Effects of exercise training on quadriceps muscle gene expression in chronic obstructive pulmonary disease

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Radom-Aizik S, Kaminski N, Hayek S, Halkin H, Cooper DM, Ben-Dov I. Effects of exercise training on quadriceps muscle gene expression in chronic obstructive pulmonary disease. J Appl Physiol 102: 1976–1984, 2007; doi:10.1152/japplphysiol.00577.2006.—Exercise capacity and training response are limited in chronic obstructive pulmonary disease (COPD), but the extent to which this is related to altered skeletal muscle function is not fully understood. To test the hypothesis that muscle gene expression is altered in COPD, we performed needle biopsies from the vastus lateralis of six COPD patients and five sedentary age-matched healthy men, before and after 3 mo of exercise training. RNA was hybridized to Affymetrix U133A Genechip arrays. In addition, peak \( O_2 \) uptake and other functional parameters (e.g., 6-min walk) were measured before and after training. The 6-min walk test increased significantly following training in both groups (53.6 ± 18.6 m in controls, \( P = 0.045 \); 37.1 ± 6.7 m in COPD, \( P = 0.002 \)), but peak \( O_2 \) uptake increased only in controls (19.4 ± 4.5%, \( P = 0.011 \)). Training significantly altered muscle gene expression in both groups, but the number of affected genes was lower in the COPD patients (231) compared with controls (573). Genes related to energy pathways had higher expression in trained controls. In contrast, oxidative stress, ubiquitin proteasome, and COX gene pathways had higher expression in trained COPD patients, and some genes (e.g., COX11, COX15, and MAPK-9) were upregulated by training only in COPD patients. We conclude that both COPD and control subjects demonstrated functional responses to training but with somewhat different patterns in muscle gene expression. The pathways that are uniquely induced by exercise in COPD (e.g., ubiquitin proteasome and COX) might indicate a greater degree of tissue stress (perhaps by altered \( O_2 \) and \( CO_2 \) dynamics) than in controls.

microarray; exercise; rehabilitation; lung disease; skeletal muscle

LUNG FUNCTIONS IN PATIENTS with chronic obstructive pulmonary disease (COPD) correlate weakly with exercise capacity (4) and do not predict exercise responses in the individual patient (25). Consequently, more recent studies have focused on muscle function abnormalities, known to occur in COPD (8, 16–18). In COPD, muscle mass, strength (5, 8, 32), and endurance (2, 23, 36) are reduced, and resting and exercise muscle metabolism are impaired (12, 26). As a result, lactic acidosis develops at lower intensities of exercise (10, 28, 40), leading to higher ventilatory requirement and early onset of muscle fatigue. Biochemical and morphological changes in the vastus lateralis muscle were demonstrated in patients with COPD (2, 11, 14, 15, 25), providing evidence that a peripheral muscle tends to shift from oxidative to glycolytic metabolism (16). This shift is associated with altered composition, a low proportion of type I fibers, a reciprocal rise in type II fibers (11, 15, 25), and reduction in capillarization (20, 42) and oxidative enzymes (2, 15). Exercise training, a core component of rehabilitation, improves exercise capacity and muscle function in COPD (3, 9, 10) but to a limited extent. Training improves cellular bioenergetics (33), increases the type I and IIa muscle fiber cross-sectional area (42), reduces lactic acidosis, and enhances oxidative capacity in COPD (24). We reasoned that the alterations in COPD muscle and the effect of training are linked to multiple molecular pathways.

We recently demonstrated marked gene alteration after training in elderly healthy men, characterized primarily by increased expression of genes involved in energy metabolism, protein amino acid dephosphorylation, and heme biosynthesis (30). Given the profound alterations in the muscle microenvironment in COPD, we hypothesized that the gene expression profile in muscle from these patients would differ in response to training compared with healthy controls. In particular, we predicted that the differences would be most marked in hypoxia-related gene pathways such as oxidative stress. To test this, we chose microarray technology, since microarrays provide an efficient way to examine groups of related genes rather than solely examining isolated, single genes. In the present study, we examined quadriceps muscle from COPD patients and controls, and we compared muscle gene expression in response to a standardized exercise training program between the two groups.

METHODS

Subjects. Six male COPD patients, aged 72 ± 2 yr, and five healthy, age-matched, sedentary men participated in the study (Table 1). The diagnosis of COPD was based on smoking history (minimum 20 pack·yr), and pulmonary function tests showing moderate-to-severe, largely irreversible airflow obstruction [forced expiratory volume in 1 s (FEV\(_1\)) 38.7 ± 3.2% of predicted value (<50% in all subjects) and diffusing capacity of the lung for carbon monoxide (DLCO) 44.4 ± 6.4% of predicted value]. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
predicted value (<70% in all subjects)]. There was no clinical evidence of exercise-limiting cardiovascular or neuromuscular disease. At the time of evaluation, the patients were in stable condition, and none had used a systemic corticosteroid for the preceding 2 mo.

The healthy controls were all nonsmokers and free of significant cardiovascular, metabolic, and musculoskeletal disorders that could limit exercise capacity or modify gene expression. “Sedentary state” was defined as no participation in regular exercise for more than once a week. After detailed explanation of the protocol, the 11 subjects gave their written consent to participate in this study, which was approved by the Sheba Medical Center, the Tel-Aviv University, and the Ministry of Health review boards. All subjects were instructed not to alter their regular activity level or dietary habits throughout the study period.

