Cardiovascular responses to graded reductions of central blood volume in normal subjects and in patients with diabetes mellitus

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Summary

1. Cardiovascular responses to graded increments of lower body negative pressure were studied in non-diabetic subjects and in patients with diabetes mellitus.

2. In all subjects, low levels of negative pressure (which did not affect significantly systemic arterial pressure) induced forearm vasoconstriction, suggesting normal function of the 'low pressure' cardiopulmonary baroreflex. However, in some diabetic patients the response to higher levels of negative pressure was abnormal, and it seems likely that although afferent mechanisms were intact there was impairment of efferent vasoconstrictor function.

3. Changes in R–R interval were linearly related to changes in systolic blood pressure induced by higher levels of negative pressure. The slope of the relationship was taken as the sensitivity of the 'high pressure' arterial baroreflex; diabetic patients showed a reduced sensitivity compared with normal subjects. Furthermore, in diabetic patients, abnormalities of R–R interval control were more common than abnormalities of vasoconstrictor function, suggesting that heart-rate control is impaired earlier than vasomotor function in diabetic autonomic neuropathy.

Key words: arterial baroreflexes, cardiopulmonary baroreflexes, forearm vasoconstriction, R–R interval.

Introduction

In normal man a reduction in central blood volume, insufficient to affect systemic arterial pressure, causes marked reflex vasoconstriction in skeletal muscle (Zoller, Mark, Abboud, Schmid & Heistad, 1972; Johnson, Rowell, Niederberger & Eisman, 1974), and an increase in central blood volume elicits reflex vasodilatation (Roddie, Shepherd & Whelan, 1957). On the basis of such observations it has been suggested that there are baroreceptors on the 'low pressure' side of the circulation responsible for modulating sympathetic vasoconstrictor outflow to skeletal muscle (Roddie et al., 1957; Zoller et al., 1972; Johnson et al., 1974; Abboud, Heistad, Mark & Schmid, 1976).

When systemic arterial pressure changes, or when carotid sinus transmural pressure is changed, there are reflex effects on heart rate and splanchnic vascular resistance (Bevegård & Shepherd, 1966; Beiser, Zelis, Epstein, Mason & Braunwald, 1970; Zoller et al., 1972; Johnson et al., 1974; Bjurstedt, Rosenhammer & Tyden, 1975; Abboud et al., 1976; Mancia, Ferrari, Gregorini, Valentini, Ludbrook & Zanchetti, 1977), indicating that the baroreceptors on the 'high pressure' side of the system contribute to these effects.

Patients with diabetes mellitus have been shown to have abnormalities of the 'high pressure' baro-
reflex affecting the heart (Low, Walsh, Huang & McLeod, 1975; Bennett, Hosking & Hampton, 1976), but the reflexes elicited by moderate changes in central blood volume have not been investigated systematically. We have used graded increments in lower body negative pressure (Brown, Goei, Greenfield & Plassaras, 1966) to produce different degrees of reduction in central blood volume and have studied the changes in forearm vascular resistance, systemic arterial blood pressure and R–R interval in normal subjects and in patients with diabetes mellitus (with and without evidence of autonomic neuropathy), in order to assess the integrity of 'low pressure' and 'high pressure' baroreflexes in such subjects.

Subjects and methods

Sixteen non-diabetic male subjects were studied; their ages ranged from 20 to 56 years (Table 1). None had any cardiovascular abnormalities or was taking drugs known to interfere with autonomic nervous function. Thirty diabetic patients (23 male, seven female) were investigated; their ages ranged from 24 to 61 years; clinical details are given in Table 1. None of the patients was on drugs that were likely to influence the results and those who were treated with insulin had had their last injections at least 6 h before the experiments took place. All subjects gave their fully informed consent to the study, which had been approved by the Ethical Committee.

