A Longitudinal, Integrated, Clinical, Histological and mRNA Profiling Study of Resistance Exercise in Myositis

Gustavo A Nader,[1,2] Maryam Dastmalchi,[2] Helene Alexanderson,[2,3] Cecilia Grundtman,[2,4] Ramkishore Gernapudi,[1] Mona Esbjörnsson,[5] Zuyi Wang,[1] Johan Rönnelid,[6] Eric P Hoffman,[1] Kanneboyina Nagaraju,[1] and Ingrid E Lundberg[2]

[1]Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC, USA; [2]Rheumatology Unit, Department of Medicine, Karolinska University Hospital Solna, Karolinska Institutet, Stockholm, Sweden; [3]Department of Physical Therapy, Karolinska University Hospital Solna, Stockholm, Sweden; [4] current address: Division of Experimental Pathophysiology and Immunology, Laboratory of Autoimmunity, Biocenter, Innsbruck Medical University, Innsbruck, Austria; [5]Division of Clinical Physiology, Department of Laboratory Medicine, Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden; [6]Unit of Clinical Immunology, Uppsala University, Uppsala University Hospital, Uppsala, Sweden

Polymyositis and dermatomyositis are orphan, chronic skeletal muscle disorders characterized by weakness, infiltrations by mononuclear inflammatory cells, and fibrosis. Until recently, patients were advised to refrain from physical activity because of fears of exacerbation of muscle inflammation. However, recent studies have shown that moderate exercise training in combination with immunosuppressive drugs can improve muscle performance. Despite the positive effects of exercise training, the molecular mechanisms underlying the exercise-associated clinical improvements remain poorly understood. The present study was designed to define, at the molecular level, the effects of resistance exercise training on muscle performance and disease progression in myositis patients. We evaluated changes in muscle strength, histology and genome-wide mRNA profiles to determine the beneficial effects of exercise and determine the possible molecular changes associated with improved muscle performance. A total of 8 myositis patients underwent a 7-wk resistance exercise training program that resulted in improved muscle strength and increased maximal oxygen uptake (VO_{2max}). Training also resulted in marked reductions in gene expression, reflecting reductions in proinflammatory and profibrotic gene networks, changes that were also accompanied by a reduction in tissue fibrosis. Consistent with the exercise-associated increase in VO_{2max}, a subset of transcripts was associated with a shift toward oxidative metabolism. The changes in gene expression reported in the present study are in agreement with the performance improvements induced by exercise and suggest that resistance exercise training can induce a reduction in inflammation and fibrosis in skeletal muscle.
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INTRODUCTION

Polymyositis and dermatomyositis are chronic, autoimmune skeletal muscle disorders characterized by proximal weakness and infiltration of mononuclear inflammatory cells. Current pharmacological treatment is based on high doses of glucocorticoids in combination with other immunosuppressive drugs. Most patients respond with improved muscle performance, but many are left with impaired muscle function and reduced health-related quality of life (1). Several factors could contribute to the sustained muscle impairment despite immunosuppressive treatments. Longitudinal studies of patients with persisting muscle weakness have demonstrated phenotypical changes of muscle tissue, including persisting major histocompatibility complex (MHC) class I expression in muscle fibers and activation markers in endothelial cells of microvessels (2). In some cases, muscle fibrosis develops, indicating repeated cycles of damage and repair. In addition, metabolic impairment occurs, leading to an acquired metabolic myopathy characterized by low levels of adenosine triphosphate (ATP) and phosphocreatine and decreased fatigue resistance (3). All these muscle features are shared by the two subsets of the disease (polymyositis and dermatomyositis). Until recently, patients were advised to refrain from physical activity because of fears of exacerbation of muscle inflammation and disease progression. However, recent studies have shown that moderate exercise in combination with immunosuppressive drugs can improve muscle performance without signs of increased muscle inflammation, suggesting that exercise represents a viable thera-

Address correspondence and reprint requests to *Ingrid E Lundberg, Rheumatology Unit D2:01, Karolinska University Hospital, Solna, SE-171 76 Stockholm, Sweden. Phone: + 46-8-5177 6087; Fax: + 46-8-5177 3080; E-mail: ingrid.lundberg@ki.se.*
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peutic intervention for autoimmune myositis patients (4,5). Therefore, understanding the molecular mechanisms underlying the exercise-induced performance improvements could yield important information for the development of novel interventions for autoimmune inflammatory myopathy patients.

The predominating molecules in muscle tissue of polymyositis and dermatomyositis patients with muscle weakness are proinflammatory cytokines and chemokines, as well as profibrotic transforming growth factor (TGF)-β. Both subsets have a similar molecular expression profile. The most consistently expressed cytokines in different phases of both polymyositis and dermatomyositis are interleukin (IL)-1 and the alarmin high-mobility group box chromosomal protein (HMGB)-1 (6–8). These cytokines have been detected in muscle tissue with a higher expression than in healthy individuals in both the early and late chronic phase of the disease, even without detectable inflammatory cell infiltrates. This occurrence suggests a potential role in muscle function impairment, similar to the negative effect of tumor necrosis factor (TNF) on muscle fiber contractility (9). Another mechanism that could lead to muscle weakness in chronic muscle inflammation is infiltration of muscle tissue by fibrosis. When present, muscle fibrosis is characterized by excessive accumulation of collagen and other extracellular matrix (ECM) components. This dynamic process is controlled by a host of processing factors responsible for enzymatic cleavage, assembly, cross-linking, elasticity and turnover of collagen. Fibrosis development involves extensive structural disorganization and remodeling of the ECM, in part owing to the altered release of fibrogenic cytokines such as TGF-β1 (10).

The aim of the present study was to define, in molecular terms, the potential mechanisms underlying the beneficial effects of resistance exercise in autoimmune inflammatory myopathy patients. Consistent with our previous findings, we show that 7 weeks of resistance exercise resulted in increased performance along with the modulations of proinflammatory and profibrotic genes. In addition, several genes associated with enhanced metabolism were also positively modulated in line with the increase in performance.