**Pulmonary function tests.** Spirometry, lung volumes, and diffusing capacity of the lung for carbon monoxide were measured according to previously described guidelines (29). Lung function was measured by spirometry (Godart-Stathham BV, water spirometer, type 15422, Holland).

**Measurements.** Peak VO₂ uptake (VO₂peak) was accepted as maximum VO₂. The anaerobic threshold was determined by the V-slope method (7) and confirmed by the traditional gas exchange criteria (7).

**Evaluation.** The subjects were evaluated during the week preceding and within 1 wk following the 12-wk training program. Incremental maximal cycle ergometry was performed using the CPX system (MedGraphics), while the subjects were breathing through a full-face mask. Expiratory fractions of O₂ and CO₂ were measured by discrete analyzers, and expired ventilation was assessed by a heated wire. VO₂ and CO₂ output were calculated breath by breath and plotted as a 10-s moving average. Electrocardiograms and percent saturation of hemoglobin were recorded throughout the tests (BCI Autocore). After resting on the cycle for 3 min and performing 3 min of unloaded pedaling, the work rate (WR) was incremented at a rate of 10–15 W/min for the patients and 20 W/min for the controls, to the point of fatigue. Six-min walk distance was measured in a 33-m bidirectional fashion. Quadriceps peak torque (Nm) was measured using isokinetic dynamometer (Biodex System II, Shirley, NY). The subjects performed five intense flexions and extensions in 60/°s velocity.

**Training program.** All subjects were trained on a cycle ergometer, 3 times/wk for 12 wk, at 80% of the predetermined individual maximal workload. During weeks 1–3, duration and intensity of the training were increased weekly by the predetermined increments until each subject was able to cycle for 45 min at the target WR that was then maintained for the remaining 9 wk.

**Biopsies.** Tru-Cut biopsies were obtained from the right vastus lateralis before the training program and 72 h after the last training session, using a 14Ga.TW*6 needle. The skin and muscle were anesthetized with 2% lidocaine, and two to four samples were taken, immediately snap-frozen in liquid nitrogen, and stored at −80°C. One sample was also used to verify its muscular origin by microscopy.

**RNA extraction.** Total RNA was extracted by means of a TRizol (Gibco-BRL) reagent, using a Polytron homogenizer (POLYTRON PT 2100, Kinematica), and purified with phenol-chloroform-isooamyl alcohol. RNA pellets were resuspended in diethyl pyrocarbonate-treated water. Integrity of RNA was determined by running a sample on 1% agarose gel. No 28S/18S band degradation was noted in all 22 samples. Purity of RNA was determined using 260/280 ratio. Only samples with ratio >1.62 were included in the analysis.

**Preparation of labeled cRNA.** The detailed protocol for preparation and microarray processing was performed as recommended by the manufacturer and is available in the Affymetrix expression manual 2003. Briefly, 4 µg of total RNA were used as a template for double-stranded cDNA synthesis. Double-stranded cDNA was synthesized with a MessageAMP aRNA kit (Ambion) using an oligo(dT) primer that contains a T7 RNA polymerase promoter site added to its 3’ end. The cDNA was purified with phenol-chloroform-isooamyl alcohol, precipitated with ethanol, and used as a template for in vitro transcription with biotin-labeled nucleotides, biotin-11-CTP and biotin-16-UTP (Enzo Diagnostics). Labeled cRNA was extracted by an RNeasy kit (Qiagen).

**Hybridization to microarray.** A total of 15-µg cRNA product in a buffer [40 mM Tris-acetate (pH 8.1), 100 mM potassium acetate, 30 mM magnesium acetate] was fragmented at 94°C for 35 min and then added to 300 µl of hybridization mix [10 mg/ml fragmented herring sperm DNA (Promega), 3 nM oligonucleotide B2, hybridization controls (biob, bioC, bioD, Cre), 50 mg/ml acetylated BSA]. Aliquots of each sample (10 µg cRNA in 200 µl hybridization mix) were hybridized to a Human U133A Affymetrix GeneChip. After hybridization, each array was washed, stained with streptavidin phycoerythrin (Molecular Probes), washed again, hybridized with biotin-labeled anti-streptavidin phycoerythrin antibodies (Vector Laboratories, Burlingame, CA), reannealed with streptavidin phycoerythrin, and scanned (Hewlett-Packard, GeneArray scanner G2500A).

**Quantitative real-time PCR.** For confirmation of microarray gene expression findings, quantitative real-time PCR (qPCR) was carried out on four genes from four major gene ontology categories. We selected two genes the expression of which was different between the two groups in the sedentary state and two whose expression was different in the trained state. To widen the range of change for validation, we selected one gene with higher expression in COPD and one with higher expression in the healthy controls at each state. These included collagen type XV, α1 (COL15A1), superoxide dismutase 2, mitochondrial (SOD2), glucose phosphate isomerase and hypoxia-inducible factor-1 (HIF-1) responsive RTP801 (HIF1-RTP801). Total RNA was extracted from the individual pre- and posttraining samples. The RNA used for qPCR was extracted separately from the RNA used

| Table 1. Physical and physiological characteristics of the healthy subjects and the patients with COPD |

