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Effects of rehabilitative exercise on peripheral muscle TNF-α, IL-6, IGF-I and MyoD expression in COPD patients

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Abstract

Background: Skeletal muscle wasting commonly occurs in patients with chronic obstructive pulmonary disease (COPD) and has been associated with the presence of systemic inflammation. The present study investigated whether rehabilitative exercise training actually decreases the levels of systemic or local muscle inflammation or mainly reverses the abnormalities associated with muscle deconditioning.

Methods: Fifteen COPD patients [(mean±SEM) FEV_1: 36±4% predicted] undertook high-intensity exercise training 3 days/week for 10 weeks. Before and after the training programme the concentration of TNF-α, IL-6 and CRP in plasma was determined by ELISA, whereas vastus lateralis mRNA expression of TNF-α, IL-6, total IGF-I and its isoform mechano-growth factor (MGF) as well as MyoD, were assessed by real-time PCR. Protein levels for TNF-α, IGF-I and MyoD were measured by Western-blot.

Results: Rehabilitation improved peak exercise work-rate (by 10±2%; p=0.004) and mean fiber cross sectional area (from 4061±254 to 4581±241 μm^2; p=0.001). Plasma inflammatory mediators and vastus lateralis expression of TNF-α and IL-6 were not significantly modified by training. In contrast, there was a significant increase in mRNA expression for IGF-I (by 67±22%; p=0.044), MGF (by 67±15%; p=0.002), and MyoD (by 116±30%; p=0.001) The increase observed at the mRNA level was also extended to protein level for IGF-I (by 72±36%; p=0.046) and MyoD (by 67±21%; p=0.012).

Conclusions: Pulmonary rehabilitation can induce peripheral muscle adaptations and modifications in factors regulating skeletal muscle hypertrophy and regeneration without decreasing the levels of systemic or local muscle inflammation.

Introduction

Inflammatory activation with increased serum levels of mediators such as tumor necrosis factor- α (TNF-α), interleukin (IL)-6 and acute phase reactant proteins such as C-reactive protein (CRP), have been described [1, 2] as important factors in the progression of chronic obstructive pulmonary disease (COPD). Although the involvement of systemic cytokines (such as TNF-α and IL-6) in peripheral muscle dysfunction has never been directly documented in COPD, the available literature strongly supports a casual relationship. [1-4] Importantly, in two studies [5-6] enhanced levels of TNF-α have been found in the skeletal muscle of COPD patients compared with age-matched healthy controls, whereas in another study local muscle TNF-α could not be detected. [7]

Recent experimental data suggest that TNF-α interferes with the action of muscle insulin-like growth factor-I (IGF-I), thereby leading to muscle atrophy. [8] In addition, TNF-α inhibits myogenic differentiation through destabilizing MyoD protein, thus interfering with skeletal muscle regeneration. [9] Furthermore, in a rodent local infusion model, IL-6 resulted in muscle atrophy characterized by preferential loss of myofibrillar protein. [10]

Skeletal muscle dysfunction is common in patients with advanced COPD and it contributes importantly to limiting their functional capacity and quality of life. [11] Morphological and biochemical changes within the vastus lateralis muscle of these patients include abnormal fiber-type distribution, reduced fiber cross-sectional areas, and decreased muscle capillarity and oxidative enzyme activities. [12] We recently reported that both constant-load and interval training modalities improve exercise capacity by reversing, at least in part, the aforementioned muscle abnormalities. [13] It remains, however, unknown whether exercise training simply reverses the abnormalities associated with muscle deconditioning or actually interferes with certain inflammatory factors, such as TNF-α and IL-6, that have been proposed to be involved in the pathogenesis of COPD-related muscle dysfunction. [1-4]

Rabinovich and colleagues [5] were the first to report that in COPD patients exercise training does not decrease the levels of systemic or local muscle TNF-α; this report however, did not provide direct evidence whether training actually slowed or even reversed the peripheral muscle abnormalities. On the other hand, although there are studies reporting no effect of exercise training on systemic levels of IL-6 [5, 14], the impact of pulmonary rehabilitation on the expression of local muscle IL-6 is unknown.

Interestingly, exercise training in healthy elderly individuals [15-18] and in patients with chronic heart failure (CHF) [19] has been shown to be associated with upregulation of mRNA expression of total IGF-I, its load-sensitive splice variant termed mechano-growth factor (MGF) and MyoD which constitute important factors for skeletal muscle hypertrophy and regeneration. The possibility that this mechanism could also be an underlying cause of muscle adaptation in COPD patients has not been investigated.

