Pilocarpine-induced sweat gland function in individuals with multiple sclerosis

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Submitted 8 August 2004; accepted in final form 27 December 2004

MULTIPLE SCLEROSIS (MS) is a demyelinating disease of the central nervous system that can disrupt autonomic pathways, leading to impaired control of thermoregulatory function. The first goal of the present investigation was to test the hypothesis that the number of activated sweat glands and sweat production in individuals with MS is reduced relative to matched healthy control subjects after cholinergic stimulation. A second goal of the investigation was to test the hypothesis that aerobic exercise training significantly increases peak aerobic capacity in MS.

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Exercise training improves sweat gland function in MS patients.

METHODS

Protocol 1: Comparison of Sweating Characteristics in Individuals With MS and Controls Subjects

Participants. Ten women with MS, recruited through physician referrals, comprised the MS group (MS-Con) (Table 1). Participants were selected on the basis of the following criteria: definite clinical diagnosis of MS, Kurtzke expanded disability status scale (EDSS) score between 3.0 and 6.0, and no history of cardiovascular, respiratory, orthopedic, metabolic, or other medical condition that would preclude participation in an aerobic training program (13, 20). Ten women with no diagnosis of MS, matched to MS participants by age, height, weight, and peak aerobic capacity (VO_{2}\text{peak}), were recruited and assigned to the control group (Con) (Table 1). Participants provided written, informed consent approved by the Institutional Review Board of the University of Utah.

Neurological evaluation. A neurological evaluation was performed on participants with MS by a board-certified neurologist specializing in MS before the beginning of the study. A thorough medical history was recorded, and participants were evaluated utilizing components of the MS minimal record of disability forms (neurological examination, incapacity status scale, Kurtzke’s functional system scales) and Kurtzke EDSS (13, 15). All individuals with MS reported existing heat sensitivity to one or more of the following conditions: high ambient temperature, high water temperature, and/or physical activity.

Exercise testing. VO_{2}\text{peak} was measured by a maximal graded exercise test performed on an air-resistance arm and leg cycle ergometer (Schwinn Air-Dyne, Boulder, CO). Four different testing protocols were used to provide appropriate workloads for individuals with varying work capacities. Participants began at a work rate of 15, 25, or 40 W with the work rate increasing 10 to 40 W every 1–2 min. During the test, oxygen consumption, respiratory exchange ratio (RER), and ventilation were recorded every 20 s via open-circuit indirect calorimetry (True Max 2400, ParvoMedics Murray, UT). Heart rate was obtained throughout the test with rating of perceived exertion (RPE) recorded after each stage and at test termination. The test was terminated when one or more of the following criteria had been met: 1) any symptoms that impaired the individual’s ability to continue or indicated a risk to safety or health, 2) volitional exhaustion, or 3) RER > 1.0.

Pilocarpine iontophoresis. Peripheral sweat production was induced by pilocarpine iontophoresis to the ventral side of the forearm, 3–4 cm distal to the antecubital space (2, 3). Individuals in MS-Con were tested on a symptomatic forearm identified during the neurological evaluation (Table 2). Individuals in Con were tested on the right forearm.

Pilocarpine (0.5% pilocarpine nitrate in solid agar gel) was iontophoresed by using a Webster sweat inducer (model 3700, Wescor, Logan, UT) to elicit maximal sweating (28). Two complete cycles of iontophoresis (each cycle equivalent to 5.5 min at 1.5 mA) were performed. An environmentally sealed sweat collection disk (Macrodust sweat collection system, Wescor) was placed over the stimulated area to collect sweat for 15 min. After sweat collection, iodine-treated linen paper with 1-cm grids was applied to the stimulated area for ~15–20 s and then scanned into a computer. Imaging software (NIH Image) was used to calculate the number of activated sweat glands per square centimeter (ASG). Total sweat volume was measured from pre- and postcollector weights. Sweat rate (SR) was calculated by dividing volume of sweat in the collector by the collector area (6.413 cm^2) and time of collection (15 min). Sweat gland output (SGO) was calculated by dividing SR by the density of glands under the collector.

Protocol 2: Effect of Training on Sweating Characteristics in Individuals with MS

Seven individuals from MS-Con agreed to participate in a supervised 15-wk exercise training program, which comprised the MS exercise group (MS-Ex) (Table 1). The individuals provided written consent to participate and were informed of their rights as outlined by the Institutional Review Board of the University of Utah. The training program was previously described by Petajan et al. (18). Individuals in the MS-Ex group participated in three supervised training sessions per week in the laboratory at an ambient temperature of 22°C. Training was performed on the same type of air resistance arm and leg cycle ergometer used during the graded exercise test. Each training session consisted of a 5-min warm-up at 30% of VO_{2}\text{peak}, 30 min of exercise at 60% VO_{2}\text{peak}, and a 5-min cooldown at 30% of VO_{2}\text{peak}.

