Terminal vessel hyperperfusion despite organ hypoperfusion in familial dysautonomia

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ABSTRACT

Patients with familial dysautonomia (FD) exhibit orthostatic hypotension as well as recumbent hypertension. In addition, during dysautonomic crises, patients have hypertensive blood pressure that is presumed to be secondary to episodic vasoconstriction, as well as swollen hands that are presumed to be secondary to vasodilatation. This discrepancy in vascular control is poorly understood, yet may provide insight into the pathophysiology of autonomic crises. To evaluate the pathological mechanisms of overall blood flow and end-organ perfusion, we assessed resting and post-ischaemic limb and skin blood flow in FD patients. In groups of 15 FD patients and 15 controls, we measured resting and post-ischaemic forearm blood flow using venous occlusion plethysmography, and superficial skin blood flow using laser Doppler flowmetry. At rest, arterial inflow was averaged from eight venous occlusion measurements and expressed as percentage volume change/min. Post-ischaemic plethysmographic inflow was determined from the peak influx during the first venous occlusion following 3 min of ischaemia. Transcutaneous forearm partial pressures of oxygen and carbon dioxide were monitored continuously. At rest, plethysmographic limb perfusion was lower in FD patients than in controls, while skin blood flow did not differ between the two groups. After ischaemia, hyperperfusion of the forearm and hand was less pronounced in FD patients than in controls, while skin blood flow was significantly higher in patients than in controls. Partial pressures of O₂ and CO₂ did not differ between the two groups. We conclude that the reduced overall limb perfusion in patients with FD is due to hypertension-induced structural changes to vessel walls, with an increase in resistance vessel rigidity. The exaggerated post-ischaemic skin perfusion in FD patients seems to be due to deficient sympathetic innervation of precapillary vessels and arteriovenous shunts and to denervation hypersensitivity of intradermal small nerve fibres. Both the reduced limb perfusion and the dysfunctional end-organ blood supply in FD patients are likely to be major contributors to the vasomotor instability observed in these subjects, particularly during periods of stress.

INTRODUCTION

In familial dysautonomia (FD), a rare autosomal recessive disorder that affects the development and survival of sensory sympathetic neurons [1–3], cardiovascular and peripheral vasomotor function are severely impaired [4,5]. Patients exhibit both orthostatic hypotension without compensatory tachycardia and recumbent hypertension. In addition, if physically or emotionally challenged, patients can suffer

Key words: familial dysautonomia, ischaemic stress, laser Doppler skin blood flow, vascular dysregulation, venous occlusion plethysmography.

Abbreviations: BMI, body mass index; FD, familial dysautonomia; PCO₂, partial pressure of carbon dioxide; PO₂, partial pressure of oxygen; PU, perfusion units; SBF, skin blood flow.

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‘dysautonomic crises’, characterized by excessive arterial hypertension, tachycardia, gastrointestinal dysfunction manifested as nausea and retching, and personality changes [4,5]. Although ultrastructural studies revealed an absence of autonomic nerve terminals in the peripheral blood vessels of FD patients [6], clinical observations suggest that there is residual sympathetic vasomotor function in such patients. We recently reported preserved, although severely reduced, vasoconstriction of skin vessels during cold pressor stimulation in FD patients [7]. During dysautonomic crises, arterial hypertension and episodic peripheral vasoconstriction are noted [4–6]. Simultaneously, there is swelling and reddening of the hands, suggesting paradoxical peripheral vasodilatation or tissue oedema during crises [4,5].

The reasons for the discrepancy between the occurrence of high blood pressure, peripheral vasoconstriction, puffy swelling of hands and deficient vasomotor terminals are poorly understood. Elucidation of the underlying pathological mechanisms might, however, contribute to further understanding of the pathophysiology of autonomic crises in FD.

Comparison of overall limb perfusion with the localized, terminal perfusion of a sympathetically innervated end-organ such as the superficial skin might contribute to a refined understanding of the pathophysiology of peripheral perfusion in FD patients. The localized blood flow in the terminal vessel bed of the dermis can be measured by means of laser Doppler flowmetry, while the overall perfusion of a limb can be assessed by venous occlusion plethysmography [8].

To clarify the pathophysiology of peripheral perfusion in FD patients, we therefore monitored simultaneously overall limb blood flow using venous occlusion plethysmography and sympathetically controlled skin perfusion at the index finger pulp using laser Doppler flowmetry under resting conditions and in response to ischaemia, a strong vasodilatory as well as sympathetic stimulus [9].