MATERIALS AND METHODS

Eight autoimmune inflammatory myopathy patients (five patients with dermatomyositis and three with polymyositis) (11) participated in a resistance exercise program at the Karolinska University Hospital, Stockholm, Sweden (12). Median age was 51 years (range, 44–61 years), and median disease duration was 4.5 years (range, 2.7–29.0 years). More detailed clinical characteristics have previously been published (12). All patients had been treated with glucocorticoids and other immunosuppressive therapies for >12 months, with some improvement of muscle function but with persisting muscle function impairment. The patients were on stable medication before and during the study and had undetected disease activity for 3 months before the beginning of the resistance exercise training program. Maximal oxygen uptake per kilogram at peak exercise (VO_{2max}) was measured according to standard procedures and was determined as the highest value recorded during the last minutes of the exercise test (13). Disease activity was measured by the clinical outcome measure myositis intention-to-treat activity index (MITAX) and by serum creatinine kinase levels (14). Serum complement 1q (C1q) was measured by kinetic nephelometry and compared with a reference curve from a pool of sera from the blood of healthy individuals. The study was approved by the local ethics committee at the Karolinska University Hospital (Solna, Stockholm, Sweden) and the Children's National Medical Center Institutional Review Board (Washington, DC, USA). All patients gave informed consent to participate.

Training Protocol

The resistance exercise training protocol was described in detail previously (12). Briefly, patients underwent a supervised resistance exercise training regimen with an intensity of 10 voluntary repetition maximum (VRM) 3 d/wk for 7 wks. The training involved five muscles groups (deltoid, quadriceps, latissimus dorsi/biceps, gastrocnemius and trunk muscles) 3 d/wk for 7 wks. The clinical and laboratory assessment as well as functional tests were defined previously (12).

Muscle Biopsies

Three to five muscle biopsy samples per patient were obtained from the vastus lateralis muscle using the percutaneous conchotome technique (15). The prebiopsy was taken 1 wk before the initiation of the training period, and the postbiopsy was taken 1 wk after the last exercise training session. The pre- and postbiopsies were taken from opposite limbs to avoid effects of successive biopsies (16) frozen in liquid nitrogen–cooled isopentane and stored at –70°C until further analysis.

RNA Preparation and Expression Profiling

Expression profiling was performed for each individual sample using Affymetrix microarrays following standard operating procedures and quality controls as reported (17). Briefly, total RNA was extracted from frozen muscle biopsies using TRIzol (Invitrogen, Carlsbad, CA, USA), cleaned and concentrated using the RNeasy Minielute Kit (Qiagen, Santa Clara, CA, USA) and quantified spectrophotometrically at 260 nm. RNA integrity was verified by agarose gel electrophoresis, and 2 μg was used for cDNA synthesis. The double-stranded cDNA was subjected to a 16-h *in vitro* transcription at 37°C in the presence of biotin-labeled nucleotides using a one-round amplification strategy. The resulting biotin-labeled cRNA was cleaned, fragmented and hybridized to whole genome Affymetrix

U133 plus 2.0 arrays. After overnight hybridizations at 45°C, microarrays were washed and stained on an Affymetrix Fluidics Station 450 and scanned on an Affymetrix GeneChip Scanner 3000 (Qiagen).

Microarray Data Analysis

All array images were visually inspected for errors and probe set grid alignment. Once the arrays passed, all stringent quality-control measures, including scaling factor <5, present calls >35% and 3′/5′ GAPDH ratios <3, were subjected to probe set absolute-intensity calculations in MAS 5 (Qiagen). In addition, all arrays were subjected to a cross-array analysis to identify any outliers using DChip (18). Statistical analysis was done using GeneSpring GX (Silicon Genetics, Redwood City, CA, USA). Additional stringent quality-control measures were performed by filtering out probe set intensities close to zero and using only probe sets flagged as "present" in half of the samples (50% P calls). Sample normalizations included a "per chip" normalization (50th percentile) and a paired sample normalization in which each subject's pretraining biopsy served as the control for the posttraining biopsy. Statistical significance was determined using a paired *t* test with an α level of 0.05 and a 1.5-fold difference in gene expression between pre- and posttraining. All data corresponding to the microarray experiments, including chip files, are publicly available via http://www.cmm.ki.se/gustavo.

Quantitative Real-Time Polymerase Chain Reaction Target Validation

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to validate the expression of selected genes obtained with the microarray platform. Briefly, cDNA was synthesized from total RNA (100 ng) from the same patient population using the cDNA archive kit (Applied Biosystems, Foster City, CA, USA). Labeled primers (FAM) were added to the samples together with 12.5 μL TaqMan Universal PCR Master Mix. Human GAPDH (VIC labeled; ABI, Seattle, WA, USA) was used as an endogenous control. Reactions were performed on the ABI PRISM 7900HT Sequence Detection System, as recommended by manufacturers. PCR amplifications were performed in triplicate, multiplexed with the endogenous control primers. Differences in gene expression were calculated using the $\Delta\Delta^{Ct}$ method, which is based on the difference in threshold cycles (Δ^{Ct}) between the target gene and endogenous control. The following primers were used: *C1S* (Hs00156159_m1), *COMP* (Hs00164359_m1), *CCL-14* (Hs00234981_m1), *CCR1* (Hs00174298_m1), *COL1α* (Hs00164004_m1), *CTGF* (Hs00170014_m1), *FGF1* (Hs00265254_m1), *IL10Rβ* (Hs00175123_m1), *IL2Rγ* (Hs00173950_m1), *IRAK3* (Hs00200502_m1), *LTBP1* (Hs00386448_m1), *RFX-2* (Hs00172177_m1), and *VAV1* (Hs00232108_m1).

