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Authors

Radom-Aizik, Shlomit
Kaminski, Naftali
Hayek, Shlomo
et al.

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

Shlomit Radom-Aizik,[1,2] Naftali Kaminski,[2,5] Shlomo Hayek,[4] Hillel Halkin,[3] Dan M. Cooper,[6] and Issahar Ben-Dov[1]

[1]*The Pulmonary Institute,* [2]*Functional Genomics Unit, and* [3]*Institute of Clinical Pharmacology and Toxicology, Sheba Medical Center, Tel Hashomer, Israel;* [4]*Department of Pediatric Orthopedics, Dana Children's Hospital, Tel Aviv Sourasky Medical Center, affiliated with the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel;* [5]*Simmons Center for Interstitial Lung Disease, Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; and* [6]*Pediatric Exercise Research Center, Department of Pediatrics, University of California, Irvine, California*

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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 = 38.7 ± 3.2% of predicted value (<50% in all subjects) and diffusing capacity of the lung for carbon monoxide = 44.4 ± 6.4% of

Address for reprint requests and other correspondence: I. Ben-Dov, The Pulmonary Institute, Sheba Medical Center, Tel-Hashomer 52621, Israel (e-mail: bendovi@post.tau.ac.il).

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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/m^2	26.4±0.7	26.0±0.6	NS	25.7±1.6	26.1±1.6	NS
FEV_1, %predicted	108.4±8.3	105.4±8.1	NS	38.7±3.2	38.8±3.1	NS
D_{LCO}, %predicted	114±4	112.8±7.7	NS	44.4±6.4	39.4±8	NS
Sa_{O_2}, %rest	97.6±0.2	97.8±0.2	NS	95.3±0.5	96.6±0.4	NS
$\dot{V}O_{2peak}$, liters	1.45±0.08	1.73±0.11	0.011	0.77±0.07	0.84±0.05	NS
$\dot{V}O_{2peak}$, %predicted	77.4±9.7	95.1±8.5	0.02	45.0±6.5	46.2±3.3	NS
AT, %predicted $\dot{V}O_{2max}$	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
Quadriceps 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; FEV_1, forced expiratory volume in 1 s; D_{LCO}, diffusion capacity for CO; Sa_{O_2}, %saturation of Hb; $\dot{V}O_{2peak}$, peak oxygen uptake; $\dot{V}O_{2max}$, 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.

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 O_2 uptake ($\dot{V}O_2$) was accepted as maximum $\dot{V}O_2$. 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 O_2 and CO_2 were measured by discrete analyzers, and expired ventilation was assessed by a heated wire. $\dot{V}O_2$ and CO_2 output were calculated breath by breath and plotted as a 10-s moving average. Electrocardiographs 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, Shirly, 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-isoamyl 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-isoamyl 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), restained 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

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 phosphate isomerase assay ID: Hs00164752_m1; HIF1-RTP801 assay ID: Hs00430304_g1). Glucoronidase-β (assay ID: Hs99999908_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 ascertained from gene ontology (GO) classifications (provided at http://www.NetAffx.com or at http://apps1.niaid.nih.gov/david/).

Statistical analysis. For statistical analysis of the microarray data, we used the Scoregene Package (available at http://www.cs.huji.ac.il/labs/compbio/scoregenes/), according to the approach to the analysis outlined by our laboratory earlier (21). To identify genes that best distinguish between the trained and untrained states, we used the Threshold Number of Misclassification (TNoM) score, as well as the traditional Student's paired *t*-test. The TNoM score counts the number of classification errors that occur between compared groups (i.e., COPD vs. controls) for each gene of the dataset. A TNoM score = 0 implies that no errors were counted and that the distinction between the analyzed groups in relation to the expression level of a specific gene is maximal. To improve the stringency of our analysis and compensate for multiple sampling, we considered genes as being significantly changed only if they had a TNoM score of 0 or 1 (i.e., none or one classification error), a two-tailed paired *t*-test *P* value <0.05 and a locus link identification.

Overabundance analysis was used to examine the number of probe sets at different *t*-test *P* values and to compare them with the expected number under the null hypothesis that the separation of the samples is random (21). Data mining, visualization, and hierarchical clustering were also performed using Spotfire DecisionSite for Functional Genomics (Somerville, MA).

Physiological data. The physiological data are presented as means and SE, and they were analyzed using the SAS software (SAS Institute, Cary, NC). The two-tailed *t*-test was applied for testing differences between quantitative parameters, and *P* values <5% were considered significant.