METHODS

Groups of 15 FD patients (nine women, six men; mean age 29.4 ± 9.9 years) and 15 healthy controls (seven women, eight men; mean age 30.2 ± 8.4 years) participated in the study. Diagnosis of FD was initially established by F. B. A. (Director of the Dysautonomia Treatment and Evaluation Center).

All study participants were non-smokers. The mean height of FD patients was 161 ± 5 cm, and that of controls was 170 ± 3 cm. However, the length of forearm and hand, the segments used for plethysmography, did not differ significantly between patients and controls. Similarly, the forearm circumference was not significantly different between patients and controls. Body mass index (BMI) was slightly, but not significantly, higher in controls (20.9 ± 0.5 kg/m²) than in FD patients (18.9 ± 0.5 kg/m²). Since adequate recruitment of controls is essential and lab and hospital staff might have a different reaction to the laboratory environment in which the procedures were carried out, we only included two members of our team in the study. The remaining 13 controls were recruited from healthy relatives and friends of the participating FD patients.

All patients with FD fulfilled accepted diagnostic criteria, which included Ashkenazi Jewish ancestry, absence of deep tendon reflexes, absence of overflow tears, absence of lingual fungiform papillae and absence of the axon flare response following intradermal histamine injection [10]. All patients had a history of supine hypertension and orthostatic hypotension, as well as episodic gastrointestinal dysfunction and dysautonomic crises. Six patients had required fundoplication and gastrostomy. All patients had the characteristic genetic haplotype, i.e. they were homozygous for the intron 20 mutation on the IKBKA gene [11]. Six patients were on a medication of up to 0.2 mg of fludrocortisone (half-life 3.5 h), five patients were taking up to 25 mg of midodrine (half-life 3–4 h) and four patients were not on any medication known to interfere with autonomic nervous system function. Only patients who were able to discontinue their medication 18 h prior to testing were included in the study.

Study participants were asked not to consume caffeine or alcohol and to stop all medications for 18 h before testing. The protocol was approved by the Institutional Review Board of New York University Medical Center. Informed consent was obtained prior to the study. For individuals less than 21 years of age, signatures were obtained from both the participant and a parent.

The test was performed in a quiet, temperature-controlled laboratory environment. The study participant rested supine for at least 30 min after arrival in the laboratory. During this period, the participant was positioned and the monitoring devices were applied, and the skin temperature was measured. The entire protocol was carried out with the subject in a supine position. All testing procedures were performed between 09.00 and 14.00 hours.

Limb blood flow at rest and after ischaemia was evaluated by means of venous occlusion plethysmography (EC6 Strain Gauge and Photo Plethysmograph; D. E. Hokanson Inc., Bellevue, WA, U.S.A.) [12–14]. A mercury-in-silastic strain gauge connected to a plethysmograph was positioned around the right forearm at the position of greatest circumference. The occlusion cuff was placed around the patient’s right arm, above the elbow. The forearm was positioned above the level of the heart by resting the elbow on foam pads and supporting the hand to ensure adequate emptying during the period of deflation [15]. We measured the change in forearm circumference during venous occlusion obtained
by inflation of the arm cuff to a pressure of 50 mmHg. The occlusion interval was 5 s, and the deflation lasted for 20 s.

The overall inflow of blood into the hand and forearm was expressed as the percentage volume change/min during venous occlusion [14]. Arterial inflow was calculated as the average arterial influx during eight artifact-free measurements. Hokanson NIVP3 Software was used for waveform capture and storage.

Arterial inflow during post-ischaemic hyperaemia was determined immediately after 3 min of ischaemia induced by inflation of the arm cuff to 200 mmHg, i.e. above systolic blood pressure (repeated blood pressure measurements confirmed that none of the study participants had systolic blood pressures exceeding 200 mmHg). After rapid release of pressure, we repeated the venous occlusion measurements as described above. Normally, ischaemia induces a rapid increase in arterial inflow into the limb. To determine the maximal post-ischaemic vasoreactivity, we evaluated the post-ischaemic response immediately after release of the suprasystolic pressure, since reactive hyperaemia is most pronounced during the first measurement after ischaemia [15].

