Peripheral vascular response to mild indirect cooling in patients with homozygous sickle cell (SS) disease and the frequency of painful crisis

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1. In homozygous sickle cell (SS) disease, skin cooling is a common precipitating factor of the painful crisis which is associated with avascular necrosis of active bone marrow. Since skin cooling does not directly induce sickling, we have investigated the nature of the reflex vascular responses to mild cooling in SS patients in a steady state of the disease and compared them with their history of painful crises.

2. Experiments were performed in Jamaica on 60 male SS patients and 30 matched control subjects with normal haemoglobin (AA) genotype. Forearm blood flow (FBF) was measured by venous occlusion plethysmography and mean arterial pressure (MAP) by a Finapres device; forearm vascular resistance (FVR) was calculated as MAP/FBF. Cutaneous erythrocyte flux in forearm and hand was monitored by a laser Doppler meter. The contralateral hand was immersed in cool water at 16°C for 2 min, 6 times, at random intervals of 0.5–3 min.

3. The first cool immersion evoked an increase in MAP, cutaneous vasoconstriction and a net increase in FVR in both AA and SS subjects. However, the direction of change in FVR varied between individuals such that 18 AA subjects showed an increase in FVR (constrictor group) while 12 showed a decrease in FVR, indicating vasodilatation in forearm muscle (dilator group). In contrast, 50 SS subjects showed an increase in FVR and only 10 showed a decrease in FVR. The proportion of subjects who showed net vasoconstriction was significantly greater in the SS than in the AA group (83% versus 60%, \( P = 0.03, \chi^2 \) test).

4. By the sixth cool stimulus, the ‘dilator’ group of AA subjects showed no change in FVR while the ‘dilator’ group of SS patients showed an increase in FVR. We suggest that forearm muscle vasodilatation was the characteristic component of the alerting/defence response to novel or noxious stimuli which habituates on repetition.

5. In the whole group of SS patients, baseline values of cutaneous vascular resistance and FVR increased between stimuli, indicating persistent vasoconstriction, and the sixth cool stimulus still evoked cutaneous vasoconstriction and a net increase in FVR. In contrast, AA subjects showed an increase in baseline FVR between stimuli, but the sixth cool stimulus had no significant effect on cutaneous vascular resistances, or FVR.

6. In SS patients there were no associations between the direction of change in FVR evoked by the first cool stimulus and forearm circumference or skinfold thickness, concentrations of haemoglobin or fetal haemoglobin. However, the frequency of painful crises was significantly greater in the ‘constrictor’ group than in the ‘dilator’ group (0.36 versus 0.12/year, \( P = 0.04, \) Mann–Whitney test).

7. These results indicate that the primary reflex vasoconstrictor response evoked by mild cooling is stronger and more persistent in SS patients than in AA subjects and is particularly strong in SS patients who are most prone to painful crises. The results are consistent with the hypothesis that skin cooling may precipitate the painful crisis by causing reflex vasoconstriction in muscle, and possibly in bone marrow, so diverting blood flow away from the active marrow.

INTRODUCTION

The painful crisis, which is characterized by severe bone pain, is a major cause of morbidity in patients with homozygous sickle cell (SS) disease: the pain results from avascular necrosis of active bone marrow. In Jamaica, skin cooling, brought about by a drop in environmental temperature, being ‘caught’...
in a shower of rain or bathing in cool water, was found to be the most common precipitating factor for the painful crisis [1, 2]. Similar observations have been made in other tropical [3] and temperate climates [4]. The mechanisms by which cooling might trigger the painful crisis are not understood.

In the past, painful crises have been attributed to random vaso-occlusion of bone blood flow by sickled or less deformable cells [5]. However, the fact that the pain is frequently bilateral and symmetrical is not consistent with the idea of random vaso-occlusion. This has led to a new hypothesis: that the neural reflex response of the peripheral vasculature to skin cooling is the trigger in that it leads to a shifting of blood flow away from the active bone marrow [1].