Accordingly, the purpose of the present study was to investigate the effect of training on the local muscle expression of IGF-I, MGF and MyoD. The effect of training on systemic (plasma TNF-α, IL-6 and CRP) and local muscle inflammation (expression of TNF-α and IL-6) was also investigated. It was hypothesized that the training-induced skeletal muscle adaptations are accompanied by increased local muscle IGF-I, MGF and MyoD expression, but not with reductions of systemic or local muscle inflammation. The latter was based on the results of previous studies in COPD showing lack of change in systemic and/or local muscle inflammation with exercise training. [5, 14]

Materials and Methods

Study population

Fifteen patients (3 female) with clinically stable COPD met the following criteria: 1) post-bronchodilator FEV_1<50% predicted and FEV_1/FVC<70% without significant post-bronchodilator reversibility (<10% FEV_1 % predicted normal) 2) optimal medical therapy according to GOLD [20] without regular use of systemic corticosteroids and 3) absence of other significant diseases that could contribute to exercise limitation. Four of these patients had also participated in a previous report. [13] Ten age-matched subjects with FEV_1> 92% predicted were also included in the present study. Patients and healthy subjects signed an informed consent that was approved by the University Ethics Committee.

Study design

Similar to our previous study [13] patients were admitted to an ongoing randomized controlled pulmonary rehabilitation trial consisting of two types of intensive exercise training (interval and constant-load) lasting for 10 consecutive weeks. Since our previous work [13] established no differences between training modalities in terms of muscle morphological characteristics, the present study aimed at determining the effect of exercise training *per se* on local muscle inflammatory and myogenic factors. Prior to and upon completion of the programme, patients were assessed for pulmonary function, exercise tolerance, and plasma inflammatory mediator levels. A muscle percutaneous biopsy was also performed on them.

Assessment

Assessment included: 1) resting pulmonary function, diffusion capacity (D_{LCO}) and subdivisions of lung volumes by body plethysmography (Medgraphics Autolink 1085D; MN, USA); 2) arterial blood analysis (ABL330; Radiometer, Copenhagen, Denmark); 3) incremental (increments of 5-10 W per min) cycle ergometer exercise (Ergoline 800; Sensor Medics, CA, USA) to the limit of tolerance (Wpeak). During the test flow rate at the mouth and gas exchange variables were recorded breath-by-breath (Vmax 229; Sensor Medics, Anaheim, CA). Cardiac frequency and percentage oxygen saturation were determined using the R-R interval from a 12-lead on line electrocardiogram (Marquette Max; Marquette Hellige GmbH, Germany) and a pulse oximeter (Nonin 8600; Nonin Medical, USA), respectively. Symptom ratings were monitored every 2-min throughout exercise using the 1-10 Borg scale. [21]

Exercise training program

Patients performed high-intensity exercise either at a constant-load (initially set at 60% Wpeak for 30-min; n=8) or at intervals of 30-s work alternated by 30-s rest (load initially set at 100% Wpeak, for 45-min, n=7). Exercise was performed on electromagnetically-braked ergometers (CatEye-Ergociser, EC-1600; Osaka, Japan) as previously described. [13, 22, 23] The workload was increased on a weekly basis as detailed below. Similarly to our previous rehabilitation studies [13, 23], the exercise prescription was designed to present patients with a similar overall training load.

Plasma inflammatory mediators

Plasma was collected using EDTA 24 hours prior to and 24 hours after the rehabilitation program. The concentration of mediators was determined by ELISA according to the manufacture's instructions (TNF-α, IL-6 and CRP: R&D systems, Minneapolis, MN, USA). Samples of healthy controls were assayed together with the patient samples. The sensitivity for the respective assays was 0.12 pg/ml for TNF-α, 0.04 pg/ml for IL-6 and 0.01 ng/ml for CRP.

Muscle biopsy

Previous work [24] has demonstrated that the mRNA content of both IGF-I and MyoD 24 h after an exercise bout is not different compared to that before the exercise bout. As such, muscle percutaneous biopsies of the right vastus lateralis muscle performed at mid-thigh (15 cm above the patella) were obtained 24 h before the first and 24 h after the last training session and analyzed blindly as previously described [13] for fiber type classification and cross-sectional areas. In brief, muscle samples were cut in two pieces. One was placed immediately into liquid nitrogen and the other was aligned under a stereoscope in order to have most of the fibers in parallel. Then it was placed in embedding compound and frozen in isopentane pre-cooled to its freezing point. All biopsies were kept at -80^{o}C until the day of

analysis. Cryostat transverse sections of 10 μm thick from the embedded samples were cut at -20°C and were stained for myofibrillar ATPase after pre-incubation at pH 4.3, 4.6 and 10.3. [25] A mean of 413 ± 39 muscle fibers were classified as type I, IIa or IIb from each sample. The cross sectional area of at least 300 fibers from each sample was measured with an image analysis system (ImagePro, Media Cybernetics Inc, Silver Spring, MD, USA) at a known and calibrated magnification. The mean cross-sectional area of all fiber-types, was calculated taking into account the relative contribution of each fiber type to total surface. [7]

