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Cardiovascular Effects of Insulin: Plasma Volume Changes in Diabetics

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Summary. The mechanism by which insulin can alter the heart-rate and blood pressure of diabetics has been studied. Insulin decreased the plasma volume of 16 diabetics without complications by an average of 190 ml, a 6% change. Nine diabetics with severe autonomic neuropathy had no significant change in plasma volume after insulin. The basal plasma volume of autonomic neuropathy patients was smaller than that of the uncomplicated diabetics, perhaps accounting for the disparity observed between these two groups of diabetics. The decrease of plasma volume could thus account for the heart rate increase of uncomplicated diabetics, but not for the induction of hypotension in those with autonomic neuropathy.

Key words: Diabetes, insulin, plasma volume, heart rate, blood pressure, autonomic neuropathy.

Insulin has been shown to have effects on the cardiovascular system even in the absence of hypoglycaemia. In normal subjects and diabetics without neuropathy intravenous insulin can cause an increase in heart rate, particularly in the upright position [1, 2]. This effect occurs within 5 to 10 min of insulin administration when the blood glucose has decreased only slightly [2].

Blood pressure changes are negligible in diabetics without complications. By contrast, in diabetics with autonomic neuropathy insulin can cause profound hypotension, particularly in the upright position [1, 3]. This effect also begins after 5 to 10 minutes and is most marked when insulin is given intravenously. It is frequently so severe that patients faint when upright [1, 3]. In a few subjects with autonomic neuropathy normal doses of subcutaneous insulin cause symptomatic postural hypotension [3]; and alteration of the timing of insulin injection can reduce the severity of the hypotension [4].

These effects may be secondary to insulin-induced changes in plasma volume. We have tested our hypothesis by measuring changes in plasma volume after intravenous insulin.

Materials and Methods

a) Patients

Male, insulin-dependent diabetics with and without autonomic neuropathy were tested (table 1).

There were 24 men without autonomic neuropathy of whom 16 were given insulin, and 8 were not. (Two subjects are included in both groups since they were tested twice, with and without insulin). The mean ages and mean durations of diabetes were comparable in the two groups. They had minimal complications: 3 had background retinopathy and one had absent ankle jerks. None had symptoms of autonomic neuropathy and the beat-to-beat variation of heart rate [5] – measured to confirm the absence of autonomic neuropathy – was normal in all of the 18 subjects tested (table 1).

The 9 diabetic men with autonomic neuropathy had a significantly longer duration of diabetes and were slightly older than those diabetics without autonomic neuropathy (Table 1). All had retinopathy, 3 had proteinuria and 8 had peripheral neuropathy usually severe (with distal numbness, previous foot sepsis and surgery), but at least with absent ankle jerks. All had one or more symptoms of autonomic neuropathy: 4 had postural hypotension (a fall of more than 30 mmHg in systolic pressure in the upright position, together with symptoms), 5 had diabetic diarrhoea, 5 had gustatory sweating [6], and one subject had had an unexpected respiratory arrest [7]. The beat-to-beat variation of heart rate was abnormal in 8 patients and low in one patient, indicating cardiac vagal neuropathy [5]. Only 2 diabetics, both with autonomic neuropathy, were on therapy other than insulin. One patient (F. W.) was on thyroxine replacement therapy; and the other (I. D.) was taking codeine phosphate and tetracycline for diarrhoea.

b) Procedure

Subjects were tested in the early morning, after an overnight fast and without taking their morning insulin. Those taking two insulin

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Table 1. Clinical data on the 3 groups of diabetics

	n	Age (years)	Dur. of diabetes (years)	No. with peripheral neuropathy	No. with retinopathy	No. with proteinuria	Beat-to-beat variation on deep breathing	
No autonomic neuropathy	24							
Insulin bolus given	16	31 (21–41)	11 (<1–27)	1	2 background	0	29 (10–44)	(n = 12)
No insulin given (control group)	8	29 (20–36)	6 (1–19)	0	1 background	0	25 (10–35)	(n = 7)
Autonomic neuropathy (All given insulin bolus)	9	37 (27–55)[a]	24 (11–38)[b]	8	4 background 5 proliferative	3	5 (1–13)[b]	(n = 9)

Mean values given for age, duration of diabetes (in years) and the beat-to-beat score; with the range given in brackets
Peripheral neuropathy defined by absence of ankle tendon reflexes
Beat-to-beat variation on deep breathing (beats/min.) = a test of cardiac vagal neuropathy (5)
[a] N. S. and [b] $P < 0.01$ (Wilcoxon rank sum test) compared diabetics with autonomic neuropathy with diabetics without autonomic neuropathy either given insulin or not given insulin

injections a day, on the day before the test, had their normal dose of insulin in the morning; and in the evening they had either their normal or a reduced dose of insulin, taken between 6 p. m. and 8 p. m. Those subjects taking once daily insulin injections usually were given their normal dose on the morning before the test (table 2). Subjects lay flat for 1 h before the start of the test and throughout the study. At least 10 min before the first estimation of plasma volume 19 G Butterfly cannulae were inserted into a vein in each forearm.