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 $\dot{V}O_2$ 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

Table 2. *Gene expression differences in the sedentary state: 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 myotonica-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

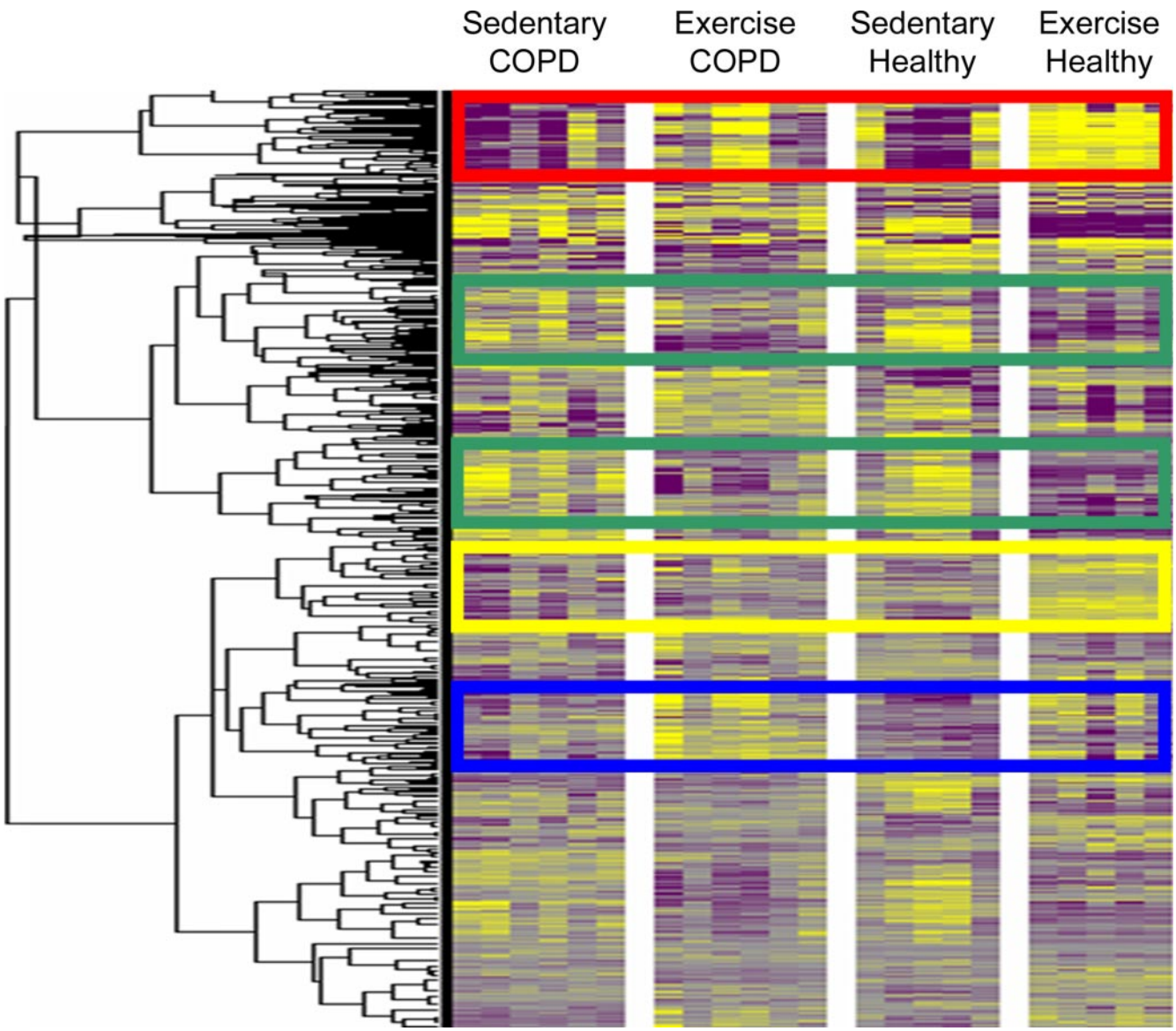

Fig. 1. Hierarchical clustering of the genes showing gene expression patterns that distinguish sedentary and trained states in chronic obstructive pulmonary disease (COPD) patients and healthy controls. Increased expressions are shown in progressively brighter shades of yellow, and decreased expressions are shown in progressively darker shades of blue. Genes were clustered using Pcluster. This figure demonstrates the similarity between the expression levels of the genes. Red box shows a cluster of genes that had higher expression after training in both groups. Green boxes show two clusters of genes that were downregulated by training in both groups. Yellow box shows a cluster of genes that had higher expression after training only in the healthy controls. Blue box shows a cluster of genes that had higher expression after training only in the COPD 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