Analysis of Biologically Relevant Gene Networks

To determine functional relationships among the identified genes, we analyzed our dataset with the Ingenuity Pathways Analysis (Ingenuity Systems®, www.ingenuity.com). From the experimental dataset, genes of interest were selected and a functional analysis was carried out. The resulting associations were computed and displayed in a series of interacting networks. The significance, which is a measure for how likely it is that genes from the dataset file participate in that function, is expressed as a *P* value. This result is calculated using the right-tailed Fisher exact test by comparing the number of user-specified genes of interest (i.e., functional analysis genes) that participate in a given function or pathway, relative to the total number of occurrences of these genes in all functional/pathway annotations stored in the Ingenuity Pathways Knowledge database. Significance in the ingenuity analyses refers to the –log (*P* value). For example, if the *P* value for a specific high-level function in one analysis is 1×10^{-10}, its significance is 10. The lower the *P* value for a function, the more significant it is.

Immunohistochemistry

Immunohistochemistry was performed as described by Grundtman *et al*. (19) in seven of eight patients because there was an insufficient amount of tissue in one patient. Primary antibodies used were monoclonal antihuman CD3 (SK7), CD4 (SK3) (both from Becton-Dickinson, San Jose, CA, USA), CD163 (Ber-MAC3, Dako Denmark A/S, Glostrup, Denmark), FOXP3 (236A/E7, eBioscience, San Diego, CA, USA), MHC class I [W6/32(5) Dako Denmark A/S], MHC class II (L243 Becton-Dickinson), IL-1α (1277-89-7) and IL-1β (2D8) (both from Immunokontakt/AMS Biotechnology [Europe], Bioggio [Lugano], Switzerland), and IL-1Ra (10309), IL-1RI (35730) and IL-1RII (832437) (all from R&D Systems, Abingdon, UK). For HMGB-1, a rabbit antibody was used (BD, Pharmingen, San Diego, CA, USA). As secondary antibodies, we used biotinylated F(ab)2 fragmented goat antimouse IgG (Caltag Laboratories, Burlingame, CA, USA) and biotinylated goat antirabbit IgG (Vector Laboratories, Burlingame, CA, USA). As negative controls, we used mouse irrelevant IgG_1 (DAK-G01), IgG_2 (DAK-G059) and negative control rabbit immunoglobulin fraction, all from Dako Denmark A/S. Coded tissue sections were evaluated by conventional microscopic assessment on whole tissue sections by two independent observers with concordant results. For quantification, we measured specific immunostaining on whole tissue sections by computed image analysis for CD3, CD163, IL-1α and HMGB-1. The results are presented as a percentage of total positively stained tissue area. For MHC class I and class II expression, the percentage of positive fibers was estimated (2). Because of variations between individual tissue samples, a significant change was defined as at least doubling

or reduction to half expression between the two biopsies (20).

Quantification of Muscle Fibrosis

Histochemical analysis was carried out on coded frozen skeletal muscle tissue sections stained by Gomori's trichrome procedure (Sigma, St. Louis, MO, USA). Digital images were obtained using light microscopy (objective 40×) and computerized image capture (Olympus C.A.S.T. Stereology System, Olympus America, Center Valley, PA, USA). Samples were analyzed randomly to avoid any bias when determining fibrosis. Connective tissue area was expressed as a ratio of the number of counted dots divided by the total number of dots on the overlay (21). In addition, collagen I content was assessed by Western blotting using an anticollagen I antibody (1:1,000, Abcam). Proteins were homogenized in radioimmunoprecipitation assay (RIPA) buffer containing 1% NP-40 and 10× of each protease and phosphatase inhibitor (Pierce, Rockford, IL, USA), separated in 10% resolving gels and transferred to nitrocellulose membranes. Protein bands were visualized by enhanced chemiluminescence (Amersham, Buckinghamshire, UK) and quantified densitometrically with Quest software (Bio-Rad, Hercules, CA, USA). The band intensities corresponding to collagen I (~95 kDa) were normalized to the intensity of the corresponding lanes in the membranes visualized after Indian ink staining (loading control).

Statistical Analyses

StatView (Abacus Concepts, Berkeley, CA, USA) was used for statistical analyses of clinical, immunohistochemistry and serum data. Because of non-normal distribution of immunological markers in muscle biopsies, nonparametric methods were used. The Wilcoxon signed-rank procedure tested for within-group changes. A parametric method was applied (Student *t* test for paired observations) to establish the level of statistical significance in VO_{2max} changes. Changes in collagen I content were calculated from the Western blot band intensities using a paired *t* test with significance established at $P < 0.05$. The Pearson moment correlation was calculated in Microsoft Excel.

RESULTS

Clinical Data

Muscle strength increased, serum levels of creatinine kinase decreased and clinical disease activity score (MITAX) improved as previously reported (12). Resistance exercise also resulted in decreased median serum levels of C1q from 141 mg/L (range, 80–330 mg/L) to 69 mg/L (range, 42–147 mg/L) ($P = 0.01$) (reference value 70–300 mg/L). A significant increase was demonstrated in maximal absolute and relative oxygen uptake (VO_{2max}) from 1.88 ± 0.3 to 2.20 ± 0.3 L × min^{-1} ($P < 0.001$) and from 26 ± 3 to 31 ± 3 mL × min^{-1} × kg^{-1} ($P < 0.001$), respectively. VO_{2max} values are means ± SD.

Global Changes in Gene Expression in Muscle Tissue after Resistance Exercise

The 7-wk training program resulted in the modulation of 265 transcripts. The predominant enriched ontologies associated with these transcripts were inflammation, fibrosis and metabolism. When these genes were cross-validated against a disease database during the ingenuity pathways analysis, we found that the majority are involved in diseases characterized by inflammation and fibrosis. Ontological analyses of selected genes revealed that 41 transcripts (15.5%) were associated with inflammatory processes, 25 transcripts (9.4%) with fibrotic processes and 7 transcripts (2.6%) with metabolic regulation. We focused on these enriched categories because they best represent the pathogenesis of autoimmune myositis (Table 1). As an initial validation of our microarray results, key representative target genes were analyzed by qRT-PCR. There was a good correlation ($R^2 = 0.62$) between the changes in gene expression detected by microarray and qRT-PCR with the changes showing both qualitative and quantitative similarity (Figure 1).