Plasma volume was measured by standard techniques [8], and was measured initially using ^{125}I-human serum albumin (Radiochemical Centre, Amersham). Thirty minutes after injection of this isotope those subjects receiving insulin were given a bolus of either 10 or 20 units of Actrapid M. C. insulin intravenously, except for one subject who was given 3 units of insulin. Forty minutes later (70 min after the first plasma volume estimation) plasma volume was measured again using ^{131}I-human serum albumin. The period of 40 min was chosen because the effect of insulin on standing pulse rate appeared to be maximal at this time [2].

With each plasma volume estimation [8] 5 μCi of isotope was injected intravenously without withdrawal of blood and the syringe weighed before and after injection. Blood samples were withdrawn at 5 min intervals from 10 to 30 min after injection of the isotope and the activity of duplicate weighed samples of plasma measured. Plasma samples and standards were counted for more than 10,000 counts and corrections made for weight of the samples and background activity. By log extrapolation the theoretical activity at time 0 (assuming instant, complete mixing) was determined and hence plasma volume at the time of the injection of the isotope could be calculated, using the following formula:

$$\frac{\text{Total counts of isotope injected}}{\text{Counts/ml of plasma at time 0.}}$$

Haematocrit was measured in triplicate at 0, 30, 70 and 100 min using the Hawksley microhaematocrit method. Blood glucose was measured at the same time intervals using the AutoAnalyzer ferricyanide method.

A total of 96 ml of blood was withdrawn during the study and only partially replaced by isotonic saline. By the time of the second plasma volume estimation 50 ml of blood, representing about 30 ml of plasma, had been withdrawn.

Thyroid uptake of radioactive iodine was blocked by daily administration of 50 mg potassium iodide, started immediately before the test and for 10 days afterwards.

Results are expressed as mean ± SD; and comparison made using Student's t test for nonpaired groups, or Wilcoxon's rank sum test for unpaired samples (where indicated).

Results

The changes in plasma volume between the first and second estimation in the 3 groups of diabetics are shown in Figure 1.

Among the 8 patients without complications who were not given insulin there was a negligible difference between the two plasma volume estimations with a mean change of − 12 ± 59 ml. In contrast the 16 diabetics without complications had a mean fall in plasma volume of 190 ± 99 ml when given insulin, significantly different ($2P < 0.001$) from the group not given insulin. There was some relation to the dose of insulin given since the 8 patients given 20 units of insulin had a mean fall in plasma volume of 231 ± 105 ml and the 7 patients given 10 units of insulin had a mean fall in plasma volume of 158 ± 79 ml, although this difference was not significant.

Of the 9 diabetics with autonomic neuropathy there was no change in plasma volume after insulin (−33 ± 67 ml) which was not different from the control group not given insulin, but significantly different ($2P < 0.001$) from the other group given insulin. One subject in this group showing a large fall in plasma volume (−168 ml) was the only one with intact ankle tendon reflexes suggesting that peripheral neuropathy was probably absent.

Table 2. Individual data on the diabetics tested, including details of insulin administration before the test

