



# Effects of Aerobic Training on Psychosocial Morbidity and Symptoms in Patients With Asthma

## A Randomized Clinical Trial

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**Background:** Asthma symptoms reduce patients' daily activities, impair their health-related quality of life (HRQoL), and increase their reports of anxiety and depression, all of which seem to be related to a decrease in asthma control. Aerobic exercise training is known to improve aerobic fitness and reduce dyspnea in asthmatics; however, its effect in reducing psychologic distress and symptoms remains poorly understood. We evaluated the role of an aerobic training program in improving HRQoL (primary aim) and reducing psychologic distress and asthma symptoms (secondary aims) for patients with moderate or severe persistent asthma.

**Methods:** A total of 101 patients were randomly assigned to either a control group or an aerobic training group and studied during the period between medical consultations. Control group patients (educational program plus breathing exercises) (n = 51) and training group patients (educational program plus breathing exercises plus aerobic training) (n = 50) were followed twice a week during a 3-month period. HRQoL and levels of anxiety and depression were quantified before and after treatment. Asthma symptoms were evaluated monthly.

**Results:** At 3 months, the domains (physical limitations, frequency of symptoms, and psychosocial) and total scores of HRQoL significantly improved only in the training group patients ( $P < .001$ ); the number of asthma-symptom-free days and anxiety and depression levels also significantly improved in this group ( $P < .001$ ). In addition, a linear relationship between improvement in aerobic capacity and the days without asthma symptoms was observed ( $r = 0.47$ ;  $P < .01$ ).

**Conclusions:** Our results suggest that aerobic training can play an important role in the clinical management of patients with persistent asthma. Further, they may be especially useful for patients with higher degrees of psychosocial distress.

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**Abbreviations:** HRQoL = health-related quality of life;  $\dot{V}O_2\text{max}$  = maximum oxygen consumption

Asthma is a chronic inflammatory disorder of the airways that causes recurring episodes of wheezing, breathlessness, chest tightness, and coughing.<sup>1</sup> The dyspnea experienced during exercise or the fear of triggering it are responsible for keeping patients with asthma from participating in sports or physical group activities<sup>2</sup> and might explain why these patients are less physically fit than their peers without asthma.<sup>3</sup>

Adults with asthma who are bothered by the symptoms report impairment in daily activities.<sup>4</sup> As a result, they feel irritated or frustrated and report limitations in their social life and deterioration in psychologic

well being, all of which lead to impairments in health-related quality of life (HRQoL).<sup>5–8</sup> In addition, increased anxiety and depression levels have been associated with decreased asthma control<sup>9</sup> and adherence to medication<sup>10</sup> and increased rates of the diagnosis of severe asthma.<sup>11</sup>

Although aerobic exercise can provoke exercise-induced bronchoconstriction in most patients with asthma, regular physical activity is included in the overall management of asthma<sup>12,13</sup> and as part of pulmonary rehabilitation programs.<sup>14,15</sup> Nevertheless, a recent metaanalysis<sup>16</sup> evaluated 13 randomized trials

performed in subjects with asthma who undertook physical training for at least 20 to 30 minutes two to three times a week over a minimum of 4 weeks. Despite the many outcomes evaluated, the only recognized effects of aerobic training were improvement in cardiovascular fitness and a decrease in dyspnea, and its effect on disease control and HRQoL remains unknown.<sup>16</sup> Although evidence supports the use of aerobic exercise training in managing the anxiety<sup>17</sup> and depression symptoms<sup>18</sup> in patients with some chronic diseases, we are not aware of any study that has evaluated the role of exercise in patients with asthma. Therefore, the aim of this study was to evaluate the effects of an aerobic training program on asthma-specific HRQoL (primary aim) and anxiety and depression scores and asthma symptoms (secondary aims) in patients with moderate or severe persistent asthma.