Resistance Exercise Downregulated Proinflammatory and Upregulated Antiinflammatory Genes

In total, 41 genes involved in inflammation changed significantly, and 34 of these were downregulated (Table 1). The genes with reduced expression could be categorized into those that are involved in T-cell activation and regulation, such as *chemokine (C-X3C motif) receptor 1* (*CCR1*), *CD44 antigen*, *IL-2Rγ*, *MHC–F* and *dedicator of cytokinesis 2* (*DOCK2*), or those involved in macrophage/monocyte activation, such as *colony stimulating factor 2 receptor β* (*CSF2RB*), *VAV1 oncogene* (*VAV1*), *interleukin receptor-associated kinase 3* (*IRAK3*) and *lipopolysaccharide-induced TNF factor* (*LITAF*). The gene for the proinflammatory cytokine HMGB-1 was significantly downregulated ($P < 0.03$), but the change did not pass the 1.5 cutoff fold change (–1.27). Other genes involved in inflammation that were downregulated were *clusterin* (*CLU*), *neurofilament light polypeptide* (*NEFL*), *CCR1*, and *prostaglandin-endoperoxide synthase 1* (*PTGS1*). Additionally, a few antiinflammatory genes were upregulated, as was *FOXP3*, a marker of regulatory T-cells. Additionally, our bioinformatic analysis revealed that several genes regulated by TNFα were negatively modulated, suggesting that they may play a role in the antiinflammatory effects of resistance exercise in polymyositis/dermatomyositis (Figure 2A). A hypothetical model of this regulatory gene network that may mediate the effects of exercise on local tissue inflammation is presented in Figure 2B.

Resistance Exercise Downregulated Profibrotic, Upregulated Antifibrotic Genes and Reduced Tissue Fibrosis

Twenty-five genes related to collagen synthesis changed significantly (Table 1). The majority of these profibrotic genes displayed decreased expression (22 of 25).

Table 1. Exercise effects on gene expression (selected genes).

Gene symbol	Gene name	Affymetrix ID	Fold-change	*P*
Inflammation genes				
ADAMTS12	*ADAM metallopeptidase with thrombospondin type 1 motif, 12*	221421_s_at	–1.6	0.037
ADAMTS5	*ADAM metallopeptidase with thrombospondin type 1 motif, 5 (aggrecanase-2)*	219935_at	–1.5	0.015
C1S	*Complement component 1, s subcomponent*	208747_s_at	–1.5	0.036
CBL	*Cas-Br-M (murine) ecotropic retroviral transforming sequence*	225231_at	–1.6	0.003
CBLB	*Cas-Br-M (murine) ecotropic retroviral transforming sequence b*	233614_at	–1.6	0.022
CCL14	*Chemokine (C-C motif) ligand 14*	205392_s_at	–2.1	0.022
CCR1	*Chemokine (C-C motif) receptor 1*	205098_at	–1.8	0.007
CD200	*CD200 antigen*	209583_s_at	–1.6	0.012
CD226	*CD226 antigen*	207315_at	1.7	0.042
CD244	*CD244 natural killer cell receptor 2B4*	234320_at	2.9	0.02
CD44	*CD44 antigen (Indian blood group)*	1565868_at	–1.6	0.05
CD81	*CD81 antigen (target of antiproliferative antibody 1)*	200675_at	–1.5	0.02
CHL1	*Cell adhesion molecule with homology to L1CAM (close homolog of L1)*	204591_at	–1.9	0.017
CHST3	*Carbohydrate (chondroitin 6) sulfotransferase 3*	209834_at	–1.5	0.031
CLU	*Clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)*	208791_at	–1.7	0.03
CSF2RB	*Colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)*	205159_at	–1.6	0.035
DOCK2	*Dedicator of cytokinesis 2*	213160_at	–2.3	0.013
FOXP3	*Forkhead box P3*	221334_s_at	2.2	0.033
HDAC2	*Histone deacetylase 2*	242141_at	–2.4	0.045
HLA-F	*Major histocompatibility complex, class I, F*	221978_at	–1.6	0.01
IL10RB	*Interleukin 10 receptor, beta*	209575_at	–1.5	0.007
IL2RG	*Interleukin 2 receptor, γ (severe combined immunodeficiency)*	204116_at	–1.5	0.027
IRAK3	*Interleukin-1 receptor-associated kinase 3*	1568830_at	–1.9	0.009
KLF4	*Kruppel-like factor 4 (gut)*	221841_s_at	–1.8	0.006
LITAF	*Lipopolysaccharide-induced TNF factor*	200706_s_at	–2.3	0.002
NLK	*Nemo-like kinase*	238624_at	–2.2	0.018
NPY2R	*Neuropeptide Y receptor Y2*	210730_s_at	2.1	0.019
OPRK1	*Opioid receptor, kappa 1*	229944_at	–3.3	0.023
PAG	*Phosphoprotein associated with glycosphingolipid microdomains 1*	225622_at	–1.5	0.016
PELI2	*Pellino homolog 2 (Drosophila)*	219132_at	–1.7	0.028
PRTN3	*Proteinase 3 (serine proteinase, neutrophil, Wegener granulomatosis autoantigen)*	207341_at	2.2	0.01
PTGS1/COX1	*Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)*	238669_at	–1.7	0.019
REL	*v-rel Reticuloendotheliosis viral oncogene homolog (avian)*	206035_at	–1.7	0.035
RFX2	*Regulatory factor X, 2 (influences HLA class II expression)*	240156_at	–1.7	0.028
RFX3	*Regulatory factor X, 3 (influences HLA class II expression)*	240867_at	1.7	0.027
RTKN	*Rhotekin*	225150_s_at	–2.1	0.04
S100A13	*S100 calcium binding protein A13*	213481_at	–2	0.048
SEMA4D	*Sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D*	1562250_at	2.4	0.05
TCIRG1	*T-cell, immune regulator 1, ATPase, H + transporting, lysosomal V0 protein A3*	204158_s_at	–2.3	0.048
TLR8	*Toll-like receptor 8*	229560_at	–1.5	0.049
VAV1	*Vav 1 oncogene*	206219_s_at	–3.5	0.031

