A REVERSIBLE VASCULAR ABNORMALITY ASSOCIATED WITH DIABETIC KETOsis

N. J. CHRISTENSEN

The Second Clinic of Internal Medicine, Kommunehospitalet, Århus University School of Medicine, Århus, Denmark

(Received 27 April 1970)

SUMMARY

1. Resting forearm blood flow was increased in untreated juvenile diabetics and a slight but significant reduction took place during insulin treatment. The peak flow after ischaemia was also increased, but no difference could be demonstrated before and after insulin administration.

2. Reactive hyperaemia was found to be considerably prolonged in the diabetic patients with moderate ketosis and mild disturbances of the blood acid-base balance as compared to non-diabetics. This abnormality disappeared after insulin treatment.

3. The prolonged blood flow recovery showed a strong association with total CO₂ in plasma. In subjects with non-ketotic metabolic acidosis of a degree similar to that seen in the diabetic patients no abnormality was found.

4. Urinary excretion of catecholamines was normal in the untreated juvenile diabetics. The observed alterations in the diabetics are similar to those observed by others in non-diabetics after administration of adrenaline or reserpine.

It is believed by many that the development of diabetic angiopathy is a consequence of the metabolic disturbances present over the years in diabetic patients despite treatment with insulin. Very few studies, however, have been concerned with the possible relationship between acute disturbances in the metabolic state and abnormal vascular function.

This is a report of the results of a study of the resting blood flow and of the rate of return of blood flow towards basal levels during reactive hyperaemia determined before treatment or after withdrawal of insulin and again after insulin treatment.

METHODS

Patients

Diabetics: Eleven male diabetic patients with a mean age of 25 years (range 18–49) were studied before treatment (cases 1, 2, 3, 5, 7, 8, 9) or after withdrawal of insulin (cases 4, 6, 10, 11).
11) and again after 2–26 days of insulin treatment. The patients demonstrated various degrees of metabolic derangement at the first examination.

Seven of the diabetic subjects had recently diagnosed juvenile diabetes. The other four had had diabetes for 3–6 years.

None of the diabetic patients was dehydrated on clinical examination at the time of the studies. Two of them had recently received 2–3 litres of isotonic sodium chloride intravenously.

Non-diabetics: Seven non-diabetic males were included in the study. Five of them were healthy medical students. One was a patient with mild steatorrhoea and one a patient with minimal pulmonary fibrosis. The mean age of this group was 27 years (range 20–44).

The same studies were performed on two of the non-diabetic males during experimental acidosis (ammonium chloride, 2 g every 4 h for 4 days) and on one patient with uraemic acidosis.

An informed consent to the procedure was obtained from all subjects examined.

Methods

The blood flow in the proximal two-thirds of the forearm was measured by venous occlusion plethysmography. The plethysmograph was water-filled and the water temperature thermostatically controlled at $34^\circ \pm 0.2^\circ$.

A blood pressure cuff 6 cm wide was applied to the arm just above the antecubital space, and a pressure of 50 mmHg was used for venous occlusion, whereas the pressure used for arterial occlusion was 250 mmHg. Circulation in the hand was interrupted during the measurement of blood flow.

The patients were studied during rest in the recumbent position in a quiet room. The temperature of the room was controlled at $23^\circ$ (range $22^\circ$–$24^\circ$).

Total CO$_2$, potassium and sodium concentrations were determined on venous blood plasma. The plasma glucose concentration was measured in capillary blood from the ear. Arterial blood was removed for determination of pH, $P_{CO_2}$ and standard bicarbonate in some patients. Haematocrit and 24 h urine excretion of catecholamines were also determined in some subjects. These measurements were all performed using standard laboratory procedures.

Resting blood flow and blood flow after short periods of ischaemia were measured. Resting blood flow was measured twice. Circulation in the forearm was then stopped for 30 s and thereafter the cuff was suddenly released. Blood flow was then measured as quickly as possible (first blood flow estimation or peak flow) and again 5 or 8 s later (second blood flow estimation). This set of four blood flow measurements as well as the resting blood flow measurements was repeated eight or more times. At the beginning of the study the second post-ischaemic flow measurement was performed alternately 5 or 8 s after release of the arterial cuff, to determine whether a logarithmic decrease in flow occurred in the immediate post-ischaemic period as reported by previous investigators (Hyman & Wong, 1968). This proved to be the case and during the latter part of the study the second post-ischaemic flow measurement was always performed at 8 s.

