.[22] All samples were centrifuged within five minutes of sampling and the plasma stored at −20°C until assay. Using the insulin analogue for the standard curve allowed measurement of its plasma concentration. The coefficients of variation of the insulin, insulin analogue, and glucagon assays were 4·2%, 7·9%, and 8·5% respectively.

The disappearance of the injected preparations from subcutaneous tissues was assessed from the amount of residual radioactivity at the injection site. External emission of γ rays was measured with a 50×57 mm thallium activated sodium iodide scintillation detector with a cylindrical lead collimator, fixed 50 mm above the skin surface. Residual radioactivity at the injection site was measured continuously for the first two hours after injection of the ^{125}I labelled preparations and thereafter for five minutes simultaneously with blood sampling. All counts were corrected for background activity and the residual activity at a given time expressed as a percentage of initial values.

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

Statistical analysis—Results are expressed as means and standard error (SE) except where stated otherwise. Individual pairs of treatments were compared by the Wilcoxon paired rank sum test.

Results

Both preparations were well tolerated by all subjects with no local or systemic adverse effects being recorded. Figure 1 shows the residual radioactivity at the injection sites after the two doses (0·05 and 0·1 U/kg) of ^{125}I labelled soluble human insulin and the equivalent doses of insulin analogue. Disappearance of the insulin analogue from the subcutis was faster than disappearance of the reference soluble human insulin, as reflected by significantly lower ($p<0·05$) levels of residual activity up to 120 and 360 minutes for the 0·05 and 0·1 U/kg doses respectively. The calculated time to 50% of the initial radioactivity was 135·4 (SE 24·5) minutes after the injection of 0·05 U soluble insulin/kg compared with 61·4 (5·2) minutes ($p<0·05$) for the analogue preparation (fig 2; table). Similarly after injection of the higher dose (0·1 U/kg) of the comparative preparations times to 50% of the initial radioactivity were 145·1 (8·1) and 67·2 (10·8) minutes ($p<0·001$) for the soluble human insulin and insulin analogue preparations respectively.

Figure 3 shows the plasma insulin, insulin analogue, and glucose concentrations after injections of the low dose (0·05 U/kg) preparations of insulin. Injection of

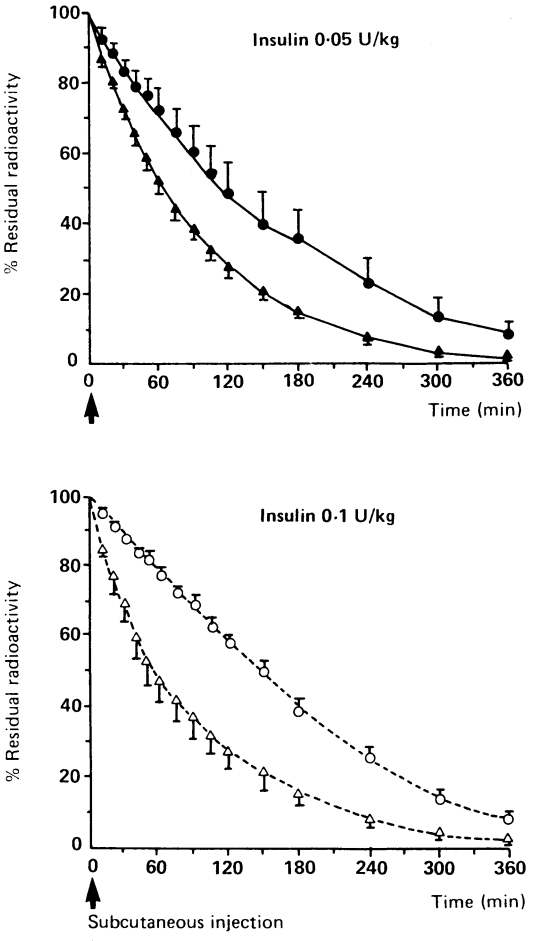

FIG 1—*Mean (SE) residual radioactivity at injection sites after dosing with ^{125}I labelled soluble human insulin (0·05 U/kg ●——●; and 0·1 U/kg ○----○) and ^{125}I labelled insulin analogue (0·05 U/kg ▲——▲; 0·1 U/kg △----△)*

