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Spence VA, Khan F, Belch JJF. *Enhanced Sensitivity of the Peripheral Cholinergic Vascular Response in Patients with Chronic Fatigue Syndrome*. Am J Med 2000; 108(9):736-739.

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### Enhanced Sensitivity of the Peripheral Cholinergic Vascular Response in Patients with Chronic Fatigue Syndrome [Brief Observations]

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Manuscript submitted July 19, 1999, and accepted in revised form March 1, 2000.

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Patients with chronic fatigue syndrome have a variety of clinical symptoms, with associated physical, psychological, and social disability. The syndrome is poorly understood; the etiology and pathophysiology are not known.

Cholinergic mechanisms are important in the control of peripheral skin perfusion (1,2) and, in part, are regulated by endothelial cells (3). We have developed a method for assessing endothelial function in which acetylcholine is delivered transdermally across intact skin using iontophoresis (4), and the vascular responses are measured by laser Doppler imaging (5). These techniques have been used in many patients with cardiovascular disease, in whom endothelial dysfunction and impaired vasodilatation are common. We have now applied this methodology to investigate cholinergic activity in patients with chronic fatigue syndrome to see if the reported evidence of central nervous system cholinergic sensitivity in these patients (6) is widespread.

## SUBJECTS AND METHODS

Patients were randomly selected from 420 members of a local chronic fatigue syndrome/myalgic encephalomyelitis self-help group. We enrolled 22 patients (7 men and 15 women; mean [ $\pm$  SD] age  $45 \pm 9$  years, range 26 to 59) who fulfilled the Centers for Disease Control criteria for chronic fatigue syndrome (7). All patients had a principal complaint of fatigue, exacerbated by physical and mental activity, for more than 6 months (illness duration  $8 \pm 5$  years, range 1 to 15). No other medical cause of



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fatigue was identified, and none of the patients had a history of vascular disease. We also enrolled 22 age-matched ( $\pm 1$  year) and sex-matched control subjects. None of the control subjects and 13 of the patients with chronic fatigue syndrome took medications. Four patients with chronic fatigue syndrome were taking amitriptyline (10 mg to 20 mg at night for pain control), 3 were taking low doses of a serotonin reuptake inhibitor, and 7 were taking nonsteroidal anti-inflammatory drugs (which were stopped for at least 10 days before the study date). All participants gave written informed consent. The local committee on medical research ethics approved the study.

## Measurement of Skin Blood Flow and Drug Delivery

We used a laser Doppler imager (Moor Instruments, Axminster, United Kingdom) that scans a low-power laser beam over the skin surface. The scanner was set to a spatial resolution of  $100 \times 100$  pixels and a scan speed of 4 ms/pixel. Moving blood in the microvasculature causes a Doppler shift, which is processed to build a color image of blood flow (termed "erythrocyte flux") in arbitrary perfusion units.

Iontophoresis was used to transport drugs in solution across intact skin. We used a protocol similar to that used in our previous studies (4,8). In brief, a circular iontophoresis electrode (inner diameter 20 mm) was attached midway on the volar aspect of the forearm. A reference electrode was placed around the wrist to complete the circuit. Both electrodes were connected to an iontophoresis controller, which provided a direct current for drug delivery.

Studies were performed in a temperature-controlled room (22°C to 23°C). Following a 20-minute equilibration period, baseline skin perfusion was measured at the volar aspect of the dominant forearm over the iontophoresis electrode. Using a 0.1-mA anodal current, a 1% solution of acetylcholine chloride (Sigma Chemical, St. Louis, Missouri) was iontophored for 10 seconds to achieve a dose of  $1 \text{ mC/cm}^2$ . The duration was increased to 20 seconds for a dose of  $2 \text{ mC/cm}^2$ , to 40 seconds for a dose of  $4 \text{ mC/cm}^2$ , and to 80 seconds for a dose of  $8 \text{ mC/cm}^2$ . Scans were performed for 2 minutes between doses. At a different site, we used a 0.1-mA cathodal current to iontophore sodium nitroprusside (David Bull Laboratories, Warwick, United Kingdom) to give doses of 1, 2, 4, and  $8 \text{ mC/cm}^2$ . Scans were performed for 4 minutes between doses.

All measurements and data analyses were blinded to patient status and were performed by a researcher who was unaware whether the subject was a patient or a control.

## Statistical Analysis

Results are expressed as mean  $\pm$  SD. Differences between patients with chronic fatigue syndrome and control subjects were compared using two-way analysis of variance (ANOVA) for repeated measures. The null hypothesis was rejected at  $P < 0.05$ .



## RESULTS

Baseline skin erythrocyte flux was similar in patients with chronic fatigue syndrome ( $22 \pm 7$  perfusion units) and control subjects ( $20 \pm 5$  perfusion units,  $P = 0.23$ ). The vascular responses to acetylcholine were significantly greater in patients with chronic fatigue syndrome than in control subjects at all 4 doses ( $P = 0.01$ , ANOVA; Figure 1). In contrast, the vascular responses to sodium nitroprusside (Figure 2) were not significantly different between the groups of subjects.

