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Evidence of local exercise-induced systemic oxidative stress in chronic obstructive pulmonary disease patients

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Evidence of local exercise-induced systemic oxidative stress in chronic obstructive pulmonary disease patients. A. Couillard, C. Koechlin, J.P. Cristol, A. Varray, C. Prefaut. ©ERS Journals Ltd 2002.

ABSTRACT: Chronic inactivity may not be the sole factor involved in the myopathy of chronic obstructive pulmonary disease (COPD) patients. One hypothesis is that exercise-induced oxidative stress that leads to muscle alterations may also be involved. This study investigated whether exercise localised to a peripheral muscle group would induce oxidative stress in COPD patients.

Eleven COPD patients (FEV_1 1.15±0.4 L (mean±SD)) and 12 healthy age-matched subjects with a similar low quantity of physical activity performed endurance exercise localised to a peripheral muscle group, the quadriceps of the dominant leg. The authors measured plasma levels of thiobarbituric reactive substances (TBARs) as an index of oxidative stress, the release in superoxide anion ($O_2^{\cdot -}$) by stimulated phagocytes as an oxidant, and blood vitamin E as one antioxidant.

Quadriceps endurance was significantly lower in the COPD patients compared with healthy subjects (136±16 s *versus* 385±69 s (mean±SEM), respectively). A significant increase in TBARs 6 h after quadriceps exercise was only found in the COPD patients. In addition, significantly higher $O_2^{\cdot -}$ release and lower blood vitamin E levels were found in COPD patients than in controls at rest. This blood vitamin E level was significantly correlated with the resting level of plasma TBARs in the COPD patients.

This study mainly showed that quadriceps exercise induced systemic oxidative stress in chronic obstructive pulmonary disease patients and that vitamin E levels were decreased in these patients at rest. The exact relevance of these findings to chronic obstructive pulmonary disease myopathy needs to be elucidated.

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The contribution of impaired peripheral muscle function to the exercise intolerance of patients with chronic obstructive pulmonary disease (COPD) has essentially been confirmed [1–5]. An important issue today is whether this myopathy is entirely due to chronic inactivity [6, 7] or if there is an intrinsic muscle disorder, since recent studies have suggested that other factors do contribute to this myopathy [8, 9, 10]. Because exercise-induced oxidative stress can induce substantial muscle alterations [11, 12], it may be one of the mechanisms involved in the myopathy of patients with COPD.

The few studies on this topic have shown that strenuous incremental cycle exercise, and even light constant cycle exercise, results in oxidative stress in COPD patients [13, 14]. However, these studies were without control groups so it was not possible to determine whether COPD patients are more susceptible to exercise-induced oxidative stress than healthy sedentary subjects. Moreover, since general exercise induces a considerable increase in such parameters as ventilation, cardiac output and so on, it was not possible to distinguish the source of oxidative stress in COPD: lung, peripheral skeletal muscle, both and/or others. To distinguish peripheral muscles as a potential source of oxidative stress, local exercise must be performed. During local exercise, work is performed almost exclusively by one muscle group, which minimises cardiac and respiratory responses, and muscle origin is more easily isolated [15].

Exercise-induced oxidative stress results from an imbalance between free radical generation, which increases during exercise, and the efficiency of antioxidant mechanisms. A deficiency in total plasma antioxidant activity has been described in COPD patients at rest, but the specific antioxidants were not determined [16]. In particular, the lack of data on the vitamin E status of COPD patients is striking because different studies have demonstrated that muscle from vitamin E-deficient rats is more prone to exercise-induced oxidative stress and damage than muscle from normal rats [17–19].

The present authors hypothesis is that local exercise-induced oxidative stress is present in COPD patients and may contribute to the myopathy of these patients. As a first step, this study was designed to investigate whether an exercise localised to the quadriceps would be able to generate oxidative stress. In addition, the authors investigated *in vitro* superoxide anion release

as a marker of oxidant production and blood vitamin E as an antioxidant.

