Orthostatic challenge reveals impaired vascular resistance control, but normal venous pooling and capillary filtration in familial dysautonomia

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ABSTRACT

Patients with familial dysautonomia (FD) frequently have profound orthostatic hypotension without compensatory tachycardia. Although the aetiology is presumed to be sympathetic impairment, peripheral vascular responses to orthostasis have not been assessed. The aim of this study was to evaluate the control of vascular responses to postural stress in FD patients. Measurements of heart rate, blood pressure, cardiac stroke volume and cardiac output (CO), by impedance cardiography, and calf-volume changes, by impedance plethysmography, were taken from nine FD patients and 11 control subjects while supine and during head-up tilt. During leg lowering, we also assessed the venoarteriolar reflex by measuring skin red-cell flux. Head-up tilting for 10 min induced sustained decreases in mean arterial pressure in the FD patients, but not in the controls. Total peripheral resistance (TPR, i.e. mean arterial pressure/CO) increased significantly in the controls (39.8 ± 6.8%), but not in the FD patients. Calf-volume changes during tilting, when normalized for the initial calf volume, did not differ significantly between the patients (4.62 ± 1.99 ml/100 ml−1) and the controls (3.18 ± 0.74 ml/100 ml−1). The vasoconstrictor response to limb lowering was present in the patients (47.7 ± 9.0% decrease in skin red-cell flux), but was impaired as compared with the controls (80.7 ± 3.4%) (P < 0.05). The impaired vasoconstriction during limb lowering and absent increase of TPR during tilting confirm that orthostatic hypotension in FD is due primarily to a lack of sympathetically mediated vasoconstriction without evidence of abnormally large shifts in blood volume towards the legs during orthostasis. This may be due, in part, to a preserved myogenic response to increased vascular pressure in the dependent vascular beds.

INTRODUCTION

Orthostatic intolerance due to autonomic nervous system dysfunction is a common disorder that severely affects the everyday lives of patients [1]. Familial dysautonomia (FD), or Riley–Day syndrome, is a rare disease, but it can be considered as a model of sympathetic dysfunction with orthostatic dysregulation during postural challenge. FD is an autosomal recessive disorder that affects the development and survival of sensory and sympathetic neurons [2,3]. In FD, sympathetic failure can be attributed to a decrease in peripheral sympathetic fibres.
and neuronal somas, for example in cervical and thoracic sympathetic ganglia, to 27–37% of the normal number [2,4,5]. FD differs from other autonomic disorders, such as pure autonomic failure, in that there are frequent episodes of supine hypertension, which possibly reflects sympathetic over-activity [6]. Patients with FD have enhanced plasma noradrenaline levels when supine, but no increase when upright [7,8]. Although a deficient sympathetic response to orthostasis is a common finding in FD [7], to date, there have been no elaborate assessments regarding the regulation of the peripheral blood vessels in FD patients.

Even in healthy subjects, the change from a horizontal to an upright posture places considerable stress on the cardiovascular system owing to pooling of blood in the veins of the dependent parts of the body [9] and an enhanced capillary filtration rate [10]. Baroreflex-mediated sympathetic activation during orthostatic stress normally results in an increase in peripheral vascular resistance, which helps to maintain blood pressure [11] and limits the extent of fluid loss from the blood vessels [12]. Furthermore, vasoconstriction in the legs is enhanced by the venoarteriolar reflex (VAR). This is a local axon reflex that responds to stretching of the venous wall by arterial vasoconstriction. The VAR is dependent on the presence and integrity of peripheral sympathetic nerve fibres; the reflex persists after acute sympathectomy, but is absent post-sympathectomy when the fibres have time to degenerate [12–16]. It has been estimated that up to 45% of the total peripheral resistance (TPR) response to orthostatic stress is due to the VAR [15].

One striking manifestation of FD is postural hypotension without compensatory tachycardia. FD patients lack appropriate catecholamine responses to orthostasis, which indicates sympathetic impairment [7,8]. Although impaired arteriolar vasoconstriction is probably the main cause of the orthostatic hypotension, excessive venous pooling and capillary filtration in the blood vessels of the lower body might also contribute. Enhanced rates of capillary fluid filtration have been associated with poor orthostatic tolerance in endurance runners and in some patients with posturally related syncope [10,17]. In FD, impairment of the baroreflex response to orthostatic hypotension has been described [18]; however, there is no direct evidence that sympathetic impairment causes an increased rate of capillary fluid filtration in the legs. Measurements of changes in leg volume in FD patients during orthostasis might clarify the role of fluid pooling in the legs, and lead to more specific and simple therapeutic interventions to counteract orthostatic hypotension, such as physical countermeasures, e.g. leg crossing or muscle pumping, [1] or compressive elastic stockings.