Excluding the 4 patients taking amitryptiline did not alter the results. The vascular response to acetylcholine was still significantly greater in the patients with chronic fatigue syndrome ( $22 \pm 7$ ,  $60 \pm 26$ ,  $105 \pm 28$ ,  $127 \pm 23$ , and  $141 \pm 21$  perfusion units) than in the control subjects ( $19 \pm 5$ ,  $37 \pm 24$ ,  $69 \pm 36$ ,  $95 \pm 35$ , and  $112 \pm 30$  perfusion units,  $P = 0.001$ ).

## DISCUSSION

The results of this study show enhanced cholinergic activity in the peripheral microcirculation of patients with chronic fatigue syndrome. This enhancement was specific for acetylcholine; the vascular responses to sodium nitroprusside were similar in patients with chronic fatigue syndrome and in control subjects.

We could not determine why the patients with chronic fatigue syndrome have acetylcholine supersensitivity in the skin microcirculation. Recent evidence in mice treated with cholinesterase inhibitors suggests that cholinergic stimulation promotes changes in the genes regulating acetylcholine metabolism (9) and that such changes are facilitated by stress (10). Whether such changes of cholinergic tone apply to the metabolism of acetylcholine in vascular endothelium, however, remains speculative. There are several ways that the acetylcholine-endothelial pathway could account for cholinergic supersensitivity. Acetylcholine produces vasodilatation through a sequence of events beginning with its binding to muscarinic receptors on the endothelial cell surface. This activates G proteins, promoting the conversion of l-arginine to nitric oxide, which diffuses into the smooth muscle cells and stimulates guanylate cyclase to produce cyclic GMP, thereby causing relaxation. As vascular responses to sodium nitroprusside were not enhanced in patients with chronic fatigue syndrome, we suggest that the enhanced vasodilator activity to acetylcholine is situated at the muscarinic M3 receptor or along the muscarinic receptor/vascular smooth muscle pathway.

Many of the symptoms of chronic fatigue syndrome, such as temperature sensitivity, gastrointestinal difficulties, problems with sleep, and orthostatic intolerance, are consistent with altered cholinergic activity. Our findings of enhanced cholinergically-mediated vasodilatation are related only to the vasoactive properties of acetylcholine within the endothelium, which are distinct from its action as a neurotransmitter. Nevertheless, the response of skin microvessels to acetylcholine is typical of blood vessels elsewhere in the body. Thus, our findings might have important implications for features of chronic fatigue syndrome that involve vascular integrity.



The patients in this study were heterogeneous and had a diverse range of symptoms and severity of presentation. We did not examine the potential effects of duration of illness or overall health at the time of testing. A study that used more specific criteria might identify differences in cholinergic sensitivity among different groups of patients with chronic fatigue syndrome, as has been seen for immune function (11,12).