The experiments were carried out between 14.00 and 16.00 hours in a temperature-controlled laboratory at 24°C. Reductions in central blood volume were achieved by subatmospheric (negative) pressure which causes blood to pool in the parts to which the negative pressure is applied (Brown et al., 1966). Subjects lay supine on a couch with the body distal to the level of the iliac crests enclosed in a box from which air could be withdrawn by a domestic vacuum cleaner; box pressure was monitored by a pressure transducer, and was adjusted by a leak in the pipe connecting the box and vacuum cleaner. The electrocardiogram was recorded from chest electrodes and the R–R intervals were derived from the ECG electronically. Blood flow in the right forearm was measured by venous occlusion plethysmography with a mercury-in-silastic strain gauge (Greenfield, Whitney & Mowbray, 1963). Systemic arterial blood pressure was measured in the left forearm (by auscultation), since it is not possible to measure blood flow and arterial pressure in the same forearm. However, there were no systematic differences in the blood pressures in the two arms so it is unlikely that problems such as localized atheroma influenced the patterns of response described in the results. Blood flow was measured every 15 s and brachial arterial pressure was measured every 30 s; forearm vascular resistance was derived by dividing mean blood pressure [(systolic blood pressure + 2 x diastolic pressure)/3] by the average forearm blood flow. Negative pressure of 5, 10, 15, 20, 25, 30, 40 or 50 mmHg was applied for a period of 1 min, with adequate rest periods between each exposure to negative pressure. (In the text increases in negative pressure mean more negative pressure.) The values for arterial pressure, forearm blood flow, forearm vascular resistance and R–R interval given in the text and Figures are the group mean values for the

<table>
<thead>
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<th>Table 1. Details of the subjects studied</th>
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<td><strong>Normal subjects</strong></td>
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<td><strong>Diabetic patients</strong></td>
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<tr>
<td><strong>Group 1</strong></td>
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<td><strong>Group 2</strong></td>
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<td><strong>Group 3</strong></td>
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<td>No. of subjects</td>
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<tr>
<td>Mean age (with range; years)</td>
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<td>Sex</td>
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<td>Mean duration of diabetes (with range; years)</td>
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<td>Treatment</td>
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<td>Insulin</td>
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<tr>
<td>Oral hypoglycaemic agents</td>
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<tr>
<td>Retinopathy</td>
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<tr>
<td>Somatic neuropathy*</td>
</tr>
<tr>
<td>Proteinuria</td>
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<tr>
<td>Postural hypotension</td>
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* Somatic neuropathy is defined here as an absence of ankle jerks together with loss of the senses of joint position, vibration and light touch.
Baroreflexes in diabetic patients

last measurements made during negative pressure. The resting figures are the means of the values immediately preceding any particular exposure to negative pressure. Although the size of the cardiovascular response elicited by negative pressure shows some variation in the same subjects on different occasions, the pattern of the response does not vary in the short term (unpublished observations). Thus the systematic differences in the patterns of response in the different groups of subjects described here cannot be attributed to random variations.

Results were analysed by Student's paired or unpaired t-test as appropriate. Slopes of regressions were compared by covariance analysis; values given are means \pm SEM.

Results
In normal subjects, progressive increases in negative pressure to 20 mmHg had no significant effect on diastolic or systolic arterial blood pressure (Fig. 1). In some diabetic subjects this response pattern was also observed, but in others a marked reduction in systolic blood pressure occurred. Inspection of our results suggested that the diabetic group should be divided into those showing a normal response (a fall in systolic blood pressure less than 10 mmHg with negative pressure of 40 mmHg) and those in whom the systolic blood pressure fell by more than 20 mmHg at this negative pressure. Within the latter group were five diabetic patients with symptomatic postural hypotension, and it was evident that these subjects behaved in a different way from those who, despite hypotension during negative pressure, had no postural hypotension and no symptoms. We therefore divided our diabetic patients into three groups on the basis of these physiological and clinical findings (Table 1):

Group 1: 18 subjects with normal systolic blood pressure response to negative pressure.
Group 2: seven subjects without postural hypotension but whose systolic blood pressure fell by more than 20 mmHg with negative pressure of 40 mmHg.
Group 3: five subjects with symptomatic postural hypotension whose systolic blood pressure fell

![Graph](image-url)

**Fig. 1.** Systolic blood pressure measured during the last 5 s of a 1 min exposure to negative pressure at the values indicated. ●, Non-diabetic subjects; ○, diabetic subjects in group 1; △, diabetic subjects in group 2; ▲, diabetic subjects in group 3.