Additional studies were performed in two diabetics before and after treatment with insulin as well as in two control subjects. The hyperaemic response was measured after 2 min periods of ischaemia. Blood flow was measured immediately after release of the arterial cuff and at 10 s intervals three or four times. Measurements were repeated four times at each examination.
Reactive hyperaemia in diabetics

Calculation. The mean value of the resting blood flow, as well as that of the first and the second post-ischaemic blood flow obtained after 30 s periods of ischaemia were calculated for each experiment. The first post-ischaemic flow was measured immediately after release of arterial occlusion and the time of measurement was called zero. The time of measurement of the second blood flow was expressed in units of 0.5 s and was determined by drawing a vertical line from the top of the first pulse wave not disturbed by any artefact down to the time marker. In all cases the standard deviation of the time of measurement of the second blood flow was less than 1 s and averaged 0.6±0.2 s.

The mean peak flow and the mean second blood flow (8 s value) were plotted on a logarithmic scale against time and the rate of return of blood flow towards basal values expressed as the half-time.

In experiments using 2 min periods of ischaemia the three to four blood flow values obtained after each single period of ischaemia were plotted on a logarithmic scale and four individual half-times were obtained in each experiment.

Conventional probability levels of significance have been used in the statistical analysis, a P value greater than 0.05 not being considered significant.

RESULTS

Resting blood flow

The mean resting blood flow obtained in seven non-diabetics and eleven diabetics appears in Table 1. In the normal subjects the mean resting blood flow averaged 2.2±0.6 ml 100 ml⁻¹ min⁻¹. In the diabetic patients it averaged 4.9±1.5 ml 100 ml⁻¹ min⁻¹ during metabolic derangement and 3.6±1.1 ml 100 ml⁻¹ min⁻¹ after insulin treatment. Both these values differ significantly from the value obtained in the non-diabetic subjects (P<0.001 and 0.01). The mean resting blood flow values obtained in the diabetics before and after insulin treatment are also significantly different (P<0.02).

Blood flow restoration after 30 s of ischaemia

All important data from the study of the restoration of blood flow after ischaemia (30 s periods) are summarized in Tables 2 and 3. In the non-diabetic subjects the peak flow averaged 12.0±3.7 ml 100 ml⁻¹ min⁻¹. The half-time averaged 7.7 s with a range between 4.9 and 12.5 s.
TABLE 2. Mean blood flow values and half times obtained after 30 s periods of ischaemia in five non-diabetics. The mean time of measurement of the second blood flow is also given.

<table>
<thead>
<tr>
<th>Case</th>
<th>Peak flow (ml 100 ml⁻¹ min⁻¹)</th>
<th>Second flow (ml 100 ml⁻¹ min⁻¹)</th>
<th>Time of measuring the second flow (s)</th>
<th>Half-time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16·4</td>
<td>4·7</td>
<td>8·7</td>
<td>4·9</td>
</tr>
<tr>
<td>2</td>
<td>9·1</td>
<td>4·1</td>
<td>8·4</td>
<td>7·5</td>
</tr>
<tr>
<td>3</td>
<td>7·4</td>
<td>4·6</td>
<td>8·5</td>
<td>12·5</td>
</tr>
<tr>
<td>4</td>
<td>14·4</td>
<td>4·7</td>
<td>8·3</td>
<td>5·2</td>
</tr>
<tr>
<td>5</td>
<td>12·5</td>
<td>6·1</td>
<td>8·5</td>
<td>8·2</td>
</tr>
<tr>
<td>Mean</td>
<td>12·0</td>
<td>4·8</td>
<td>8·5</td>
<td>7·7</td>
</tr>
<tr>
<td>SD ±</td>
<td>3·7</td>
<td>0·8</td>
<td>0·2</td>
<td>3·1</td>
</tr>
</tbody>
</table>

In the diabetic patients peak flow was greater than in the normals, but no significant difference was seen before and after treatment.

It appears from Table 3 that the half-times were considerably prolonged during uncontrolled conditions in six of the nine diabetic subjects studied. This increase averaged approximately 100%. In the other three patients (cases 7, 8 and 9) the half-times were nearly identical in the two situations, i.e. during metabolic derangement and after insulin treatment.