Quantitative real-time PCR

Total RNA was extracted from 30 mg muscle biopsies using an RNeasy Fibrous Tissue (Qiagen, West Sussex, UK). Subsequently, total RNA was quantified (260 nm) and adjusted to a concentration of 1μg/μl. The cDNA was synthesized using 1 μg of total RNA from each sample, using a SuperScript First–Strand Synthesis System (Invitrogen, Carlsbad, CA) for RT-PCR according to the manufacturer's instructions. Two micro liters of each cDNA sample were used as template for the amplification reaction using SyBR greenER qPCR Supermix Universal. Primer sequences for TNF-α, IL-6, IGF-I total (IGF-IEabc), MGF (IGF-IEc) and MyoD, are given in Table 1 (see online supplement). PCR amplifications were performed in triplicates in a Chromo4 Detector and PTC-200 Peltier Thermal Cycler and analysed with Opticon software 2.03 (MJ Research, Massachusetts, USA). The threshold cycle (C_T value) was chosen as the first amplification cycle giving a signal above background. To calculate the relative quantity of the respective genes, the $\Delta\Delta C_T$ method was used. [26] Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression (Table 1) was used for normalization. [27] More information on real-time PCR procedures is given on the online supplement.

Muscle protein immunoblotting

Vastus lateralis muscle biopsies stored at -80ºC were homogenized in 10 volumes (wt/vol) of a lysis buffer as previously described. [26] Protein concentration was determined by using DC protein assay (BioRad, Hercules, CA). Samples were subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer to a polyvinylidene fluoride membrane (PVDF) (Millipore Corp. Bedford, MA). Immunoblotting was carried out by using primary antibodies raised against MyoD (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), total IGF-I (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), and TNF-a (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA). To validate equal protein loading among the various lanes, PVDF membranes were striped and re-probed with a monoclonal anti-sarcomeric α-actinin antibody (1:1000, Sigma-Aldrich, St. Louis, MO). [28] All bands were visualized using Chemiluminescent Substrate (Pierce, Rockford, IL). Data was digitalised and quantified by densitometric analysis. More information on protein immunoblottig is given on the online supplement.

Statistical analysis

The minimum sample size was calculated based on 80% power and a two-sided 0.05 significance level using the Statistica 7.0 statistical program. Sample size capable of detecting a change of 3 pg/ml for the TNF-α and IL-6 plasma levels, and of 30% for the TNF-α mRNA expression was estimated using data obtained from a previous study [5] and the following standard deviations: TNF-α: 9 pg/ml, IL-6: 7 pg/ml, and TNF-α mRNA: 25 TNF-α/18S.The critical sample size was estimated to be 14 patients. Data are presented as mean±SD for the subject characteristics and as mean±SEM for all other variables. Mean differences between pre- and post-rehabilitation (with 95% confidence intervals - CI) are given for the plasma inflammatory mediators and the local muscle mRNA and protein expression. Comparisons of baseline plasma inflammatory levels, demographic and lung function characteristics between COPD and healthy subjects were performed using an unpaired t-test. Pre- and post-training comparisons were performed using the Student's paired t-test (exercise testing data, muscle morphological characteristics, plasma and local muscle TNF- α and IL-6) and the Wilcoxon signed rank test (plasma CRP and local muscle mRNA expression of IGF-I, MGF and MyoD) depending on whether data were normally distributed or not, respectively. One sample t-test was used to identify whether the pre-to

post rehabilitation differences in protein levels were significant. The level of significance was set at $p<0.05$.

Results

Baseline patient characteristics

Patients were characterized by severe airflow limitation, hypoxemia without CO_2 retention at rest, reduced diffusing capacity and lung hyperinflation, as reflected by increased FRC (Table 2). Age and BMI were not significantly different between COPD patients and healthy controls, (Table 2).

Table 2: Demographic and lung function characteristics of COPD patients (n = 15) and age-matched healthy subjects (n = 10).

	COPD	Healthy
Age (yr)	66 (7)	61 (5)
BMI (kg/m^2)	25.9 (2.7)	28.8 (2.6)
FEV_1 (l) [% pred]	0.94 (0.44) [35.7 (16.4)]	2.86 (0.8) [94.7 (5.4)] *
FVC (l) [% pred]	2.47 (0.82) [71.3 (20.0)]	3.76 (0.90) [101.3 (11)] *
FEV_1/FVC (%)	36 (11)	77 (4) *
D_{LCO} (% pred)	40.2 (16.8)	81.2 (5.9) *
TLC (l) [% pred]	7.92 (1.24) [126 (9)]	6.16 (0.21) [95 (3)] *
FRC (l) [% pred]	5.83 (0.76) [172 (14)]	3.22 (0.11) [96 (4)] *
RV (l) [% pred]	4.52 (0.82) [209 (30)]	1.93 (0.09) [88 (7)] *
IC (l) [% pred]	2.09 (0.60) [72 (10)]	2.96 (0.18) [92 (5)] *
PaO_2 (mmHg)	65.1 (9.5)	–
$PaCO_2$ (mmHg)	39.8 (2.6)	–
pH	7.44 (0.20)	–
SaO_2 (%)	92.8 (2.2)	–

Values are means (SD). * Significant difference compared to COPD $p<0.05$ (unpaired t-test). D_{LCO}: diffusion capacity, TLC: total lung capacity, FRC: functional residual capacity, IC: inspiratory capacity.