Few studies on the effect of cooling on the peripheral vasculature have been performed in SS patients. Hatch et al. [6] reported that the cold pressor test, elicited by immersion of one hand in water at 4°C for 30 s while monitoring the vascular resistance of the contralateral forearm (FVR) by venous occlusion plethysmography, did not change FVR in patients with sickle haemoglobinopathy, but increased FVR in control subjects, indicating forearm vasoconstriction. In another study, the cold pressor test evoked no change in arterial pressure in 30 patients with sickle haemoglobinopathy, but substantially increased arterial pressure in 30 control subjects [7]. In both studies it was concluded that reflex vasoconstriction is impaired in sickle cell anaemia. However, these results are difficult to interpret in relation to the hypothesis mentioned above. Firstly, both studies included patients who had sickle cell trait or other haemoglobinopathies, as well as those who were homozygous for sickle cell disease. Secondly, in both studies approximately half of the patients and controls were female and no information is provided on the stage in the menstrual cycle at which the experiments were performed: vasoconstrictor responses are impaired in the luteal phase [8]. Thirdly, the cold pressor test constitutes a noxious as well as a cool stimulus and therefore has the potential to evoke two different patterns of response simultaneously.

Our recent study performed in Birmingham, U.K., on 12 SS patients of West Indian ancestry and on West Indian and Caucasian control subjects with normal haemoglobin (AA) genotype illustrates this last point and indicated that further experiments were required [9, 10]. Thus, we found that mild cooling of the hand in water at 16°C increased FVR in the contralateral arm, as measured by plethysmography, indicating net vasoconstriction in the forearm (skin and skeletal muscle) in approximately half of the AA subjects. This response persisted from the first to the sixth cool stimulus when the cooling was repeated in an experimental session. The remaining AA subjects showed a decrease in FVR in response to the first stimulus which decreased in magnitude on repetition of the stimulus. These results were consistent with the view that mild cooling evokes a primary vasoconstriction in the skin and skeletal muscle of the forearm that can be overcome by the muscle vasoconstriction of the ‘alerting response’: the alerting response includes generalized vasoconstriction, but vasodilatation in muscle is evoked by novel or noxious stimuli and habituates on repetition of the stimulus [9, 11]. In contrast, all 12 SS patients showed an increase in FVR from the first through to the sixth cool stimulus [10]. This consistency and persistency of the forearm vasoconstrictor response in the SS patients, despite their perception of the cooling as a noxious stimulus, suggested that the primary muscle vasoconstrictor response to cooling is particularly strong in SS patients. We proposed that if a corresponding large reflex vasoconstriction occurs in bone in response to cooling this might explain the association between cooling and the painful crisis [10].

Since it is impossible to directly measure changes in bone blood flow in human subjects, the present study was undertaken to further explore our hypothesis by examining the vascular responses to cooling in larger groups of SS patients and AA subjects in Jamaica, and by correlating the vascular responses of the SS patients with their history of painful crises. Since painful crises were found to be more common in SS patients with mild rather than severe anaemia [12, 13] and since the concentration of fetal haemoglobin (HbF) has been variously reported to show no association, negative or positive correlation with painful crisis frequency [12–14], we studied subgroups of SS patients with a range of haemoglobin (Hb) and HbF concentrations. A brief report of this study has already been made [15].

MATERIALS AND METHODS

Subjects

The SS patients attended the Sickle Cell Clinic of the University Hospital of the West Indies, Kingston, Jamaica. The study group of 60 individuals comprised males aged 26.4 ± 0.9 years (mean ± SEM, range 16–40 years) living in Kingston and with a history of painful crises. They were randomly selected to provide six subgroups with low, medium or high Hb (<7.5, 7.5–8.5 and >8.5 g/dl respectively) and low or high HbF (<4.5 and >4.5% respectively, as shown in Table 1). Hb >8.5 g/dl was found to be a risk factor for painful crises [12], while HbF = 4.5% is the average value for SS patients monitored at the University Hospital of the West Indies. The patients were normotensive with no overt signs of infection. All of the patients were in a steady state, i.e. they had not had a painful crisis for at least 4 weeks before the study and had not had a blood transfusion within the previous 3 months.