| Healthy Subjects (n = 5) | COPD (n = 6) |
|-------------------------|-------------|
| **Sedentary** | **Trained** | **P value** | **Sedentary** | **Trained** | **P value** |
| **Age, yr** | 70 ± 2 | — | 72 ± 2 | — |
| **BMI*, kg/m²** | 26.4 ± 0.7 | 26.0 ± 0.6 | NS | 25.7 ± 1.6 | 26.1 ± 1.6 | NS |
| **FEV₁, %predicted** | 108.4 ± 8.3 | 105.4 ± 8.1 | NS | 38.7 ± 3.2 | 38.8 ± 3.1 | NS |
| **DLCO, %predicted** | 114 ± 4 | 112.8 ± 7.7 | NS | 44.4 ± 6.4 | 39.4 ± 8 | NS |
| **SaO₂, %rest** | 97.6 ± 0.2 | 97.8 ± 0.2 | NS | 95.3 ± 0.5 | 96.6 ± 0.4 | NS |
| **V̇O₂peak, liters** | 1.45 ± 0.08 | 1.73 ± 0.11 | 0.011 | 0.77 ± 0.07 | 0.84 ± 0.05 | NS |
| **V̇O₂peak, %predicted** | 77.4 ± 9.7 | 95.1 ± 8.5 | 0.021 | 45.0 ± 6.5 | 46.2 ± 3.3 | NS |
| **AT, %predicted V̇O₂max** | 49.4 ± 5.5 | 61.3 ± 5.2 | 0.0001 | 36.1 ± 8.2 | 40.4 ± 6.9 | NS |
| **6-min Walk, m** | 631.4 ± 28.0 | 685 ± 39.9 | 0.045 | 458.6 ± 23.4 | 495.6 ± 25.7 | 0.002 |
| **Quadriiceps peak torque, 60/°s, Nm** | 148.8 ± 13.7 | 144.7 ± 9.4 | NS | 119.9 ± 10.9 | 136.2 ± 12.3 | 0.003 |

Values are means ± SE; n, no. of subjects. COPD, chronic obstructive pulmonary disease; BMI, body mass index; FEV₁, forced expiratory volume in 1 s; DLCO, diffusion capacity for CO; SaO₂, %saturation of Hb; V̇O₂peak, peak oxygen uptake; V̇O₂max, maximum oxygen uptake; AT, anaerobic threshold; NS, not significant. P was determined by paired t-test (two tails). *Weight did not change significantly during the study.
for microarrays. One microgram of RNA was reverse transcribed using the Reverse Transcription Reagents kit (Invitrogen), according to the manufacturer’s instructions, using random primers in a 20-μl reaction. The qPCR analysis was performed with the ABI PRISM 7900HT Sequence Detection System (TaQMan; Applied Biosystems) by using TaqMan Universal PCR Master Mix and Assays-on-Demand Gene Expression probes (Applied Biosystems) (COL15A1, assay ID: Hs00266332_m1; SOD2 assay ID: Hs00167309_m1; glucose phosphatase isomerase assay ID: Hs00164752_m1; HIF1-RTP801 assay ID: Hs00430304_g1). Glucoronidase-β (assay ID: Hs9999908_m1) was used as an endogenous control in the real-time PCR (RT-PCR).

Data analysis. The arrays were scaled to an average intensity of 150 units and analyzed with Affymetrix Microarray Suit version 5 (MAS5). The microarray data have been deposited in the GEO database (http://www.ncbi.nlm.nih.gov/geo/; series accession number = GSE1786). Only probe sets that reached a signal value ≥20 and a present call by MAS5 criteria in at least one array were selected for the analysis. Overall, 11,333 and 11,064 out of 22,283 probe sets represented on the array met these criteria in the COPD patients and in the healthy controls, respectively. The general approach to analysis has been described by our laboratory elsewhere (26).

Gene annotation. Known or putative gene functions were determined from gene ontology (GO) classifications (provided at http://www.ncbi.nlm.nih.gov/geo/; series accession number = GSE1786). Only probe sets that reached a signal value ≥20 and a present call by MAS5 criteria in at least one array were selected for the analysis. Overall, 11,333 and 11,064 out of 22,283 probe sets represented on the array met these criteria in the COPD patients and in the healthy controls, respectively. The general approach to analysis has been described by our laboratory elsewhere (26).

RESULTS

Subjects. The physical and physiological data on the study participants appear in Table 1. The two groups were matched for all parameters, with the exception of lung function.

Physiological effects of training. As expected, training led to a significant improvement in the exercise capacity of the healthy subjects. Both peak VO2 and the anaerobic threshold were significantly improved. Improvement was limited, however, for the COPD patients, with only the 6-min walk and quadriceps peak torque having improved to a level of significance (Table 1).

Sedentary state. In the sedentary state, the expression of 271 genes could be differentiated between the two groups. Table 2 lists some of the differentiating genes that are of interest (the complete list of differentiated genes is shown in supplementary Tables S1 and S2; the online version of this article contains the supplemental data). Genes that regulate metabolism, collagen synthesis, and muscle contraction had higher expression in the sedentary controls, while, importantly, genes that are linked to oxidative stress, heat shock protein, and proteolysis had higher expression in the sedentary COPD patients.