## MATERIALS AND METHODS

### Patients

A total of 101 (79 women/22 men) patients between 20 to 50 years old with moderate or severe persistent asthma were recruited at a university hospital. Asthma diagnosis was based on the Global Initiative for Asthma.<sup>1</sup> Patients were under medical treatment of  $\geq 6$  months and considered clinically stable (ie, no crises and changes in medication for  $\geq 30$  days). Patients with cardiovascular, pulmonary, or musculoskeletal diseases that would impair exercise training were excluded from the study. The Clinics Hospital ethics committee approved the study, and patients signed an informed consent form.

### Experimental Design

The study was performed between two medical consultations to avoid changes in medication. Patients were randomized (by drawing lots) into a control group ( $n = 51$ ) or a training group ( $n = 50$ ) (Fig 1). Both groups completed a 4-hour educational program and were taught breathing exercises. The training group patients completed an aerobic training program based on maximum oxygen consumption ( $\dot{V}O_2\text{max}$ ). Before and after the intervention, patients underwent pulmonary function and cardiopulmonary

exercise testing. Both groups completed questionnaires to quantify asthma-specific HRQoL and anxiety and depression levels. Daily asthma symptoms were evaluated monthly.

**Educational Program:** Both groups completed an educational program that consisted of two classes held once a week, each lasting 2 hours. The core activity was based on an educational videotape, *ABC of Asthma*,<sup>19</sup> including information about asthma pathophysiology, medication skills, self-monitoring techniques, and environmental control and avoidance strategies.<sup>1,13,19</sup> Patient doubts were elucidated with an interactive discussion.

**Breathing Exercise Program:** Both groups were taught yoga breathing exercises, including *Kapalabhati* (fast expiratory breathing exercise followed by passive inhalation); *Uddhiyana* (full exhalation followed by a forced inspiration performed without air inhalation [apnea]); and *Agnisara* (full exhalation followed by a sequence of retractions and protrusions of the abdominal wall in apnea).<sup>20,21</sup> A 30-min session was performed twice a week for 3 months, and every exercise was executed in sets of three with 2 min of exercise intercalated with 60 sec of rest.

**Aerobic Training Program:** Training group patients completed an aerobic training program for 30 min per session twice a week for 3 months. Aerobic exercise was initiated at 60% of  $\dot{V}O_2\text{max}$  in the first 2 weeks and then increased to 70%  $\dot{V}O_2\text{max}$ .<sup>13</sup> The intensity was increased by 5% if the patient maintained two consecutive exercise sessions without symptoms. Salbutamol (200  $\mu\text{g}$ ) was used 15 min before exercise if peak flow was  $< 70\%$  of the patient's best value.

### Assessments

Asthma-specific HRQoL was assessed by a four-domain questionnaire<sup>22</sup> consisting of 11 physical limitation questions, two frequency of symptoms questions, 11 socioeconomic questions, and seven psychosocial questions, with maximum scores of 33, 6, 11, and 7 points, respectively. Every domain was converted to percentages, with lower scores representing better HRQoL.

Depression level was evaluated with the Beck Depression Inventory<sup>23</sup> validated to Portuguese.<sup>24</sup> The Beck Depression Inventory consists of 21 assertions, with each score ranging from 1 to 3. The total score classifies the individual as having no depression (0-9), mild-to-moderate depression (10-18), moderate-to-severe depression (19-29), or severe depression ( $> 29$ ).

Anxiety levels were evaluated by using the State-Trait Anxiety Inventory<sup>25</sup> validated to Portuguese.<sup>24</sup> The State-Trait Anxiety Inventory consists of two scales: state anxiety (a transitory state of tension depending on the living condition) and trait anxiety (the individual's personality in the face of an acute threatening situation). Every scale consists of 20 assertions scored on a scale of 1 to 4. Total scores  $< 33$ , from 33 to 49, and  $> 49$  indicate mild, moderate, and high levels of anxiety, respectively.

Clinical asthma symptoms were quantified by a daily diary of cough, diurnal or nocturnal dyspnea, wheezing, and use of relief medication. A day free of asthma symptoms was considered when a patient did not report any symptoms. All patients were familiarized with the diary during the 30 days before the study. The patients filled out the diary during the follow-up period, and symptom-free days were quantified monthly.