The 30 male AA subjects were selected from the control group of a cohort study who had been followed from birth [16], or were recruited as volun-
Table 1. The six subgroups of SS patients based on total haemoglobin (Hb) and foetal haemoglobin (HbF). Values are expressed as means ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Hb level</th>
<th>Hbf level</th>
<th>Hb (g/dl)</th>
<th>Hbf (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>Low</td>
<td>Low</td>
<td>6.3 ± 0.28</td>
<td>2.2 ± 0.55</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Low</td>
<td>High</td>
<td>6.8 ± 0.25</td>
<td>6.98 ± 0.92</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>Medium</td>
<td>Low</td>
<td>7.9 ± 0.09</td>
<td>2.03 ± 0.27</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Medium</td>
<td>High</td>
<td>8.00 ± 0.13</td>
<td>7.3 ± 0.73</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>High</td>
<td>Low</td>
<td>9.5 ± 0.19</td>
<td>2.06 ± 0.34</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>High</td>
<td>High</td>
<td>9.4 ± 0.18</td>
<td>8.51 ± 0.86</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td></td>
<td></td>
<td>9.5 ± 0.28</td>
<td>2.06 ± 0.34</td>
</tr>
</tbody>
</table>

Recordings

Experiments were performed as in the U.K. study [9, 10] using equipment described recently [17]. Briefly, the experiments were performed in a quiet room which was thermostatically maintained at 25°C (relatively humidity 45%). The subjects rested on a comfortable couch for 30 min during which the recording devices were attached. All recording equipment was positioned behind the subject in order to minimize visual and auditory stimuli. All equipment was positioned behind the subject in order to minimize visual and auditory stimuli. All studies were conducted between 09.00 h and 12.00 h to avoid the effect of diurnal variations in the haemodynamic variables [17].

Total forearm blood flow (FBF) was recorded from the left arm using strain-gauge, venous occlusion plethysmography with the arm at heart level. During the period of recording a sphygmomanometer cuff wrapped around the wrist was inflated to 200 mmHg to prevent blood flow to the hand. Arterial pressure was monitored continuously from the middle finger of the left hand with a Finapres instrument (Ohmeda 2300; Biomedical International Corp., Miami, FL, U.S.A.). Cutaneous erythrocyte flux was recorded continuously from the left forearm (FCRCF) and hand (HCRCF) with a dual-channel laser Doppler meter and probes (MBF3D and P1 respectively; Moor Instruments, Axminster, Devon, U.K.), the probes being placed adjacent to the strain gauge on the forearm and on the thenar eminence of the hand.

Data were collected on a Macintosh computer with Labview 2 (version 2.2, National Instruments Corp., Austin, TX, U.S.A.). Mean arterial pressure (MAP) and heart rate (HR) were computed beat by beat from the pressure trace and displayed continuously with FCRCF and HCRCF. FBF was calculated by the computer from the initial slope of the increase in forearm circumference over 3 to 4 heart beats after venous occlusion: the slope was determined manually by aligning two markers on the computer monitor along the recording of strain-gauge output. Calibration of the FBF recording was performed on the arm at the beginning and end of each session. Total FVR, cutaneous vascular resistance in the forearm and hand (FCVR and HCVR respectively) were calculated as MAP divided by FBF, FCRCF or HCRCF respectively.

Protocol

The protocol was explained to all subjects and they gave their informed consent. The study was conducted in accordance with the Declaration of Helsinki (1989) and was approved by the Ethical Committee of the University Hospital of the West Indies.

The protocol was that used in our U.K. studies [9, 10]. The SS patients and AA subjects were asked not to drink tea, coffee or alcohol, smoke or engage in heavy physical exercise on the morning of the study. Throughout the protocol the right hand was immersed to the wrist in water at a thermoneutral temperature of 33°C except when it was transferred to a second water bath containing cool water at 16°C (see below). There was an initial equilibration period of 30 min, which allowed the recorded variables to stabilize so that successive recordings varied by less than approx. 5%. Then, three measurements were made at 2 min intervals of each of the recorded variables: MAP, HR, FCRCF and HCRCF were recorded immediately before the sphygmomanometers were inflated for measurement of FBF. After a random interval of 0.5–3 min, the right hand was transferred from the thermoneutral bath to the bath containing cool water for 2 min. A measurement was made of all variables within the last 30 s of the cool immersion as just described. The hand was then returned to the thermoneutral bath and three more sets of measurements were made at 2 min intervals. This protocol of cool immersion after a randomized time interval, followed by measurements made during the recovery period, was repeated for a further five cycles.

At the end of the experimental session, each subject was weighed on a lever balance to the nearest 0.1 kg while wearing light trousers, but no shoes. Height was measured with a standing stadiometer accurate to 0.1 cm. Forearm skinfold thickness at the site of the strain gauge was measured to the nearest 0.1 cm using skinfold calipers and forearm circumference was measured at the same site with a standard tape measure. Each subject also answered a short questionnaire to determine their association of pain with cooling: they were asked to indicate whether they never, sometimes, usually or always experienced pain when cold.