The aim of the present study was to determine the role of the vascular resistance response and the influence of the VAR, as well as blood-volume shifts to the legs in FD patients during orthostatic challenge. We therefore measured changes in total peripheral vascular resistance, as well as leg volume changes, during head-up tilting in FD patients.

**METHODS**

Nine FD patients (four women and five men; mean age 28 ± 3 years) and 11 healthy, age-matched control subjects (four women and seven men; mean age 26 ± 1 years) participated in the study. The mean height of the FD patients was 155 ± 4 cm, while that of the controls was 178 ± 2 cm. The mean body mass of the FD patients was 50 ± 3 kg, while that of the controls was 71 ± 4 kg. The mean body-surface area, calculated according to the DuBois Formula \((0.007184 \times \text{height}^{0.725} \times \text{weight}^{0.425})\), was 1.46 ± 0.06 m\(^2\) in the FD patients and 1.87 ± 0.05 m\(^2\) in the controls \((P < 0.01)\).

All FD patients were ambulatory and were tested during their annual routine examination at the Dysautonomia Treatment and Evaluation Center, New York University School of Medicine. Among the criteria used to establish the diagnosis of FD were Ashkenazi Jewish ancestry, absent or diminished deep tendon reflexes, absence of overflow tears, absence of fungiform papillae of the tongue, and absence of an axon flare-response after intradermal histamine injection [3,19,20]. Furthermore, many patients exhibited signs frequently seen in FD, such as delayed development, failure to thrive, decreased corneal reflex, increased sweating, postural hypotension, skin blotching and hypertensive crises associated with stress [3,19,20]. None of the FD patients had compromised renal function. All FD patients had typical mutations of the IKKBAP gene, as described previously [21].

Controls were selected from among the staff and students of the University of Erlangen-Nuremberg. None of them had clinical signs or symptoms of autonomic dysfunction, orthostatic intolerance or any other condition that affects the cardiovascular system. Controls and patients were asked to refrain from caffeine and alcohol for 24 h prior to the procedures, and to only have a light breakfast at least 3 h before the measurement. They were instructed to drink normally before the tests. Only patients who were able to discontinue their drug treatment (fludrocortisone or midodrine) for 18 h before testing were included in the study. None of the participants were taking any other medication affecting cardiovascular control or the autonomic nervous system.

Studies were carried out in a room in which the temperature was maintained at a comfortable level \((22 ± 1 °C)\).

The study protocol was approved by the Institutional Review Board of New York University School of
Procedures

Orthostatic stress testing

After giving an initial history and undergoing a full neurological examination, subjects were introduced to the laboratory and underwent at least 35 min of supine rest. Then, we continuously recorded heart rate with a 5-lead ECG and measured non-invasive blood pressure by radial arterial tonometry at the wrist (Colin-Pilot™, Colin Medical, San Antonio, TX, U.S.A.). Cardiac stroke volume was measured by impedance cardiography (Cardioscreen, Medis GmbH, Ilmenau, Germany), using electrodes attached to the neck and thorax. This technique has been described previously [22,23] and has been extensively validated against echocardiographic methods [24,25] and the invasive thermodilution and direct Fick methods [26,27].

Calf-volume changes were determined using impedance plethysmography [10], a technique based on the fact that when the fluid volume of a tissue segment increases, its electrical impedance decreases proportionately [28]. Recording electrodes were placed on the lateral malleolus and lateral aspect of the knee to define a calf segment. To calculate the initial volume of the calf, we measured the length and made measurements of the circumference at 3-cm intervals [10]. An impedance monitor (Cardioscreen, Medis GmbH, Ilmenau, Germany) supplied a 1-mA, 50-Hz current, which was passed between electrodes placed 5 cm outside the recording electrodes. Changes in the impedance of the calf segment enclosed by the recording electrodes were measured and stored for analysis.

Recordings were made during a 5-min baseline period at supine rest and then during 10 min of head-up tilting at 60°. A strap was placed across the knees of the subjects in order to provide support and to avoid activation of the muscle pump of the legs by movement, which might affect the measured responses. Blood pressure and heart rate were continuously assessed online so that the test could be terminated if there were any signs of impending syncope.

VAR

A laser Doppler probe (Perimed™, Stockholm, Sweden) was placed at the distal, dorsal part of the right foot and superficial skin red-cell flux was measured in arbitrary perfusion units (p.u.). After a 5-min baseline recording in the supine position, the VAR was elicited by lowering the foot to 40 cm below heart level for 5 min and the maximum decrease in skin red-cell flux recorded. The VAR was calculated as the percentage decrease in skin red-cell flux compared with the baseline value [29]. This procedure was repeated once and the average value was taken as the VAR index [29].