**TABLE 2. Resting cardiovascular variables in the groups of subjects studied**

<table>
<thead>
<tr>
<th></th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Forearm blood flow (ml min(^{-1}) 100 ml(^{-1}))</th>
<th>Forearm vascular resistance</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic (n = 16)</td>
<td>125 ± 3.0</td>
<td>81 ± 2.0</td>
<td>5.1 ± 0.8</td>
<td>19.3 ± 3.0</td>
<td>76 ± 3.8</td>
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<tr>
<td>Diabetic</td>
<td></td>
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</tr>
<tr>
<td>Group 1 (n = 18)</td>
<td>131 ± 2.4</td>
<td>83 ± 2.0</td>
<td>3.8 ± 0.6</td>
<td>27.4 ± 3.3</td>
<td>82 ± 2.3</td>
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<tr>
<td>Group 2 (n = 7)</td>
<td>146 ± 7.0</td>
<td>88 ± 3.0</td>
<td>2.7 ± 0.5</td>
<td>39.8 ± 6.5</td>
<td>86 ± 3.3</td>
</tr>
<tr>
<td>Group 3 (n = 5)</td>
<td>168 ± 12.8</td>
<td>91 ± 5.0</td>
<td>4.1 ± 0.6</td>
<td>30.0 ± 8.9</td>
<td>87 ± 4.2</td>
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by more than 20 mmHg with negative pressure of 40 mmHg. (In group 3 the mean postural fall in systolic blood pressure was 38 ± 5 mmHg, and all subjects became dizzy. This group included the eldest subject studied, but his results did not affect the mean disproportionately).

Table 2 shows the resting cardiovascular status of the various groups of subjects studied. There was a significant \( (P < 0.05) \) difference between the systolic blood pressures, forearm blood flows, calculated forearm vascular resistance and heart rates of the non-diabetic subjects and those of the diabetic subjects considered as a single group. Those diabetic subjects who showed a fall in systolic blood pressure on exposure to negative pressure (groups 2 and 3) had a significantly \( (P < 0.05) \) higher resting systolic blood pressure than those who did not (group 1). Furthermore, it appeared that those diabetic subjects with the highest systolic blood pressure showed the largest falls in systolic blood pressure on exposure to negative pressure (Fig. 1). This raised the possibility that the results were due to hypertension and not diabetes. However, within groups 2 and 3 there were six subjects with a mean systolic blood pressure of 140 ± 4-0 mmHg who showed a fall of 48 ± 6-2 mmHg in systolic blood pressure on exposure to negative pressure of 50 mmHg. Their resting systolic blood pressure was not significantly different from that of six subjects within group 1 (143 ± 2-0 mmHg) whose systolic blood pressure fell by only 14 ± 2-6 mmHg on exposure to negative pressure of 50 mmHg.

Fig. 2 shows the effects of stepped increases in negative pressure on forearm blood flow. In all groups of subjects the most marked changes in forearm blood flow occurred with negative pressure over the range 5–20 mmHg. With negative pressure above this range the decrement in forearm blood flow tended to level out.

Mean brachial arterial blood pressure divided by forearm blood flow provides an estimate of forearm vascular resistance. Thus on a graph where these two variables are plotted as the axes, it is possible to draw a series of isopleths corresponding to different values of forearm vascular resistance. In Fig. 3 are shown values for mean blood pressure and forearm blood flow during and after various negative pressures. It is clear that, in all groups, forearm vasoconstriction occurred during negative pressure and forearm vasodilatation occurred after negative pressure (Brown et al., 1966).

In the normal subjects and in the diabetic sub-

![Fig. 2. Forearm blood flow measured during the last 5 s of a 1 min exposure to negative pressure at the values indicated. ○, Non-diabetic subjects; ○, diabetic subjects in group 1; Δ, diabetic subjects in group 2; ▲, diabetic subjects in group 3.](image-url)
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Vascular resistance (arbitrary units)

FIG. 3. Corresponding values for forearm blood flow and mean blood pressure in non-diabetic subjects (●), diabetic subjects in group 1 (○), diabetic subjects in group 2 (Δ) and diabetic subjects in group 3 (▲). The arrows indicate the resting values for the two variables in the various groups; to the left of the arrows in sequence the points correspond to the values measured during the last 5 s of a 1 min exposure to negative pressure of 5, 10, 15, 20, 25, 30, 40 or 50 mmHg respectively. The points to the right of the arrows are those representing the lowest vascular resistances seen after negative pressures of 10, 20 and 40 mmHg in sequence respectively. Broken lines represent vascular resistance isopleths; where vascular resistance changes independently of mean blood pressure the points are disposed parallel to the x axis; where flow falls passively with a fall in driving pressure the points tend to lie along the resistance isopleths.

FIG. 4. Relationship between systolic blood pressure and R–R interval measured during the last 5 s of a 1 min exposure to negative pressure of 5, 10, 15, 20, 25, 30, 40 or 50 mmHg in non-diabetic subjects (●), diabetic subjects in group 1 (○), diabetic subjects in group 2 (Δ) and diabetic subjects in group 3 (▲). In every case the longest R–R interval represents the resting state. With low negative pressures there was little change in systolic blood pressure or R–R intervals; however, except in diabetic subjects of group 3 the two variables were significantly \( P < 0.001 \) correlated; \( r \) is the correlation coefficient.
groups. Diabetic subjects in group 3 showed no significant change in R–R interval on exposure to negative pressure (Fig. 4).