The effects of training on gene expression. Training induced a remarkable change in gene expression in both groups. Hierarchical clustering of the genes clearly distinguished between the sedentary and trained states in both the COPD and the healthy controls. The program identified clusters of genes whose expression had increased and decreased as a result of training (Fig. 1). To ascertain the biological relevance of these results, we performed the overabundance test and compared between the two states in each group. There were 906 and 1,239 probe sets that achieved a t-test P value <0.05 in the COPD patients and controls, respectively. This number is considerably higher than the expected 564 and 552 probe sets under the null hypothesis that separation of groups is random. In the COPD group, 401 probe sets had a TNoM score of 0 or 1, out of which 231 also had a paired t-test P value <0.05. For the healthy controls, 1,002 probe sets had a TNoM score of 0 or 1, out of which 573 also had a paired t-test P value <0.05. Figure 2 describes the number, the percentage, and the functional classification of the genes that had higher expression after training in the two groups. Genes that had higher expression after training only in the COPD patients but did not change significantly in the healthy controls vs. patients

| Category/Gene                  | Gene Symbol | Expression Ratio (Healthy Sedentary/COPD Sedentary) | P Value (t-test) |
|-------------------------------|-------------|-----------------------------------------------------|-----------------|
| Collagen                      |             |                                                     |                 |
| Collagen, type IV, α3 (Goodpasture antigen) | COL4A3      | 2.14                                                 | 0.038           |
| Collagen, type IV, α2         | COL4A2      | 1.58                                                 | 0.025           |
| Collagen type XV, α1 (COL15A1)| COL15A1     | 1.48                                                 | 0.030           |
| Muscle contraction and development |           |                                                     |                 |
| FXYD domain containing ion transport regulator 1 | FXYD1       | 1.27                                                 | 0.035           |
| Caldesmon 1                   | CALD1       | 1.52                                                 | 0.023           |
| Dystrophia myotonic-protein kinase | DMPK       | 1.32                                                 | 0.026           |
| Myosin, heavy polypeptide 1, skeletal muscle, adult | MYH1       | 1.77                                                 | 0.023           |
| Myosin, light polypeptide 4, alkali; atrial, embryonic | MYL4       | 1.21                                                 | 0.043           |
| Response to oxidative stress |             |                                                     |                 |
| Superoxide dismutase 2, mitochondrial (SOD2) | SOD2        | 0.61                                                 | 0.027           |
| Hypoxia-inducible factor prolyl 4-hydroxylase | PH-4        | 0.87                                                 | 0.049           |
| Heat shock protein activity |             |                                                     |                 |
| Heat shock 90-kDa protein 1, α | HSP90AA1    | 0.81                                                 | 0.032           |
| Heat shock 27-kDa protein 1   | HSPB1       | 0.69                                                 | 0.001           |
| Ubiquitin protein degradation system |           |                                                     |                 |
| Ubiquitin protein ligase E3A   | UBE3A       | 0.77                                                 | 0.034           |

Table 2. Gene expression differences in the sedentary state: healthy controls vs. patients
healthy controls are of special interest (Table 3). Among them are genes linked to response to stress (MAPK-9, MAPKAPK-3), proteolysis (ubiquitin specific protease 15, ubiquitin-conjugating enzyme E2G 1, RAD23 homolog B), and cyclooxygenase (COX) (COX11, COX15). The transcripts of these genes were 35–90% more abundant in the trained state of the COPD patients.

**Trained state.** Of the total of 462 genes that were differentiated between the two groups in the trained state, 245 had higher expression in the trained controls than in the trained COPD patients, and 217 had higher expression in the trained COPD patients than in the trained controls; the complete list of genes is shown in the supplementary Tables S3 and S4. Many of the genes with higher expression in the trained state in the healthy controls regulate energy pathways. Table 4 is a partial list of the genes linked to energy pathways, with higher expression in the healthy controls in the trained state compared with COPD patients. Table 5 lists genes that are of major interest, since their expression in the trained state was higher in the COPD group. Transcripts of genes that are related to adaptive response to hypoxia (HIF-1 responsive RTP801), ubiquitin proteasome pathway (proteasome 26S subunit ATPase 4, proteasome 26S subunit non-ATPase 4, proteasome 26S subunit non-ATPase 1), and COX (COX11, COX15) were 35–85% more abundant in the trained COPD group compared with the trained controls.

**RT-PCR verification of specific gene.** We verified the directional change as well as the magnitude of the change for four
DISCUSSION

This study shows that, while patients with COPD and healthy sedentary subjects presented a distinct gene expression response after endurance training, these responses differed quantitatively and qualitatively. Quantitatively, fewer genes were significantly changed in patients with COPD (231 genes) compared with healthy controls (573 genes). Qualitatively, genes associated with oxidative stress, ubiquitin proteasome, and COX pathways were distinctly induced only in patients with COPD, potentially reflecting the specific molecular response of the muscle to exercise in these patients and suggesting additional mechanisms for exercise limitation in COPD.

Comparison between the two groups in the sedentary state. In the sedentary state, the expression of only 271 genes was different between the two groups. The relatively small difference between the patients and the healthy controls in the pretraining state may have resulted from our selection of only patients with stable COPD and testosterone levels and no use of systemic corticosteroid. This difference, however, increased markedly (to 462 genes) after training (see below).