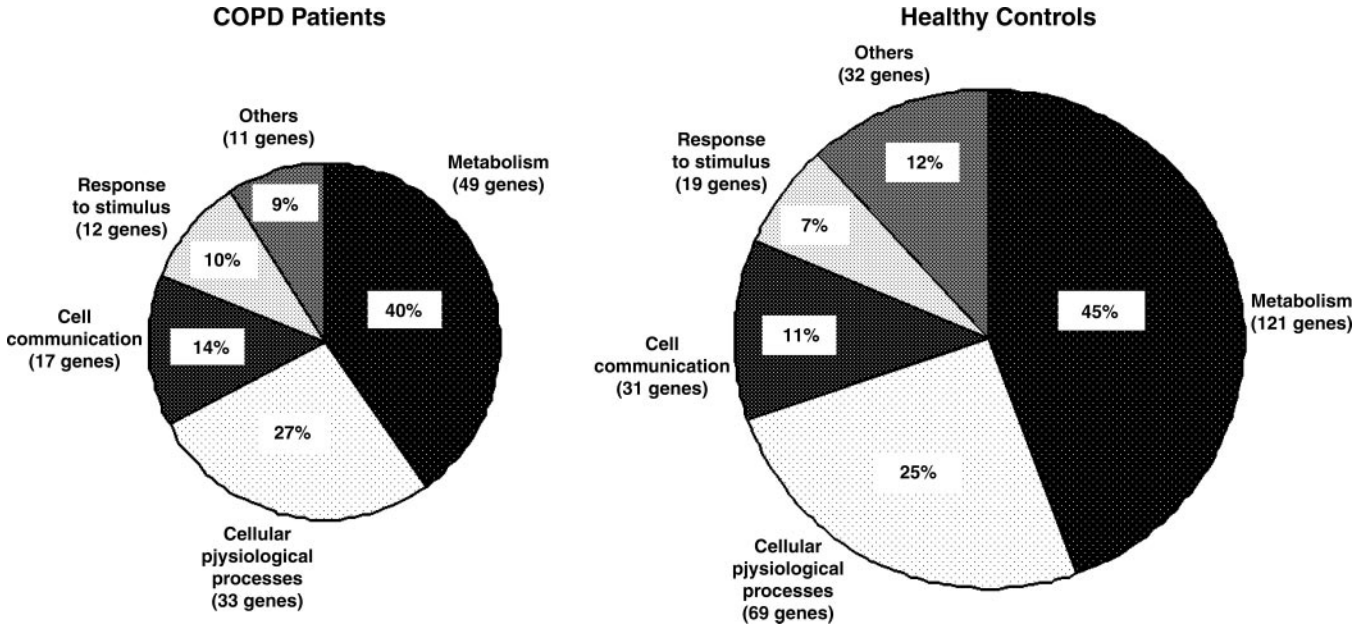

Fig. 2. Genes the expression of which increased after training and that could be classified according to functional classification in individual groups. Only genes that could be classified were included.

genes from four major gene ontology categories. Figure 3 displays the comparison between the microarrays and RT-PCR for these genes.

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) COPD	Expression Ratio (Healthy Trained/Healthy Sedentary)	*P* Value (*t*-test) Healthy
Response to stress					
Mitogen-activated protein kinase 9	MAPK-9	1.41	0.041	0.9	0.70
Mitogen-activated protein kinase-activated protein kinase 3	MAPKAPK-3	1.36	0.014	1.25	0.18
Oxidative phosphorylation (respiratory gaseous exchange)					
COX11 homolog, cytochrome-*c* oxidase assembly protein (yeast)	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
Ubiquitin protein degradation system					
Ubiquitin specific protease 15	USP15	1.39	0.007	1.29	0.12
Ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, *C. elegans*)	UBE2G1	1.48	0.022	1.28	0.17
RAD23 homolog B (*S. cerevisiae*)	RAD23B	1.89	0.048	1.59	0.12
Negative regulation of cell proliferation					
TGF-β inducible early growth response	KLF10	1.76	0.037	0.7	0.27

TGF-β, transforming growth factor-β.

Table 4. *Partial list of the genes of which expression was higher in trained controls compared with trained COPD patients*

Category/Gene	Gene Symbol	Expression Ratio (Healthy Trained/COPD Trained)	*P* Value (*t*-test)
Oxidative phosphorylation			
NADH dehydrogenase (ubiquinone) 1α subcomplex, 5, 13 kDa	NDUFA5	1.39	0.014
NADH dehydrogenase (ubiquinone) 1β subcomplex, 7, 18 kDa	NDUFB7	1.44	0.016
NADH dehydrogenase (ubiquinone) (NADH-coenzyme Q reductase)	NDUFS8	1.76	0.023
ATPase, H^+ transporting, lysosomal V0 subunit a isoform 1	ATP6V0A1	1.23	0.015
Inorganic pyrophosphatase 2	PPA2	1.32	0.019
Citrate cycle (TCA cycle)			
Succinate dehydrogenase complex, subunit D, integral membrane protein	SDHD	1.46	0.017
Fumarate hydratase	FH	2.33	0.007
Malate dehydrogenase 2, NAD (mitochondrial)	MDH2	1.19	0.035
Glycolysis/gluconeogenesis and ATP synthesis			
Glucose phosphate isomerase	GPI	1.28	0.006
Fructose-1,6-bisphosphatase 1	FBP1	1.5	0.022
ATPase, H^+ transporting, lysosomal V0 subunit a isoform 1	ATP6V0A1	1.23	0.015

