INSULIN DISAPPEARANCE AFTER INTRAVENOUS INJECTION AND ITS EFFECT ON BLOOD GLUCOSE IN DIABETIC AND NON-DIABETIC CHILDREN AND ADULTS

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SUMMARY

1. Porcine insulin (0.1 i.u./kg) was injected intravenously into diabetic and non-diabetic subjects.

2. In each group insulin produced a fall in blood glucose within 5 min which was exponential and which continued until approx. 99% of the insulin had left the plasma.

3. In the diabetic subjects both the insulin values and blood glucose fell more slowly than in the controls, when expressed as a proportion of the fasting value. However, both insulin and glucose continued to fall for a longer period, with the result that there was a total fall of blood glucose of 67% in the diabetics and 70% in the non-diabetics. Though in absolute terms considerably more glucose disappeared in diabetics than in the normal subjects, this could be related to the higher initial glucose concentrations in the former.

4. It is suggested that not only does insulin act very rapidly, but it is also rapidly inactivated.

Key words: insulin, glucose.

It has previously been shown that in non-diabetic children intravenously administered insulin has a very rapid effect on blood glucose, and glucose concentrations fall exponentially to reach a nadir between 20 and 25 min, by which time almost all of the administered insulin has left the circulation (Stimmler, Mashiter, Boucher & Meadow, 1969). The aim of the present study was to extend these observations to diabetic children and normal and diabetic adults.

METHODS

Blood glucose was measured in an auto-analyser by using the glucose oxidase method (Wincey & Marks, 1961). Radioimmunoassayable insulin concentrations were determined by the double-antibody technique described by Morgan & Lazarow (1963).

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Subjects

All tests were performed with the informed consent of the subjects, and in the case of the children, of their parents.

Four groups of subjects were investigated, details of whom are given in Tables 1–4, deposited as *Clinical Science* Tables 42/2, 42/3, 42/4 and 42/5 with the Librarian at the Royal Society of Medicine.

1. Thirteen non-diabetic adults, seven males and six females aged 17–65 years (mean 34 years). Their mean percentage of ideal weight was 98.9%.

2. Thirteen children aged 4–13 years (mean 8 years, all prepubertal). Mean percentage of ideal weight 107.7%. These children were being investigated for short stature, but none of them proved to have hypopituitarism.

3. Twelve patients in whom diabetes had developed after puberty, with ages ranging from 17–61 years (mean 41 years). None of these subjects had had insulin therapy. Their mean percentage of ideal weight was 106.1%.

4. Twelve diabetic children, aged 4–13 (mean 8 years, all prepubertal). Seven of these children had received soluble insulin therapy for between 2 and 10 days to control initial ketosis. No insulin was administered for 15 h before the test. Their mean percentage of ideal weight was 97%.

Insulin tests

Porcine insulin (0.1 i.u./kg) body weight was injected intravenously after an initial blood sample had been taken from a cannula inserted into the antecubital vein in the other arm. Blood samples were then obtained after 5 min and subsequently at 2.5 min intervals for 20 min. This was followed by sampling at 5 min intervals for the next 25 min and then at 10 min intervals for the last 0.5 h of the test.

RESULTS

Changes in blood glucose

As expected, the fasting blood glucose concentrations of diabetic subjects were significantly greater than those of their respective normal controls. After intravenous insulin the absolute fall in blood glucose was significantly greater ($P<0.001$) in diabetics (mean 112 mg/100 ml) than in normal subjects (mean 54 mg/100 ml). Individual responses to insulin varied considerably but within each group the total fall in glucose was significantly correlated ($P<0.001$) with the fasting glucose concentrations (Fig. 1). When considered in relative terms (as a percentage of the fasting value), the proportional fall in glucose in diabetics ($67\pm14\%$) was similar to that of the controls ($70\pm10\%$), the difference being non-significant ($P<0.2$).

