Intravenous Insulin has no Effect on Transcapillary Escape Rate of Albumin and on Plasma Volume in Short-term Juvenile Diabetics

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SUMMARY

The permeability of the microvasculature was studied for the first 50 min after an intravenous injection of between 6 and 10 U of crystalline insulin in seven juvenile diabetics whose mean age was 22 yr. No patient had clinical signs of neuropathy or microangiopathy. The following variables were determined before and after the insulin injection: plasma volume, PV (^{125}I-labeled and ^{131}I-labeled human serum albumin), plasma protein concentration, hematocrit, blood pressure, and pulse rate. PV was measured 40, 45, and 50 min after the insulin injection. Transcapillary escape rate of albumin (TER), defined as the fraction of intravascular mass of albumin that passes to the extravascular space per unit of time, was determined after insulin from the disappearance of the intravenously injected ^{125}I-labeled human serum albumin. The mean blood glucose concentration decreased from 15.1 to 10.8 mmol/L at 50 min after the insulin injection. Blood pressure remained unchanged, while a significant increase in pulse rate occurred after the insulin administration. A reduction in PV, calculated after a mixing period lasting 10 (−3.9%), 15 (−2.3%), and 20 (−1.7%) min, was found after the insulin injection. The last-mentioned difference is not significant statistically. The venous hematocrit remained unchanged; this variable was measured so accurately that a 1% decrease in plasma volume could be excluded with 99% confidence. Plasma protein concentration also remained unchanged. Except the one patient with an extremely high TER (10.6%/h), the remaining six patients had normal TER values after the insulin injection (mean, 5.8; range, 4.5–7.2%/h) compared with our previously investigated control group (mean, 5.4; range, 3.8–7.2%/h) and a comparable group of six short-term juvenile diabetics investigated during good (mean, 5.8; range, 4.4–7.2%/h) and poor (mean, 6.9; range, 5.7–7.7%/h) metabolic regulation (these patients did not receive insulin before the TER determination). In agreement with a previous study, thus, we found a significant reduction in plasma volume and in intravascular mass of protein after a small dose of crystalline insulin intravenously. But the present finding of an unchanged hematocrit and a normal TER does not support the suggestion made in the previous study, viz. that there is an increased transfer of fluid and albumin out of the vascular system. The most likely explanation for the above-mentioned reductions is bad mixing (pooling), probably a result of the insulin-induced increase in adrenergic nervous activity, causing vasoconstriction and reduced peripheral blood flow, as demonstrated previously. DIABETES 28:282–286, April 1979.

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Recently, Gundersen and Christensen[1] found that 6 to 8 U of crystalline insulin given intravenously induced an 8 to 9% reduction in plasma volume and in intravascular albumin mass after 45 min in five juvenile diabetics. They interpreted the findings as evidence that insulin, directly or secondarily to its metabolic and/or hemodynamic effects, increases the transfer of fluid and albumin out of the vascular system. Such an effect of insulin is of considerable interest, because in this way insulin might contribute to the development of the diabetic microangiopathy by increasing the extravasation of plasma protein, with subsequent protein deposition in the microvascular wall, according to Lendrum's concept of plasmatic vasculosis.[2,4,5]

For further evaluation of the above-mentioned findings, we measured the transcapillary escape rate of albumin (TER), plasma volume, plasma protein concentration, and hematocrit in short-term juvenile diabetics after an intravenous injection of crystalline insulin. The present results, in particular the constancy of the hematocrit after insulin, have led us to doubt the interpretation of Gundersen and Christensen. Our data, rather, suggest that, after i.v. insulin, the plasma volume determination is in error because of incomplete mixing of the indicator used.

MATERIAL AND METHODS

Seven insulin-dependent, juvenile diabetics (four girls and three boys), aged 14 to 34 yr (mean, 22 yr), all of whom had been informed of the nature of the study before giving their consent, were investigated. The duration of their diabetes ranged from 0.5 to 10 yr, mean 4 yr. None of the patients had clinical signs of neuropathy or microangiopathy. Ketone bodies in the urine were not present in any patient. The last dose of insulin was given in the morning the day before the investigation.

The patients were studied in the morning after at least 12 h of fasting and one hour of rest in the supine position. The patients were covered with blankets, and the arm used for sampling was heated to 37°C by an electric blanket. The temperature of the room was about 22°C.