Spirometry (SensorMedics 229; SensorMedics Corp; Homestead, FL) was performed before and after the inhalation of 200  $\mu\text{g}$  of salbutamol, and technical procedures were followed as recommended by the American Thoracic Society and European Respiratory Society.<sup>26</sup> Predicted normal values were those proposed by Knudson et al,<sup>27</sup> and a 12% and 200-mL increase in FEV<sub>1</sub> from baseline characterized a positive response to the bronchodilator.

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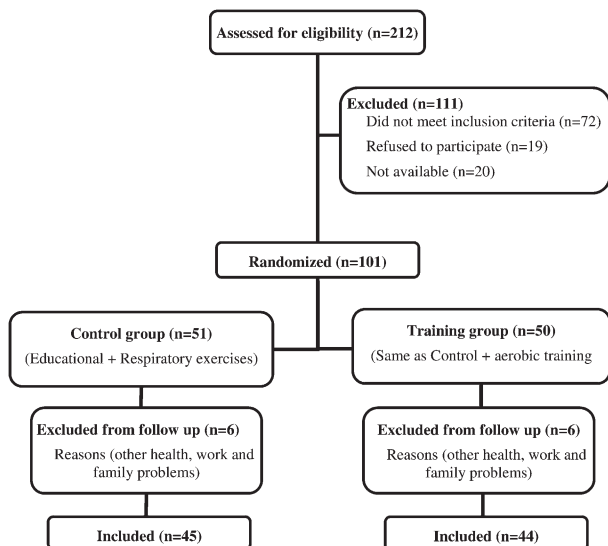


FIGURE 1. Study flow diagram.

Cardiopulmonary exercise testing was evaluated by a symptom-limited treadmill test on a digital computer-based exercise system (SensorMedics 229) with breath-by-breath analysis according to the Balke-modified protocol.<sup>25</sup> Aerobic impairment was classified according to Cooper et al.<sup>29</sup>

#### Statistical Analysis

Normality was evaluated by using the Kolmogorov-Smirnov test, and data were presented as medians (95% CI). The Mann-Whitney *U* test was used to compare baseline nonparametric data (age, BMI, and corticosteroid dosage), and the  $\chi^2$  test was used to evaluate gender and bronchodilator response at baseline. HRQoL (primary outcome) and asthma symptoms were assessed by two-way repeated-measures analysis of variance followed by a Holm-Sidak post hoc test. Anxiety and depression levels were evaluated by the McNemar test. Linear correlation analysis was evaluated by the Spearman test. The statistical significance level was set at 5% for all tests. All analyses were performed with statistical software (Sigmapstat 3.5; Systat Software Inc.; Chicago, IL).

## RESULTS

Twelve patients (6 control group, 6 training group) withdrew from the study because of health problems other than asthma, scheduling difficulties, or personal

**Table 1—Baseline Anthropometric Data, Bronchodilator Response, and Corticosteroid Consumption in Patients With Persistent Asthma**

Anthropometric Data	Control Group (n = 45)	Training Group (n = 44)
Gender, <sup>a</sup> female (male)	35 (10)	39 (5)
Age, <sup>b</sup> y	39.5 (23.5-47.0)	39.0 (22.0-47.9)
BMI, <sup>b</sup> kg/m <sup>2</sup>	24.5 (20.0-27.3)	25.2 (18.0-29.0)
Bronchodilator responders <sup>a</sup>	12 (27%)	7 (16%)
Budesonide dosage, <sup>b</sup> $\mu$ g/d	800 (400-1,300)	800 (400-1,200)

Data are presented as median (95% CI), unless otherwise indicated. *P* values were not significant.

<sup>a</sup> $\chi^2$  test.