Haematological indices were measured in a Coulter Counter (Coulter S plus 4; Coulter Electronics Inc., Hialeah, FL, U.S.A.). Packed cell volume was determined as microhaematocrit by centrifugation; mean cell haemoglobin and mean cell haemoglobin concentration was determined by calculation. HbF was measured by alkali denaturation [18].
Statistical analyses

For the anthropometric and haematological data, mean values ± SEM were calculated for SS patients and AA controls, except in the case of forearm skinfold thickness which was not normally distributed and is therefore expressed as median (range). For cardiovascular data, the averages of the first three resting values for each variable were used as baseline values. For SS patients and AA controls, MAP, HR, FBF and HCRCF were normally distributed and were therefore expressed as means ± SEM; FVR, FCRCF, FCVR and HCVR were not normally distributed and so were expressed as median (range). Comparisons between the baseline values of SS patients and AA subjects were made using Student's unpaired t-test; for the variables that were not normally distributed this was done after log transformation.

Comparison within SS patients and within AA subjects of the baseline values recorded before the first and sixth cool immersion was made by Student’s paired t-test and showed that the baseline of some variables changed over this time (see below). Thus, the changes evoked in all variables by the cool immersions were expressed in absolute terms, rather than as a percentage of baseline. Within SS patients and AA subjects, comparisons between baseline and values recorded at the second minute of cool immersion were made by Student’s paired t-tests, while comparisons of changes between groups were made by Student’s unpaired t-test.

Multivariate regression was used to compare the baseline values and the changes evoked in all variables by cool immersion within the six subgroups of SS patients.

Determination of painful crisis history

Painful crisis was defined as bone pain severe enough to require a clinic visit and prescription of a narcotic analgesic. Multiple visits within a 14 day period were counted as a single event. Painful crisis frequency was calculated from clinic notes as crises/year over the 10 years preceding the study in 49 SS patients and over 6.7 ± 0.7 years in the remaining patients who did not have 10 years of observation. The incidence rate of painful crisis per year was calculated as the number of patients who had painful crises during the period of observation divided by the total crisis-free time before the first crisis. The Sickle Cell Clinic is the primary health provider for these patients, and on each visit specific enquiry is made about episodes of bone pain since the last visit. It is therefore reasonable to assume that the data are complete and reliable.

The proportions of AA subjects and SS patients who showed net forearm vasoconstriction in response to cooling (see below) and the incidence rate of painful crisis in SS patients who showed forearm vasoconstriction and dilatation (see below) were compared by χ² test, while pain crisis frequency and direction of forearm vascular response were compared by Mann–Whitney test. For all statistical analyses, P < 0.05 was taken as significant.

RESULTS

The haematological data for the SS patients and AA subjects (see Table 2) confirmed the lower erythrocyte count, haemoglobin concentration and haematocrit expected in SS patients. The anthropometric data showed the SS and AA subjects were of similar height, but the SS patients had lower body weight, forearm circumference and forearm skinfold thickness (Table 3). Baseline HR, FBF, HCRCF and FCRCF were higher, while FVR, FCVR and HCVR were lower in SS patients than in AA subjects (Table 4).

Responses evoked by repeated cooling

The changes evoked in all variables in response to the first cool immersion for SS patients and AA subjects are shown in Fig. 1. In SS patients, there were

<table>
<thead>
<tr>
<th>Variable</th>
<th>AA (n = 30)</th>
<th>SS (n = 60)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>174.0 ± 1.1</td>
<td>174.6 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.3 ± 1.6</td>
<td>55.5 ± 1.3</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Circumference forearm (cm)</td>
<td>26.3 ± 0.3</td>
<td>24.4 ± 0.3</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Forearm skinfold thickness (mm)</td>
<td>4.0 (3.1–5.6)</td>
<td>3.7 (2.6–5.4)</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Table 2. Comparison of haematological variables in SS patients and AA subjects. Values are expressed as means ± SEM. P values indicate significant difference between AA subjects and SS patients; NS, no significant difference.