Data acquisition and analysis

The signals of electrocardiographic R–R interval and arterial pressure were digitized at a sampling rate of 300 Hz, fed to a computer and stored for off-line analysis. A computer program identified the peak of each R wave and constructed time series of R–R interval, systolic, mean and diastolic blood pressure. The impedance cardiography and leg impedance signals were fed to a second computer and analysed using commercially available software (Multiscreen 4.1, Medis GmbH, Ilmenau, Germany) to obtain beat-to-beat stroke volume values. Cardiac output (CO) was calculated as the product of heart rate and stroke volume. For each subject, we calculated the cardiac index as the cardiac output per square metre of body surface. TPR was calculated as mean arterial pressure (MAP) divided by CO.

Calf-volume changes were calculated from the relative changes in calf impedance [10]. To account for variations in calf size among the patients and controls, we normalized the calf-volume changes by expressing them as changes per 100 ml of baseline calf volume, i.e.

\[
\text{normalized calf-volume change} = \frac{\text{volume change}}{\text{initial calf volume}} \times 100
\]

Head-up tilting typically results in an initial rapid increase in the volume of the calf, which is mainly due to venous pooling, followed by a slower, almost linear increase due to capillary fluid shifts [10]. For each subject, we applied linear regression to the slow volume change between the 4- and 10-min timepoints of head-up tilting and took the net capillary filtration rate as the slope of this line. To calculate the initial volume change due to venous pooling, we reverse-extrapolated the regression line and noted the value at the start of head-up tilting.

Statistical analysis

All values are reported as means ± S.E.M., unless otherwise stated. Comparisons of the responses between controls and FD patients were made using Student’s t tests, or by Mann–Whitney U test when the assumptions for parametric testing were not fulfilled. A significance level of \( P < 0.05 \) was applied.

RESULTS

In the supine position, CO values were significantly lower \( (P < 0.01) \) in the FD patients than in the controls, but supine heart rate was significantly higher \( (P < 0.01) \) in the FD patients compared with the control group (Table 1). Resting cardiac index (CO/body-surface area) and supine arterial blood pressures did not differ significantly between the two groups (Table 1).
Table 1  Haemodynamic parameters recorded while supine and in the final minute of head-up tilting
BP, blood pressure; TPR, total peripheral resistance; HUT, head-up tilt. *P < 0.05, †P < 0.01 (controls versus FD patients); ‡P < 0.01 (supine versus HUT).

<table>
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<tr>
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<th>Controls Supine</th>
<th>HUT</th>
<th>FD patients Supine</th>
<th>HUT</th>
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<tbody>
<tr>
<td>Heart rate (beats \cdot min^{-1})</td>
<td>61.4 ± 1.8</td>
<td>82.0 ± 2.9†</td>
<td>71.8 ± 1.9†</td>
<td>78.3 ± 2.4†</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>137 ± 8</td>
<td>74 ± 5†</td>
<td>78 ± 10†</td>
<td>57 ± 6</td>
</tr>
<tr>
<td>CO (l \cdot min^{-1})</td>
<td>8.32 ± 0.42</td>
<td>5.98 ± 0.32†</td>
<td>5.52 ± 0.71†</td>
<td>4.43 ± 0.46*</td>
</tr>
<tr>
<td>Cardiac index (l \cdot min^{-1} \cdot m^{-2})</td>
<td>4.47 ± 0.25</td>
<td>3.20 ± 0.16†</td>
<td>3.79 ± 0.48</td>
<td>3.02 ± 0.27</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>119 ± 4</td>
<td>111 ± 4</td>
<td>121 ± 8</td>
<td>83 ± 6†</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>65 ± 2</td>
<td>69 ± 3</td>
<td>71 ± 7</td>
<td>47 ± 6†</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>83 ± 3</td>
<td>83 ± 3</td>
<td>85 ± 8</td>
<td>58 ± 6†</td>
</tr>
<tr>
<td>TPR (arbitrary units)</td>
<td>10.2 ± 0.5</td>
<td>14.1 ± 0.7†</td>
<td>16.8 ± 2.1†</td>
<td>13.8 ± 1.6</td>
</tr>
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During head-up tilting (Figure 1), the two groups showed similar relative decreases in cardiac index (FD patients, 15.84 ± 6.37% versus controls, 27.81 ± 2.27%); however, the FD patients showed an immediate and sustained decrease in systolic, mean and diastolic blood pressure (P < 0.01). In contrast, in the controls, the blood pressure during tilting did not differ significantly from when the subjects were supine (Figure 2). No participant had any signs or symptoms of presyncope during the head-up tilt manoeuvre.