Discussion

The present study has confirmed that, during and after negative pressure, forearm blood flow can change without any significant change in mean blood pressure. It is known that during and after negative pressure there are changes in central blood volume (Brown et al., 1966); thus our observations are consistent with the view that changes in forearm vascular resistance are largely due to excitation or inhibition of forearm vasoconstrictor fibres by afferent signalling from 'low pressure' cardiopulmonary receptors (Roddie et al., 1957; Zoller et al., 1972; Johnson et al., 1974; Abboud et al., 1976).

However, the present results do not provide definite proof that this is so, since we did observe small changes in systolic blood pressure with low negative pressures. Given that the reflex loop we were assessing was 'closed', then any deviation of systolic blood pressure from the set level would immediately be sensed by arterial baroreceptors and if the reflex responses were effective then systolic blood pressure might not have been seen to have changed significantly at the time when we measured it. However, such an objection cannot be raised to the findings of Zoller et al. (1972), since these workers measured intra-arterial blood pressure continuously and demonstrated that forearm blood flow could change independently of any change in systolic blood pressure.

Assuming that the changes in forearm vascular resistance, particularly with low negative pressures, were largely due to cardiopulmonary baroreflexes, then in some diabetic patients (group 1) these reflex responses appeared similar to those seen in the non-diabetic control subjects. However, in the patients in group 2, exposure to negative pressure above 20 mmHg did not provoke further increments in forearm vascular resistance, even though these higher negative pressures caused a fall in systemic arterial blood pressure. But the maximum vasoconstriction seen in this group of subjects was greater than that seen in any others, which raises the possibility that the pattern of response observed was not due to a neuronal dysfunction, but an inability of the resistance vessels to constrict further.

The pattern of response seen in the diabetic subjects with postural hypotension (group 3) was clearly abnormal. In this group there was a significant fall in systolic blood pressure even with low negative pressures and yet the proportional increase in forearm vascular resistance was not as great as that seen in the groups of subjects who showed no fall in systolic blood pressure. If there was a fall in systolic blood pressure then the fall in central blood volume, and hence the stimulus for cardiopulmonary receptors, must have been maximal. On this basis it seems reasonable to conclude that the integrity of the cardiopulmonary baroreflex was impaired in those diabetic subjects who had postural hypotension. That a response occurred at all indicates that there was some afferent input and it may be that the efferent system was abnormal. The reduction in the vasoconstriction evoked by negative pressure could have been due to a loss of vasomotor fibres (Grover-Johnson & Kim, 1976), the remaining ones being incapable of producing a maximal effector response even at high levels of activity. It is also possible that the marked fall in systolic blood pressure caused a centrally mediated suppression of vasoconstrictor activity in skeletal muscle vascular beds, or a concurrent activation of vasodilator mechanisms as is seen in the vasovagal response (Barcroft & Edholm, 1945). The absence of bradycardia in these subjects in spite of marked falls in systolic blood pressure is consistent with a loss of cardiac vagal influences (see below).

We have noted elsewhere that forearm vascular resistance is elevated in diabetic subjects and that this does not appear to be attributable simply to hypertension (Bennett, Hosking & Hampton, 1979).

The high resting values for forearm vascular resistance in the diabetic subjects could be due to increased vasoconstrictor tone, but this is unlikely since the phenomenon was also observed in diabetic subjects in group 3 who showed an impaired vasoconstrictor response to negative pressure. Furthermore, the diabetic subjects showed a less-marked forearm vasodilatation after negative pressure and, since this is due to inhibition of noradrenergic vasomotor tone (Brown et al., 1966), then it is likely that the raised forearm vascular resistance was due to changes in the vasculature. It is of interest that in congestive heart failure there is an increased forearm vascular resistance, partly due to an increased stiffness of vessel walls attributable to elevated sodium and water contents (Mason, Zelis, Longhurst & Lee, 1977). It may be that such a disturbance occurs in diabetes; another possibility is that the raised vascular resistance is due to angiopathy (Kohner, 1977).
The absence of changes in R–R interval at low negative pressures when there were no changes in systolic blood pressure, and the linear relationship between R–R interval and systolic blood pressure when systolic blood pressure was lowered during negative pressure, are consistent with the suggestion that arterial rather than cardiopulmonary baroreceptors are responsible for controlling heart rate (Zoller et al., 1972; Johnson et al., 1974; Mancia et al., 1977). However, the application of negative pressure reduces right and left atrial distension, which, in the dog at least, would tend to inhibit cardiac sympathetic activity (Linden, 1975). It is possible therefore that, in man, the changes in R–R interval seen with negative pressure are due to interaction of opposing influences from cardio-pulmonary and systemic arterial baroreceptors. With the present method, systolic blood pressure and R–R interval were measured during the last 5 s of a 1 min exposure to negative pressure. At this time a steady state had been reached and thus the changes seen in R–R interval were probably largely due to an arterial baroreceptor-mediated inhibition of vagal, and excitation of sympathetic, cardiac efferents in the non-diabetic subjects (Pickering, Gribbin & Sleight, 1972; Korner, West, Shaw & Uther, 1974). This differs from the situation in which systolic blood pressure is changed acutely and the immediate changes in R–R interval are measured, a technique which emphasizes vagal effects (Smyth, Sleight & Pickering, 1969; Pickering et al., 1972; Korner et al., 1974). With this latter method, Pickering et al. (1972) found that changes in R–R interval were greater when systolic blood pressure was raised than when it was lowered. Comparison of our present results with our previous findings (Bennett et al., 1976) shows they are in agreement with those of Pickering et al. (1972), although our data were collected from different groups of subjects on the two occasions.