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>VO_{2}\text{peak}, ml/kg⁻¹ min⁻¹</th>
<th>EDSS</th>
<th>EDSS Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>10</td>
<td>44.0 (9.1)</td>
<td>166.8 (7.3)</td>
<td>77.3 (23.7)</td>
<td>26.9 (6.9)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>MS-Con</td>
<td>10</td>
<td>44.7 (8.0)</td>
<td>165.8 (7.1)</td>
<td>70.8 (16.1)</td>
<td>26.0 (5.9)</td>
<td>5.2 (1.3)</td>
<td>3.0–6.5</td>
</tr>
<tr>
<td>MS-Ex</td>
<td>7</td>
<td>44.5 (7.4)</td>
<td>169.4 (6.8)</td>
<td>75.5 (17.1)</td>
<td>25.0 (7.0)</td>
<td>4.4 (1.5)</td>
<td>3.0–6.0</td>
</tr>
</tbody>
</table>

Values are means (SD); n, no. of subjects; VO_{2}\text{peak}, peak aerobic capacity; Con, control subjects; MS-Con, individuals with multiple sclerosis; MS-Ex, subgroup of MS-Con who participated in a 15-wk exercise training program; EDSS, expanded disability scale score; NA, not applicable.
training ASG, SR, and SGO were identified and compared with pretraining values.

Statistical Analysis

Unpaired \( t \)-tests were performed on ASG, SR, and SGO comparing MS-Con and Con groups. Paired \( t \)-tests were used to compare Base and Wk15 differences in V˙O\(_2\) peak, SR, ASG, and SGO in the MS-Ex group. Statistical significance was accepted at \( P \leq 0.05 \). All values are expressed as means ± SD.

RESULTS

Protocol 1: Comparison of Sweating Characteristics in Individuals with MS and Con

No differences were observed in V˙O\(_2\) peak, peak heart rate, RER, or RPE between MS-Con and Con at the termination point of the maximal graded exercise test (Table 3). No differences were observed in ASG (Fig. 1A) between MS-Con [106.3 glands/cm\(^2\) (SD 20.3)] and Con [109.5 glands/cm\(^2\) (SD 15.7)]. SR (Fig. 1B) for MS-Con [0.18 mg·cm\(^{-2}\)·min\(^{-1}\) (SD 0.08)] was significantly lower (\( P < 0.05 \)) than Con [0.27 mg·cm\(^{-2}\)·min\(^{-1}\) (SD 0.10)]. Similarly, SGO (Fig. 1C) was also significantly lower (\( P < 0.05 \)) in MS-Con [1.74 g·gland\(^{-1}\)·min\(^{-1}\) (SD 0.79)] compared with Con [2.43 g·gland\(^{-1}\)·min\(^{-1}\) (SD 0.69)].

Protocol 2: Effect of Training on Sweating Characteristics in Individuals With MS

All seven MS-Ex participants completed the training protocol with no changes in neurological status. Clinical and treatment status remained unchanged throughout the training period. Fifteen weeks of aerobic training significantly increased (\( P < 0.05 \)) V˙O\(_2\) peak (Table 4, Fig. 2). However, exercise training did not alter ASG, SR, or SGO (Table 4, Fig. 2).

DISCUSSION

The primary finding of this study is that both SR and SGO were significantly reduced in untrained individuals with MS compared with similarly inactive control subjects. No differences were observed in the number of active sweat glands between groups, indicating that a similar number of sweat glands in individuals with MS were responsive to pilocarpine administration compared with healthy controls. These results are in agreement with Vas (27), who suggested that postganglionic sympathetic nerves and sweat glands were responsive to peripheral cholinergic stimulation in individuals with MS. These results suggest that diminished sweat function observed in individuals with MS is due to reduced SGO rather than diminished sweat gland recruitment. By carefully matching each individual with MS to a healthy control with similar V˙O\(_2\) peak, initial cardiorespiratory fitness did not impact the observed differences in sweat function between groups.

Diminished areas of sweating in individuals with MS were previously visually identified by using quinazarin powder ap-

Table 3. Peak physiological variables for Con and MS-Con during maximal graded exercise testing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Con</th>
<th>MS-Con</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V˙O(_2) peak, ml·kg(^{-1})·min(^{-1})</td>
<td>26.9 (6.9)</td>
<td>26.0 (5.9)</td>
<td>0.770</td>
</tr>
<tr>
<td>Peak heart rate, beats/min</td>
<td>172.2 (13.8)</td>
<td>166.2 (15.7)</td>
<td>0.376</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>1.10 (0.09)</td>
<td>1.07 (0.06)</td>
<td>0.284</td>
</tr>
<tr>
<td>Rating of perceived exertion</td>
<td>18.2 (1.7)</td>
<td>17.3 (1.9)</td>
<td>0.461</td>
</tr>
</tbody>
</table>

Values are means (SD).