<sup>b</sup>Mann-Whitney *U* test.

problems. Eighty-nine patients completed the study (45 control group, 44 training group). Before the study, both groups had similar distributions with regard to sex, age, BMI, daily dose of corticosteroids ( $P > .05$ ) (Table 1), asthma-specific HRQoL ( $P > .05$ ) (Table 2), anxiety and depression levels ( $P > .05$ ) (Table 3), asthma symptoms ( $P > .05$ ) (Fig 2), aerobic capacity ( $P > .05$ ) (Fig 3), and pulmonary function ( $P > .05$ ) (Table 4). Patients from both groups maintained the same bronchodilator and corticosteroid dosage throughout the treatment.

#### HRQoL

After training, improvements in the physical limitations, frequency of symptoms, and psychosocial domains and total asthma-specific HRQoL score occurred only in the training group ( $P < .001$ ) (Table 2). In contrast, the socioeconomic domain scores were similar in both groups before and after the study (Table 2).

#### Anxiety and Depression

At baseline, 94% of patients from both groups presented moderate or severe scores of either trait or state anxiety. Only patients with asthma who participated in the aerobic training program showed a reduction in state anxiety levels ( $P < .001$ ) (Table 3). No difference was observed in the final levels of trait anxiety in both groups. Scores from 71% of patients

**Table 2—Asthma-Specific Health-Related Quality of Life Score of Adult Patients With Asthma Before and After the Treatment Program**

HRQoL Domain	Control Group (n = 45)		Training Group (n = 44)		<i>P</i> Value
	Before	After	Before	After	
Physical limitation	57.8 (34.4-89.4)	66.7 (41.1-90.9)	57.6 (40.1-93.3)	31.6 (13.0-69.7) <sup>a</sup>	< .001
Symptom frequency	66.7 (41.6-83.3)	66.7 (39.6-87.5)	66.7 (41.6-88.3)	41.6 (22.5-66.7) <sup>a</sup>	< .001
Socioeconomic	57.0 (42.8-85.7)	71.4 (35.2-100.0)	63.6 (27.3-93.6)	54.5 (15.5-90.9)	> .05
Psychosocial	54.1 (14.2-85.7)	71.4 (14.3-100.0)	57.14 (14.3-100.0)	28.6 (10.0-71.4) <sup>a</sup>	< .001
Total score	61.3 (36.5-79.1)	66.0 (34.3-83.0)	61.8 (33.9-84.5)	39.8 (25.0-63.3) <sup>a</sup>	< .001

Data are presented as median (95% CI). HRQoL = health-related quality of life.

<sup>a</sup> $P < .05$  compared with the intragroup value obtained at baseline and control group, two-way repeated-measure analysis of variance test.

**Table 3—Proportion of Anxiety and Depression Levels of Adult Patients With Asthma Before and After the Treatment Program**

Anxiety and Depression Levels	Control Group (n = 45)		Training Group (n = 40)		P Value
	Before	After	Before	After	
STAI score					
State score					
Mild to moderate (< 50)	36 (80)	39 (87)	28 (64)	42 (95) <sup>a</sup>	< .001
Severe (≥ 50)	9 (20)	6 (13)	16 (36)	2 (5) <sup>a</sup>	< .001
Trait score					
Mild to moderate (< 50)	31 (69)	37 (82)	39 (89)	39 (89)	> .05
Severe (≥ 50)	14 (31)	8 (18)	5 (11)	5 (11)	> .05
BDI score					
Absent (< 10)	14 (31)	25 (56)	12 (27)	32 (73) <sup>a</sup>	< .001
Mild to moderate (10 to 18)	21 (47)	14 (31)	22 (50)	5 (11) <sup>a</sup>	< .001
Moderate to severe (> 18)	10 (22)	6 (13)	10 (23)	7 (16) <sup>a</sup>	< .001

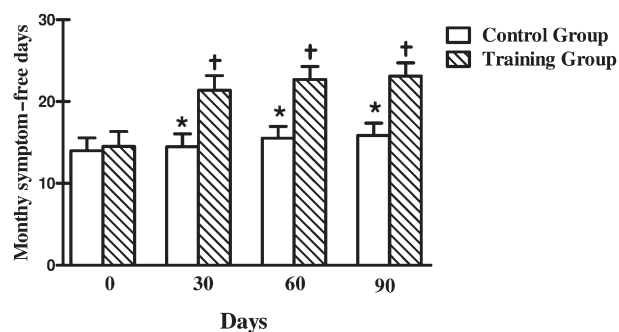
Data are presented as No. (%). BDI = Beck Depression Inventory; STAI = State-Trait Anxiety Inventory.