Exercise training program

Examination of the mean training intensity (interval = 115±15% and constant-load = 70±5% of baseline Wpeak) revealed that the total amount of work sustained during interval and constant-load training was comparable. Mean training intensity increased progressively throughout the rehabilitation program such that at weeks 3, 6 and 10 corresponded for interval training to 100±13%, 115±13%, and 130±16% of baseline Wpeak, and for constant-load training to 62±3%, 70±5%, and 81±5% of baseline Wpeak respectively.

Exercise capacity

After rehabilitation there was a significant improvement in peak values of external work rate, oxygen uptake, lactate threshold and minute ventilation (Table 3).

Table 3 Peak exercise responses to pre-training and post-training in COPD patients (n=15)

	Pre-training	Post-training
Work Rate (W)	50 (6)	55 (6) *
$\dot{V}O_2$ (ml/kg/min)	12.1 (1.1)	13.3 (1.4) *
LT (ml/kg/min)	8.5 (0.6)	10.7 (2.4) *
$\dot{V}_E$ (l/min)	35.9 (3.2)	37.1 (3.1) *
*f*b (breaths/min)	31 (2)	34 (2) *
V_T (l)	1.15 (0.08)	1.16 (0.09)
SpO_2 (%)	93 (1)	90 (1)
RER	0.98 (0.04)	0.99 (0.04)
Dyspnoea (Borg)	3.8 (0.4)	4.4 (0.4)
Leg fatigue (Borg)	3.9 (0.4)	3.6 (0.5)

Mean (SEM); *Definition of abbreviations:* WR = work rate; $\dot{V}O_2$ = oxygen uptake; LT = lactate threshold; $\dot{V}E$ = minute ventilation; fb = breathing frequency; V_T = tidal volume; SpO_2% = percentage arterial oxygen saturation; RER = Respiratory exchange ratio
* Significant difference compared with before training $p<0.05$ (paired t-test).

Skeletal muscle adaptations

The proportion of type I and type IIa fibers did not change significantly after training, whereas the proportion of type IIb fibers was significantly (p=0.001) reduced (Table 4). Following rehabilitation the cross-sectional area of each fiber type was significantly increased (Table 4). Hence, the mean fiber cross-sectional area also increased significantly (p=0.001) (from 4061±254 to 4581±241 μm^2).

Table 4 Effect of training on vastus lateralis characteristics in COPD patients (n=15)

	Pre-training	Post-training
Fiber-type distribution (%)		
Type I	37 (4)	39 (4)
Type II a	49 (4)	53 (5)
Type II b	14 (3)	8 (1) *
Cross sectional area (μm^2)		
Type I	4606 (332)	5170 (372) *
Type II a	4225 (295)	4711 (264) *
Type II b	3236 (199)	3693 (209) *

Mean (SEM); * Significant difference compared with before training $p<0.05$ (paired t-test).

Plasma TNF-α, IL-6 and CRP

In patients with COPD pre-training plasma concentration of TNF-α (1.44±0.17 pg/ml), IL-6 (5.68±0.97 pg/ml) and CRP (0.97±0.19 mg/dl) was significantly (p=0.001) greater (by 3, 8 and 7-fold, respectively), compared to healthy subjects (0.53±0.12 pg/ml, 0.76±0.17 pg/ml and 0.15±0.04 mg/dl, respectively) (Figure 1). Following rehabilitation in COPD the mean difference (95 % CI) in TNF-α [-0.10 (-0.34, 0.13) pg/ml], IL-6 [-0.59 (-2.8, 1.7) pg/ml] and CRP [-0.15 (-0.7, 0.6) mg/dl] was not significant (Figure 2).

Skeletal muscle mRNA expression

The mRNA contents were normalized for GAPDH [24, 27] and the results are given in relative units. Following exercise training local muscle TNF-α mRNA expression was not significantly (p=0.73) changed relative to pre-training (Figure 3A: from 1.24±0.19 to 1.37±0.26) corresponding to a mean difference (95 % CI) from baseline of 10±22% (-36, 57%). Neither IL-6 mRNA expression was significantly (p=0.50) changed post-training (Figure 3B: 1.38±0.22 to 1.29±0.22). The mean difference (95 % CI) from baseline was -2±12 (-29, 25%).