Name	Age (yrs.)	Dur.[n] of diabetes (yrs.)	% ideal body wt.[a]	Usual evening dose of insulin[b]	Evening dose of insulin before test[b]	Basal blood glucose mmol/l	Actrapid insulin bolus given at test	Change in plasma volume (ml)[c]
Diabetics without autonomic neuropathy								
JS	33	3	+11.1	NPH24 om	–	13.6	20	−425
WH	30	5	−19.6	SI28, NPH24	SI28, NPH24	13.8	20	− 75
AC	25	12	−13.4	SI44, NPH12	SI44	17.1	20	−301
PB	28	17	−10.2	SI36	SI36	16.8	20	−262
BF	29	18	− 4.2	SI24, NPH16	SI24, NPH16	16.2	20	−165
MW	38	27	+13.3	SI6, NPH8	SI6	14.0	20	−250
TG	34	18	− 1.6	SI28, NPH8	SI20	17.0	20	−180
BP	38	10	−10.0	SI8, NPH16	SI12	18.2	20	−192
MB	29	16	− 9.4	SI12, NPH8	SI12	13.6	10	−258
AA	30	8	−17.9	SI20	SI16	8.5	10	− 89
SL	21	<1	− 0.3	SI8, ST20	SI8, ST20	11.4	10	−174
CC	29	2	− 1.7	SI16	SI16	10.9	10	−272
WL	22	17	− 9.4	SI16, NPH10	SI16, NPH4	10.2	10	−119
RH	41	4	−16.7	SI8, NPH8	SI8	8.4	10	− 75
KG	35	8	−14.8	NPH16 om	–	10.6	10	−121
MT	36	6	−15.7	SI18	SI16	12.8	3	− 78
TM	20	2	− 8.0	SI20	SI20	5.4	–	− 76
JP	31	1	+ 4.3	LR10 om	–	8.2	–	− 35
CK	27	4	−16.2	SI8, NPH8	SI8, NPH8	5.0	–	+105
JL	30	9	− 9.0	SI20	SI20	11.8	–	− 3
AS	31	1	− 6.2	SI4, NPH16	SI4, NPH12	3.0	–	− 53
WS	25	19	−18.9	SI44, PZI44 om	–	5.4	–	+ 29
MJ	36	6	−15.0	SI18	SI18	8.5	–	− 59
KG	35	8	−10.1	NPH24 om	–	9.3	–	− 2
Diabetics with autonomic neuropathy								
FW	55	32	−11.4	R28	R16	10.4	20	− 36
ID	40	15	[d]	SI8	SI4	21.8	20	− 9
RL	32	13	−10.7	SI24, PZI24 om	SI12	15.3	20	+ 79
MP	29	11	− 3.7	SI4, NPH8	SI4, NPH4	11.5	10	− 28
JW	27	23	− 1.8	SI24	SI24	12.0	10	−168
MT	28	17	− 0.8	SI8, NPH20	SI8, NPH16	19.4	10	+ 11
PH	33	30	[e]	SI12	SI8	20.6	10	− 50
IS	44	38	− 2.1	SI8, NPH10	SI8, NPH10	14,7	10	− 76
ED	41	33	− 5.7	SI28	SI20	8.3	10	− 19

[a] Metropolitan Life Insurance Co. Statistical Bulletin, Nov.–Dec. 1959

[b] In patients on once-daily insulin the morning dose (o. m.) is given instead (Patient R. L. omitted the PZI on the day before the study). Insulin abbreviation: SI = soluble insulin; NPH = Isophane; R = Rapitard; LR = LeoRetard; PZI = Protamine Zinc insulin

[c] Change in plasma volume = difference between first and second plasma volume estimation

[d] Left mid-thigh amputation

[e] Height below minimum shown in Life Tables

Two diabetics without complications were tested on two separate occasions. The first showed a fall in plasma volume of 59 ml without insulin; and after 3 units of insulin a fall of 78 ml. The second, without insulin showed a fall of 2 ml; and after 10 units of insulin a fall of 174 ml.

Comparing haematocrits at 0 and 70 min there was a mean change of only − 0.3 ± 0.9% (n = 5) in the control group not given insulin. The group without complications given insulin had a mean change in haematocrit of +0.8 ± 1.2% (n = 16) ($2P < 0.05$). The mean haematocrit change in diabetics with autonomic neuropathy was − 0.4 ± 1.1% (n = 7), similar to the control group not given insulin, but significantly different ($2P < 0.05$) from the other group given insulin.

In the two groups given insulin the mean fall in blood glucose over 40 min (from 30 to 70 min) was the same at 4.9 mmol/l.

Mean plasma volume was 46.2 ± 5.4 ml/kg body weight in the diabetics without complications (n = 21) (Figure 2). Diabetics with autonomic

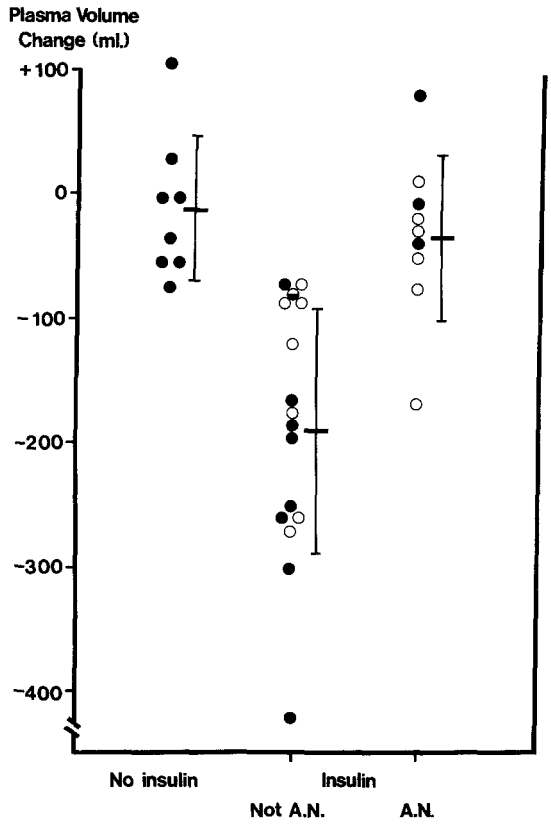

Fig. 1. Changes in plasma volume between first and second plasma volume estimation: in diabetics without complications (not A. N.) and with autonomic neuropathy (A. N.). In diabetics given insulin solid circles represent 20 units of insulin, open circles 10 units and half solid circle 3 units