<sup>a</sup>*P* < .05 compared with the intragroup value obtained at baseline; McNemar test.

from both groups reflected low, moderate, or severe depression (43, 18, and 2, respectively) before the study. At the end, only patients from the training group showed a reduction in depression levels (*P* < .001) (Table 3).

### Asthma Symptoms

On average, both groups had 14 days without asthma symptoms (training group, 14 d/mo [95% CI, 7.1-27.3 d/mo] vs control group, 13 d/mo [95% CI, 6-24 d/mo]) (Fig 2). The control group showed a slight increase in the asthma-symptom-free days after 30, 60, and 90 days (average, 16 d/mo). Nevertheless, the training group showed a significant increase in the number of days without asthma symptoms after 30 days (23.5 days; 95% CI, 7.1-27.3 days; *P* < .001) that was maintained after 60 and 90 days of aerobic training (24 days each; *P* < .001) (Fig 2). Five patients (control group, n = 4; training group, n = 1) visited the emergency department, and eight (control group, n = 7; training group, n = 1) had asthma exacerbations during the study.



**FIGURE 2.** Asthma symptoms during the study period. Data are presented as mean number of symptom-free days/month (SD). Time points are 0 days (1 month before treatment), 30 days (first month of treatment), 60 days (second month of treatment), and 90 days (third month of treatment). \**P* < .05 compared with baseline. <sup>†</sup>*P* < .05 compared with baseline and control group (two-way repeated-measure analysis of variance).

### Aerobic Capacity and Pulmonary Function

At baseline, 55% (49/89) of the patients had  $\dot{V}O_2$ max values < 70% of predicted normal values. After the study, only the training group showed an increase in  $\dot{V}O_2$ max compared with the control group (*P* < .001) (Fig 3), without changes in the pulmonary function (Table 4). The training group also showed a moderate relationship (*r* = 0.66; *P* < .001) between baseline aerobic capacity and its improvement  $\{[(\dot{V}O_2$ max posttraining – pretraining)/pretraining] × 100\}. A positive response to training (improvement in  $\dot{V}O_2$ max, ≥ 10%) was found in 37 (84%) patients who were considered responders.

### Linear Relationship Among Outcomes

There was a moderate linear relationship between baseline anxiety (*r* = 0.52; *P* < .001) (Fig 4A) and depression (*r* = 0.62; *P* < .001) (Fig 4B) scores that improved after aerobic training (ie, the worse the baseline score, the better the improvement after aerobic training). Similar results were observed in asthma-specific HRQoL (*r* = 0.56; *P* < .001) (Fig 4C) and  $\dot{V}O_2$ max (Fig 3B). A positive linear relationship also was observed between improvement in the  $\dot{V}O_2$ max and the number of days without asthma symptoms (*r* = 0.47; *P* = .001).

## DISCUSSION

The present study shows that an aerobic training program in adults with moderate-to-severe persistent asthma improves asthma-specific HRQoL and reduces anxiety and depression levels and asthma symptoms. These benefits were associated with baseline values, suggesting that the patients who started with worse psychosocial levels demonstrated greater improvement.