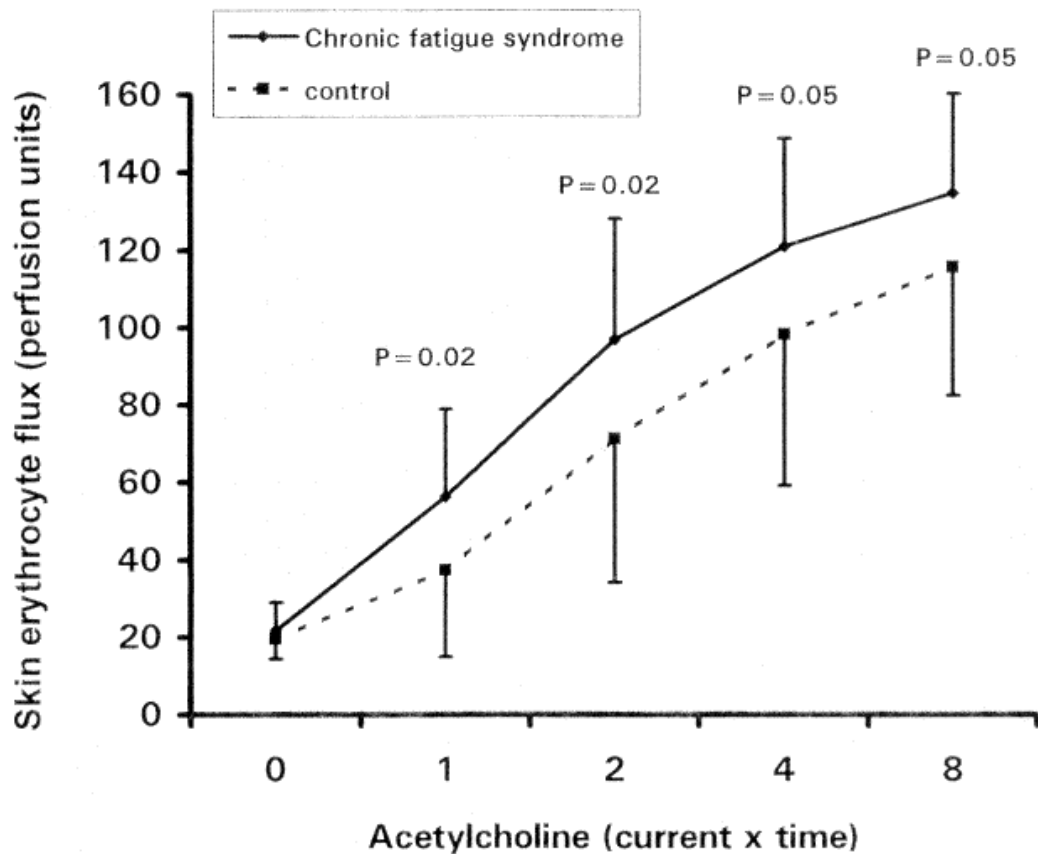
## ACKNOWLEDGMENT

We are grateful to the Myalgic Encephalomyelitis Association Moray Firth group and Tenovus Scotland for their support. We thank Jenny Cooper for her technical assistance.

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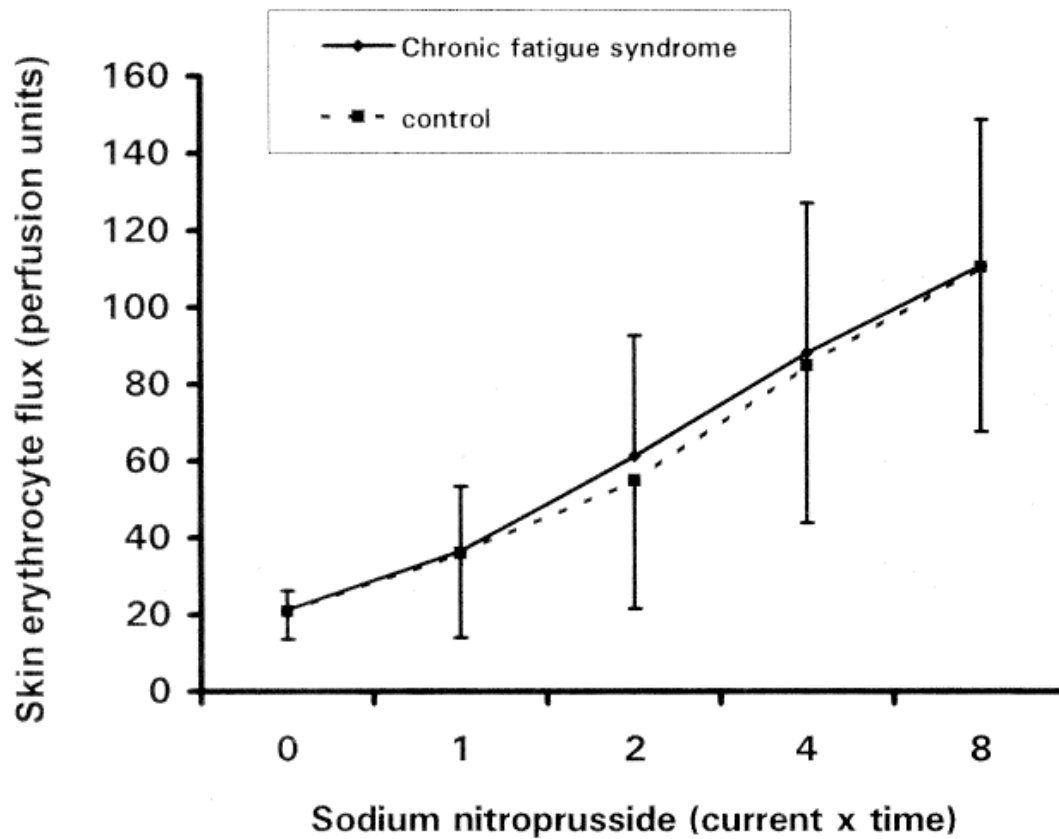




**Figure 1.** Skin vascular responses to iontophoresis of acetylcholine in patients with chronic fatigue syndrome (n = 22) and control subjects (n = 22). Vascular responses were significantly enhanced in patients with chronic fatigue syndrome ( $P = 0.01$ , ANOVA).  $P$  values on the graph refer to posthoc testing. Error bars represent  $\pm 1$  SD.

From: Spence: Am J Med, Volume 108(9).June 15, 2000.736-739





**Figure 2.** Skin vascular responses to iontophoresis of sodium nitroprusside in patients with chronic fatigue syndrome (n = 22) and control subjects (n = 22). There were no significant differences between the groups. Error bars represent  $\pm 1$  SD.

*From:* Spence: Am J Med, Volume 108(9).June 15, 2000.736-739