Methods

Study population

Subjects. This study included 23 male subjects. Eleven were COPD patients, all exsmokers, selected on the basis of moderate-to-severe COPD according to American Thoracic Society guidelines [20]. All patients had irreversible obstructive airway disease (forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) <60% of predicted values and <10% improvement of FEV_1 after inhalation of a bronchodilating agonist), no hypoxaemia and they were clinically stable. They had not experienced respiratory tract infection or exacerbation of their disease for $\geq$4 weeks prior to the study. None were on oral corticosteroid therapy, although a few inhaled steroids. All patients received anticholinergic or β_2-agonists as bronchodilator therapy. To avoid potentially confounding effects, subjects with known muscle disorders, cardiac failure, diabetes mellitus, and alcoholism were excluded.

Twelve healthy nonsmokers, age-matched, with only sedentary activities at the time of the study, volunteered to serve as control subjects. These healthy subjects were recruited from among friends of members of the laboratory. All participants were questioned on their dietary habits to ensure that none were taking antioxidants or vitamin supplements. In addition, all had a body mass index <35. The study had the approval of the local ethics committee. Written consent was obtained from all participants after they had received a complete explanation of the purposes and protocol of the study.

Definition of recruitment criteria. Lung function. All patients and healthy volunteers underwent spirometry using a whole body plethysmograph (Transmural Bodybox 2800; Sensormedics, Yorba Linda, CA, USA). Measurements included FVC and FEV_1. Tiffeneau's ratio (FEV_1/FVC) was then calculated. The values obtained were compared to the theoretical values of QUANJER *et al.* [21].

Level of physical activity. Physical activity (PA) was assessed with a PA questionnaire adapted for older retired adults [22]. The questionnaire scored the past year's household activities, sports activities, and other physically active leisure-time activities and gave an overall PA score. The subjects were asked to describe the type of activity, hours per week spent on it, and period of the year in which the activity was normally performed. All activities were classified according to posture and movement. An intensity code based on net energetic costs of activities according to BINK *et al.* [23] was used to classify each activity. This questionnaire provides a reliable and valid method for classifying the activity level of older subjects as high, medium, or low. With this method, subjects who obtained a score of 9 were classified as having low physical activity, and thus as "sedentary".

Local muscle exercise

Maximum voluntary contraction (MVC) of the quadriceps for each leg was assessed using an exercise bench (Banc de Koch; Genin Medical, Les Angles, France). Using the same bench, the endurance of the quadriceps of the dominant leg, which was used for local muscle exercise, was then assessed. MVC and quadriceps endurance were assessed according to the technique of SCHERRER *et al.* [24] modified by SERRES *et al.* [5].

Quadriceps femoris endurance exercise was performed by execution of maximal knee extensions against weights corresponding to 40% of MVC with a pace of 12 movements·min^{-1} imposed with an audio signal (metronome) until exhaustion. The dynamic knee extension was performed quickly, immediately followed by passive flexion and then by rest before the next extension. The subjects were instructed to release muscles just after maximal extension, without maintaining static contraction or resisting when weights were set back. The active part of the movement was thereby considered to be the load lift. The duration of rest was always greater than the duration of movement. The test was stopped when the subjects could no longer respect the required maximal extension or frequency two consecutive times despite verbal encouragement. Since a subject's motivation may determine attainment of maximal effort, the same investigator supervised the endurance test and gave the same encouragement to all subjects.

The duration, called the "limit time" (T_{lim}, expressed in s), was then recorded. The dyspnoea score was measured at rest and immediately after exercise on a visual analogic scale.

Protocol

The subjects were instructed to abstain from strenuous physical activity 3 days before and on the day of local muscle exercise. After an overnight fast, and without breakfasting, the subjects arrived at the laboratory between 8:00–9:00 h and underwent spirometric testing. They then responded to the physical activity questionnaire and resting blood samples (T_{rest}) were taken. Next, strength was evaluated and the subjects were familiarised with the endurance test procedures by performing five consecutive dynamic knee extensions. They then performed the local muscle exercise. The second blood samples were taken immediately at the end of the local exercise (T_{end}) and the third, 6 h later (T_{6h}).

Assessment of oxidative stress parameters

Blood samples were drawn in heparinised tubes. Reactive oxygen species production was immediately determined on a 100 μL aliquot of blood. Plasma was obtained by centrifugation (1200×*g* for 10 min at 4°C) and stored at -80°C until analysis.

Products of lipid peroxidation as markers of oxidative stress. Levels of lipid peroxidation were determined

by assessment of thiobarbituric reactive substances (TBARs) using a fluorimetric method previously described by YAGI [25]. The reproducibility calculated as the coefficient of variation was 4.64%.