The effect of training in each group. As expected, training led to significantly improved exercise capacity in the healthy subjects, but the improvement was limited in the patients. These phenotype changes were accompanied by corresponding

Table 3. Genes that had higher expression after training only in COPD patients

| Category/Gene                        | Gene Symbol | Expression Ratio (COPD Trained/COPD Sedentary) | P Value (t-test) | Expression Ratio (Healthy Trained/Healthy Sedentary) | P Value (t-test) |
|--------------------------------------|-------------|-----------------------------------------------|-----------------|----------------------------------------------------|-----------------|
| Response to stress                   |             |                                               |                 |                                                    |                 |
| Mitogen-activated protein kinase 9   | MAPK-9      | 1.41                                          | 0.041           | 0.9                                                | 0.70            |
| Mitogen-activated protein kinase 3   | MAPKAPK-3   | 1.36                                          | 0.014           | 1.25                                               | 0.18            |
| Oxidative phosphorylation (respiratory gaseous exchange) | COX11    | 1.61                                          | 0.014           | 1.26                                               | 0.11            |
| COX15 homolog, cytochrome-c oxidase assembly protein (yeast) | COX15 | 1.43                                          | 0.006           | 1.06                                               | 0.57            |
| COX15 homolog, cytochrome-c oxidase assembly protein (yeast) | USP15    | 1.39                                          | 0.007           | 1.29                                               | 0.12            |
| Ubiquitin protein degradation system | UBE2G1      | 1.48                                          | 0.022           | 1.28                                               | 0.17            |
| Ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, C. elegans) | RAD23B  | 1.89                                          | 0.048           | 1.59                                               | 0.12            |
| Negative regulation of cell proliferation | KLF10    | 1.76                                          | 0.037           | 0.7                                                | 0.27            |

TGF-β, transforming growth factor-β.
changes in gene expression in the quadriceps muscles. In the healthy controls, 573 genes were affected by training (258 were upregulated and 315 were downregulated), while in the COPD group only 231 genes were affected (107 were upregulated and 124 were downregulated). Despite the different magnitudes in the number of genes, the upregulated genes in the two groups were linked to the same functional categories (Fig. 2).

**Why were more genes affected in the healthy subjects?** One explanation for our findings lies in the difference in training intensity between groups. Both groups were trained at 80% of the predetermined peak WR, but the absolute intensity differed. The COPD group and the healthy controls were trained at a level of ~60 and 100 W, respectively. Because the duration of training was similar, the total work of the healthy controls was higher. Even though these differences in intensities may have contributed to the smaller number of genes affected in the COPD group, these corresponding training levels represent the typical training intensity settings usually encountered in standard rehabilitation programs for COPD patients and exercise programs for healthy individuals. We believe that a dose-response effect between training intensity and gene expression cannot account for some of the differences in gene expression between the two groups, because some of the changes were unique to the COPD group.

**Genes that had higher expression after training only in the COPD group and/or genes whose expression was higher in trained COPD patients compared with trained controls.** These genes are of special interest, since they may account for the limited effect of training in COPD. Some genes that are connected to the response to stress and to the ubiquitin protein degradation pathway were upregulated by training only in the COPD patients (Table 3). In addition, we found some genes that had higher expression in trained COPD patients compared with trained controls (Table 5).

**Oxidative stress during training in COPD.** The COX11 and COX15 genes were upregulated by training only in the COPD group (Table 3), and their expression was higher in the trained COPD patients (Table 5). COX15 may be involved in the biosynthesis of heme A, while COX11 exerts its effect at a terminal stage of cytochrome-c oxidase synthesis, which is the terminal complex of the electron transport chain. The adaptive role of COX elevation was suggested in previous studies that showed increased COX activity in skeletal muscle under conditions of tissue hypoxia, such as in peripheral arterial insufficiency (22) and in healthy individuals at moderate altitudes (31). In keeping with these observations, Sauleda et al. (34) found that the activity of COX was increased in COPD patients, particularly in those with chronic respiratory failure.

### Table 5. Genes the expression of which was higher in trained COPD patients compared with trained controls

| Category/Gene | Gene Symbol | Expression Ratio (COPD Trained/Healthy Trained) | P Value (t-test) |
|---------------|-------------|-------------------------------------------------|-----------------|
| Adaptive response to hypoxia | DDIT4 | 1.86 | 0.010 |
| Proteasome | PSMC4 | 1.59 | 0.035 |
| | PSMD4 | 1.51 | 0.048 |
| | PSMD1 | 1.36 | 0.011 |
| Development | ID2 | 2.53 | 0.003 |
| | JARID2 | 1.28 | 0.009 |
| Negative regulation of cell proliferation | KLF11 | 1.48 | 0.018 |
| Oxidative phosphorylation | COX11 | 1.68 | 0.030 |
| | COX15 | 1.82 | 0.016 |

HIF-1, hypoxia-inducible factor-1.
They also found a strong inverse relationship between the degree of arterial hypoxemia and both the activity of COX and the expression of some mtDNA genes (34). Another gene that was expressed significantly higher in trained COPD patients compared with trained controls is HIF-1-RTP801 (Table 5). Shoshani et al. (37) reported the identification and cloning of a novel HIF-1-dependent gene, RTP801, which is ubiquitously expressed in multiple human tissues at low levels, but its transcription is sharply increased both in vitro and in vivo in response to hypoxia.

The higher expression of COX and of HIF-1-RTP801 in trained COPD patients compared with healthy controls may reflect the fact that training had exposed the patients to muscle hypoxia, a potentially deleterious effect with a potential role in limiting the benefit of training and thereby evoking some special adaptive mechanism. Resting oxygen saturation was mildly reduced in our COPD group (Table 1), and two of the six patients underwent training with oxygen supplementation, because their saturation was below 92%. It is likely that the training encouraged more self-initiated activity during the day (with no supplemental oxygen) that could have contributed to upregulation of COX and HIF-1-RTP801 gene expression. Moreover, Soguel et al. (39) showed that patients with moderate-to-severe COPD may develop transient oxygen desaturation during daily activities associated with motion or irregular breathing, such as walking, washing, and eating. An increase in COX activity would help to sustain metabolic flux rates by increasing the blood-to-mitochondrion Po2 gradient and facilitating oxygen diffusion and tissue extraction (41).