In each group of subjects the mean fall of glucose was exponential from 5 min after insulin until the nadir was achieved (Fig. 2). The nadir was reached 24±4 min in the non-diabetics and 50±20 min in the diabetics. The mean rate of fall of the blood glucose was significantly slower in diabetic subjects than in normal subjects. For diabetic adults the rate of fall was 3·3 %/min; for normal adults 6·3 %/min; for diabetic children 2·6 %/min and for non-diabetic children 5·6 %/min. Complete information on individual glucose values is given in Tables 5–8, lodged with the Librarian of the Royal Society of Medicine as *Clinical Science* Tables 42/6, 42/7, 42/8 and 42/9.
Effect of insulin on blood glucose

**Fig. 1.** Total fall in blood glucose produced by 0·1 unit of insulin/kg related to fasting blood glucose concentrations. ▲, Non-diabetic children; ●, non-diabetic adults; △, diabetic children; ○, diabetic adults. (r = 0·84, P < 0·001.)

**Fig. 2.** Semilogarithmic plot of mean blood glucose against time from 5 min up to the nadir. D, Diabetic; N, non-diabetic; vertical bars, ± SEM.
The plasma insulin concentrations for each sample from every subject are given in Tables 9–12, also lodged with the Librarian at the Royal Society of Medicine as Clinical Science Tables 42/10, 42/11, 42/12 and 42/13. The mean insulin concentrations at each time-interval for each group are plotted in Fig. 3.

Though there was much divergence in individual results, the mean insulin concentrations fell more slowly for each diabetic group than that of the non-diabetic controls (Fig. 3), and mean values were significantly different from 10 to 40 min (the degree of significance varied at different times from $P<0.05$ to $P<0.001$). The difference between the two curves is more marked between the diabetic and control groups of children than between the corresponding adults.

Attempts were made to interpret our results in terms of a one or two pool system by using methods similar to those employed by Silvers, Farquhar, Lerner & Reaven (1970). In essence this involves the use of a least-squares technique to fit exponential functions to the individual sets of results. This was done by using a simplex method of function minimization (Nelder & Mead, 1965) in a computer program. Although the sets of results could be fitted satisfactorily by the various functions, some of the parameters found were often biologically meaningless.
Effect of insulin on blood glucose

For example, an extrapolation of the lines of best fit to zero time often lead to negative values, thus implying negative compartmental sizes.

Stern, Farquhar, Silvers & Reavon (1968) drew attention to the difficulty in performing this sort of analysis and suggested a solution which did not use the concept of pool sizes. Their technique involves the calculation of the 'irreversible loss rate from the sampled pool' by using isotopically labelled insulin as a marker. In the current study we used unlabelled insulin and therefore could not distinguish between endogenous and exogenous insulin. The technique of Stern et al. (1970) could not therefore be applied to this study.

In both the non-diabetic and diabetic groups there was a significant relationship between the time at which insulin concentrations fell below 25 μunits/ml and the time of the lowest glucose concentration obtained in that subject (Fig. 4). It can be calculated, assuming the plasma volume to be 30–40 ml/kg body weight (Gregerson & Stewart, 1939), that when the plasma insulin has fallen to 25 μunits/ml then 99% of the exogenous insulin will have left the plasma. Thus at the time of the minimum blood glucose concentration not more than 1% of the injected insulin remains in the circulation. This time (for insulin to reach 25 μunit/ml), as well as that of the glucose nadir, was found to occur significantly later in each diabetic group than in the controls (\(P<0.05\) for adults and \(P<0.001\) for children).

The slower glucose disappearance observed in diabetics is therefore associated with slower disappearance of insulin.
DISCUSSION