Vastus lateralis total IGF-I mRNA expression was significantly (p=0.044) increased after training (from 1.02±0.07 to 1.77±0.40) or by 67±22% (CI: 3, 117%) (Figure 4A). MGF mRNA expression was also significantly (p=0.002) increased after training (from 0.92±0.16 to 1.50±0.27) or by 67±15% (CI: 34, 100%) (Figure 4B). Similarly, local muscle MyoD mRNA expression was significantly (p=0.001) upregulated in response to training (from 1.18±0.22 to 2.20±0.33) or by 116±30% (CI: 51, 181%) (Figure 4C).

Skeletal muscle TNF-α protein

To test whether the changes observed at the mRNA level were also extended to protein level, muscle tissue was analysed by Western blotting. Representative Western blots are shown in Figure 5. Based on densitometric analysis, it was determined that expression of TNF-α was not significantly (p=0.74) changed [9±24% (CI: -49, 66%)] after rehabilitation. In contrast, post-training, there was a significant increase in the expression of both IGF-I [by 72±36% (CI: -12, 155%); p=0.046] and MyoD [by 67±21% (CI: 18, 116%); p=0.012] (Figure 5).

Discussion

Although the involvement of systemic inflammation in peripheral muscle dysfunction has never been directly documented in COPD, the available literature strongly supports a casual relationship. [1-4] Regular exercise training is known to partially reverse the peripheral muscle abnormalities [12, 13], though it is uncertain whether this is the result of reversing the effects of deconditioning alone or if muscle re-conditioning actually decreases the peripheral muscle inflammation. The results of the present study demonstrate that lower-limb exercise training induces significant adaptations in vastus lateralis fiber size and typology without reducing the systemic inflammatory mediator levels or the local muscle expression of TNF-α and IL-6. As these skeletal muscle adaptations were accompanied by significant increase in factors regulating skeletal muscle hypertrophy and regeneration (namely local muscle total IGF-I, MGF and MyoD expression), it is suggested that the peripheral muscles of COPD patients retain adequate plasticity for remodeling in response to exercise training.

In the present study we investigated the effects of peripheral muscle training on specific systemic inflammatory mediator levels (i.e.: TNF-α, IL-6 and CRP) which have been proposed to be associated with muscle dysfunction in COPD. [1- 4] Compared to healthy age-matched controls our patients exhibited significantly elevated plasma levels for TNF-α (3-fold), IL-6 (8-fold) and CRP (7-fold). The fold increase of these mediators found pre-rehabilitation in our COPD patients relative to healthy controls, are in line with those previously reported in patients with stable COPD of similar severity and body mass index. [2, 29, 30] Although lack of change in systemic levels of TNF-α and IL-6 with exercise training has been previously reported [5, 14], to our knowledge this is the first study in COPD patients to document lack of effect of rehabilitation on systemic CRP levels. This is an interesting finding since CRP has been implicated as a marker for impairment in exercise capacity and respiratory distress. [2]

With the present rehabilitation program, local muscle TNF-α mRNA and protein levels were not significantly changed, thus expanding on the results by Rabinovich et al. [5] whose patients exhibited unchanged levels of muscle TNF-α mRNA expression upon completion of a similar endurance training program. In addition, the current study is the first to our knowledge to examine the effects of training on vastus lateralis muscle IL-6 mRNA expression in patients with COPD. Lack of change in local muscle IL-6 mRNA expression corroborates previous results in healthy individuals that exercise training does not impact on local muscle Il-6 mRNA expression. [31]

In the study by Rabinovich et al. [5], lack of training-induced downregulation of muscle TNF-α mRNA expression was attributed to the lower muscle antioxidant capacity in COPD compared to healthy controls. [32] Furthermore, Mercken et al. [33] have shown that patients with COPD when compared to aged-matched healthy subjects are characterized by increased systemic and pulmonary oxidative stress markers after endurance exercise, which although tend to be reduced after pulmonary rehabilitation they remain at higher levels compared to healthy controls. Since muscle inflammation, tissue hypoxemia and oxidative stress are strongly associated in COPD [3, 4] it can be suggested that within the present training program the repeated intense exercise sessions in chronically hypoxemic patients may have exaggerated muscle oxidative stress, thus preventing a significant reduction in local muscle TNF-α and IL-6 mRNA expression.

Animal studies have shown that local muscle TNF-α and IL-6 expression impairs the ability of IGF-I to promote protein synthesis [10, 34] and inhibits the expression of critical muscle-specific transcription factors such as MyoD [35], thus resulting in skeletal muscle atrophy. It is therefore interesting to note that the significantly training-induced upregulation of both muscle IGF-I and MyoD mRNA expression and changes in fibre morphological characteristics occurred despite the finding that muscle TNF-α and IL-6 mRNA expression was not significantly decreased after training. A possible explanation of these findings is that regular work overload is a powerful stimulus modulating an increase in factors that regulate myofibrils hypertrophy, thereby exceeding the potentially negative impact of TNF-α and IL-6 on muscle architecture. On the other hand, exercise training in patients with CHF and healthy elderly humans [36, 37] has been shown to decrease skeletal muscle TNF-α mRNA

expression and induce muscle fibre hypertrophy, thereby suggesting that training may reduce the inhibitory effect of TNF-α on muscle protein synthesis. In CHF, exercise training has also been shown to reduce the local muscle mRNA expression of IL-6. [36] The duration of the training program lasting for 6 months in CHF [36] and/or the mode of exercise (resistance) in healthy elderly humans [37] may account for the differences between those two studies and the current one with regard to the effects of training on muscle inflammation.