Table 3. Comparison of some anthropometric variables in SS patients and AA subjects. Values are expressed as means ± SEM or median (range). P values indicate significant difference between AA subjects and SS patients; NS, no significant difference.
Table 4. Comparison of baselines of cardiovascular variables in SS patients and AA subjects. Values indicate significant difference between AA subjects and SS patients; NS, not significantly different. Values are expressed as mean ± SEM or median (range). pu, perfusion unit.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AA (n = 30)</th>
<th>SS (n = 60)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>85.9 ± 1.8</td>
<td>83.1 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>61.7 ± 2.2</td>
<td>68.8 ± 1.2</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>FBF (ml min(^{-1}) 100 ml(^{-1}))</td>
<td>2.4 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>HCRCF (pu)</td>
<td>136.4 ± 23.4</td>
<td>209.1 ± 15.3</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>FVR (mmHg ml(^{-1}) min(^{-1}) 100 ml(^{-1}))</td>
<td>5.7 (10.0–33.3)</td>
<td>23.5 (7.0–72.0)</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>FCVR (pu)</td>
<td>5.7 (10.0–33.3)</td>
<td>23.5 (7.0–72.0)</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>FCVR (mmHg/pu)</td>
<td>14.7 (2.5–106.3)</td>
<td>3.9 (1.0–12.5)</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>HCVR (mmHg/pu)</td>
<td>1.0 (0.2–37.7)</td>
<td>0.5 (0.2–7.7)</td>
<td>( P &lt; 0.001 )</td>
</tr>
</tbody>
</table>

highly significant changes from baseline in all variables except FCRCF. Thus, they showed an increase in MAP and HR, while FVR, FCVR and HCVR also increased, indicating respectively, net vasoconstriction in total forearm circulation (skin and skeletal muscle) and in the skin of the forearm and hand. Reflecting this vasoconstriction, FBF and HCRCF decreased. AA subjects tended to show the same pattern of response, but the changes were less pronounced; they showed a significant rise in MAP, no change in HR, an increase in FVR and HCVR, but no change in FCVR. Comparisons of the changes between genotypes showed that the change in FBF was greater in SS patients than in AA subjects (\( P < 0.05 \)), while the change in HCVR was greater in AA subjects (\( P < 0.01 \)).

Between the first and sixth cool stimulus the baseline levels of MAP, FVR, FCVR and HCVR increased significantly in SS patients, indicating long-lasting vasoconstriction in the forearm as a whole and in the skin of the forearm and hand. This was accompanied by decreases in FBF, FCRCF and HCRCF, while HR fell significantly. In contrast, in AA subjects there was a significant increase in baseline MAP, but of the regional vascular resistances, only baseline FVR increased significantly (Fig. 2). Despite the changes in baselines, the SS patients showed similar responses to the sixth cool immersion as to the first immersion, except HCVR did not change significantly in the sixth immersion (Fig. 1). In contrast, in the AA subjects, only HCRCF still showed a significant decrease in response to the sixth cool stimulus (Fig. 1).

The descriptions given so far concern the mean data for the SS and AA groups. However, within each genotype, some individuals showed an increase in FVR in response to the first cool stimulus, while others showed a decrease in FVR to the first stimulus even though the direction of change in the other variables was comparable in the group as a whole. As in our previous studies [9, 10], we have termed these groups ‘constrictor’ and ‘dilator’ respectively. As found previously, there was no indication that the direction of the change in FVR was dependent on whether the recording was made from the dominant or non-dominant side (J. Mohan and J. M. Marshall, unpublished work). Of the 60 SS patients, 50 showed an increase in FVR to the first immersion and only 10 showed a decrease, while among the AA subjects, 18 showed an increase and 12 showed a decrease in FVR. The proportion of SS patients in the ‘constrictor’ group (83%) was significantly greater than the proportion of AA subjects in the ‘constrictor’ group (60%; \( P = 0.03, \chi^2 \) test).

In the ‘constrictor’ group of SS patients, baseline FVR increased between the first and sixth immersion, but the sixth cool immersion still evoked a significant increase in FVR from the new baseline (Fig. 3). The ‘dilator’ group of SS patients showed no significant change in baseline FVR between the first and sixth immersion, but in the sixth immersion there was a significant increase in FVR, indicating the net forearm vasodilatation had reversed to net forearm vasoconstriction. Similarly, the ‘constrictor’ group of AA subjects showed an increase in baseline FVR between the first and sixth immersion, but unlike the ‘constrictor’ group of SS patients, the change in FVR evoked by the sixth immersion did not reach significance (Fig. 3). The ‘dilator’ group of AA subjects showed no change in baseline FVR between the first and sixth immersions, but in contrast to the ‘dilator’ group of SS patients they showed no significant change in FVR in response to the sixth cool stimulus; some of these AA subjects still showed a net decrease in FVR.