Human serum albumin labeled with ^{125}I or ^{131}I (codes MIAK and MIMS, Institute for Atomic Energy, Kjeller, Norway) was used for determination of plasma volume and TER. These tracer preparations contain less than 1% of free radioactive iodide and have been demonstrated to be virtually non-denatured by metabolic studies.[6,7] About 10 μCi ^{125}I-labeled albumin was injected in one arm vein, and 10 blood samples of 6 ml each were drawn from a catheter placed in an antecubital vein of the other (heated) arm at 10, 15, 20, 30, 35, 40, 50, 60, 65, and 70 min after the injection. Immediately after the 20-min blood sample had been taken, 6 to 10 U of crystalline insulin was given intravenously.

The transcapillary escape rate of albumin (TER) was determined during the first 50 min after the insulin injection. The procedure for the calculation of TER during unsteady state has been described in detail previously.[4,8] The activity of ^{125}I-labeled albumin in plasma, measured in duplicate, was expressed as: [counts/min × (100-hematocrit)]/hematocrit, thereby eliminating the influence of possible plasma volume changes during the sampling period. This ratio is based on the relationship between plasma volume (PV) and erythrocyte volume (EV): PV = [EV × (100-hematocrit)]/hematocrit. Assuming constant EV during the sampling period, we obtain a relative measure of intravascular tracer as indicated. The initial disappearance of the tracer can be represented by a monoexponential curve during the sampling period (see figure 3). The rate constant k_1 is a measure of TER, which is expressed as the percentage of the intravascular mass of albumin leaving the intravascular space per hour. The k_1 values were calculated by the least-squares method with the use of a desk computer. The TER results were compared with previously obtained results in 28 normal adults[4] and in six short-term juvenile diabetics in good and poor metabolic control.[9] None of the diabetics had ketone bodies in the urine, and they had received their last insulin 24 h before the TER determination.

Plasma volume. PV (ml) was determined from the injected amount of tracer (measured by weighing) and from the plasma radioactivity (measured in duplicate) sampled after a mixing period of 10, 15, and 20 min. ^{125}I-labeled albumin was used before insulin, and, exactly 30 min after insulin, 10 μCi of ^{131}I-labeled albumin was injected. Due to extravasation of tracer albumin during the mixing period, each of the above-mentioned plasma volumes were corrected by 2%. Withdrawn blood was not replaced, but plasma volumes, determined at 40, 45, and 50 min after insulin, were corrected with the calculated loss of 25 ml.

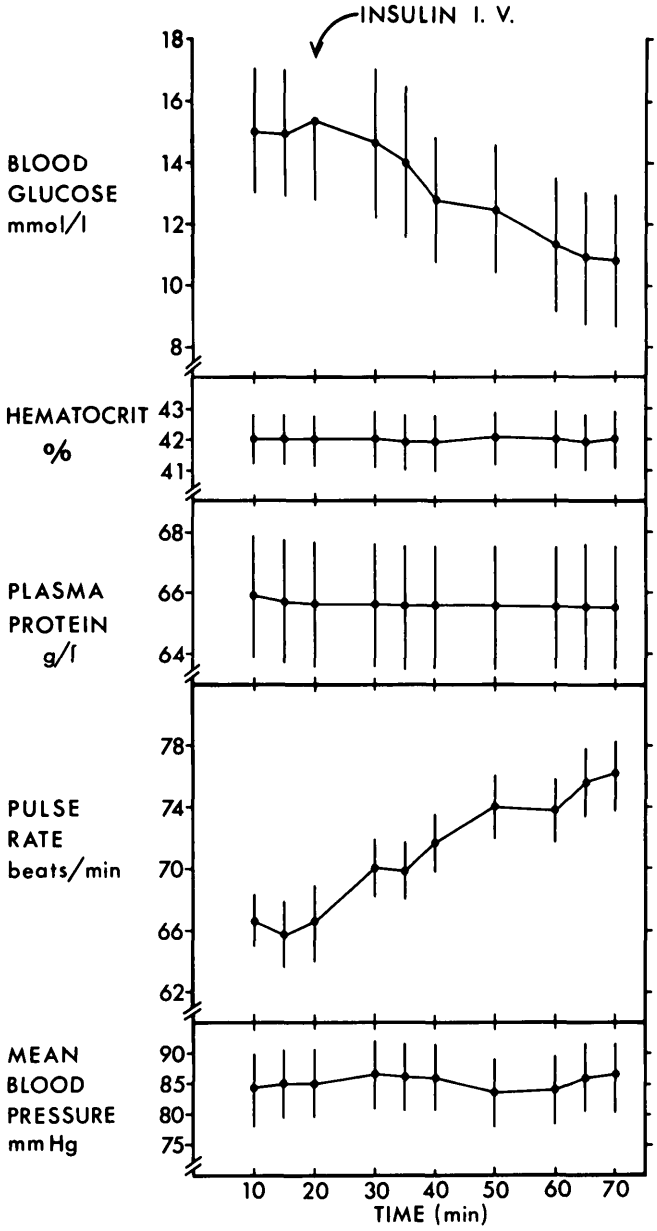

FIGURE 1. Blood glucose, hematocrit, plasma protein, pulse rate, and mean blood pressure before and after intravenously injected insulin and seven short-term juvenile diabetics. Mean and SEM.