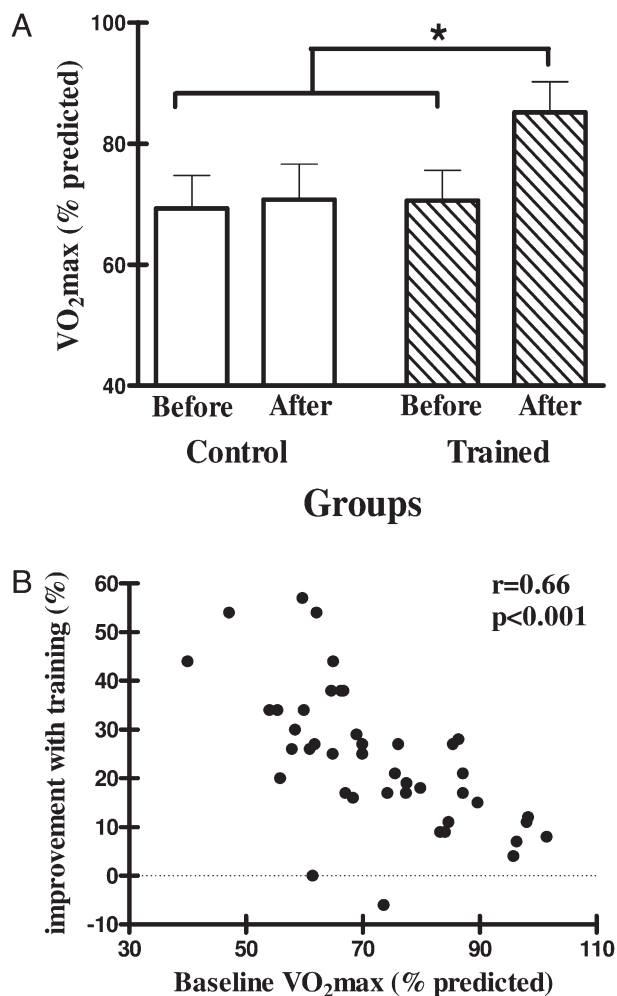


FIGURE 3.  $\dot{V}O_2$ max before and after the study (A). Linear relationship between maximum oxygen consumption at baseline and percentage improvement after training in 44 patients with moderate-to-severe asthma as evaluated by the Spearman correlation test (B). \* $P \leq .05$  compared with the intragroup value obtained before the study and with the control group (two-way repeated-measure analysis of variance test).  $\dot{V}O_2$ max = maximum oxygen consumption.

### Psychosocial Factors

The constant apprehension of asthmatic patients in experiencing breathlessness due to an asthma crisis has a deleterious effect on their HRQoL, impairs

sleep, and makes it difficult for them to perform regular daily activities.<sup>7</sup> Our results show that only patients who participated in aerobic training showed an improvement in aerobic conditioning and physical limitations, frequency of symptoms, and psychosocial domains. To our knowledge, this study is the first randomized clinical trial to show that aerobic training improves HRQoL in adult patients with asthma. The only other study evaluating such benefits<sup>30</sup> included patients with COPD and other types of intervention rather than aerobic training alone, and it was not included in the metaanalysis<sup>16</sup> because the experimental design was considered flawed.

Asthma symptoms cause sleep disturbance, irritability, and anxiety<sup>31</sup> that impair patients' HRQoL. In contrast, when patients are symptom free, their HRQoL is comparable to or even better than the population average.<sup>4</sup> Several studies have described a strong correlation between HRQoL and subjective perception of asthma severity.<sup>4,32</sup> Interestingly, we observed a positive correlation between improvement in the psychosocial HRQoL domain and days without asthma symptoms in the training group; therefore, we suggest that improvement in exercise capacity reduces impairment in daily activities and improves social life and HRQoL.

Our patients presented higher baseline levels of anxiety and depression than a healthy population,<sup>24</sup> and only those submitted to aerobic training showed a reduction of those baseline levels. The decrease in the state anxiety without changes in trait anxiety observed in our training group suggests that improvement in physical fitness can attenuate patients' fear of an acute crisis but does not modify their perception of asthma as a chronic disease. Previous studies have shown that depression symptoms in patients with asthma reduce their disease control and adherence to clinical treatment.<sup>9,10</sup> Therefore, we propose that aerobic training can be used as an important support to improve patient adherence to medical treatment. In addition, we observed that the reduction in the anxiety and depression levels induced by exercise training was positively associated with the baseline values (Fig 4),