Continued on next page

Increased expression was found in the *latent TGF-β1 binding protein 1* (*LTBP-1*), which is a negative regulator of TGF-β (for example, an antifibrotic gene). Consistent with the decrease in tissue fibrosis genes, collagen gene expression was also reduced (*COL1A1*, *COL14A1*, *COL5A2*) (Table 1). The functionally relevant *TGF-β receptors 1* and *2* (*TGFBR 1* and *2*) did not pass the fold-change cutoff (1.36- and 1.15-fold) but did reach statistical significance ($P < 0.02$ and $P < 0.04$, respectively). Again, our bioinformatic analysis indicated that several genes regulated by TGF-β were downregulated,

Table 1. ***Continued.***

Fibrosis genes				
ADAM12	*ADAM metallopeptidase domain 12 (meltrin alpha)*	226777_at	–3.7	0.004
FBN2	*Fibrillin 2 (congenital contractural arachnodactyly)*	203184_at	–3.2	0.014
VIM	*Vimentin*	1555938_x_at	–3.1	0.045
MGP	*Matrix Gla protein*	238481_at	–2.6	0.012
HGF	*Hepatocyte growth factor (hepapoietin A; scatter factor)*	210755_at	–2.3	0.045
EDIL3	*EGF-like repeats and discoidin I-like domains 3*	225275_at	–2.3	0.01
COMP	*Cartilage oligomeric matrix protein*	205713_s_at	–2.1	0.049
CHRD	*Chordin*	211248_s_at	–2.1	0.029
CALCRL	*Calcitonin receptor-like*	234996_at	–2	0.004
FGF1	*Fibroblast growth factor 1 (acidic)*	205117_at	–2	0.016
COL14A1	*Collagen, type XIV, α 1 (undulin)*	212865_s_at	–1.8	0.031
COL1A1	*Collagen, type I, α 1*	1556499_s_at	–1.7	0.04
PLOD2	*Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2*	202620_s_at	–1.7	0.04
MAOA	*Monoamine oxidase A*	212741_at	–1.6	0.022
LRRFIP1	*Leucine rich repeat (in FLII) interacting protein 1*	227391_x_at	–1.6	0.001
NCAM1	*Neural cell adhesion molecule 1*	212843_at	–1.6	0.034
LOXL1	*Lysyl oxidase-like 1*	203570_at	–1.6	0.045
CSPG2	*Chondroitin sulfate proteoglycan 2 (versican)*	204619_s_at	–1.6	0.015
CTGF	*Connective tissue growth factor*	209101_at	–1.6	0.022
NID	*Nidogen 1*	202007_at	–1.5	0.021
COL5A2	*Collagen, type V, α 2*	221729_at	–1.5	0.046
MFAP4	*Microfibrillar-associated protein 4*	212713_at	–1.5	0.022
LTBP1	*Latent transforming growth factor β binding protein 1*	1566267_at	1.5	0.034
PREP	*Prolyl endopeptidase*	229644_at	1.7	0.038
MMP16	*Matrix metallopeptidase 16 (membrane-inserted)*	208166_at	2.7	0.041
Metabolism genes				
ALDH1L1	*Aldehyde dehydrogenase 1 family, member L1*	1559766_at	–2.6	0.008
AK3	*Adenylate kinase 3-like 1*	230630_at	–2.3	0.039
ACACA	*Acetyl-coenzyme A carboxylase alpha*	212186_at	–1.5	0.046
ATP2C1	*ATPase, Ca^{++} transporting, type 2C, member 1*	209935_at	–1.5	0.02
UCP3	*Uncoupling protein 3 (mitochondrial, proton carrier)*	219827_at	1.6	0.005
UQCRC2	*Ubiquinol-cytochrome c reductase core protein II*	239465_at	1.6	0.039
ATP1B2	*ATPase, Na^+/K^+ transporting, β2 polypeptide*	204311_at	1.8	0.025

and a negative modulator of TGF-β (*LTBP-1*) was induced by resistance exercise (Figure 3A). A hypothetical model of this regulatory gene network leading to decreased fibrosis is displayed in Figure 3B. Consistent with this model, a marked decrease in connective tissue (nonmuscle) area was observed (Figure 4A, B), which reflected the significant 24.7 ± 8.2% decrease in collagen I content (Figure 4C, D).

Resistance Exercise Upregulated Genes Involved in Oxidative Metabolism and Downregulated Genes Involved in Lipid Biosynthesis

Resistance exercise resulted in the modulation of genes involved in energy metabolism (Table 1). This is reflected by a reduction in genes involved in lipid biosynthesis, for example, *acetyl-coenzyme A carboxylase α* (*ACACA*) and an increased expression of the mitochondrial genes, for example, *ATPase Na^+/K^+ transporter* (*ATP1B2*).

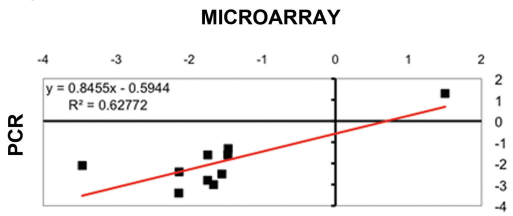
Immunohistochemistry Results from Muscle Tissue after Resistance Exercise

A low degree of inflammation with scattered T-cells and macrophages was found in muscle biopsies in six of seven patients, and this was unchanged after the resistance exercise period. One patient who had several inflammatory cell infiltrates displayed a >100% reduction in expression of $CD3^+$ T-cells and $CD163^+$ macrophages by computerized image analysis and also a decreased extranuclear HMGB-1 expression (data not shown). Proinflammatory cytokines and receptors, previously detected in muscle tissue of myositis patients (IL-1α, IL-1β, IL-1 receptor [R] I and IL-1-RII), were present in most biopsies with a low expression and were unchanged after training. Generally, <5% of the fibers expressed MHC class I and II, and there was no statistically significant change after the resistance exercise period.