Fig. 1 illustrates the difference between blood flow restoration before and after treatment. The left side of the figure shows plethysmographic curves (peak flow and the 8 s value) obtained in case 1 during metabolic derangement after a single period of ischaemia. The right side of the figure shows a similar set of plethysmographic curves obtained in the same patient after insulin treatment. The first flow or peak flow is nearly identical in the two situations. The second flow is approximately doubled before treatment.

![Fig. 1. Plethysmographic curves obtained after a single period of ischaemia in case 1 before treatment (a) and after treatment (b). Peak flow was 20.7 and 19.0 ml 100 ml⁻¹ min⁻¹ respectively. The second flow was 11.8 and 5.9 ml 100 ml⁻¹ min⁻¹ respectively. Abscissa: Time in s.](image-url)
Table 3. Mean blood flow values obtained after 30 s periods of ischaemia in nine diabetic patients studied during metabolic derangement (B) and after insulin treatment (A). The half-time is expressed in s. The half-time obtained before treatment is also expressed as a percentage of the value obtained after treatment with insulin. The patients have been divided into two groups depending on whether total plasma CO₂ was reduced (cases 1-6) or normal (cases 7-9) during metabolic derangement.

<table>
<thead>
<tr>
<th>Case</th>
<th>Peak flow (ml 100 ml⁻¹ min⁻¹)</th>
<th>Second flow (ml 100 ml⁻¹ min⁻¹)</th>
<th>Time of measuring the second flow (s)</th>
<th>Half-time (s)</th>
<th>Half-time (%)</th>
<th>Total CO₂ in plasma (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>18.9</td>
<td>17.2</td>
<td>11.2</td>
<td>5.9</td>
<td>8.5</td>
<td>8.1</td>
</tr>
<tr>
<td>2</td>
<td>13.4</td>
<td>16.4</td>
<td>8.3</td>
<td>7.0</td>
<td>10.7</td>
<td>8.1</td>
</tr>
<tr>
<td>3</td>
<td>20.8</td>
<td>19.6</td>
<td>14.9</td>
<td>10.8</td>
<td>8.1</td>
<td>8.0</td>
</tr>
<tr>
<td>4</td>
<td>17.7</td>
<td>25.1</td>
<td>12.8</td>
<td>9.8</td>
<td>8.4</td>
<td>8.1</td>
</tr>
<tr>
<td>5</td>
<td>12.6</td>
<td>12.3</td>
<td>9.6</td>
<td>8.0</td>
<td>8.1</td>
<td>8.5</td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>10.4</td>
<td>7.5</td>
<td>4.8</td>
<td>8.4</td>
<td>8.5</td>
</tr>
<tr>
<td>Mean</td>
<td>15.6</td>
<td>16.8</td>
<td>10.7</td>
<td>7.7</td>
<td>8.7</td>
<td>8.2</td>
</tr>
<tr>
<td>SD±</td>
<td>4.2</td>
<td>5.3</td>
<td>2.8</td>
<td>2.3</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>14.6</td>
<td>13.0</td>
<td>7.0</td>
<td>5.6</td>
<td>8.7</td>
<td>8.5</td>
</tr>
<tr>
<td>8</td>
<td>24.6</td>
<td>16.9</td>
<td>13.2</td>
<td>7.6</td>
<td>7.7</td>
<td>8.0</td>
</tr>
<tr>
<td>9</td>
<td>18.3</td>
<td>22.6</td>
<td>4.9</td>
<td>7.8</td>
<td>8.4</td>
<td>8.6</td>
</tr>
<tr>
<td>Mean</td>
<td>19.2</td>
<td>17.5</td>
<td>8.4</td>
<td>7.0</td>
<td>8.3</td>
<td>8.4</td>
</tr>
<tr>
<td>SD±</td>
<td>5.1</td>
<td>4.8</td>
<td>4.3</td>
<td>1.2</td>
<td>0.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

All patients
Mean | 16.8                          | 17.1                            | 8.6                                 | 8.3          | 7.5                       |
SD±  | 4.5                           | 4.8                             | 0.9                                 | 0.3          | 2.5                       |
It was noted that a highly significant association could be established between the half-time measured during uncontrolled conditions and the total CO₂ in plasma. This is shown in Fig. 2. The relationship tends to be curvilinear. The regression coefficient differs significantly from zero ($P<0.002$). No association could be established between the resting blood flow and the total plasma CO₂ or between the peak flow and the total CO₂.

![Graph](image)

**Fig. 2.** Ordinate: Half-time obtained in diabetic patients during untreated conditions expressed as a percentage of the half-time obtained in the same patients after insulin treatment. Abscissa: Total plasma CO₂ in mmol/l obtained during untreated conditions.