Hematocrit was determined in each sample in triplicate by a 30-min centrifugation at 1500 g. In each sample the total plasma protein concentration was read refractometrically with a T. S.-meter (American Optical Company). None of the patients had interfering hyperlipemia. Blood glucose was measured by a glucose-oxidase method on an AutoAnalyzer. Blood pressure and pulse rate were measured 10 times during the investigation.

Wilcoxon's nonparametric test for paired and unpaired comparison was used for statistical analysis.

RESULTS

Figure 1 shows the blood glucose concentration, hematocrit, plasma protein concentration, pulse rate, and mean blood pressure before and after the i.v.-injected insulin. Blood glucose concentration decreased gradually from 15.1 mmol/L to 10.8 mmol/L 50 min after insulin. The venous

hematocrit remained completely unchanged, averaging 42.0 before and also after insulin. A small and statistically insignificant decrease (−0.8%) in plasma protein concentration occurred after insulin. The pulse rate increased gradually from 66 beats/min to 75 beats/min. The mean arterial blood pressure remained practically constant.

Figure 2 shows that six of the seven diabetics studied in this series have normal TER values when compared with the values we obtained previously in normals and in a comparable group of six short-term juvenile diabetics who were in good (mean blood glucose = 7.1; range, 4.1–10.5 mmol/L) and poor (mean blood glucose = 15.1; range, 10.5–20.8 mmol/L) metabolic regulation. One diabetic in the present series (case 6) had an extremely high TER of 10.6%/h.

Figure 3 shows a semilogarithmic plot of the initial plasma disappearance of ^{125}I-albumin compiled from all diabetics except case 6. The rate constant of the plasma disappearance curve is nearly identical before and after the insulin injection, viz. −0.249 (preinsulin, 0–20 min), −0.249 (postinsulin, 0–20 min), and −0.261 (postinsulin, 0–50 min). These rate constants correspond to TER values of 5.8, 5.8, and 6.0% per hour, respectively.

Figure 3 also shows the ^{131}I-albumin concentrations measured 10, 15, and 20 min after injection of the tracer. The much steeper disappearance rate of this tracer than of the previously injected ^{125}I-albumin is evident and will be commented on in the DISCUSSION.

Plasma volume before and 40, 45, and 50 min after insulin is compared in Table 1. PV was calculated after a mixing period of 10, 15, and 20 min. A slight reduction in PV, calculated after a 10 (−3.9%), a 15 (−2.3%), and a 20 (−1.7%) min mixing period was found after the insulin injection. The last-mentioned difference is not statistically significant from zero.

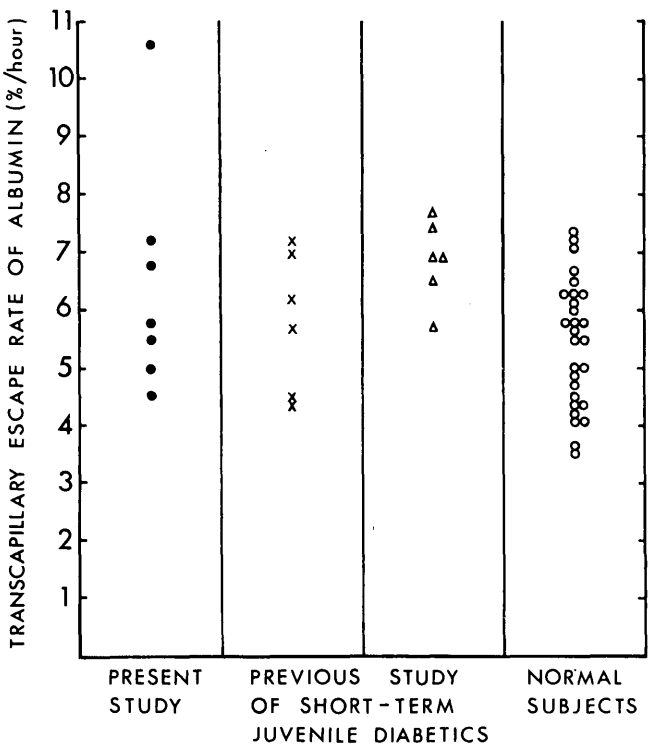

FIGURE 2. TER in the present study (●) compared with values obtained previously in short-term juvenile diabetics during good (×, mean blood glucose = 7.1 mmol/L) and poor (△, mean blood glucose = 15.1 mmol/L) metabolic regulation and in normal subjects (○).