Table 4. Effects of 15 wk of aerobic exercise training on aerobic capacity, sweat gland density, sweat rate, and sweat gland output in individuals with multiple sclerosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Base</th>
<th>Wk15</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V˙O(_2) peak, l/min</td>
<td>1.86 (0.75)</td>
<td>2.10 (0.67)*</td>
<td>0.025</td>
</tr>
<tr>
<td>Activated sweat gland density (ASG), gland/cm(^2)</td>
<td>106.3 (18.2)</td>
<td>109.8 (20.6)</td>
<td>0.365</td>
</tr>
<tr>
<td>Sweat rate, mg·cm(^{-2})·min(^{-1})</td>
<td>0.18 (0.10)</td>
<td>0.19 (0.10)</td>
<td>0.442</td>
</tr>
<tr>
<td>Sweat gland output, ( \mu g )·gland(^{-1})·min(^{-1})</td>
<td>1.68 (0.90)</td>
<td>1.86 (1.08)</td>
<td>0.357</td>
</tr>
</tbody>
</table>

Values are means (SD) for 7 subjects in MS-Ex group. Base, pretraining; Wk15, after 15 wk of aerobic exercise training. *\( P < 0.05 \) vs. Base values.
plied to the skin during a passive heat stress (5, 17, 27). This technique does not separate central (i.e., neural) from peripheral (i.e., sweat gland) abnormalities. Therefore, local administration of pilocarpine, a cholinergic agent, was used in this study to evaluate and quantify peripheral sweat gland function (activated glands and sweat production) independent of central nervous system control (11).

Although growing evidence demonstrates the benefits of physical activity in the management of MS, many individuals with the disease remain sedentary (16, 19, 29). Complete inactivity (14 days of bed rest) has been shown to decrease maximal acetylcholine-induced sweat rate without altering the number of activated sweat glands (6). Decreased reliance on sweating as a heat dissipation mechanism leads to changes in the sweat gland, including reduced gland size and decreased cholinergic sensitivity (24). Conversely, maximal cholinergic sweat function is preserved in individuals who exercised throughout bed rest (6). Training has been shown to improve the secretory activity of the human sweat gland independent of neural control in healthy subjects (2–4, 10). Therefore, it was hypothesized that exercise training might be a viable intervention for increasing cholinergic sensitivity and glandular output in individuals with MS. Although a significant increase in aerobic capacity was attained, SR and SGO were unchanged in individuals with MS after 15 wk of aerobic training.

Individuals exercised on an air-resistance ergometer that produced greater air movement with increasing work rate. This air movement could have cooled individuals during exercise, thereby decreasing the thermal stimulus. However, our previous work with individuals with MS utilizing an identical training protocol in similar ambient conditions resulted in an average core temperature increase of 1.0°C during the 40-min exercise bout (29). This magnitude of thermal stimulus induced via exercise has previously been shown to produce adaptations in sweat function (22). In addition, Sawka et al. (25) suggested that exercise training intensities should exceed 50% maximal oxygen uptake for \( \geq 1 \) wk for thermoregulatory adaptations to occur, with optimal adaptations occurring in 8–12 wk. The present study’s exercise training regime is comparable to these guidelines (i.e., 60% \( \dot{V}_O_2 \) peak for 15 wk). We cannot exclude the possibility that adaptations in sweating could be observed with higher intensity and/or duration training. However, increasing intensity and/or duration of training sessions in this population is difficult because of acute exercise-induced worsening of clinical symptoms.

Areas of the sympathetic nervous system (hypothalamic area and posterior tract of the spinal cord) that control thermoregulatory functions are susceptible to demyelination in individuals with MS (24). Diminished sweat function observed in MS patients, while peripheral in origin, are suggestive of central nervous system impairments causing conduction abnormalities and even neuronal loss within the descending sudomotor pathways due to the disease (1, 27). This insufficient sympathetic input to sweat glands could lead to peripheral adaptations such as 1) reduced cholinergic sensitivity, 2) downregulation of muscarinic receptors, and/or 3) atrophy of sweat glands contributing to the diminished SR and SGO observed in this study. Central nervous system impairments may also account for the minimal peripheral sweat gland adaptations observed after exercise training in MS patients.

**Limitations**

Despite no significant increases in cholinergic sweat function after exercise training in this study, four of seven individuals in MS-Ex demonstrated small increases in SR after exercise training. Pilocarpine iontophoresis is an index of maximal cholinergic sweating and may not be reflective of exercise SR or less than maximal SR (9). It remains unclear whether exercise training in individuals with MS could improve sweat function during exercise or during passive heat exposure. This warrants further investigation. Regardless of the lack of improvement in maximal sweat function, exercise training should be encouraged as a therapeutic strategy in the management of the disease given the positive physiological and psychological benefits to individuals with MS (18).
Summary and Conclusions

Individuals with MS have diminished sweat gland function compared with healthy control subjects. Improvements in heat dissipation mechanisms such as sweating could be beneficial to individuals with MS by maintaining a safe core temperature and decreasing heat related signs and symptoms. Moreover, 15 wk of aerobic exercise training did not evoke any changes in sweat gland function in individuals with MS. If sweat function cannot be improved through exercise training, strategies to avoid excessive heat exposure, such as cooling or precooling, must be emphasized to individuals with MS.

ACKNOWLEDGMENTS

The authors express their appreciation to Cynthia Schindler and Robert Schaffer for technical assistance and to Dr. Manabu Shibasaki and Dr. David M. Keller for valuable comments and suggestions regarding the manuscript. The considerable time and effort of the participants are greatly appreciated.

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