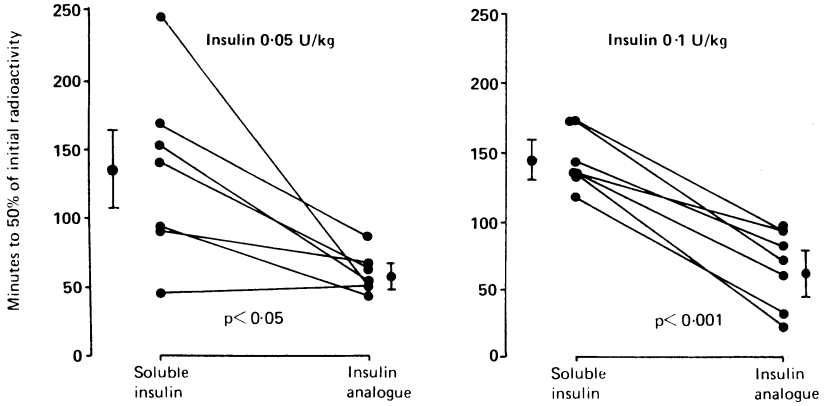

FIG 2—*Times (minutes) to 50% of initial radioactivity at injection sites for soluble human insulin and insulin analogue at doses of 0·05 and 0·1 U/kg in seven normal subjects. Mean values expressed with SE (bars)*

Time in minutes to 50% of initial radioactivity (time to 75% of initial radioactivity in parentheses) at injection site for soluble human insulin and insulin analogue

	Soluble insulin		Insulin analogue	
Subject No	0·05 U/kg	0·1 U/kg	0·05 U/kg	0·1 U/kg
1	95 (54)	118 (44)	45 (18)	34 (14)
2	170 (87)	174 (79)	87 (38)	96 (35)
3	141 (61)	174 (111)	65 (30)	73 (36)
4	47 (18)	134 (63)	52 (25)	25 (11)
5	154 (73)	136 (54)	57 (27)	62 (23)
6	93 (39)	143 (65)	68 (28)	84 (29)
7	246 (125)	136 (73)	56 (23)	95 (40)
Mean	135·4 (65·3)	145·1 (69·7)	61·4 (27·0)	67·2 (26·9)
SD	64·9 (34·6)	21·4 (21·6)	13·7 (6·1)	28·4 (11·2)
SE	24·5 (13·1)	8·1 (8·2)	5·2 (2·3)	10·8 (4·2)

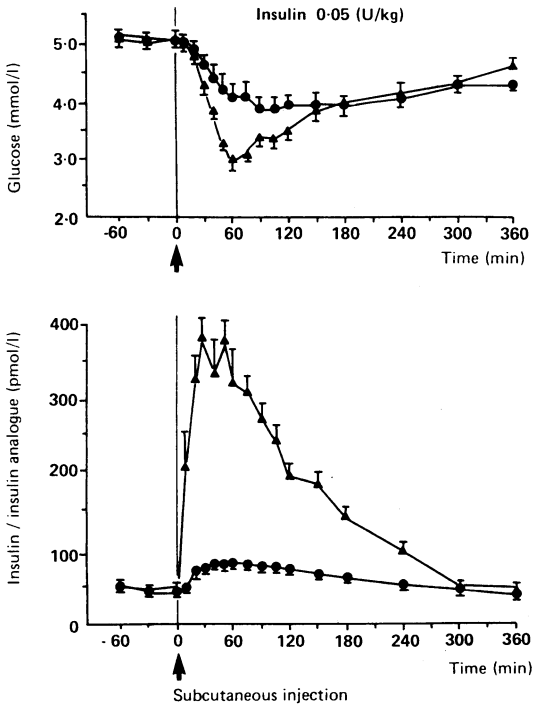

FIG. 3—*Plasma glucose, insulin, and insulin analogue concentrations after injections of soluble insulin (●——●) and insulin analogue ▲——▲) 0·05 U/kg. Bars are SE*