We found clear-cut differences in the slope of the regression of R–R interval on systolic blood pressure (i.e. ‘baroreflex sensitivity’) for non-diabetic and diabetic subjects. Moreover, the progressive reduction in baroreflex sensitivity seen in diabetic subjects appeared to be associated with an increasing inability to maintain systolic blood pressure on exposure to negative pressure. Obviously if shortening of R–R interval is an important contributor to the maintenance of systolic blood pressure during negative pressure then reduced baroreflex sensitivity would necessarily be associated with impaired arterial pressure regulation.

There is good evidence that cardiac vagal responses may be impaired in diabetic subjects (Wheeler & Watkins, 1973; Bennett, Hosking & Hampton, 1975; Lloyd-Mostyn & Watkins, 1975; Low et al., 1975); thus it is possible that the reduced baroreflex sensitivities seen in the diabetic subjects in groups 1 and 2 were due to increasing degrees of vagal impairment.

The almost total loss of arterial baroreflex effects on the heart in the subjects in group 3 could be explained by a loss of vagal and sympathetic cardiac control (Bennett et al., 1975; Lloyd-Mostyn & Watkins, 1975). These subjects were those with postural hypotension and it is likely that the dysfunction of cardiac control would contribute to this problem, but it is not clear to what extent splanchic vasoconstrictor impairment is involved (Low et al., 1975). Vascular degenerative changes affecting the carotid sinuses might also contribute to the problem by interfering with baroreceptor afferent signalling.

One complicating factor noted in the results is the presence of a significantly raised supine systolic blood pressure in some diabetic patients. Since it is known that non-diabetic hypertensive patients may show a reduction in high pressure baroreflex sensitivity (Bristow, Honour, Pickering, Sleight & Smyth, 1969; Korner et al., 1974; Mancia, Ludbrook, Ferrari, Gregorini & Zanchetti, 1978), it must be accepted that hypertension in diabetic patients could contribute to baroreflex abnormalities. However, these subjects are only hypertensive when supine; thus it is not clear how important a contributory factor this is. Clearly it is not the only factor since we found in the present study sub-groups of diabetic subjects who showed marked falls in systolic blood pressure on exposure to negative pressure, but whose resting systolic blood pressure was not different from that of subjects whose systolic blood pressure was little affected by negative pressure. Furthermore, postural hypotension has not been reported as a complication in untreated hypertensive patients without diabetes mellitus.

The patterns of response to negative pressure in the diabetic subjects in groups 2 and 3 correspond to those in previous observations on postural hypotension associated with orthostatic tachycardia and postural hypotension associated with a fixed heart rate (Bennett et al., 1975). Interestingly, one subject we have studied repeatedly for 5 years has in that time moved from the former to the latter category, probably due to a progressive cardiac sympathetic neuropathy.

The present study has shown that arterial baro-
reflexes affecting the heart may be impaired in diabetic subjects who show a normal pattern of vaso- 

tensor response to cardiopulmonary baroreceptor inhibition. This is likely to be due to the early in-

volvement of the cardiac vagus in diabetic autono-

mous neuropathy (Wheeler & Watkins, 1973; Bennett et al., 1975). More extensive autonomic in-

volvement gives rise to abnormalities in cardiopulmonary baroreceptor reflexes. We believe that 

the present techniques provide a useful and sen-

sitive means of describing the dysfunctions that 

occur.

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