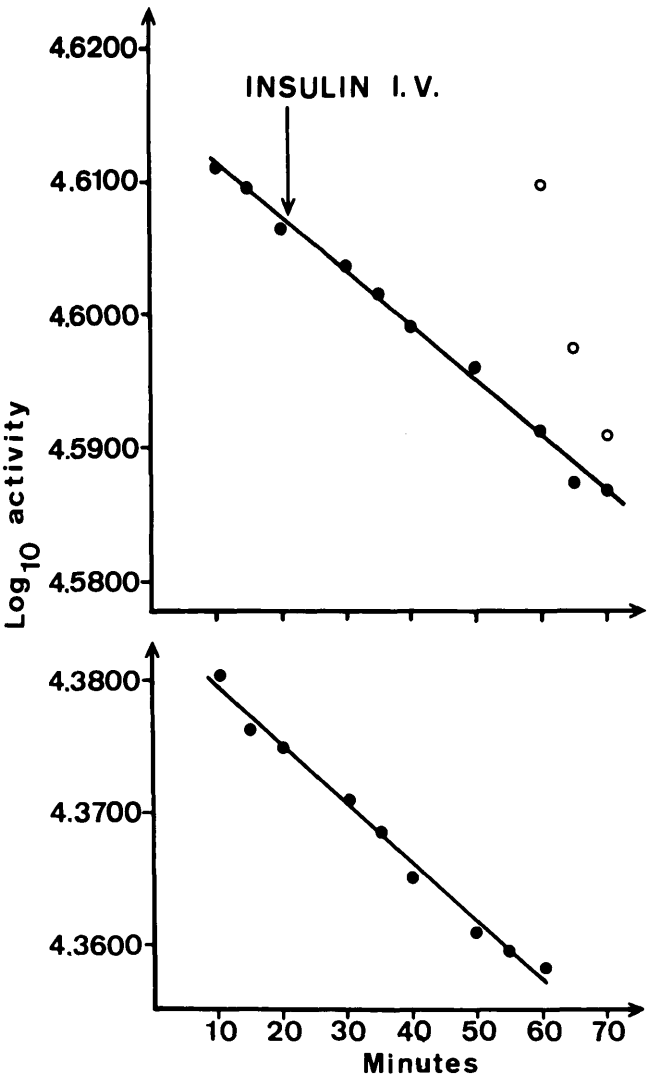

FIGURE 3. A semilogarithmic plot of the initial disappearance of tracer albumin (^{125}I) from plasma compiled from six of the presently studied diabetics (top) as compared with our previous results in 12 control subjects (bottom). The observations fit a straight line. Note that the concentration of the postinsulin tracer (^{131}I-albumin, ○) decreases about three times faster than that of the preinsulin tracer (^{125}I/albumin).

DISCUSSION

We strived to use a protocol as close as possible to that of Gundersen and Christensen.[1] It should be stressed, however, that we did not include their 7-min period of body tilt between the two PV determinations.

The mean age and the duration of diabetes were nearly the same in the two studies. The mean preinsulin blood glucose value was 15.1 mmol/L in the present study and 11.4 mmol/L in the study of Gundersen and Christensen. None of the patients in either series had ketonuria. We investigated girls and boys, while only boys were studied by Gundersen and Christensen.

A prerequisite for obtaining correct results concerning plasma volume and the endogenous TER are that the

TABLE 1
Plasma volume before and after intravenous insulin injection in short-term juvenile diabetics

	Plasma volume (ml)					
	10 min*		15 min*		20 min*	
Subject	I†	II†	I	II	I	II
1	2440	2515	2475	2570	2520	2525
2	2970	2850	3020	2965	3030	3015
3	2920	2735	2895	2880	2870	2930
4	2535	2395	2565	2490	2585	2595
5	3440	3405	3460	3340	3535	3430
6	2085	1915	2160	1945	2150	2020
7	2720	2545	2715	2645	2760	2615
Mean	2730	2623	2756	2691	2779	2733
P Value	<0.05		<0.05		NS	

* = Plasma volume calculated at 10, 15, and 20 min after tracer albumin injection.
†I = Before insulin; II = 40, 45, and 50 min after insulin injection.

organism does not distinguish between labeled and endogenous albumin. In a recent, detailed discussion of the metabolic properties of the tracers used in the previous and the present study (they were the same), it was concluded that the metabolic behavior of the tracers was satisfactory.[7]