Determination of superoxide anion release by stimulated phagocytes. Superoxide anion ($O_2^{\cdot -}$) release by stimulated phagocytes was measured at Trest and Tend according to a method derived from VACHIER *et al.* [26]. The specific probe used was lucigenin (at a final concentration of 1.5×10^{-4} M) and the luminescence was recorded at 37°C by means of a 125I LKB Wallac Luminometer (Wallac Co., Turku, Finland). The reproducibility calculated as the coefficient of variation was 6.50%.

Determination of plasma vitamin E. Blood vitamin E was measured by high-performance liquid chromatography according to the method previously described by CACHIA *et al.* [27]. The sum of triglycerides and cholesterol was assessed using a routine enzymatic method (Boehringer Mannheim, Meyland, France). The reproducibility calculated as the coefficient of variation was of 5.6%.

Statistical analysis

Values are reported as mean±SEM. Unpaired t-tests were performed to compare COPD patients with controls. If the equal variance test failed, a Mann-Whitney U-test was used. Correlations between continuous variables were made using simple linear regression. Two-way analysis of variance followed by Tukey's pairwise multiple comparison procedure was used to determine differences between COPD patients and controls in $O_2^{\cdot -}$ production, blood vitamin E, and TBARs at rest and immediately/6 h after exercise. Significance was set at the 0.05 level.

Results

Anthropometric data

There were no significant differences between the COPD and control groups for anthropometric data (table 1). Differences between the two groups could be observed for spirometric function, with the COPD group showing moderate-to-severe airflow obstruction with an FEV1 of 41±3.4% (mean±SEM) pred. As expected, both groups were sedentary since they had a similar low level of PA (table 1).

Muscle performance

Analysis of peripheral muscle performance showed no significant difference in MVC between the COPD and control groups (28±2.8 *versus* 29.75±1.7 kg, respectively). In contrast, muscle endurance (Tlim) was significantly lower in the COPD patients compared with the healthy subjects, with the COPD Tlim being nearly three-fold lower than control Tlim (136±16 *versus* 385±69 s, respectively; $p<0.01$). At the end of exercise, dyspnoea was assessed in five subjects in each group. Between rest and immediate end of exercise, the average dyspnoea scores changed from 0.45±0.2 to 2.08±0.6 and from 0.1±0.07 to 2.8±0.7, respectively, in COPD patients and healthy subjects.

Table 1. – Characteristics of the study population

	COPD	Controls	Statistical difference
Subjects n	11	12	
Age yrs	65±3.6	58±1.5	NS
Height cm	168±2.9	171±2.7	NS
Weight kg	73±4.9	77±4	NS
BMI $kg\cdot m^2$	25.81±1.2	26±0.8	NS
FEV1 L	1.15±0.11	3.29±0.2	***
FEV1 % pred	41±3.4	102±5.3	***
Physical activity score	7.6±1.9	6.6±1.1	NS

Data are presented as mean±SEM unless otherwise indicated. BMI: body mass index; FEV1: forced expiratory volume in one second; COPD: chronic obstructive pulmonary disease; pred: predicted. ***: $p<0.001$. NS: nonsignificant difference between groups.

Rest oxidative stress variables

Rest levels of plasma TBARs were higher at rest in the COPD patients (fig. 1). This difference between groups was notable but did not reach statistical significance. $O_2^{\cdot -}$ by stimulated phagocytes was significantly higher in the COPD patients at rest ($p<0.05$) (table 2). There was a positive and significant correlation in COPD patients between $O_2^{\cdot -}$ release and TBARs ($r=0.73$, $p<0.01$). Absolute values of blood vitamin E at rest were significantly lower in the patients ($p<0.05$) (table 2). Both groups had similar levels of triglycerides (TG) and cholesterol. There was