Table 4—Pulmonary Function of Adult Patients With Asthma Before and After the Treatment Program

Variable	Control Group (n = 45)		Training Group (n = 44)		P Value
	Before	After	Before	After	
FEV <sub>1</sub> , L	2.3 (1.4-3.19)	2.4 (1.4-3.4)	2.3 (1.3-3.2)	2.3 (1.0-3.4)	.485
% predicted	84.0 (53.5-105.3)	80.0 (49.8-101.9)	78.0 (48.0-107.7)	82.6 (40.0-109.9)	.929
FVC, L	3.3 (2.3-4.3)	3.2 (2.3-4.3)	3.1 (2.3-4.1)	3.2 (2.0-4.1)	.510
% predicted	91.0 (70.8-108.8)	90.1 (64.3-111.7)	93.0 (74.5-116.3)	93.0 (68.0-114.2)	.192
FEV <sub>1</sub> /FVC	72.0 (49.3-86.3)	72.0 (54.0-85.4)	73.0 (53.4-85.0)	72.0 (50.1-92.0)	.657
FEF <sub>25-75%</sub> , L/s	1.7 (0.6-3.4)	1.4 (0.4-3.7)	1.8 (0.6-3.9)	1.7 (0.4-4.3)	.683
% predicted	46.0 (17.4-100.9)	46.0 (14.3-105.8)	50.0 (19.0-104.0)	53.0 (16.0-110.0)	.171

Data are presented as median (95% CI) after two-way repeated-measure analysis of variance. FEF<sub>25-75%</sub> = forced expiratory flow, midexpiratory phase.

suggesting that patients presenting higher psychosocial distress should experience greater benefits from aerobic training.

### Asthma Symptoms

Few controlled and randomized studies have evaluated the benefits of aerobic training on asthma symptoms,<sup>33</sup> so the effect of aerobic exercise remains poorly known.<sup>16</sup> The present study showed that an improvement in aerobic fitness increases the number of days without asthma symptoms after 30 days of training, and this effect was maintained until the end of the treatment program. It is worth mentioning that the improvement in asthma symptoms might be related to

improvement of aerobic capacity because we observed a positive relationship between both outcomes in the training group ( $r = 0.47$ ;  $P < .01$ ). A very recent study also has shown that regular physical activity is associated with reduced risk of an exacerbation in women with asthma.<sup>34</sup> Although merely speculative, we believe that the reduction in asthma symptoms due to aerobic training might be explained by a reduction in minute ventilation during mild-to-moderate daily activities.

### Aerobic Capacity in Asthma

Our training group showed an increase of 5.7 mL/kg/min in  $\dot{V}O_2$ max that fully agrees with the findings of a recent metaanalysis (5.5 mL/kg/min).<sup>16</sup> Although improvement in physical fitness was not the main goal in our study, these results will significantly reinforce future metaanalyses because we have the largest sample of patients with asthma submitted to aerobic training presented in the literature.<sup>16</sup>

As was previously observed in children with asthma,<sup>35</sup> we verified that the improvement in  $\dot{V}O_2$  max is inversely related to the baseline values (Fig 3B). Interestingly, the linear relationship coefficient observed in our study was similar to those obtained by Neder and coworkers<sup>35</sup> ( $r = -0.66$  vs  $r = -0.72$ , respectively). Taken together, these results suggest that patients with asthma and lower levels of aerobic capacity would experience greater benefits from exercise and should be selected to participate in these programs.

Certain limitations of this study should be noted. First, only about 50% of patients interviewed participated in the study, which may not represent the entire population with asthma. Second, the evaluation of outcomes and the rehabilitation program were performed by the same investigators; however, the HRQoL questionnaires and the anxiety and depression inventories were filled out by each patient. Third, both groups were not exposed to a similar amount of attention during the intervention session; however, we did attempt to equalize baseline asthma knowledge, which could explain the reduction of asthma symptoms in the control group. This addition may represent an improvement compared with other studies where control groups only received usual care.<sup>16</sup> Finally, patients were followed for a short-term period, and further studies are required to understand long-term effects.