By the end of tilting, the heart rate increase from baseline was significantly higher in the controls than the FD patients (20.6 ± 2.3 beats \cdot min^{-1} versus 6.5 ± 1.4 beats \cdot min^{-1} respectively; P < 0.01). TPR increased by 39.8 ± 6.8% (P < 0.01) in the controls, but decreased by 14.4 ± 6.9% (P = 0.07) in the FD patients (Figure 3). TPR was significantly lower in the FD patients than in controls during the entire tilting procedure (P < 0.05).

Calf-volume changes

Initial resting calf volumes were significantly higher in the controls than in the FD patients (2429 ± 233 ml versus 1467 ± 116 ml respectively; P < 0.01). Head-up tilting induced a significant increase in calf volume, which was characterized by a rapid initial increase, followed by a slower and sustained change (Figure 4) that was almost linear, in both the control group (r = 0.97 ± 0.01) and the patient group (r = 0.97 ± 0.01). The calf-volume changes after 10 min of tilting, when normalized for supine calf volume, did not differ significantly between controls and FD patients (3.18 ± 0.74 ml \cdot 100 ml^{-1} versus 4.62 ± 1.99 ml \cdot 100 ml^{-1} respectively; P = 0.47).
Orthostatic responses in familial dysautonomia

**Figure 3** Responses of TPR and heart rate to a 10-min head-up tilting
TPR and heart rate (HR) are indicated as the percentage change from baseline. FD patients had an impaired HR response to tilting, and failed to increase their TPR, in contrast with the large increase observed in the controls. Significant differences between controls and patients are denoted by *(P < 0.05)* and **(P < 0.01)*.

**Figure 4** Time-course of calf-volume increases during 10 min of head-up tilting
To account for the calf-size difference between patients and controls, we normalized the volume changes by expressing them per 100 ml of calf volume. Although the FD patients tended to have a slightly greater increase in calf volume, the difference between controls and patients was not significant.

The rate of capillary filtration, which was calculated as the slope of the linear part of the volume change between the 4- and 10-min timepoints of head-up tilting, was greater in the FD patients \((0.26 \pm 0.18 \, \text{ml} \cdot \text{min}^{-1} \cdot 100 \, \text{ml}^{-1})\) than in the controls \((0.18 \pm 0.04 \, \text{ml} \cdot \text{min}^{-1} \cdot 100 \, \text{ml}^{-1})\), but the difference was not significant \((P = 0.60)\). The extent of venous pooling, which was calculated by reverse-extrapolation of this line to the start of head-up tilting, also did not differ significantly \((P = 0.75)\) between controls \(1.62 \pm 0.48 \, \text{ml} \cdot 100 \, \text{ml}^{-1}\) and FD patients \(1.96 \pm 0.98 \, \text{ml} \cdot 100 \, \text{ml}^{-1}\).

**VAR**
Lowering the right leg by 40 cm for 5 min caused the red-cell flux at the dorsum of the foot to decrease significantly from \(10.54 \pm 2.29 \, \text{p.u.}\) to \(1.50 \pm 0.23 \, \text{p.u.}\) \((P < 0.01)\) in the controls, and from \(9.58 \pm 1.54 \, \text{p.u.}\) to \(3.96 \pm 0.58 \, \text{p.u.}\) \((P < 0.01)\) in the FD patients; however, the VAR index (the percentage decrease in red-cell flux) was significantly lower in the FD patients than in the controls \((47.7 \pm 4.0\%\) versus \(80.7 \pm 1.5\%\) respectively; \(P < 0.01\)).

**DISCUSSION**
During orthostatic challenge, we found that the FD patients were unable to maintain their blood pressure or to generate an adequate reflex tachycardia, which confirmed results from previous studies [8,30]. We demonstrated that there is also a lack of TPR response, yet the FD patients did not develop excessive pooling of blood in the legs or an enhanced fall in CO during tilting.