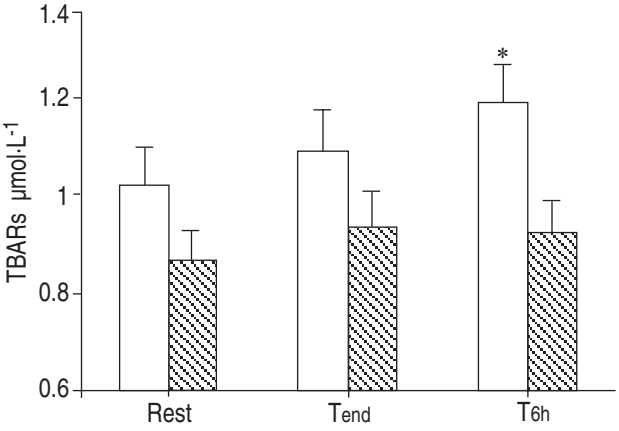

Fig. 1. – Levels of plasma products of lipid peroxidation at rest, at the end of exercise (Tend) and 6 h after the end of exercise (T6h). □: chronic obstructive pulmonary disease (COPD) patients; ▨: controls. Local exercise induced a significant increase in plasma levels of lipid peroxidation products 6 h after the end of exercise in COPD patients ($p<0.05$). Plasma level of thiobarbituric reactive substances (TBARs) was unchanged in the healthy subjects after local exercise.

Table 2. – Values of superoxide anion ($O_2\cdot^-$) release by stimulated phagocytes at rest (Trest) and end (Tend) of exercise and blood vitamin E (Vit. E) at Trest, Tend and 6 h (T6h) postexercise in chronic obstructive pulmonary disease (COPD) patients and controls

	$O_2\cdot^-$ IU		Vit. E mg·L^{-1}		
	Trest	Tend	Trest	Tend	T6h
Controls	385.9±66.3	407.9±69.2	27.7±1.28	28.9±1.5	28.5±1.8
COPD	794.5±189*	759±179*	23.3±1.2*	21.55±1.09**	20.45±1.26**

Values are expressed as mean±SEM. COPD patients: n=11; controls: n=12. *: $p<0.05$ and **: $p<0.01$ compared with control values.

only a positive and significant relationship between the sum of TG+cholesterol and vitamin E in the control group (r=0.66). There was a negative and significant correlation at rest between TBARs and blood vitamin E level in the COPD patients (r=-0.69; $p<0.05$) (fig. 2). No correlation was found between blood vitamin E and TBARs at rest in the healthy subjects.

Local muscle exercise

As shown in figure 1, quadriceps exercise induced a significant increase in plasma products of lipid peroxidation in the COPD patients only 6 h after exercise ($p<0.05$). Exercise had no significant effects on blood vitamin E level or $O_2\cdot^-$ release in either group. No significant correlation was found between resting blood level of vitamin E and TBARs 6 h after exercise in either patients with COPD (r=-0.50, p=0.11) or healthy subjects (r=0.23).

Discussion

The major findings of this study were: 1) a significant increase in blood markers of oxidative stress in COPD patients performing an exercise localised to a peripheral muscle group, the quadriceps of the dominant leg; and 2) a significantly lower blood vitamin E level at rest in these patients.

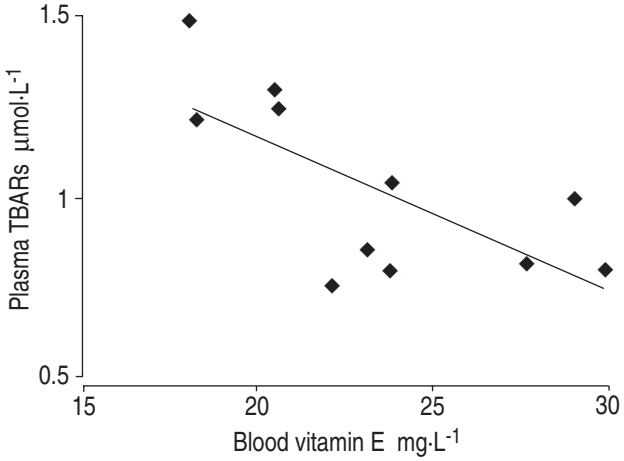

Fig. 2. – Significant correlation was found at rest between blood vitamin E and markers of oxidative stress (thiobarbituric reactive substances (TBARs)) in chronic obstructive pulmonary disease patients (r=-0.69; $p<0.05$).

Methodology

Physical activity. A specific physical activity questionnaire adapted for older retired adults with independent lifestyles was chosen. Although a questionnaire is not a pure objective tool, the chosen method provided a reliable and sufficient method to demonstrate that both groups had the same low level of physical activity.