**Fig. 3.** Comparison of quantitative PCR (qPCR) and microarray (AffyU133A) expression data for four genes: COL15A1, superoxide dismutase 2, mitochondrial (SOD2), glucose phosphate isomerase (GPI), and hypoxia-inducible factor-1 (HIF-1)-RTP801. For the microarray intensity, data for COL15A1 and SOD2 are shown as 1/300 of actual value, for GPI as 1/1,500, and for the HIF-1-RTP801 as 1/150. Both qPCR and microarray results showed concordant directional as well as proportional changes.

**Exposure to proinflammatory cytokines during training in COPD.** MAPK-9 is activated both by environmental stress and proinflammatory cytokines by phosphorylating a number of transcription factors. MAPKAPK-3 is activated both by mitogens and by stress-inducing agents or proinflammatory cytokines (27, 38, 43). Skeletal muscle can recover rapidly if the inflammatory response is limited, but prolonged systemic inflammation may lead to muscle wasting. Inflammation impacts on muscle protein metabolism in two ways: first, by increasing demand for amino acids to synthesize acute phase proteins in the liver, and second, by the direct effects of circulating proinflammatory cytokines on muscle protein synthesis and degradation. The combination of these direct and indirect effects could explain why interventions, such as increased dietary intake and exercise, aimed at reversing muscle wasting in COPD, have had only limited success (19).

**Genes linked to proteolysis had higher expression after training only in the COPD group and not in healthy controls.** The expression of genes linked to the ubiquitin-proteasome pathway was higher after training only in COPD patients, and/or their expression was higher in trained COPD compared with trained controls. Ubiquitin-conjugating enzyme E2G 1 catalyzes the covalent attachment of ubiquitin to other proteins and may be involved in the degradation of muscle-specific proteins that are widely expressed mainly in skeletal muscle.
The proteasome (prosome, macropain) 26S subunit ATPase-4 (PSMC4) is involved in the ATP-dependent degradation of ubiquitinated proteins. The proteasome (prosome, macropain) 26S subunit non-ATPase-1 (PSMD1) acts as a regulatory subunit of the 26 proteasome, which is involved in the ATP-dependent degradation of ubiquitinated proteins. Proteasomes are distributed throughout eukaryotic cells at a high concentration and cleave peptides in an ATP/ubiquitin-dependent process in a nonlysosomal pathway. Gomes-Marcondes and Tisdale (13) showed that mild oxidative stress increases protein degradation in skeletal muscle by causing an increased expression of the major components of the ubiquitin-proteasome pathway.

An inhibitor of DNA binding 2, dominant negative helix-loop-helix protein (ID2), may be an inhibitor of tissue-specific gene expression. Selman and Leeuwenburgh (35) suggested that activation of the expression of ID-2 and other proteolytic caspases (caspase-3, -7, -8, and -10) may be responsible for the initiation of muscle protein degradation.

The role of the ubiquitin-proteasome pathway in causing muscle wasting is stronger in some conditions, e.g., trauma and acidic renal failure, than it is in others, e.g., Cushing’s syndrome. There are, as yet, no data on the role of this pathway in muscle wasting in COPD (19). Our findings suggest that this pathway may contribute to the limiting training effect that we had observed in COPD patients.

Acidosis and hypoxemia may coexist in severe, acute exacerbations of COPD. These conditions may stimulate muscle proteolysis via the ubiquitin-proteasome pathway (6). Thus acidosis may contribute to a dramatic enhancement of muscle proteolysis via the ubiquitin proteasome pathway in patients with COPD (24). Agusti et al. (1) showed that increased apoptosis occurs in skeletal muscle of underweight COPD patients and that this may contribute to limiting their exercise tolerance.

A proposed mechanism that may explain the limited training effect in COPD compared with healthy subjects. The limited influence of training on COPD stems from the fact that oxygen stress increases and acidosis develops at low levels of muscle activity during exercise. In addition, there is evidence of upregulation of genes, which are activated by exposure to inflammatory cytokines, and the activation of proteolytic mechanisms. Therefore, it is likely that training produces predominantly an anabolic influence among healthy people, while muscle activity simultaneously activates anabolic and catabolic pathways in COPD patients, with the end result of a limited training effect.

Study limitations. The aim of this study was to identify the differences in gene expression between the pre- and posttraining states that may contribute to the limitation of exercise capacity in these states in patients with COPD compared with healthy matched subjects. Ethical and safety considerations limited the amount of tissue that could be obtained. Therefore, we could not determine the dynamics of the changes over time, or whether the genes that had changed as a result of training at the RNA level had also changed at the protein level. Although it could be argued that a change in the mRNA of a single gene will not be translated into a change in its protein expression levels, it is most likely that a coordinated change in the expression of a group of genes that belongs to a certain family or to a functional group will cause such a change. As in every study involving microarrays and a relatively small number of samples, it is possible that the detection of some of the genes was spurious. We attempted to minimize the number of spurious genes by using the intersection of the t-test and TNoM. Having said that, the genes associated with exercise are significantly enriched with biologically meaningful annotations and belong to relevant biological processes, suggesting that the majority of them are, indeed, relevant. Even though a change in a single gene may be spurious, a coordinated change in a group of genes, however, is probably biologically meaningful. Our results were obtained in a prospective experimental setting from carefully characterized groups of patients with COPD and healthy sedentary elderly men, and they could provide a framework for future animal and human experiments. The availability of our raw microarray data allows other investigators to reanalyze them and generate new hypotheses to test in the laboratory setting.