The failure of FD patients to generate a significant heart-rate response during tilting suggests an impaired sympathetic response, but might also be related to the relative tachycardia displayed by the FD patients when supine. The lack of TPR adjustment suggests that FD patients lack a sufficient efferent vasoconstrictor response. This is consistent with the reported profound reduction in the numbers of sympathetic neurons and vasomotor fibres in FD [2,4,5], as well as the impaired catecholamine response to tilting [7,8]. The partially preserved, albeit reduced, decrease of skin red-cell flux during limb-lowering suggests that the arterial resistance vessels in the dependent regions maintain some ability to constrict in response to an increase in venous pressure. The vasoconstriction during limb lowering might indicate a partial preservation of the VAR in FD; however, this seems unlikely given the sympathetic impairment in FD. A large component (40–50%) of the normal vasoconstrictor response to limb lowering is attributable to a myogenic mechanism, completely independent of any vascular innervation [12–14,16]. Our finding that FD patients showed approx. 50% of the normal vasoconstrictor response to leg lowering fits with the hypothesis that the VAR is absent in FD and that only local myogenic responses contribute to the vasoconstriction.

Although peripheral vasoconstrictor dysfunction might be expected to result in an increase in fluid shifts to the legs during orthostatic stress, the calf volumes of the FD patients increased only slightly more than those of the controls, and changes in cardiac index were actually slightly less in the FD patients. Even in healthy subjects, blockade of the sympathetic ganglia that innervate the lower-body vasculature increases the volume pooled in the legs during orthostasis only by a negligible amount [31]. This finding confirms other studies that failed to
find exaggerated orthostatic pooling of blood in the lower limbs of patients with autonomic dysfunction [32–34]. Evidently, fluid shifts to the legs do not make an important contribution to the pathophysiology of orthostatic hypotension in the FD patients; however, we cannot exclude the possibility that a smaller increase in hydrostatic pressure in the lower limbs of FD patients (due to their smaller size) might also have limited the amount of capillary-fluid loss, compared with control subjects.

Our results are similar to findings in patients with pure autonomic failure (PAF) who, during tilting, show decreases in CO that are similar to those in controls, together with a decrease in TPR and blood pressure [35]. Patients with multiple system atrophy (MSA) also show postural hypotension during tilting, but with unchanged TPR [35]. Chandler and Mathias [35] suggested that the different responses to tilting in PAF and MSA patients might be influenced partly by the site of the lesion – PAF is characterized more by peripheral nerve involvement, while in MSA, the lesion is central [36]. FD is considered to be a disease of peripheral nerve deficiency, and the similarity of our results to findings in PAF patients is in agreement with the suggestion that responses to tilting might be influenced partly by the site of the lesion [35].

There is, however, some evidence, from functional neuroimaging studies, of central nervous involvement in FD [37]. Despite this, FD patients seem able to adapt their cerebral vessels to changes in blood pressure, which possibly indicates preserved central sympathetic activation [30].

The role of discordant peripheral vascular responses and the shift of blood to other vessel beds needs further consideration. Our group showed that FD patients have a preserved, though reduced, ability to increase their blood pressure upon painful cold stimulation [38], despite an absence of pain-induced peripheral vasoconstriction. We concluded that this discrepancy might be due to discordant responses of the peripheral vessels and other vessel beds, particularly the splanchnic bed. The splanchnic circulation is highly compliant and contains approx. 25% of the total blood volume [39]. Normally, there is a baroreflex-mediated sympathetic constriction of splanchnic veins and arterioles [39] during orthostasis, which assures that blood pressure is maintained upon tilting. Splanchnic denervation leads to orthostatic intolerance [40,41]. While the sympathetic dysfunction in FD does not seem to lead to increased fluid pooling in the legs, it is likely that impaired sympathetic innervation of the splanchnic bed partially accounts for the orthostatic hypotension. This assumption is supported by the beneficial effects of abdominal compression on maintaining blood pressure in patients with orthostatic intolerance [42].

Orthostatic hypotension can be alleviated by enhancing arterial vasoconstriction with pharmacological agents such as the α-agonist midodrine [20,43,44]. Although pharmacological pressor agents clearly have a potential role in the treatment of orthostatic hypotension in FD and other disorders, the possible side effects, such as augmentation of recumbent hypertension [45,46], make it desirable to also consider non-pharmacological interventions, such as expansion of blood volume by salt loading [47]. The potential benefits of salt loading, however, should also be weighed against the risks of worsening propensity to supine hypertension [48].

In conclusion, we have shown that in FD patients, orthostatic hypotension is not caused by enhanced pooling of blood in the lower body. Although impaired, there is a local vasoconstriction in response to leg lowering in FD, which might partially protect against oedema in the legs; however, the lack of an overall increase in TPR results in a decrease in orthostatic blood pressure. The findings of this study have implications for the treatment of orthostatic hypotension in FD and other autonomic diseases because they emphasize the importance of the general increase in TPR during orthostasis, even though venous pooling and capillary filtration of fluid to the legs do not seem to contribute to orthostatic intolerance, despite sympathetic impairment.

REFERENCES