In tandem with our previous findings [13] rehabilitative training induced a significant improvement in the cross sectional areas of all fiber types. Although it is known that the muscles of COPD patients can hypertrophy in response to endurance training [11-13], the coupling to local growth factor expression has not been demonstrated. Hence, the present study is the first to show that muscle fibre hypertrophy was accompanied by significant upregulation of IGF-I (both at mRNA and protein levels) that is known to play an important role in the hypertophic adaptation of muscle to overload. [17, 18] The magnitude of increase in IGF-I mRNA expression (by ~70%) in our COPD population is similar to that reported in patients with CHF after endurance training (by ~80%) [19], though it is lower compared to the increase reported after resistance training (by ~300 to 500 %) in healthy elderly. [17, 18]

Interestingly, the present study also demonstrated a significant increase (by ~70%) in the expression of one of the three isoforms of IGF-I, namely the MGF that is known to be sensitive to mechanical loading and to be upregulated after resistance training in healthy elderly (increase in mRNA by ~160%). [15] Hence, it is suggested that the muscles of COPD patients are able to up-regulate MGF mRNA in response to a period of endurance training.

The expression of MyoD is known to be highly induced after resistance training in healthy elderly individuals [16], but to the best of our knowledge an increase in MyoD expression has not been previously reported after an endurance training program in humans. MyoD is expressed in muscle satellite cells and mature myofibers and has been implicated in mediating the process of cell proliferation and differentiation for subsequent muscle regeneration and hypertrophy. [38] Accordingly, it can be hypothesized that the repeated bouts of muscle loading within the present training program caused significant upregulation of MyoD mRNA expression due to the activation of satellite cells for muscle repair/regeneration [38] The increase in local muscle MyoD protein levels was of smaller magnitude compared to the mRNA expression. It has been suggested that owing to a number of posttranscriptional controls (e.g., RNA splicing, RNA editing, blocked nuclear export, subcellular localization, negative translational control), a close relationship between mRNA and protein would not be expected. [9, 39],

Study limitations

The invasive nature of the muscle biopsies precluded a parallel, aged-matched, healthy control, training group which would allow comparisons in training-induced changes in local muscle inflammatory and growth factors between healthy subjects and COPD patients. However, in the study of Rabinovich et al. [5], where such a control group was available, exercise training did not significantly modify local muscle TNF-α mRNA in healthy subjects. In addition, another study [40] involving endurance exercise training in healthy elderly individuals, confirmed that this type of training does not impact on local muscle TNF-α protein levels. Moreover, IGF-I, MGF and MyoD mRNA levels have been shown to increase after resistance training in healthy subjects, aged-matched with those of our study. [15,-17]

Although our previous work [13] established no differences between training strategies in terms of muscle morphological characteristics, it is possible that the effectiveness of one of the two training modalities might be superior in terms of local muscle expression of growth and myogenic regulatory factors. However, in the present study there was not sufficient power within the two modalities to look for such differences. Future studies including larger sample size within each training group will allow such a comparison.

In conclusion, the results of the present study demonstrate that in the absence of a decrease in systemic or local muscle TNF-α and IL-6, endurance exercise training in COPD induces peripheral muscle adaptations that are accompanied by up-regulation of factors regulating skeletal muscle hypertrophy and regeneration.

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Figure Legends

Figure 1 Individual (circles) and mean group (lines) values of plasma levels of (A) C-reactive protein (CRP), (B) tumor necrosis factor-α (TNF-α), and (C) interleukin-6 (IL-6) in patients with COPD (n=15; closed circles) and healthy age-matched subjects (n=10; open circles).

Figure 2 Individual (circles) and mean (±SEM) group (triangles) values of plasma levels of (A) C-reactive protein (CRP), (B) tumor necrosis factor-α (TNF-α), and (C) interleukin-6 (IL-6) pre- and post-training in patients with COPD (n=15) .

Figure 3 Individual (circles) and mean (±SEM) group (triangles) effects of training on vastus lateralis mRNA expression of (A) tumor necrosis factor-α (TNF-α), and (B) IL-6 (n=15). Data are normalized for GAPDH mRNA. * $p < 0.05$ significantly different from pre-training values.