In conclusion, our results suggest that aerobic training can play an important role in the clinical management of patients with moderate or severe persistent asthma by improving HRQoL, decreasing anxiety and depression levels, and decreasing asthma symptoms. Future studies of patients with asthma presenting with higher degrees of psychologic distress are required to evaluate the benefits of aerobic training in this population.

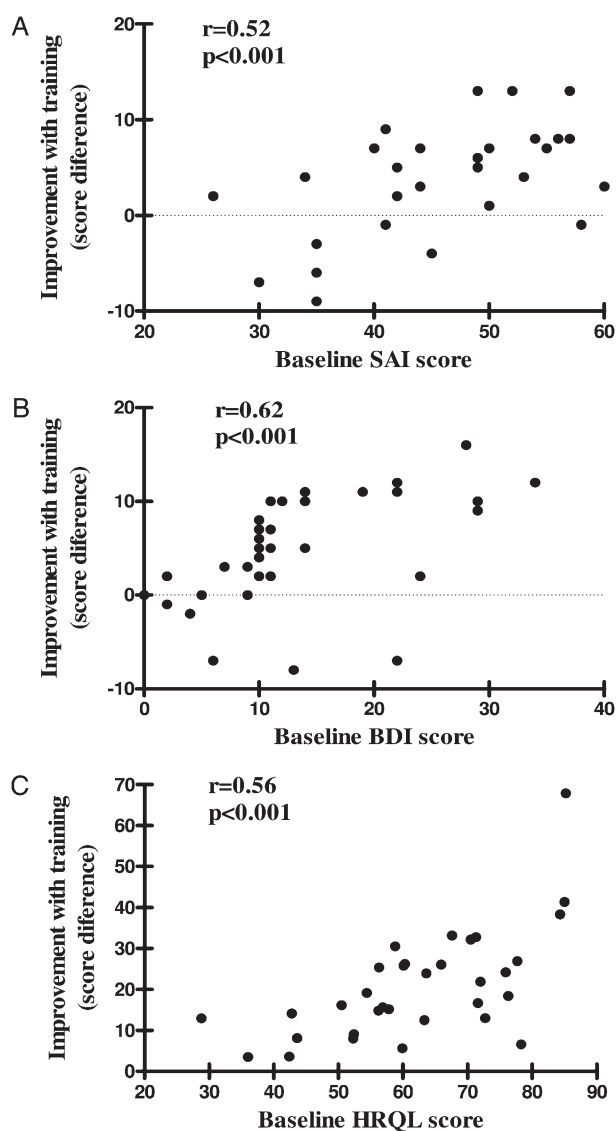


FIGURE 4. Linear relationship between baseline score of state anxiety (A), depression (B), and health-related quality of life (C) and their improvement after training in 44 patients with moderate-to-severe asthma as evaluated by the Spearman correlation test. BDI = Beck Depression Inventory; HRQoL = health-related quality of life; SAI = State Anxiety Inventory.

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**Author contributions:** *Mr Mendes:* contributed to manuscript writing, the study concept and design, data acquisition, and data analysis and interpretation.

*Ms Gonçalves:* contributed to manuscript writing, the study concept and design, data acquisition, and data analysis and interpretation.

*Dr Nunes:* contributed to manuscript revision and the study concept and design.

*Dr Saraiva-Romanholo:* contributed to manuscript revision, data acquisition, and data analysis and interpretation.

*Dr Cukier:* contributed to manuscript revision, the study concept and design, and data analysis and interpretation.

*Dr Stelmach:* contributed to manuscript revision, the study concept and design, and data analysis and interpretation.

*Dr Jacob-Filho:* contributed to manuscript revision and data analysis and interpretation.

*Dr Martins:* contributed to manuscript revision, the study concept and design, and data analysis and interpretation.

*Dr Carvalho:* contributed to manuscript writing, the study concept and design, and data analysis and interpretation.

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