Localized superficial skin blood flow (SBF) was assessed at the index finger pulp and not at the forearm, as responses to skin vasomotor reflexes at the forearm reflect a combination of vasoconstrictor and vasodilator influences, while the skin vessels at the index finger pulp are innervated only by vasoconstrictor fibres [16,17]. SBF was monitored continuously before, during and after ischaemia using a Periflux™ laser Doppler instrument (Perimed, Stockholm, Sweden). The laser light probe emits a divergent narrow band of light at a wavelength of approx. 780 nm with an intensity of ≤ 0.8 mW [18]. The volume measured in the skin is a hemisphere with an approximate radius of 1 mm. However, the instrument does not measure perfusion in absolute values (ml·min⁻¹·g⁻¹), since the measured volume is tissue dependent and not exactly known. Therefore, after calibration of the instrument with a motility standard according to the manufacturer, flow was measured in arbitrary perfusion units (PU) [18].

SBF and diastolic arterial blood pressure were recorded continuously from the left radial artery at the wrist using non-invasive arterial tonometry (Colin Pilot™, Colin Medical Instruments Corp., San Antonio, TX, U.S.A.) [19]. The tonometer consists of an array of 31 equally spaced piezoresistive pressure transducers, an automated positioning system, and signal conditioning and initial calibration by oscillometric cuff measurement of brachial artery blood pressure [19].

Transcutaneous partial pressures of carbon dioxide (Pco₂) and oxygen (Po₂) (Radiometer, Copenhagen, Denmark) were monitored continuously at the forearm, with the probe placed below the plethysmography strain gauge.

A commercially available statistical program (SYSTAT, Evanston, IL, U.S.A.) was used for statistical calculations. ANOVA was used for assessment of differences between the groups. Moreover, differences between patient and control values were evaluated by the Mann–Whitney U-test. The Wilcoxon test was used to evaluate differences between values assessed at baseline and after ischaemia. The level of significance was set at P < 0.05.

RESULTS

During venous occlusion, the average arterial blood inflow was significantly lower in FD patients (4.6 ± 1.8 %/min) than in controls (6.1 ± 1.4 %/min) (Mann–Whitney U-test, P < 0.05; ANOVA, P < 0.05). Similarly, post-ischaemic arterial hyperperfusion was lower in the FD patients than in the controls, with a maximum volume change during the first post-ischaemic influx peak of 12.8 ± 5.0 %/min in the patients and 16.9 ± 2.9 %/min in the controls (Mann–Whitney U-test, P < 0.05; ANOVA, P < 0.05). Figure 1 shows exemplary curves for the overall arterial inflow of one FD patient and one control subject at baseline and after ischaemia.

In contrast, the SBF of controls increased to 266.1 ± 102.5 PU (Mann–Whitney U-test, P > 0.05; ANOVA, P > 0.05). During the 3 min of ischaemia, SBF decreased to minimum values of 4.7 ± 3.1 PU in the FD patients and 3.2 ± 0.9 PU in the controls (Mann–Whitney U-test, P < 0.05; ANOVA, P < 0.05). After ischaemia, SBF increased rapidly in both groups. However, post-ischaemic hyperperfusion was significantly greater in patients than in controls (Mann–Whitney U-test, P < 0.05; ANOVA, P < 0.05). In patients, there was a SBF overshoot to 964.4 ± 421.7 PU. In contrast, the SBF of controls increased to 266.1 ± 65.1 PU, values that were slightly, but not significantly, above their baseline SBF (Wilcoxon test, P > 0.05). Figure 2 shows the SBF of patients and controls at baseline and after 3 min of ischaemia.

Supine blood pressure was significantly higher in FD patients (systolic, 125.8 ± 6.3 mmHg; diastolic, 86.2 ± 3.8 mmHg) than in control subjects (systolic, 105.8 ± 6.6 mmHg; diastolic, 75.2 ± 3.8 mmHg) (Mann–Whitney U-test, P < 0.05; ANOVA, P < 0.05). At rest, transcutaneous Pco₂ or Po₂ did not differ between patients and controls (Mann–Whitney U-test, P > 0.05). Baseline Po₂ was 75.5 ± 12.1 mmHg in the patients and 79.5 ± 13.9 mmHg in the controls. During 3 min of ischaemia, Po₂ decreased to minimum values of 3.8 ± 1.8 mmHg in patients and 2.7 ± 1.8 mmHg in controls (Wilcoxon test, P < 0.05). Similar to the
Figure 1  Typical curves for the overall inflow into the forearm and hand measured by means of venous occlusion plethysmography in one FD patient and one control subject at baseline (upper panels) and after 3 min of ischaemia (lower panels)

Note the different values on the y axes at baseline and after ischaemia.