Figure 4 Individual (circles) and mean (±SEM) group (triangles) effects of training on vastus lateralis mRNA expression of (A) IGF-I, (B) MGF and (C) MyoD (n=15). Data are normalized for GAPDH mRNA. * $p < 0.05$ significantly different from pre-training values.

Figure 5 (A) Representative pre-and post-training Western blots for four patients for local muscle (A) TNF-α, (B) IGF-I and (c) MyoD are shown relative to (D) α-actinin antibody. Data quantified by densitometric analysis.

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Figure 1

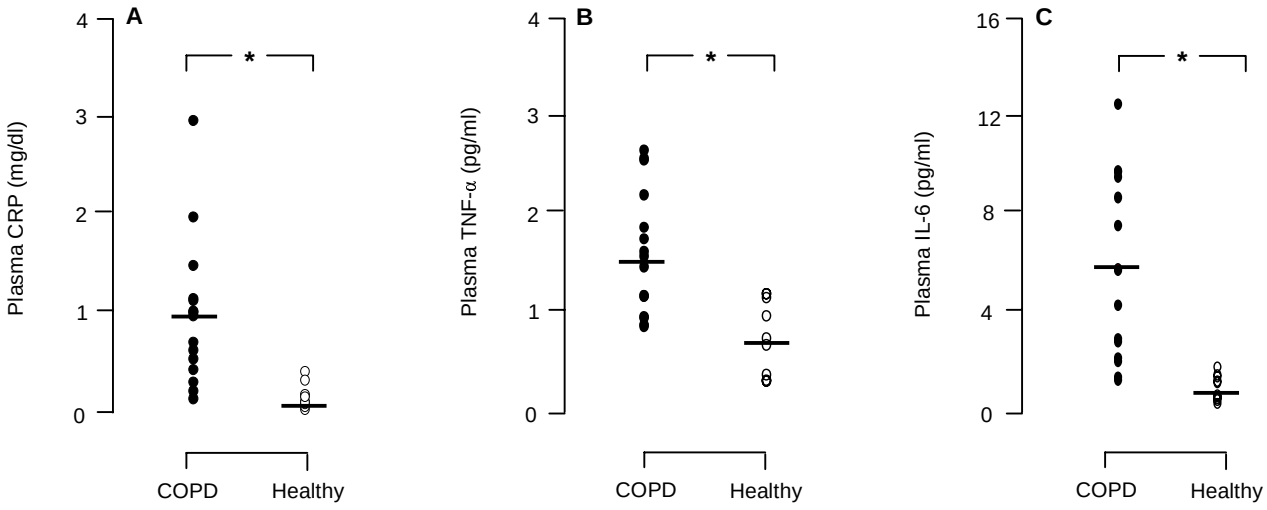
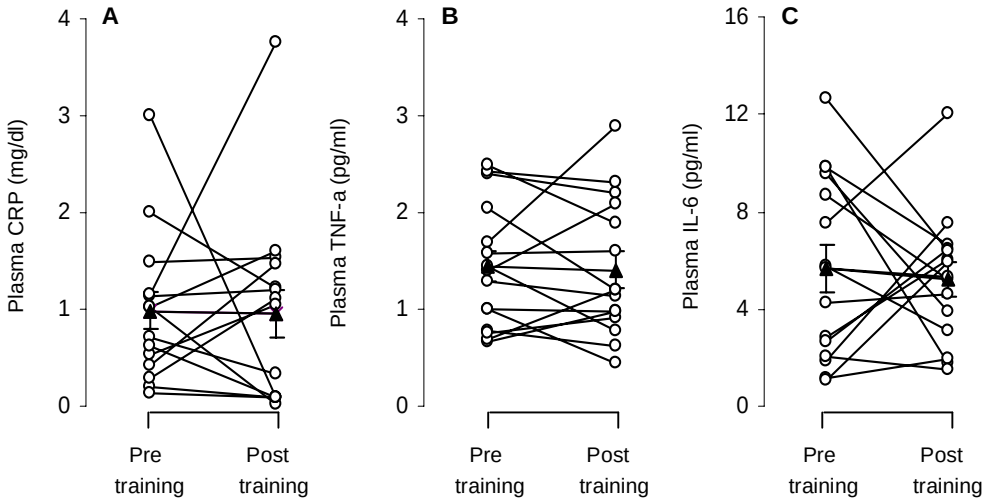