Standard bicarbonate, pH and $P_{CO_2}$ were determined on samples of arterial blood obtained from cases 4, 5 and 6. As expected a mild, partially compensated metabolic acidosis was demonstrable. pH averaged 7.36 before treatment and 7.42 after treatment, standard bicarbonate 18 and 24 mEq/l and $P_{CO_2}$ 29 and 38 mmHg, respectively.

Table 3 is arranged so that the six cases with reduced total CO₂ during metabolic derangement are listed in the upper section, and the three cases with normal CO₂ are shown in the lower. It appears that the half-time obtained in the upper six patients averaged 16.8 s in uncontrolled conditions against 8.0 s after insulin treatment. This difference is significant ($P<0.001$). A significant difference was found between the mean half-times obtained in the normal subjects and the diabetic patients with moderate ketosis ($P<0.002$). After treatment the mean values were nearly the same in these two groups.

Multiple regression analysis was carried out using data from all the nine diabetics with the half-time obtained during metabolic derangement expressed as a percentage of the half-time obtained after treatment with insulin as the variable. The three invariables were the resting blood flow and the peak flow expressed in the same way as the half-time and the total plasma CO₂. Only the last mentioned factor was significant ($P<0.02$).

When the half-time was calculated after subtraction of the resting blood flow value from the
peak flow and the second blood flow, it averaged 9·1 s in patients with mild acid-base abnormalities and 4·7 s after treatment. This difference is also significant ($P<0.01$).

No association could be established between serum sodium concentration or potassium concentration and the half-time. The glucose concentration in the blood averaged 384±43 mg/100 ml in the diabetics before treatment and 160±66 mg/100 ml after treatment. The halftime was not correlated with the plasma glucose concentration. Blood pressure was normal in all the diabetic patients and no difference was found before and after treatment with insulin.

Table 4 shows the half-times in the two normal subjects during metabolic acidosis induced by ammonium chloride ingestion and after normalization of the acid-base balance. The pH, standard bicarbonate and the $PCO_2$ obtained in arterial blood on the day of examination are also given in the Table. During ammonium chloride treatment the total $CO_2$ in plasma declined to 15 mmol/l at 48 h. It can be seen that a partially compensated metabolic acidosis of a degree similar to that in the three diabetic patients (cases 4, 5 and 6) was present in both subjects. No prolongation of the half-time could be demonstrated in these two subjects during experimental acidosis. It should be mentioned that the duration of the experimental acidosis was at least as long as the duration of the acid-base disturbances present in two of the diabetic patients (cases 3 and 6) both of whom showed a marked abnormality and in whom the duration of ketosis was known.

The half-time was also studied in one patient with metabolic acidosis due to chronic renal insufficiency. The total $CO_2$ in venous plasma was 15 mmol/l. The half-time was 7·2 s, i.e. it was not prolonged.

**Blood flow restoration after 2 min periods of ischaemia**

The results obtained in the two diabetics are illustrated in Fig. 3. Mean half-times were 21·6 and 15·1 s during metabolic derangement and 7·8 and 7·3 s after institution of therapy. In the two non-diabetics mean half-times were 10·1 and 7·9 s.

Both diabetic patients had moderate ketosis and slight acid-base abnormalities during metabolic derangement.

![Fig. 3.](image-url)
Table 4. Mean blood flow values and half-times obtained in the two non-diabetic subjects during experimental acidosis (B) and after normalization of the acid-base balance (A). pH, standard bicarbonate and $P_{co_2}$ are also given.

<table>
<thead>
<tr>
<th>Case</th>
<th>Resting blood flow (ml 100 ml$^{-1}$ min$^{-1}$)</th>
<th>Peak flow (ml 100 ml$^{-1}$ min$^{-1}$)</th>
<th>Second flow (ml 100 ml$^{-1}$ min$^{-1}$)</th>
<th>Time of measurement of second flow (s)</th>
<th>Half-time (s)</th>
<th>pH</th>
<th>Standard bicarbonate (mEq/l)</th>
<th>$P_{co_2}$ (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 B</td>
<td>2.4</td>
<td>14.0</td>
<td>4.4</td>
<td>8.5</td>
<td>5.1</td>
<td>7.30</td>
<td>17</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>16.4</td>
<td>4.7</td>
<td>8.7</td>
<td>4.9</td>
<td>7.38</td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td>2 B</td>
<td>1.1</td>
<td>9.5</td>
<td>2.7</td>
<td>8.5</td>
<td>4.5</td>
<td>7.30</td>
<td>17</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>9.1</td>
<td>4.1</td>
<td>8.4</td>
<td>7.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Reactive hyperaemia in diabetics

It appears that the same abnormality is present in these patients as it was when shorter periods of ischaemia were employed.