Association between response to cooling and other factors

There was no correlation in either SS patients or AA subjects between baseline FVR and the direction of change in FVR in response to the first cool stimulus (Fig. 3). Furthermore, there was no relationship between forearm circumference or skinfold thickness and whether the subject (SS or AA) showed net forearm constriction or dilatation in response to the first immersion. Thus, in SS patients, forearm circumference was 24 ± 0.31 and 24.87 ± 0.81 cm in the ‘constrictor’ and ‘dilator’ groups respectively, and in the AA subjects it was 25.92 ± 0.42 and 26.85 ± 0.49 cm respectively. Forearm skinfold thickness was 3.73 ± 0.08 and 3.86 ± 0.21 mm in the ‘constrictor’ and ‘dilator’ groups of SS patients, and 3.90 ± 0.09 and 4.36 ± 0.22 mm respectively in the AA subjects.

There were also no significant relationships between the baselines of the haemodynamic variables and the levels of Hb and HbF in the six haematological subgroups of SS patients. Nor were there any relationships between the change in FVR evoked by the first cool immersion and the level of Hb or HbF. Thus, the mean change in FVR in those with low HbF was 5.6 ± 1.3, 6.8 ± 2.8 and 9.4 ± 2.6.
mmHg ml⁻¹ min⁻¹ 100 ml⁻¹, in the low, medium and high Hb subgroups respectively, while the change in FVR in those with high HbF was 16 ± 14, 1.4 ± 0.6 and 3.9 ± 2.6 mmHg ml⁻¹ min⁻¹ 100 ml⁻¹ in the low, medium and high Hb subgroups respectively. There were 0, 4 and 1 'dilators' in the low

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**Fig. 1.** Cardiovascular responses evoked in AA subjects and SS patients by the first and sixth cool stimulus. Columns show mean change from baseline (± SEM) at the second minute of cooling. Open columns, AA subjects; shaded columns, SS patients. Left and right hand side, first and sixth cool stimulus respectively. Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001 compared with baseline.
HbF subgroups respectively and 2, 1 and 1 'dilators' in the high HbF subgroups respectively.

Vascular responses and painful crisis

In the 'constrictor' group of SS patients, 29/50 (58%) reported by questionnaire that they normally experienced pain in response to cooling, compared with 4/10 (40%) of the dilator group of SS patients (not significant, \( \chi^2 \) test). Thus, there was no obvious association between the perception of pain on cooling and the vascular response. However, the objective analysis of painful crises revealed a notable correlation with the vascular response. Thus, the total number of painful crises recorded in the clinic notes over the period of observation was 168, and of these 160 occurred in the 'constrictor' group and 8 occurred in the 'dilator' group, these occurring in 38 of the 50 'constrictors' and in 4 of the 10 'dilators'. The frequency of painful crises for the 'constrictors' was 0.36±0.10/year and this was 3-fold greater than the frequency for the 'dilators' (0.12±0.06/year; Mann–Whitney test, \( P = 0.04 \)). The incidence rate (per 100 years) of painful crisis in the 'constrictors' (12.65) was more than 2-fold that in the 'dilators' (5.46); this difference did not reach statistical significance (\( P = 0.08, \chi^2 \) test).

The number of painful crises per year in those with low HbF was 0.25±0.1, 0.19±0.08 and 0.66±0.35 in the low, medium and high Hb subgroups respectively, and in those with high HbF, there were 0.30±0.14, 0.08±0.04 and 0.29±0.1 crises per year in the low, medium and high Hb subgroups respectively. The group with the highest Hb and lowest HbF tended to have the highest painful crisis frequency, but any differences in painful crisis

![Fig. 2. Changes in cardiovascular baselines between the first and sixth cool stimulus in AA subjects and SS patients. Graphs show mean values ± SEM recorded for each variable before the first and before the sixth cool stimulus as indicated below the graphs. ■, AA subjects; ○, SS patients. In some cases SEM is within symbol. *P < 0.05, **P < 0.01, ***P < 0.001: significant differences between values recorded before first and sixth cool stimulus.](image-url)
frequency among the six subgroups or associations between absolute change in FVR and painful crisis frequency did not reach statistical significance.