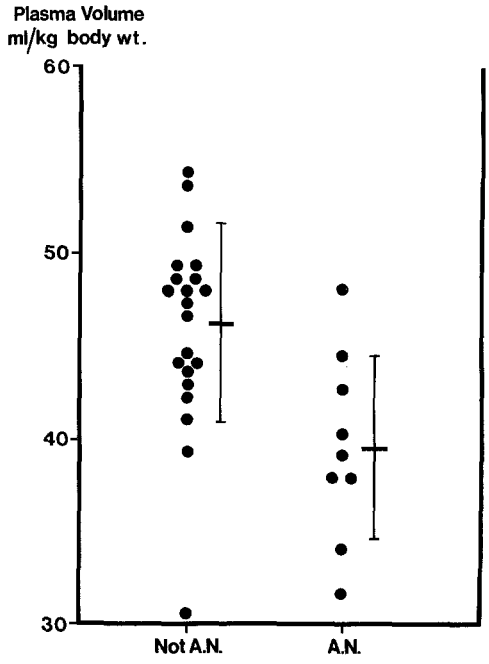

Fig. 2. Plasma volume in diabetics without complications (not A. N.) an diabetics with autonomic neuropathy (A. N.)

neuropathy had a smaller mean plasma volume of 39.5 ± 5.0 ml/kg body weight (n = 9) ($2P < 0.005$).

Only one subject developed hypoglycaemic symptoms and that was 8 min before the end of the experiment.

Blood pressure was measured at 5 or 10 min intervals from 30 min before until 40 min after insulin administration (until the second plasma volume estimation) in 5 diabetics without complications given insulin and 5 diabetics with autonomic neuropathy. The autonomic neuropathy group had a mean fall in systolic blood pressure of −8.6 ± 10.2 mmHg after insulin administration which was not significantly different from a negligible change in the uncomplicated group.

Discussion

Insulin-induced changes in heart-rate and blood pressure are potentially important in diabetics who receive daily injections of insulin. The mechanisms are not fully understood. We have shown, however, that intravenous insulin in essentially uncomplicated diabetics causes a decrease in plasma volume of between 75 and 425 ml, with a mean of 190 ml – a 6.0% change in plasma volume. There were parallel although smaller changes in haematocrit. (With haemoconcentration the proportional change in haematocrit is always less than the change in plasma volume [9, 10]. In contrast, diabetics with severe autonomic neuropathy showed no appreciable change in plasma volume after insulin.

Gundersen and Christensen [11] found an appreciable fall in plasma volume following small doses of intravenous insulin. The fall in plasma volume probably causes the increase in heart rate since there is a very close relationship between the fall in plasma volume and the rise in plasma noradrenaline.

The decrease of plasma volume could be apparent rather than real because of vascular pooling, but we believe that there is a true fall of plasma volume because insulin has been shown to cause a fall in the intravascular mass of albumin [11], and because we found a fall in haematocrit.

It has been suggested that insulin alters the function of endothelial cells either directly or indirectly thereby increasing the transfer of fluid and albumin out of the vascular system. Morphological work in diabetic rats has shown changes in endothelial vesicular transport after insulin which supports this hypothesis [12]. The fall in blood glucose is unlikely itself to be the cause of the plasma volume changes as they do not occur in the patients with autonomic neuropathy in whom there were similar falls in blood glucose.

The absence of insulin-induced changes in plasma volume in diabetics with autonomic neuropathy was unexpected, particularly in view of the known marked hypotensive effect of insulin in these patients. The reasons for this disparity between diabetics with and without autonomic neuropathy are not clear but this work shows that diabetics with autonomic neuropathy had smaller plasma volumes, and this might limit transfer of fluid out of the intravascular compartment.

Long-term diabetics with microangiopathy have decreased plasma volumes and increased capillary permeability [13] and this could account for the decreased plasma volume in our patients with autonomic neuropathy since they all had evidence of microangiopathy. However, autonomic neuropathy may itself contribute to the smaller plasma volume: diabetics with autonomic neuropathy have impaired renin responses [14]; and subjects with idiopathic orthostatic hypotension, another disorder with widespread autonomic neuropathy, also have smaller plasma volumes than normal [15].

The hypotensive effect of insulin in patients with autonomic neuropathy must therefore have some cause other than a change in blood volume. Recent work with rats suggests that insulin can attenuate the vasoconstrictor response of blood vessels to noradrenaline [16]; and we suggest that a local effect of insulin of blood vessel tone is the most likely reason for its hypotensive action in diabetics with autonomic neuropathy.

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