Summary. Three months of training induced a remarkable change in gene expression in both study and control groups. The effect was larger among the healthy controls, and most of the changing genes in the two groups belong to the same biological pathways and had meaningful expected biological annotation. Some of the genes affected by training, however, were unique to COPD, among them genes that are linked to response to stress, proteolysis, and COX, and these may have a role in limiting the effect of training on patients with COPD.

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References

1. Agusti AGN, Sauleeda J, Miralles C, Gomez C, Togores B, Sala E, Batle S, Busquets X. Skeletal muscle apoptosis and weight loss in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 166: 485–489, 2002.
2. Allaire J, Maltais F, Doyon JF, Noel M, LeBlanc P, Carrier G, Simard C, Jobin J. Peripheral muscle endurance and the oxidative profile of the quadriceps in patients with COPD. Thorax 59: 673–678, 2004.
3. Ambrosino N, Strambini S. New strategies to improve exercise tolerance in chronic obstructive pulmonary disease. Eur Respir J 24: 313–322, 2004.
4. Antonucci R, Berton E, Huertas A, Laveneziana P, Palange P. Exercise physiology in COPD. Monaldi Arch Chest Dis 59: 134–139, 2003.
5. Baarends EM, Schols AM, Mostert R, Wouters EF. Peak exercise response in relation to tissue depletion in patients with chronic obstructive pulmonary disease. Eur Respir J 10: 2807–2813, 1997.
6. Bailey JL, Wang X, England BK, Price SR, Ding X, Mitch WE. The acidosis of chronic renal failure activates muscle proteolysis in rats by augmenting transcription of genes encoding proteins of the ATP-dependent ubiquitin-proteasomal pathway. J Clin Invest 97: 1447–1453, 1996.
7. Becker WH, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol 60: 2020–2027, 1986.
8. Belardin S, LeBlanc P, Whittom F, Carrier G, Jobin J, Béliveau R, Maltais F. Peripheral muscle weakness in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 158: 629–634, 1998.
9. Casaburi R. Skeletal muscle dysfunction in chronic obstructive pulmonary disease. Med Sci Sports Exerc 33: S662–S670, 2001.
10. Casaburi R, Patessio A, Iolfi L, Zamaboni S, Donner CF, Wasserman K. Reductions in exercise lactic acidosis and ventilation as a result of exercise training in patients with obstructive lung disease. *Am Rev Respir Dis* 143: 9–18, 1991.

11. Debriare R, Cote CH, Hould FS, LeBlanc P, Maltais F. In vitro and in vivo contractile properties of the vastus lateralis muscle in males with COPD. *Eur Respir J* 21: 273–278, 2003.

12. Engelen MP, Schols AM, Does JD, Gosker HR, Deutz NE, Wouters EF. Exercise-induced lactate increase in relation to muscle substrates in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 162: 1697–1704, 2000.

13. Gomes-Marcondes MC, Tisdale MJ. Induction of protein catabolism and the ubiquitin-proteasome pathway by mild oxidative stress. *Cancer Lett* 180: 69–74, 2002.

14. Gosker HR, Kubat B, Schaart G, van der Vusse GJ, Wouters EF, Schols AM. Myopathological features in skeletal muscle of patients with chronic obstructive pulmonary disease. *Eur Respir J* 22: 280–285, 2003.

15. Gosker HR, van Mameren H, van Dijk PJ, Engelen MP, van der Vusse GJ, Wouters EF, Schols AM. Skeletal muscle fibre-type shifting and metabolic profile in patients with chronic obstructive pulmonary disease. *Eur Respir J* 19: 617–625, 2002.

16. Gosker HR, Wouters EF, van der Vusse GJ, Schols AM. Skeletal muscle dysfunction in chronic obstructive pulmonary disease and chronic heart failure: underlying mechanisms and therapy perspectives. *Am J Clin Nutr* 71: 1033–1047, 2000.

17. Gosselink R, Decramer M. Peripheral skeletal muscles and exercise performance in patients with chronic obstructive pulmonary disease. *Monaldi Arch Chest Dis* 53: 419–423, 1998.

18. Gosselink R, Troosters T, Decramer M. Peripheral muscle weakness contributes to exercise limitation in COPD. *Am J Respir Crit Care Med* 153: 976–980, 1996.

19. Jagoe RT, Engelen MP. Muscle wasting and changes in muscle protein metabolism in chronic obstructive pulmonary disease. *Eur Respir J* 22: 525–635, 2003.

20. John J, Maltais F, Doyon JF, LeBlanc P, Simard PM, Simard AA, Simard C. Chronic obstructive pulmonary disease: capillarity and fiber-type characteristics of skeletal muscle. *J Cardiopulm Rehabil* 18: 432–437, 1998.

21. Kaminski N, Friedman N. Practical approaches to analyzing results of microarray experiments. *Am J Respir Cell Mol Biol* 27: 125–132, 2002.