DISCUSSION

Exercise training has beneficial effects on systemic inflammation, as determined by a reduction in selective serum markers such as IL-6, C-reactive protein and

A)



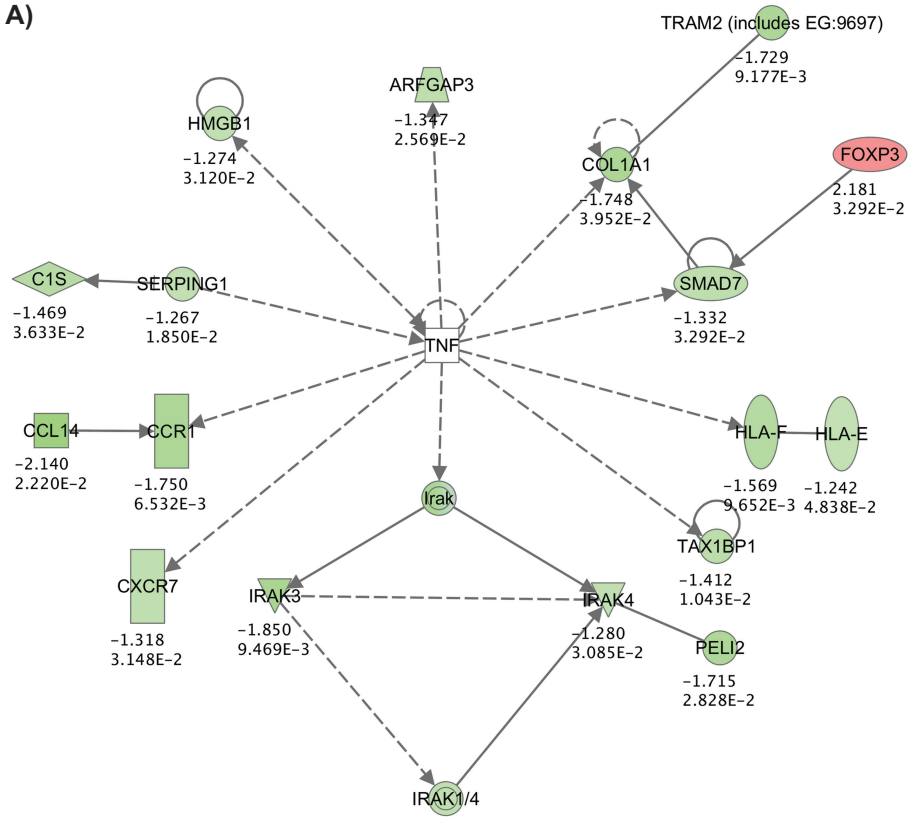
B)

Gene	Microarray	PCR
C1S	-1.5	-1.3
CCL14	-2.1	-2.4
CCR1	-1.8	-2.8
IL10R-β	-1.5	-1.6
IL2R-γ	-1.5	-1.5
RFX2	-1.7	-3
VAV1	-3.5	-2.1
COL1A1	-1.7	-1.6
COMP	-2.1	-3.4
CTGF	-1.6	-2.5
LTBP1	1.5	1.3

Figure 1. Validation of selected target gene by qRT-PCR. The PCR results suggest a good correlation (R^2 = 0.62) between the changes in gene expression detected by microarray and qRT-PCR.

TNF in healthy individuals (22). However, little is known about the effects of exercise training on inflammation in patients suffering from chronic inflammatory disorders. In the present study, we demonstrate that 7 weeks of resistance exercise modulated the expression of genes involved in inflammation, fibrosis and metabolism in muscle from polymyositis/dermatomyositis patients. These postexercise changes in gene expression may explain the improved muscle performance observed in our patients, and seem to agree with the conclusion that resistance exercise can improve muscle performance in polymyositis/dermatomyositis patients without disease exacerbation by posi-

A)



B)

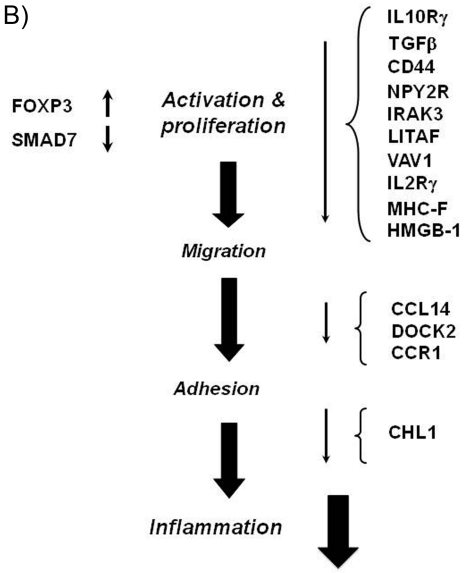


Figure 2. (A) Identification of an exercise-induced gene network involved in tissue inflammation using the Ingenuity Pathways Analysis knowledge database. Several genes regulated by TNFα were negatively modulated by resistance exercise, suggesting that they may play a role in the antiinflammatory effects of exercise in myositis patients (see text for further explanations). (B) Model of exercise-induced reduction in inflammation in autoimmune myositis patients. Based on the experimental expression data generated by the microarray analysis, we developed a hypothetical model in which three discrete steps in the regulation of tissue inflammation can be identified, suggesting that exercise could reduce inflammation by downregulating the expression of genes involved in activation and proliferation of T-cells and macrophages, migration and adhesion. (Panel A image: © 2000-2010 Ingenuity Systems, Inc. All rights reserved.)