the soluble human insulin resulted in an increase in plasma insulin concentration from 40 (SE 4) pmol/l during the basal period to a peak of 90 (8) pmol/l at 40-60 minutes, followed by a slow decline. By contrast, injection of the low dose of analogue produced a much faster rise in plasma concentrations, from a basal value of 50 (6) pmol/l to a peak of 390 (25) pmol/l at 30 minutes, thereafter declining gradually. Plasma insulin analogue concentrations were significantly greater than plasma insulin concentrations at between 10 and 150 minutes ($p<0·05$). The hypoglycaemic response to soluble human insulin 0·05 U/kg was a decline in plasma glucose concentration from a basal 5·1 (SE 0·1) mmol/l to a nadir of 3·9 (0·2) mmol/l at 90 minutes. At the same dosage insulin analogue resulted in a faster and greater decline in plasma glucose concentration, from 5·1 (0·1) mmol/l before the injection to a nadir of 3·0 (0·2) mmol/l at 60 minutes. Plasma glucose concentrations were significantly lower after the analogue at 40, 60, and 120 minutes ($p<0·05$).

Figure 4 displays the plasma insulin, insulin analogue, and glucose concentrations after the higher dose (0·1 U/kg) of soluble human insulin and insulin analogue. Injection of soluble insulin led to a rise in

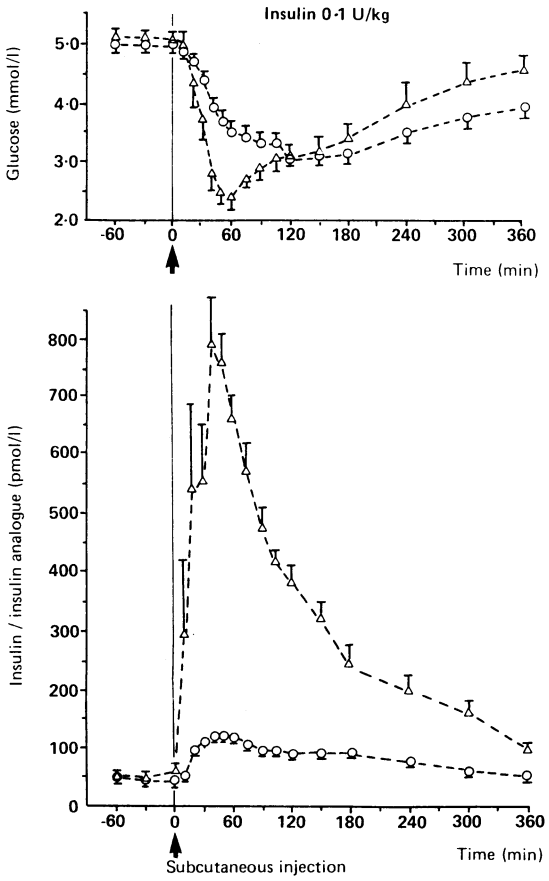

FIG 4—*Plasma glucose, insulin, and insulin analogue concentrations after injections of soluble insulin (○----○) and insulin analogue △----△) 0·1 U/kg. Bars are SE.*

plasma concentration from 40 (SE 5) pmol/l during the basal period to a peak of 130 (9) pmol/l at 40 minutes. Injection of insulin analogue resulted in a much faster and larger rise in plasma insulin analogue concentration, from 70 (10) pmol/l before injection to a peak of 800 (70) pmol/l at 40 minutes. Plasma concentrations of the analogue were significantly greater than those of immunoreactive insulin from 40 minutes onwards ($p<0·05$). The much higher plasma concentration of the insulin analogue reflected its two to three times faster rate of absorption and 20-30% slower metabolic clearance rate than human insulin (A Vølund, personal communication).

Plasma glucose concentration declined from 5·1 (SE 0·1) mmol/l to a nadir of 3·1 (0·1) mmol/l at 120 minutes after injection of soluble human insulin 0·1 U/kg. By contrast, a faster and greater hypoglycaemic effect was noted in response to the injection of insulin analogue, the plasma glucose concentration declining from 5·1 (0·2) mmol/l before injection to a nadir of 2·4 (0·2) mmol/l at 60 minutes. Plasma glucose concentrations were significantly lower after injection of the high dose of analogue between 40 and 75 minutes ($p<0·05$).