Figure 2  SBF at the index finger pulp at baseline and after ischaemia in FD patients and controls

SBF at baseline was similar in patients and controls. However, after 3 min of ischaemia, there was a pronounced overshoot of SBF in FD patients that significantly exceeded the response of controls. Note the different values on the y axes at baseline and after ischaemia.

transcutaneous oxygen levels at rest, the transcutaneous $P_{CO_2}$ values at rest did not differ between patients (43.2 ± 5.6 mmHg) and controls (38.4 ± 5.8 mmHg). Ischaemia induced a significant increase in $P_{CO_2}$ in both patients (53.5 ± 5.1 mmHg) and controls (51.6 ± 5.2 mmHg) (Wilcoxon test, $P < 0.05$).

DISCUSSION

Our study shows that both limb blood flow and end-organ perfusion of the skin are compromised in patients with FD at rest and after ischaemic challenge.

Theoretically, the patients’ medication – fludrocortisone and midodrine – might have contributed to the findings of reduced arterial inflow and increased SBF in our patients. However, drug effects such as salt retention or potassium depletion, or direct autonomic effects, seem to be rather unlikely in our patients, since we only tested patients after they had discontinued fludrocortisone or midodrine for at least 18 h.

Alternatively, the differences between findings in FD patients and controls might have been due to discrepant physical and/or psychological characteristics of the two groups. Laboratory or hospital staff might have a different reaction to the laboratory environment in which the procedures were carried out. Therefore we enrolled mainly healthy relatives and friends of the participating FD patients as controls. We only included two members of our team into the study, and we feel confident that this small percentage has not biased our results. Moreover, physical parameters such as the greater BMI and height in controls than in patients might have had some influence on the results.
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on the results. However, the differences in BMI were not significant, and the length and circumference of the forearm did not differ to a degree that was likely to significantly bias the plethysmographic results.

The finding of reduced limb perfusion in FD patients not only under resting conditions but also after ischaemia was somewhat unexpected. As sympathetic vasomotor control is reduced in the FD patients [4,5], one might expect that limb perfusion at baseline and especially with ischaemia-induced vasodilatation would be higher in the patients than in the controls. However, perfusion values measured by venous occlusion plethysmography depend largely on the rigidity and resistance of the small-vessel bed, and not so much on sympathetic vasomotor control [12]. The reduced limb perfusion in FD patients, therefore, is most probably due to an increase in small vessel rigidity. FD patients are exposed repeatedly to clinically overt and disabling orthostatic hypotension. More importantly, they have to compensate for a chronic and possibly more damaging exposure to arterial hypertension when supine, i.e. during sleep or rest [8,20,21]. Chronic arterial hypertension alters vessel morphology and promotes sclerosis of the vessel walls [22], resulting in increased vessel rigidity and vascular resistance [15,23–25].

Post-ischaemic limb hyperperfusion depends particularly on the minimum vascular resistance, which correlates with the media/lumen ratio of resistance vessels [15,26,27]. Post-ischaemic hyperaemia is considered to be an indirect consequence of the structure of resistance vessels [15], and is decreased in patients with chronic arterial hypertension in relation to the structural changes in their resistance vessels [15,23–25].

From the plethysmographic findings, we conclude that forearm and hand blood flow are reduced in the FD patients because of structural – most likely hypertension-induced – changes in the resistance vessels. In a study evaluating cerebral blood flow regulation in FD patients during rapid fluctuations in blood pressure, we also obtained findings suggesting increased rigidity of intracerebral resistance vessels [21].

Unlike the overall blood flow into the forearm and hand measured by plethysmography, the localized end-organ perfusion of the skin, as determined by laser Doppler flowmetry, was slightly higher in FD patients than in controls at baseline. The difference was even more pronounced if skin perfusion was related to the overall influx into the arm and hand, which was 25 % lower in the patients than in the controls. Weiser et al. [4] also reported increased baseline skin perfusion in FD patients, and concluded that dysfunctioning or a decrease in the number of sympathetic neurons and α-adrenoreceptors accounted for the higher SBF.

Laser Doppler flowmetry reflects not only blood flow in the superficial, 0.2–0.4 mm-deep capillary loops entering the papillae of the corium, but also blood flow in the arterioles, arteriovenous shunts and venules supplying and draining the capillaries and forming microvessel layers in the upper and mid-dermis and in the deeper skin [28–30]. In contrast with the capillary blood flow, perfusion of the arterioles and arteriovenous shunts depends on sympathetic vasoconstrictor function [29,31].