The haematocrit was also determined at the time of the studies. In the non-diabetics the values were 46 and 42 and in the diabetics 44 and 43 before treatment and 42 and 46 after treatment.

The urinary excretion of adrenaline and noradrenaline was determined in the diabetics before and after insulin treatment and the results compared with values obtained in a larger group of normal subjects. No tendency to increased adrenaline values was observed in the diabetics during untreated conditions and no change whatsoever was observed in the two metabolic situations.

DISCUSSION

The present study demonstrates that a vascular abnormality of post-ischaemic blood flow recovery is present in diabetic patients with ketosis and mild acid-base abnormalities. The rate of return towards basal flow levels is approximately 50% of normal values. Treatment with insulin reverses the abnormality. Peak flow is approximately the same in the two conditions. Resting blood flow is increased in untreated diabetics and a slight but significant decrease takes place during insulin treatment.

The mechanism of the vascular changes is not clear. It is obvious that the prolongation of the half-time is associated with diabetic ketosis and mild disturbances of acid-base balance. However, the results obtained in the subjects with experimental acidosis and the single patient with uraemic acidosis suggest that the abnormality is not the simple consequence of a change in pH, $P_{CO_2}$ or bicarbonate.

The possibility that prolongation of the recovery phase could be due to dehydration must be considered. This seems however unlikely because none of the patients was dehydrated on clinical examination and because an increase in whole blood viscosity will decrease the resting blood flow and particularly the peak flow (Bollinger & Lüthy, 1968). In the present study, resting blood flow and peak flow were greater in diabetics than in normal subjects. Furthermore, this explanation seems improbable as the haematocrit values in two patients in whom the half-time was considerably prolonged showed slight and inconsistent changes.

The physiological mechanism behind the vascular response to ischaemia is not known. Obviously, therefore, it is difficult to explain the mechanism of the change in the restoration of blood flow after ischaemia in the diabetic patients.

The recovery phase in normal subjects is known to be longer after exercise than after ischaemia even at the same peak flow. The speed of recovery is not changed by anoxia (Dornhorst & Whelan, 1953). Hyperaemia is prolonged in patients with occlusive peripheral vascular disease, but in these patients peak flow is also considerably reduced. It is interesting that the recovery phase after ischaemia in the forearm can be increased by infusion of adrenaline (Dornhorst & Whelan, 1953; Abrams, Barker & Butterfield, 1965). The resting blood flow is also increased whereas the peak flow is slightly reduced. The same alterations can be induced by depletion of the noradrenaline stores by reserpine (Abrams et al., 1965). Accordingly the vascular abnormalities present in the ketotic diabetic patients might be explained on the basis of an increase in circulating adrenaline or by a depletion of the noradrenaline stores. The finding of normal adrenaline excretion in the urine in two patients demonstrating a prolonged half-time does not support the first explanation. Further studies of blood and tissue catecholamines in diabetics are now in progress.
Patients with severe ketosis demonstrate a high cardiac output but a normal or low blood pressure (Howarth, McMichael & Sharpey-Shafer, 1948). The vascular dilatation which must exist in such patients might represent an exaggeration of the vascular changes found in the present study.

The observation that diabetic patients have an increased resting blood flow has been made by other investigators.

Butterfield & Whichelow (1965) noted a high resting blood flow in the forearm in a group of juvenile diabetics with a duration of disease between 7 and 12 years. Alexander, Teusen & Mitzkat (1968) also found a significantly higher resting blood flow in various parts of the extremities in diabetics, although the increase was very small.

Increased blood flow has also been observed in adipose tissue (Steen & Svanborg, 1968) and insulin treatment is accompanied by a decrease.

In conclusion it can be said that functional changes in the vascular system occur in diabetics as a direct consequence of alterations in the metabolic state. Accordingly these changes are also present in patients with a recently diagnosed, untreated diabetes and disappear when insulin is administered. The significance of these observations in the pathogenesis of the blood vessel disease of long-term diabetics has still to be explored.

REFERENCES