**DISCUSSION**

The results of the present study have confirmed and extended the findings of our previous studies [9, 10, 17]. In particular, the present study indicates that SS patients are more likely than AA subjects to show net vasoconstriction in the forearm when a novel, but mild, cool stimulus is applied to the contralateral hand. The results also indicate that those SS patients who show net forearm vasoconstriction in response to cooling have a 3-fold greater frequency of painful crisis than those SS patients who show net forearm vasodilatation. This strengthens the view that the avascular necrosis that characterizes the painful crises can be triggered by the reflex vasoconstrictor response to cooling [10]. In the discussion that follows we have elaborated on these points and have considered some of the factors that may be responsible for the associations we have observed.

In our U.K. study [10], in our recent Jamaican study [17] and in the present study, peripheral vascular resistances recorded in the forearm vasculature as a whole (skin plus skeletal muscle) by plethysmography and in forearm and hand skin by laser Doppler flowmetry were lower in SS patients than in

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![Graphs showing changes in FVR](image-url)

**Fig. 3.** Changes in FVR evoked by the first and sixth cool stimulus in the 'constrictor' and 'dilator' groups of AA subjects and SS patients. Each column shows mean ± SEM of FVR before (open columns) and during (shaded columns) the first and sixth cool stimulus as indicated below columns (1, 6). Top, FVR values in whole group; middle, FVR values in 'constrictors'; bottom, FVR values in 'dilators'. Significant values are shown for the 'constrictor' or 'dilator' groups for the first cool stimulus because we selected the groups (see text). Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001 compared with control; ††P < 0.01, †††P < 0.001 compared with baseline.
AA subjects. This peripheral vasodilatation may be a compensatory response to anaemia in general or to sickle cell anaemia in particular [6, 17]. Our recent Jamaican study [17] and the present study demonstrated that the peripheral vascular resistances were lower and the peripheral blood flows were higher in both SS patients and AA subjects than they were in the U.K. [10] even though all measurements were made under comparable laboratory conditions, when the subjects had equilibrated in a room at 24°C for at least 30 min. The simplest explanation for these discrepancies is that in Jamaica, both the SS patients and AA subjects were showing a long-lasting peripheral vasodilatation in response to the much higher temperatures outside of the laboratory.

Despite the differences in the baseline values in the U.K. and Jamaica, a first immersion of one hand in cool water produced comparable responses in the AA subjects of the present study and of the U.K. study: a rise in MAP, net vasoconstriction in the forearm and in the cutaneous circulation of the contralateral hand. Moreover, just as in our U.K. study, the AA subjects could be divided into a ‘constrictor’ group who showed a net increase in FVR and a ‘dilator’ group who showed a net decrease in FVR. Since the increase in cutaneous vascular resistance of the forearm was small, we can conclude that the net increase and decrease in FVR in these groups reflected vasoconstriction and vasodilatation respectively in the underlying skeletal muscle. Since in the ‘dilator’ group, the net decrease in FVR had disappeared by the sixth cool immersion, it is reasonable to conclude that they showed a predominating muscle vasodilatation of the alerting response which is evoked by a novel stimulus and habituates on repetition [10, 11]. A new finding of the present study was that the ‘constrictor’ group of AA subjects showed an increase in baseline FVR between the first and sixth cool immersion. This suggests the muscle vasoconstrictor response to cooling was not only larger in magnitude in these subjects, but persisted between stimuli. This may have been more obvious in the present study than in our U.K. study simply because the vasculature was relatively more dilated (see above).

The whole group of SS patients showed a very similar pattern of response to cooling to that recorded in SS patients in our U.K. study: a rise in MAP and HR with strong vasoconstriction in the forearm vasculature as a whole and in the skin of the forearm and hand. That 10 of the 60 SS patients in the present study showed net vasodilatation in the forearm whereas the remainder showed net forearm vasoconstriction, is inconsistent with our finding in the U.K. study that all SS patients showed net forearm vasoconstriction, for the latter was carried out on only 12 SS patients. Rather, the present study indicates that a minority of SS patients can behave just like up to 50% of AA subjects and show the muscle vasodilatation of the alerting response to a novel cool stimulus. A larger number of patients in the present study now allows the firm conclusion that the proportion of individuals who show net forearm vasoconstriction in response to mild cooling is significantly greater in SS patients than in AA subjects. The important question, from a physiological and pathological point of view, is why should this be?