Several mechanisms are likely to contribute to enhanced intradermal blood flow at rest in patients with FD. Structural changes in vessel walls due to the above-mentioned longstanding recumbent hypertension and resulting increase in vessel wall rigidity might prevent adequate constriction of arterioles and arteriovenous shunts at rest and in response to ischaemia-induced hyperperfusion. A far more important cause of skin hyperperfusion at baseline and after ischaemia is likely to be the deficiency of sympathetic vasomotor innervation in FD patients, as discussed by Weiser et al. [4]. The decrease in the number of sympathetic neurons demonstrated in the cervical and thoracic ganglia of FD patients [32,33] and the deficiency of sympathetic nerve terminals in vessel walls [6] explain the higher skin perfusion at rest and after ischaemia [4]. Although laser flow values are rather high at baseline, capillary skin perfusion might be poor in FD patients because of inadequate closure of sympathetically innervated arteriovenous shunts. Such malperfusion of the end-organ tissue would contribute to trophic alterations seen at the finger and toe tips and the nails of FD patients [5,34]. Similar phenomena occur in patients with diabetes with peripheral and autonomic neuropathy [35–39]. Despite increased SBF, diabetic patients develop skin ulcers and nail dystrophy because of reduced vasoconstrictor tone and increased arteriovenous shunting [35–37].

After the period of ischaemia, the FD patients had a pronounced overshoot in skin perfusion to values three times higher than baseline flow, while the SBF of the controls was only slightly above their resting values. The discrepancy between the significant post-ischaemic increase in SBF and the limited increase in forearm and hand perfusion seems to reflect different pathological mechanisms of vascular dysfunction in FD.

While the limited post-ischaemic hyperperfusion of the forearm and hand in FD patients can be attributed to increased rigidity and restricted distensibility of the entire vessel bed resulting from the longstanding recumbent hypertension, the local overshoot of skin perfusion in these patients seems to be due primarily to a lack of sympathetic vasomotor control [2,6,32,40]. Deficient activation of sympathetic vasomotor fibres results in a lack of counter-regulation to the post-ischaemic dilatation of arteriovenous shunts and of precapillary arterioles [6]. While healthy subjects showed rapid counter-regulation of post-ischaemic hyperperfusion and re-establishment of flow close to baseline, FD patients seem to be unable to adequately increase sympathetic vasoconstrictor tone after ischaemia-induced vasodilatation.
In addition, post-ischaemic hyperperfusion in patients with FD might result not only from compromised sympathetic vasoconstriction, but also from hypersensitivity of denervated intracutaneous nerve fibres to ischaemia [41,42]. Ischaemia-induced acidosis causes neuropeptide release from primary afferent neurons innervating the skin [43,44]. In FD patients, the number of peripheral thinly myelinated A δ-fibres and unmyelinated C-fibres is markedly reduced, and this declines further with age [2,3,45]. Therefore capsaicin-sensitive afferents of FD patients might be more sensitive to protons because of increased excitability of neuropathic fibres [46]. Denervation hypersensitivity with exaggerated responses to sympathomimetic and parasympathomimetic agents has been described previously in patients with FD [6,47,48]. Consequently, the release of peptides such as calcitonin gene-related peptide (CGRP), neurokinin A and substance P might be enhanced, thus resulting in pronouced vasodilatation in the patients [46,49,50]. Calcitonin gene-related peptide, the most active vasodilator neuropeptide released under conditions of acidosis, mediates dilatation of cutaneous arterioles via a direct action on vascular smooth muscles and independently of nitric oxide or prostaglandins [51].

To summarize, vasomotor control in patients with FD seems to be compromised in at least two ways. Limb perfusion is diminished at rest and in response to stressful conditions such as ischaemia, most probably because of chronic recumbent hypertension that leads to consecutive generalized structural changes in vessel walls. In addition, there is an alteration to end-organ perfusion, probably with increased arteriovenous shunting and reduced perfusion of capillaries. This malperfusion of the end-organ might, to some extent, result from structural changes at the level of the shunts, but seems to be caused primarily by the deficient sympathetic vasomotor control of precapillary arterioles and arteriovenous shunts due to small-fibre neuropathy in FD patients. Finally, denervation hypersensitivity of intracutaneous afferent C-nerve fibres is likely to further augment vasodilatation in response to ischaemic stress [43,44,46].

We assume that both the reduced perfusion of the limb and the dysfunctional blood supply to the skin, i.e. to the end-organ, are major contributors to the vasomotor instability of FD patients. Particularly when challenged, the peripheral vascular system of FD patients fails to respond adequately. Consequently, FD patients are at particular cardiovascular risk during periods of cardiovascular stress, such as increased physical effort, that induce relative ischaemia and concomitant sympathetic challenge.

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REFERENCES