Quadriceps endurance. To study muscle endurance, local exercise consisting of dynamic knee extensions at a regular pace was chosen, similar to the model described by ANDERSEN *et al.* [15] for study of isolated exercising muscle in humans. Using this model, electromyographic recordings taken from different muscles in addition to exercising quadriceps indicated that only the quadriceps was active during the local exercise. Furthermore, this local exercise minimised ventilatory response [15]. In the present study, the increase in dyspnoea as an index of ventilation after local exercise was of small amplitude. Thus, the advantage of this model is that all the measurements concerning oxidative stress markers are closely related to changes in the working muscle group. In an experiment consisting of repetitive tests with the same technique, Tlim was found to be highly reproducible [28].

Markers of oxidative stress. A global and clinical method was used to assess the parameters of oxidative stress: plasma lipid peroxidation by measurement of TBARs. This is a nonspecific oxidative stress index that should be used with caution because under oxidative stress conditions, malondialaldehyde, hydroperoxides and certain carbohydrates and amino acids may yield products that could react with thiobarbituric acid [29–31]. Determination of TBARs in biological samples thus reflects at least effective peroxidation or an index of peroxidisability. For this reason, TBARs should be used on a group rather than individual basis to assess oxidative stress [29]. Finally, the fluorimetric method used in this study is less technically difficult than others (magnetic resonance or isoprostane measurement) and sufficiently sensitive and reproducible to provide a valid estimation of oxidative stress [25].

Quadriceps endurance and physical activity

As previously mentioned, an important issue concerning the peripheral muscle dysfunction in patients with COPD is whether it is entirely due to chronic inactivity and muscle deconditioning or if there is an intrinsic muscle disorder. In a previous study on this topic [8], COPD patients and normal subjects were not matched for quantified physical activity levels and it was therefore necessary to confirm the multifactorial aspect of COPD myopathy. The present study showed that T_{lim} was nearly three-fold lower in the COPD patients compared with healthy subjects with the same level of physical activity. This result indicated clearly that in addition to chronic inactivity, other phenomena are involved in the muscle dysfunction of these patients.

Oxidative stress at rest

In the present study the resting difference in plasma levels of TBARs between the two groups was of some magnitude, but this did not reach statistical significance. This result contrasts with that of PRATICO *et al.* [32], who described a significant increase in resting urinary isoprostane levels as markers of oxidative stress in patients with COPD compared with age-matched healthy subjects. However, the discrepancy between results may be attributable to a difference in disease severity in terms of exacerbation and hypoxaemia, with patients of the present study less severe than the patients in the study by PRATICO *et al* [32].

The present results showed that $O_2\cdot^-$ release by *in vitro*-stimulated phagocytes was significantly higher at rest in COPD patients compared with controls. This result is in agreement with that of RAHMAN *et al.* [16], who showed that the release of $O_2\cdot^-$ from stimulated phagocytes was greater in COPD patients than in age-matched healthy subjects and greatest in those patients with an exacerbation. These results may suggest a continued oxidant burden originating from phagocytes in patients with COPD.

One of the main results of the present study was that the level of blood vitamin E, a nonenzymatic antioxidant, was significantly lower in the patients compared with controls. To the best of the authors' knowledge, such a low level in COPD has never been reported. It is well known that blood vitamin E level is strongly correlated with cholesterol and TG levels [33]. Yet although a lower level was noted in the patients, the two groups had similar levels of TG and cholesterol. In addition, a significant relationship between the sum of TG+cholesterol and vitamin E was found, as expected, in the healthy subjects, but not in the COPD patients. Therefore, the lower blood vitamin E level in the COPD patients does not seem to be due to an abnormality in lipid absorption, which suggests that in these patients it may be regulated by other mechanisms. The patients' lower blood vitamin E level is of interest in relation to COPD muscle dysfunction because a previous study in animals demonstrated that vitamin E deficiency can cause myopathy [17]. As previously mentioned, vitamin E deficiency can induce skeletal muscle histological changes [34] associated with necrosis of type 1 muscle fibres [19]. Moreover, since vitamin E serves as a lipid soluble biological antioxidant with high specificity for loci of potential lipid peroxidation, a vitamin E deficiency increases the susceptibility of many subcellular membranes to oxidative damage [18]. In agreement with this, it was observed that the lower blood vitamin E level was inversely and significantly correlated with resting plasma levels of oxidative stress measured by TBARs. This suggests that decreased blood vitamin E could be an indicator of oxidative stress in COPD patients. Finally, the results may suggest that antioxidative mechanisms are able to compensate the oxidant burden at rest in the study's stable patients. In this sense, the lower blood vitamin E level observed in the study patients could be a response to the possible oxidant burden originating from phagocytes. However, this hypothesis remains speculative and needs further investigation.