Gundersen and Christensen used a 10-min mixing period and calculated a 9% reduction in plasma volume 45 min after i.v. injection of between 6 and 8 U of insulin (mean blood glucose reduction, 5.4 mmol/L). We calculated plasma volume both before and after insulin using a mixing period of 10, 15, and 20 min. This corresponds in the postinsulin measurement to 40, 45, and 50 min after the injection. As clearly demonstrated in Table 1 the plasma volume reduction after insulin diminished by prolonging the mixing period, viz. −3.9% (10 min), −2.3% (15 min), and −1.7% (20 min). *This reduction in calculated plasma volume can be due to either extravasation of fluid or bad mixing (pooling).*

In order to differentiate between these two possibilities we analyzed the hematocrit data: the finding of a constant hematocrit rules out fluid extravasation; the hematocrit was measured so accurately that the standard error of the difference between the three triplicate determinations before insulin and those measured 40, 45, and 50 min after insulin was only 0.19%. *This means that a 1% decrease in plasma volume, had it occurred, would have been detected with 99% confidence.* This conclusion is only valid, of course, if the hematocrit can be regarded as a fully reliable indicator of plasma volume changes. The gradual decrease in plasma glucose concentration of 4.3 mmol/L would correspond to a reduction of 1.4% in plasma osmolality if uninfluenced by any opposing factors. Since the erythrocyte acts like an ideal osmometer, an approximately 1.0% swelling of the red cells would immediately ensue, according to the classic work of Ponder.[10] But, since the intraerythrocytic glucose is in equilibrium with that of the plasma, this swelling will persist only one or two minutes after a step change. It is unlikely that the reduction in arm blood flow (about 30%) reported by Gundersen and Christensen[1] affects hematocrit, since a fivefold change in leg blood flow does not induce significant changes in leg hematocrit.[3] Thus, we found no evidence to incriminate the validity of the hematocrit as an indicator of plasma volume alterations (fluid extravasation).

Evidence of incomplete mixing 10 min after injection of labeled albumin in the postinsulin period was also obtained by analyzing the *rate of disappearance* of the two albumin tracers. As shown in Figure 3 the concentration of the postinsulin tracer (^{131}I-albumin) decreases 10 min after injection about three times faster than the preinsulin tracer (^{125}I-albumin) that was mixed in the plasma volume for one hour. Since the ^{125}I-albumin curve is linear, there was no indication of measurable recirculation of this tracer. Hence the discrepancy in disappearance rate must be due to incomplete mixing (pooling) after insulin injection.

Gundersen and Christensen calculated the extravasation rate of tracer albumin by measuring the total plasma radioactivity (plasma volume by 10 min values × plasma radioactivity/ml) before and 45 min after insulin. Using their values, a mean TER of 11.7%/h can be calculated. By using the same approach in our studies, we calculated the TER value to be 10.4%/h [measured TER was 6.5%/h and PV (10 min) was 3.9%]. But this approach is valid, of course, only if the calculated PV reduction reflects fluid extravasation.

In the present study, TER was determined by measuring the relative plasma radioactivity, i.e., counts/min/ml × (100-hematocrit)/hematocrit. No correction was applied, since the hematocrit remained constant for the first 50 min after insulin. The TER values were, for six of our patients, within the range of our previously investigated control group and short-term juvenile diabetic group.[4,9] We have no explanation for the high TER value found in the last patient. Theoretically, it is possible that we overlooked a small increase in TER, since we did not measure TER before the insulin injection. If this was the case the slope of the plasma disappearance curve of ^{125}I-albumin should be steeper during the postinsulin period than it was in the preinsulin period. However, we found that the plasma disappearance curve of ^{125}I-albumin, followed from the start of the investigation, fits a straight line in a semilogarithmic plot during the preinsulin and postinsulin periods (Figure 3), in complete agreement with previous observations in normal man.[4] The rate constant (slope) of the plasma disappearance curve of ^{125}I-albumin and, thus, of TER is nearly identical during the preinsulin and postinsulin periods as mentioned previously. This finding led us to conclude that insulin does not enhance the total extravasation rate of albumin (TER).

Using a 10 min mixing period, we found a significant but smaller reduction in calculated plasma volume and intravascular mass of protein after insulin, in agreement with Gundersen and Christensen.[1] But the present finding of an unchanged hematocrit and an unchanged and normal TER does not support their suggestion of an increased transfer of fluid and albumin out of the vascular system. The most likely explanation of the above-mentioned reduction is bad mixing (pooling), probably a result of the demonstrated increase in adrenergic nervous activity that causes vasoconstriction and reduced peripheral blood flow.[1]

ACKNOWLEDGMENT
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