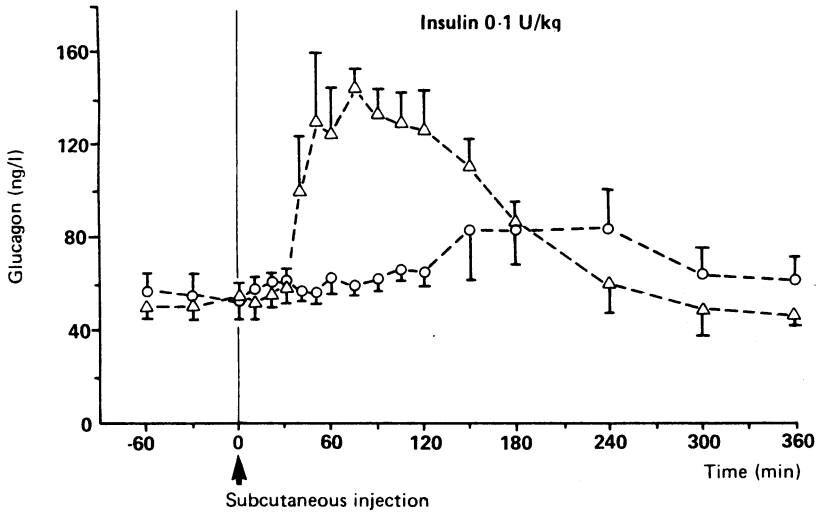

FIG 5—*Plasma glucagon concentrations in response to subcutaneous injection of soluble human insulin (○———○) and insulin analogue (△———△) 0·1 U/kg. Bars are SE*

A faster rate of recovery was noted after the insulin analogue, as reflected in higher plasma glucose concentrations at 240 and 300 minutes ($p<0·05$). The more dramatic hypoglycaemic response with the insulin analogue resulted in a greater counterregulatory effect represented by the glucagon response (fig 5). The glucagon concentration was significantly greater after the insulin analogue from 60 to 105 minutes ($p<0·05$).

Discussion

This is the first study in human subjects to record the rapid absorption of a monomeric insulin analogue from subcutaneous tissue. By comparison with soluble human insulin the absorption of the analogue was substantially faster at both doses. The more rapid disappearance of the ^{125}I labelled insulin analogue was paralleled by a faster rise in plasma insulin analogue concentrations when compared with soluble human insulin. Onset of the hypoglycaemic effect resulting from this rapid absorption was significantly faster with the monomeric insulin analogue.

The events between subcutaneous injection of soluble insulin and its appearance in the bloodstream are far from elucidated. It has been suggested that after subcutaneous injection of soluble insulin transport within the interstitial space and absorption across the capillary membrane are dependent on diffusion.[13-15] Furthermore, it has been postulated that the insulin hexamer, which is the predominant configuration in commercially available neutral soluble insulin preparations,[12] dissociates into the monomeric or dimeric form before absorption across the capillary membrane.[14 16] The delay in reaching peak rates of absorption and plasma concentrations after subcutaneous injection of soluble insulin may therefore be due to the dissociation of the insulin hexamer into monomers or dimers at the injection site and the attainment of a diffusion equilibrium across the capillary membrane.[13-16] No delay in absorption occurred with the monomeric insulin analogue, resulting in a rapid rise in plasma concentrations and an earlier and more pronounced hypoglycaemic effect. In an attempt to reduce the initial delay in the absorption of subcutaneously injected soluble insulin and minimise postprandial hyperglycaemia soluble insulin is injected some 30 minutes before a meal, which may often be inconvenient and impracticable.[10 11] Attempts to increase the initial rate of absorption include local massage at the injection site[23] and the use of jet injection devices[24 25] or sprinkler needles.[26]

Our findings show that in normal subjects the disubstituted monomeric insulin analogue is absorbed substantially faster from subcutaneous tissue than the currently available soluble human insulin preparation Actrapid HM. The insulin analogue is therefore a potential candidate for rapid insulin delivery after subcutaneous bolus injection.

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