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 ones 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			
HIF-1 responsive RTP801	DDIT4	1.86	0.010
Proteasome			
Proteasome (prosome, macropain) 26S subunit, ATPase, 4	PSMC4	1.59	0.035
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	PSMD4	1.51	0.048
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	PSMD1	1.36	0.011
Development			
Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	ID2	2.53	0.003
Jumonji, AT rich interactive domain 2	JARID2	1.28	0.009
Negative regulation of cell proliferation			
TGF-β inducible early growth response 2	KLF11	1.48	0.018
Oxidative phosphorylation			
COX11 homolog, cytochrome-*c* oxidase assembly protein (yeast)	COX11	1.68	0.030
COX15 homolog, cytochrome-*c* oxidase assembly protein (yeast)	COX15	1.82	0.016

HIF-1, hypoxia-inducible factor-1.

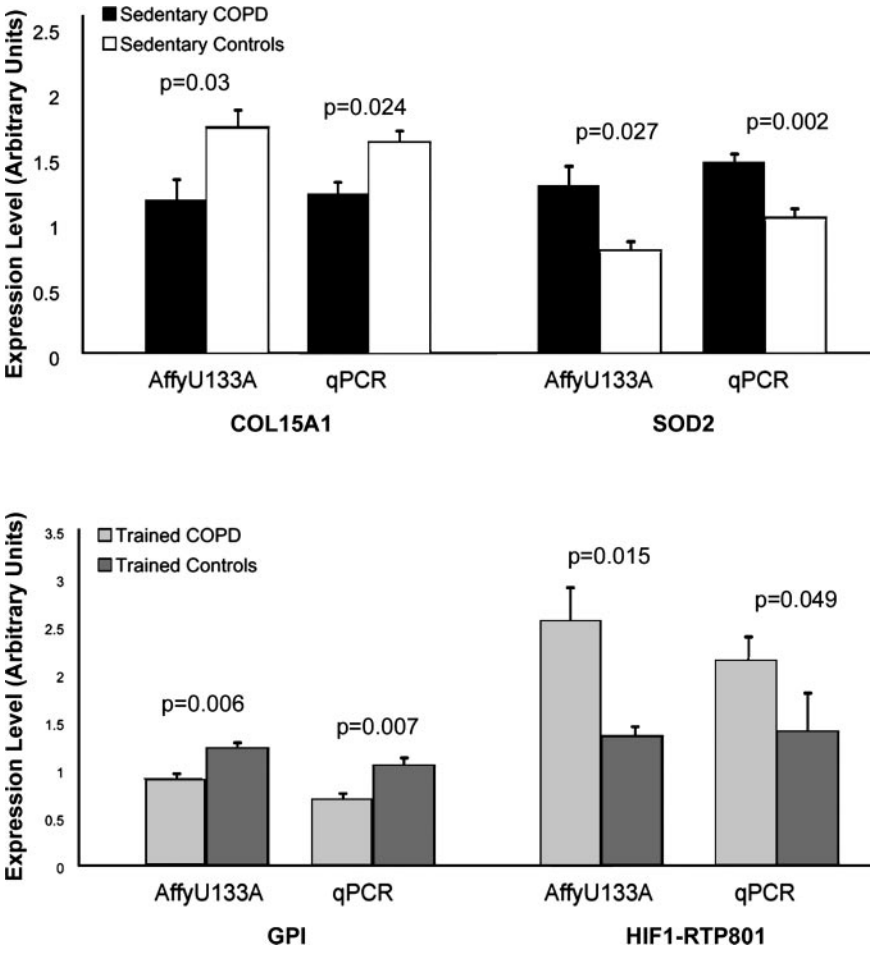

Fig. 3. Comparison of quantitative PCR (qPCR) and microarray (AffyU133A) expression data for four genes: COL15A1, superoxide dismutase 2, mitochondrial (SOD2), glucose phosphate isomerase (GPI), and hypoxia-inducible factor-1 (HIF-1)-RTP801. For the microarray intensity, data for COL15A1 and SOD2 are 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.

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 P_{O_2} gradient and facilitating oxygen diffusion and tissue extraction (41).

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 acidotic 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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