Local exercise-induced oxidative stress

Previous studies have shown that strenuous and/or light exercise induces systemic oxidative stress in COPD patients, which suggests that these patients may be frequently exposed to lipid peroxidation and muscle damage in their daily living activities [13, 14]. However, as mentioned, the few studies on this topic have focused on whole body exercise and did not identify the specific source of this exercise-induced oxidative stress (*e.g.* lungs, heart, liver, *etc.*). The present observation of a significant increase (18%) in plasma products of lipid peroxidation in COPD patients 6 h after local quadriceps exercise indicates exercise-induced oxidative stress in the patients, but not in the controls. The absence of oxidative stress in controls after this local exercise is not surprising since oxidative stress (12% increase in blood lipid peroxidation) occurs in healthy subjects when they perform a half-marathon run [35, 36]. The present study indicated clearly that the patients with COPD presented a greater susceptibility to local exercise-induced oxidative stress. Moreover, as exercise localised to the quadriceps was used without notable respiratory response, the authors suggest that a part of the local exercise-induced oxidative stress assessed in the patients' plasma originated from this muscle. However, the possibility that the COPD increase in lipid peroxidation originated from sources other than the quadriceps (heart, liver, *etc.*) cannot be completely ruled out. In fact, the best way to identify a specific muscle origin of exercise-induced oxidative stress would be by needle biopsy of the quadriceps. If confirmed, this result may be of clinical relevance since muscle oxidative stress generated during exercise might be one of the mechanisms of the peripheral muscle dysfunction described in patients with COPD.

Two mechanisms linking exercise and oxidative stress are increased pro-oxidant activity and inadequate antioxidant activity. The activity of $O_2\cdot^-$ release in circulating phagocytes was unchanged after local exercise in the COPD patients. This result is not

surprising since the circulating catecholamines generated during local exercise are not sufficient to induce an increase in phagocyte activation [37]. In this sense, the local exercise-induced oxidative stress in COPD patients may not be attributable to increased phagocyte $O_2\cdot^-$ release in response to exercise. However, the present study did not investigate other mechanisms of free radical production, such as an altered mitochondrial respiratory chain or xanthine oxidase activity. HEUNKS *et al.* [14] showed that both exercise-induced glutathion oxidation and elevation in lipid peroxides were prevented by allopurinol treatment, a xanthine oxidase inhibitor. This strongly suggests that xanthine oxidase, mainly localised in capillary endothelium, is involved in the exercise-induced oxidative stress in COPD patients.

Apart from increased muscular oxidants, a decrease in antioxidative defences may have contributed to the oxidative stress induced by exercise. In this study, the correlation between resting blood vitamin E levels and TBARs 6 h after exercise did not reach statistical significance and local exercise had no significant effects on COPD blood vitamin E levels. These results suggest that vitamin E was not involved in the exercise-induced oxidative stress in these patients. However, it is also possible that the oxidant burden induced by exercise exceeded vitamin E capacity, which was already significantly decreased at rest. In this sense, the lower resting blood vitamin E levels in these patients, probably associated with other altered antioxidants, may have enhanced the exercise-induced oxidative stress. On this topic, ENGELEN *et al.* [38] showed a significant decrease in quadriceps glutathione (GSH) level in patients with emphysema, and RABINOVICH *et al.* [39] reported that patients with COPD had a reduced ability to adapt to endurance training, as reflected by a lower capacity to synthesise GSH. These recent studies suggest that at least another antioxidant system may be exceeded during exercise and involved in local exercise-induced oxidative stress in these patients.

In conclusion, the present study showed that local exercise induces oxidative stress in chronic obstructive pulmonary disease patients and it also revealed a significant decrease in blood vitamin E in these patients. Further studies based on needle muscle biopsies are needed to confirm the muscular origin of exercise-induced oxidative stress. If these preliminary findings are indeed confirmed, the next step will be to determine whether and how this muscle oxidative stress is involved in the myopathy of chronic obstructive pulmonary disease patients.

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