22. Lundgren F, Dahllof AG, Schersten B, Bylund-Fellenius AC. Muscle enzyme adaptation in patients with peripheral arterial insufficiency: spontaneous adaptation, effect of different treatments and consequences on muscle metabolism and exercise tolerance in COPD. *Am J Respir Cell Mol Biol* 22: 228–232, 2003.

23. Mador MJ, Bozkanat E, Kufel TJ. Quadriceps fatigue after cycle exercise in patients with COPD compared with healthy control subjects. *Arch Phys Med Rehabil* 83: 1573–1580, 2002.

24. Maltais F, Jobin J, Maltais C, Schersten T, Bylund-Fellenius AC. Muscle enzyme adaptation in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 162: 1697–1704, 2000.

25. Maltais F, Sullivan MJ, LeBlanc P, Duscha BD, Schachat FH, Follman C, Blank JM, Jobin J. Altered expression of myosin heavy chain in the vastus lateralis muscle in patients with COPD. *Eur Respir J* 13: 850–854, 1999.

26. Maltais F, Jobin J, Sullivan MJ, Bernard S, Whittom F, Killian KJ, Desmeules M, Belanger M, LeBlanc P. Metabolic and hemodynamic responses of lower limb during exercise in patients with COPD. *J Appl Physiol* 84: 1573–1580, 1998.

27. McLaughlin MM, Kumar S, McDonnell PC, Van Horn S, Lee JC, Livy GP, Young PR. Identification of mitogen-activated protein (MAP) kinase-activated protein kinase-3, a novel substrate of CSBP p38 MAP kinase. *J Biol Chem* 271: 8488–8492, 1996.

28. Polkey MI. Muscle metabolism and exercise tolerance in COPD. *Chest* 121: 1315–1355, 2002.

29. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Pedrin R, Vernolet JC. Lung volumes and forced ventilatory flows. Report World Lung Party Standardization of Lung Function Tests, European Community for Steel and Coal Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 16: 5–40, 1993.

30. Radom-Aizik S, Hayek S, Shahrar I, Rechavi G, Kaminski N, Ben-Dov I. Effects of aerobic training on gene expression in skeletal muscle of elderly men. *Med Sci Sports Exerc* 37: 1680–1696, 2005.

31. Reynafarje B. Myoglobin content and enzymatic activity of muscle and altitude adaptation. *J Appl Physiol* 17: 301–305, 1962.

32. Rochester CL. Exercise training in chronic obstructive pulmonary disease. *J Rehabil Res Dev* 40: 59–80, 2003.

33. Sala E, Roca J, Marrades RM, Alonso J, Gonzalez De Susso JM, Moreno A, Barbera JA, Nadal J, de Jover L, Rodriguez-Roisin R, Wagner PD. Effects of endurance training on skeletal muscle bioenergetics in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 159: 1726–1734, 1999.

34. Sauleda J, Garcia-Palmer F, Wiesner RJ, Tarraga S, Haring I, Tomas P, Gomez C, Saus C, Palou A, Agusti AG. Cytochrome oxidase activity and mitochondrial gene expression in skeletal muscle of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 157: 1413–1417, 1998.

35. Selman C, Leeuwenburgh C. The role of IGF and apoptosis during skeletal muscle remodeling. *Am J Physiol Regul Integr Comp Physiol* 284: R538–R539, 2003.

36. Seres I, Gautier V, Varray A, Prefaut C. Impaired skeletal muscle endurance related to physical inactivity and altered lung function in COPD patients. *Chest* 113: 900–905, 1998.

37. Shoshani T, Faerman A, Mett I, Zelin E, Tenne T, Gorodin S, Moshel Y, Elbaz S, Budanov A, Chajut A, Kalinski H, Kamer I, Rosen A, Mor O, Keshet E, Leshkowitz D, Einat P, Sklalter R, Feinstein E. Identification of a novel hypoxia-inducible factor 1-responsive gene, RFP801, involved in apoptosis. *Mol Cell Biol* 22: 2283–2293, 2002.

38. Simkhovich BZ, Abdilshoo B, Poizat C, Hale SL, Edes LH, Klener RA. Gene activity changes in ischemically preconditioned rabbit heart gene: discovery array study. *Heart Dis* 4: 63–69, 2002.

39. Soguel Schenkel N, Burdet L, de Muralt B, Fitting JW. Oxygen saturation during daily activities in chronic obstructive pulmonary disease. *Eur Respir J* 9: 2584–2589, 1996.

40. Sue DY, Wasserman K, Moricca RB, Casaburi R. Metabolic acidosis during exercise in patients with chronic obstructive pulmonary disease. *Am J Physiol Regul Integr Comp Physiol* 284: R538–R539, 2003.

41. Soguel Schenkel N, Burdet L, de Muralt B, Fitting JW. Oxygen saturation during daily activities in chronic obstructive pulmonary disease. *Eur Respir J* 9: 2584–2589, 1996.

42. Witten F, John J, Simard PM, LeBlanc P, Simard C, Bernard S, Belleau R, Maltais F. Histochemical and morphological characteristics of the vastus lateralis muscle in patients with chronic obstructive pulmonary disease. *Med Sci Sports Exerc* 30: 1467–1474, 1998.

43. Zakowski V, Kermans G, Killian K, Rapp UR, Ludwig S. Mitogen-activated 3p kinase is active in the nucleus. *Exp Cell Res* 299: 101–109, 2004.