Figure 2



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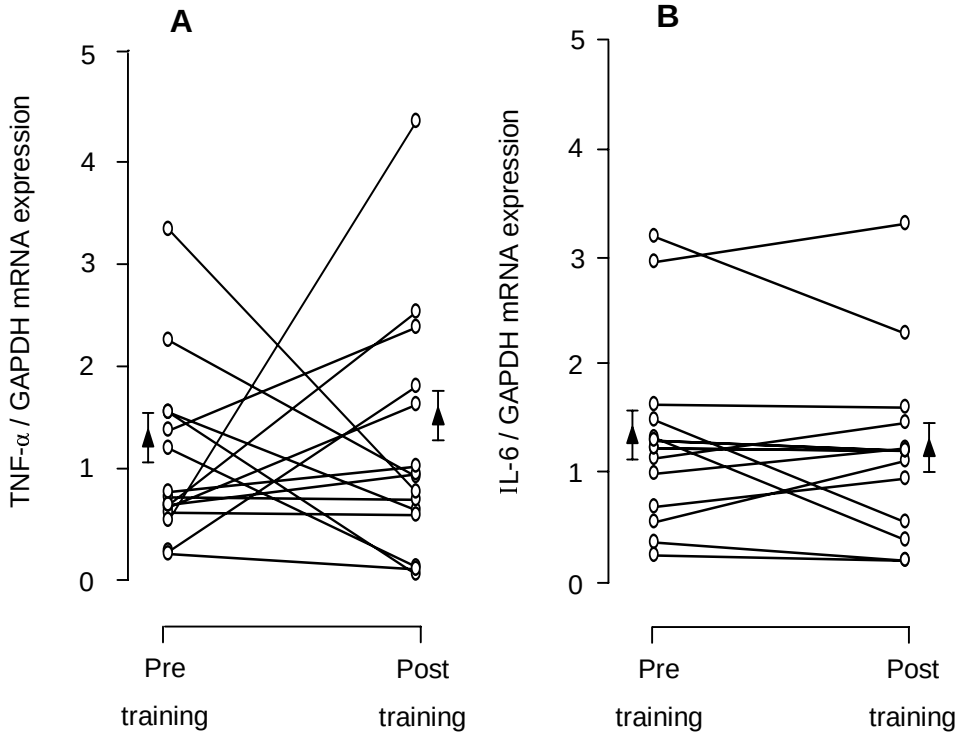

Figure 4

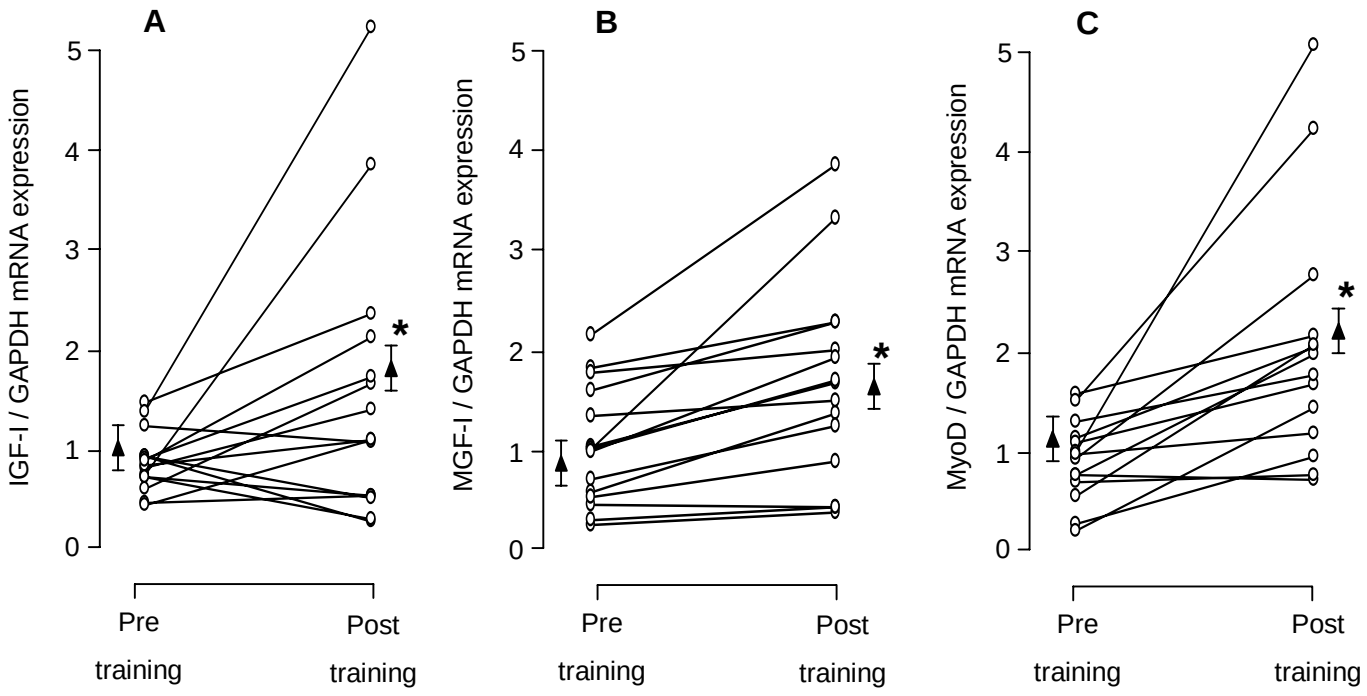

Figure 5