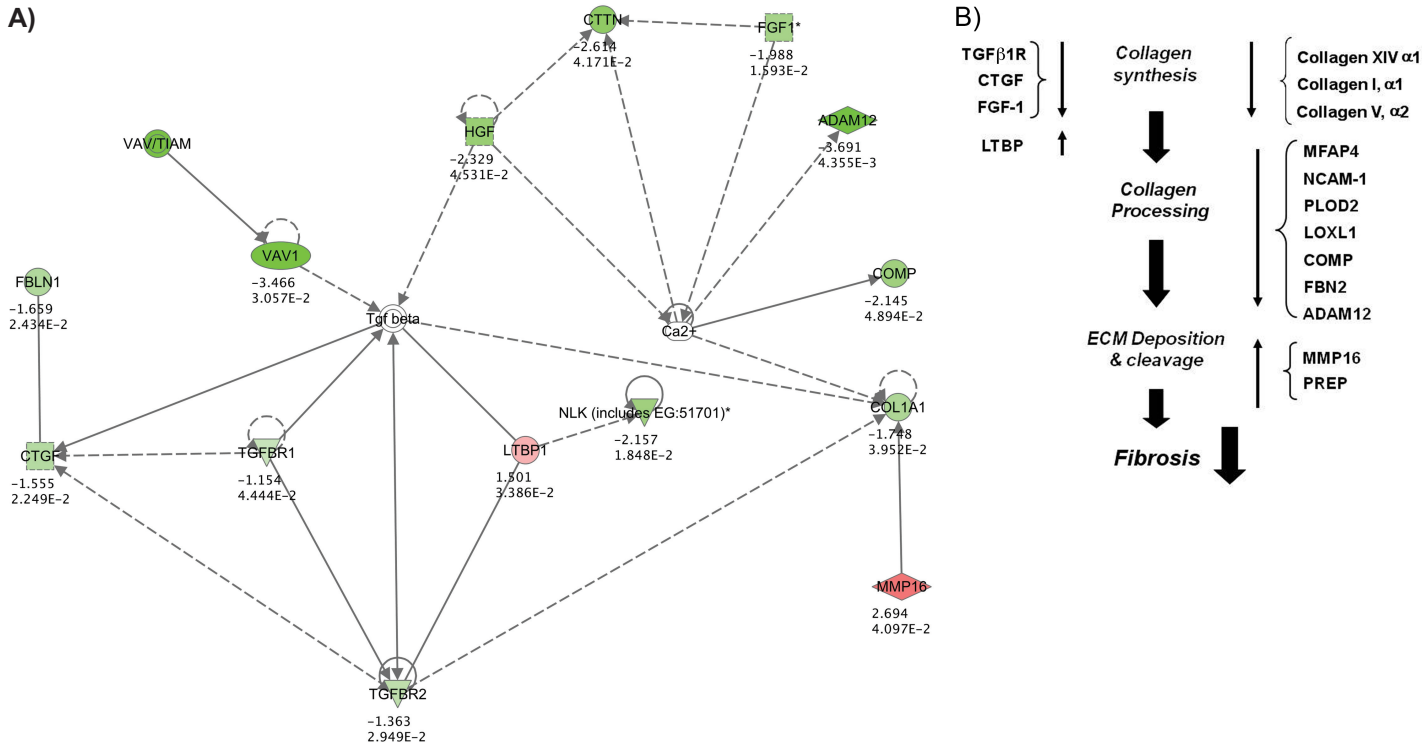


Figure 3. (A) Identification of an exercise-induced gene network involved in tissue fibrosis using the Ingenuity Pathways Analysis knowledge database. Several genes regulated by TGF-β were negatively modulated, and a negative modulator of TGF-β (*LTBP1*) was induced by exercise in a manner consistent with the reduction in tissue fibrosis and improved performance in myositis patients (see text for further information). (B) Model of exercise-induced reduction in fibrosis in autoimmune myositis patients. Collagen synthesis is suppressed because of a reduction in growth factor production and an increase in inhibitory molecules such as *LTBP1*. The reduction in collagen processing likely contributes to the decrease in ECM deposition. This, together with an increase in ECM degradation by collagen-cleaving enzymes and matrix metalloproteases, will result in a reduction in tissue fibrosis. (Panel A image: © 2000-2010 Ingenuity Systems, Inc. All rights reserved.)

tively modulating genes involved in the disease process.

The modified expression pattern of genes involved in inflammation suggests that resistance exercise induced a coordinated reduction of proinflammatory transcripts and an increase in anti-inflammatory transcripts. For example, *PTGS1*, previously found to be chronically upregulated in myositis and refractory to conventional immunosuppressive treatment (23), was reduced after training, and the upregulation of *FOXP3* was consistent with the downregulation of *SMAD7*-dependent signaling, suggesting a coordinated modulation of T-cell activity (24). Similarly, the upregulation of *NPY2R*, a negative effector of monocytes (25), further supports a resistance exercise–mediated antiinflammatory effect. These findings suggest that resistance exercise can improve muscle function in myositis patients by modulating inflammation-associated gene expression in skeletal muscle and seem to agree with previous reports from chronic heart failure and frail elderly men and women, where exercise training resulted in a significant reduction in the local expression of inflammatory molecules such as TNFα (26,27).

Although a reduction in TNFα mRNA was not detectable in our patients, the interpretation of a TNF-related gene network as the target for the local immunosuppressive effects of resistance exercise is consistent with a role in the overall decrease in disease activity after training (Figure 2B). In the present study, serum C1q levels were reduced after resistance exercise and likely modulated by the reduced expression of *C1S* mRNA. Altogether, our findings support the interpretation that resistance exercise can reduce the expression of proinflammatory genes in autoimmune myositis patients. The lack of a significant decrease at the protein level of inflammatory molecules may be explained by the low expression before exercise and the low sensitivity of immunohistochemistry for quantitative measures. Another possibility is that the observed changes in mRNA levels do not reflect changes in protein synthesis.