It might be that the muscle vasodilatation of the alerting response is less readily evoked in SS patients. This is unlikely since, in response to a novel sound stimulus, net forearm vasodilatation was just as common and just as large in magnitude in SS patients and in AA subjects [15, 20]. A second possibility is that mild cooling is less likely to provide an adequate stimulus to the brainstem regions that mediate the alerting response in SS patients. This is very unlikely as, in our previous study [10], SS patients gave the cool stimulus a high score for unpleasancess on a rising scale of 0 to 10 whereas AA subjects gave it a zero score. Moreover, in the present study, over 50% of the SS patients reported by questionnaire that they usually felt pain when cold. Thus, by association they had good reason to perceive the experimental cool stimulus as noxious.

The third and most likely possibility is simply that the vasoconstrictor response to cooling is generally stronger in SS patients and is even exaggerated relative to that seen in the ‘constrictor’ group of AA subjects. The very fact that muscle vasoconstrictor response to cooling predominated over the muscle vasodilatation of the alerting response in the whole group of SS patients from the first through to the sixth immersion, is consistent with this idea. So too is the fact that in the whole group of SS patients, the baseline total vascular resistance in the forearm and cutaneous vascular resistance of the forearm and hand all increased significantly between the first and the sixth immersion, suggesting the vasoconstrictor responses were generally more persistent in the SS patients. Moreover, despite these increases in baseline, the SS patients still showed further increases in FVR, FCVR and HCVR in response to the sixth cool immersion, whereas this was not the case for the whole group of AA subjects. Furthermore, if we consider separately the ‘constrictor’ and ‘dilator’ groups, the ‘constrictor’ group of SS patients still showed an increase in FVR in response to the sixth cool immersion despite the substantial increase in baseline FVR, while in the ‘dilator’ group the decrease in FVR reversed to a significant increase in FVR by the sixth immersion: neither was the case in the AA subjects for their forearm vascular responses to the sixth immersion were smaller and variable in direction.

The conclusion that the vasoconstrictor response to mild cooling is greater in SS patients than in AA subjects apparently contrasts with the published results on the cold pressor test (see Introduction). However, the results of previous studies may have been affected by the inclusion of patients with other haemoglobinopathies, the sickle cell trait and/or by
inclusion of female SS patients (see Introduction). Moreover, if the SS patients found the more severe cooling of the cold pressor test more noxious than the AA subjects (see above), it could be that the muscle vasodilatation of the alerting response more effectively competed with the vasoconstrictor response to cooling in the SS patients.

The vasoconstrictor response to mild cooling may be stronger in SS patients than in AA subjects because they have less insulating subcutaneous fat. Although there was no significant correlation between the skinfold thickness on the forearm and the change in FVR evoked by the first cool immersion, it was generally the case that the skinfold thickness in the forearm and other body regions [17] was substantially smaller in SS patients than in AA subjects. The SS patients may therefore be more vulnerable to the local effect of cooling upon peripheral thermoreceptors and to any consequential response to cooling in the SS patients.

That there was no obvious correlation between the change in FVR and the concentration of Hb and HbF among the SS patients is not surprising in view of the discussion above, for there is no reason to suppose that either factor would affect the extent of body cooling induced by the stimulus, or the reflex response to it. However, the tendency for the sub-group with the highest Hb and low HbF to have the highest frequency of painful crises is consistent with previous reports that relatively high Hb is a risk factor for the painful crisis and that high HbF offers some degree of protection (see Introduction and [12, 14]). Indeed, our new finding that the frequency of painful crises is higher among those who showed an increase in FVR in response to mild cooling raises the question of whether there is a causal link between reflex vasoconstriction, high Hb, low HbF and painful crises. If strong vasoconstriction in skin and muscle in response to cooling is accompanied by reflex vasoconstriction in active bone marrow and a decrease in bone blood flow [10], a reasonable hypothesis is that the risk of sickling and vaso-occlusion leading to necrosis and pain is greater in those SS patients who have the highest concentration of erythrocytes, but who do not have a high level of HbF to inhibit polymerization of haemoglobin S [21]. An extension of this hypothesis would be that any situation that leads to a strong reflex increase in sympathetic activity to skeletal muscle and skin carries a greater risk for initiating the painful crisis in SS patients who have high Hb and low HbF. The practical extension of the present study is that a simple test of the forearm vascular response to mild cooling may identify those SS patients who are at greatest risk of painful crisis after skin cooling.

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