The fall in blood glucose concentration was exponential between 5 and 25 min after insulin administration in both diabetic and non-diabetic subjects. This implies that the decrease in blood glucose concentration is more rapid initially, when the amount of glucose is high, and becomes progressively slower as the nadir is approached. The percentage fall of glucose per minute, however, remains constant between the 5 and 25 min period. The effect of intravenously administered insulin on the blood glucose concentration is already maximal 5 min after its administration. There is, therefore, no suggestion from the present results that any cumulative effect of insulin on blood glucose occurs between leaving the blood and reaching the tissues. We have observed an association between the time at which the glucose nadir occurs and the time at which all but 1% of the administered dose of insulin has left the blood, both in diabetics and normal controls. The remaining 1% is equivalent to a plasma insulin concentration of 25 \( \mu \text{unit/ml} \) which is the upper limit of the fasting concentrations in this study. It would seem possible, therefore, that the blood glucose ceases to fall when almost all the administered dose of insulin has left the blood and that which remains approximates to a fasting value. It could be argued that the blood glucose ceases to fall in normal subjects when homeostatic substances such as glucagon, cortisol, adrenaline and growth hormone are secreted to counteract hypoglycaemia and as the diabetic patients do not become hypoglycaemic, the homeostatic mechanisms are not activated to the same degree as in normal subjects. This could be a cause of the later glucose nadir in the diabetics. Defects of glucose homeostasis, however, occur in panhypopituitary subjects. The glucose nadir in such patients has been shown to occur at the same time as in normal subjects by Fraser, Allbright & Smith (1941) although, having attained its nadir, the glucose concentration remains low rather than returning to the fasting value. The prolonged fall of glucose in diabetic subjects is associated with a lower rate constant for glucose disappearance than that found in normal controls. This results in a total fall from the fasting value which is proportionately similar in both diabetic and non-diabetic subjects.

The slower disappearance of insulin in the diabetics leads to delay in reaching an insulin value of 25 \( \mu \text{unit/ml} \), but it is not possible from the present results to say whether this is in any way causally related to the lower rate constant for glucose disappearance from the blood. Insulin disappearance from plasma, particularly after a bolus injection as in the present study, must be the result of several events. These will include diffusion into the extracellular space, uptake by the tissues and inactivation. The diffusion phase is especially liable to vary for individual subjects as it may depend on cardiac output, the plasma and extracellular concentration of insulin, and is affected by any alteration in the distribution of blood to different body tissues. It is not surprising, therefore, that objective analysis of insulin disappearance curves (using a computer) failed to provide a general multiple exponential expression suitable for all individual subjects, though in non-diabetic children the insulin concentrations fall so rapidly that the results can be expressed by a single exponential after subtraction of fasting values (Stimmler \textit{et al.}, 1969). However, Orskov & Christiensen (1969) considered that the semilogarithmic plot of insulin concentrations with time became linear at a point which varied from individual to individual occurring between 13 and 25 min after insulin injection. These authors do not state how the time of onset of linearity was assessed, but the time chosen materially affected their calculated half-times of insulin disappearance. For example, it
can be shown from their combined data on young adults (normal and diabetic) that there is a significant positive correlation between the half time of the linear part of the curve and the time at which linearity was thought to begin \((r = 0.72, P < 0.01)\). Further, for every minute's delay in reaching linearity, the half-time was lengthened by approx. 0.5 min. This may account for their failure to observe any difference in the half-time of insulin disappearance between their normal and diabetic groups. Since only a proportion of diabetics show any marked impairment of insulin disappearance, the variable incidence of such patients could also account for the discrepancy between Orskov & Christensen's (1969) studies and our own.

Differences in insulin disappearance are most significant between diabetic and non-diabetic children. This suggests that impairment of insulin disappearance is either more common or more severe in diabetic children.

It is not possible from our results to elucidate whether the observed delay in insulin disappearance follows a delay in distribution, e.g. due to differences in capillary permeability, differences in the sizes of ‘insulin pools’ or to differences in breakdown of insulin between diabetic and non-diabetic subjects.

It is possible that the delayed disappearance of insulin from the plasma could contribute to the apparently increased concentrations of insulin found in some diabetics (Yalow & Berson, 1960), and to its prolonged elevation in such subjects after oral glucose (Chiles & Tzagournis, 1970). Possibly the slower removal of insulin together with its delayed effect on glucose could account for the reactive hypoglycaemia observed in some mild diabetics (Sussman, Stimmmer & Birenboim, 1966).

It does not seem likely that impaired insulin clearance could be a major factor in the production of diabetes mellitus though it may be one of a number of aetiological factors.

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