The second largest group of genes that was changed with resistance exercise was genes involved in fibrosis. Muscle fibrosis may over time expand and increase relative to the contractile tissue resulting in weakness and decreased performance. In the present study, 7 weeks of resistance exercise was sufficient to produce a significant reduc-

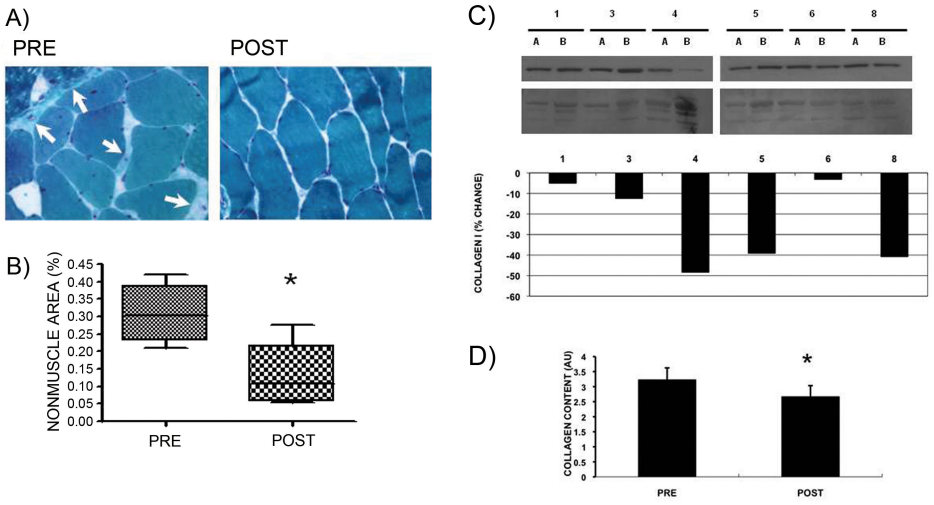


Figure 4. Exercise training reduced fibrosis in myositis muscles. Resistance exercise training resulted in a significant reduction in nonmuscle area infiltrates. (A) Gross morphological analysis via Gomori trichrome staining pre- and posttraining (fibrotic areas are indicated by arrows). (B) Quantitative analysis of tissue sections demonstrates a decrease in nonmuscle area. (C) Type I collagen protein levels in pre- and posttraining biopsies in individual patients. (D) Type I collagen levels were reduced significantly after resistance training (n = 6; *P = 0.01). AU, arbitrary units. Data are means ± SEM.

tion of tissue fibrosis, as determined by gross morphological examination and the specific decrease of collagen I levels. Consistent with this finding, posttraining analysis of gene expression revealed that resistance exercise also suppressed the expression of a profibrogenic molecular signature consisting of genes involved in collagen synthesis, processing and extracellular matrix assembly and deposition (network depicted in Figure 3). Our bioinformatics analysis identified that a subset of transcripts modulated by resistance exercise is associated with TGF-β signaling (Figure 3A). Furthermore, the increased expression of *LTBP-1*, a negative modulator of TGF-β (28), supports the interpretation that downregulation of a TGF-β–associated gene network may mediate the effects of resistance exercise by decreasing muscle fibrosis, not only by suppressing the synthesis of collagen and other ECM components, but also by increasing collagenolysis and elastolysis by matrix metalloproteinases, such as *matrix metallopeptidase 16* (*MMP16*), and other collagen-cleaving enzymes, such as *prolyl endopeptidase* (*PREP*) (Figure 3B).

Noticeably, resistance exercise exerted a modulatory effect on both networks, but the precise mechanisms by which this occurred, or whether they can modulate one another in this particular condition, remain to be elucidated. For example, depletion of T lymphocytes in *mdx* mice resulted in a reduction in muscle fibrosis, suggesting a direct link between muscle inflammation and the aberrant accumulation of ECM (29).

Finally, we found that resistance exercise improved the metabolic profile of myositis muscle. Our training protocol resulted in a shift in the muscle's metabolic gene profile toward a more oxidative state. Both *AK3* and *ATP1B2* were reduced after training, suggesting a decreased reliance on immediate energy production mechanisms (30). Conversely, the increased expression of transcripts coding for respiratory enzymes *UCP3* (a mitochondrial proton carrier) and *UQCRC2* and the decreased *ACACA* expression suggest that training may have stimulated the activity of oxidative metabolism and decreased lipid biosynthesis, respectively. The increase in oxidative gene expression is in line with the observed increase in VO_{2max}. A similar effect on VO_{2max} was recently reported after strength training in elderly men (31).

A potential limitation of the study is the absence of a nontrained control group. However, we feel that the repeated muscle biopsy design, where each individual served as its own control, compensated for the absence of a separate control group and was better suited for the microarray analysis, since this design reduces the effects of genetic heterogeneity on gene expression (32). Repeat muscle biopsies also compensated for the low subject number and, together with the high stringency microarray analysis, contributed to a final reliable dataset from which some selected genes could be confirmed by qRT-PCR and could functionally begin to explain the effects of resistance exercise on both inflammation and fibrosis outcomes. Furthermore, we did not find any differences in gene expression profiles between polymyositis and dermatomyositis patients, which is in agreement with previously published data on muscle cytokines and chemokines despite differences in inflammatory cell infiltrates. This finding could also suggest that, in the chronic phase of disease with a low degree of inflammation, shared disease mechanisms could contribute to the impaired performance between the myositis subsets.

In conclusion, resistance exercise may restore muscle function in autoimmune myositis patients by reducing inflammation and tissue fibrosis and by improving metabolic homeostasis, events that were reflected by the corresponding molecular signatures represented by the reduced expression of proinflammatory and profibrotic gene networks and by the increased expression of oxidative metabolism genes. In addition, our results demonstrate that high-throughput analysis of skeletal muscle gene expression can provide useful information for the identification of novel disease biomarkers and potential targets for pharmacological interventions of autoimmune myositis patients.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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