Impaired Expression of Notch Signaling Genes in Aged Human Skeletal Muscle

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Notch signaling is essential for myogenesis and the regenerative potential of skeletal muscle; however, its regulation in human muscle is yet to be fully characterized. Increased expression of Notch3, Jagged1, Hes1, and Hes6 gene transcripts were observed during differentiation of cultured human skeletal muscle cells. Furthermore, significantly lower expressions of Notch1, Jagged1, Numb, and Delta-like 1 were evident in muscle biopsies from older men (60–75 years old) compared to muscle from younger men (18–25 years old). Importantly, with supervised resistance exercise training, expression of Notch1 and Hes6 genes were increased and Delta-like 1 and Numb expression were decreased. The differences in Notch expression between the age groups were no longer evident following training. These results provide further evidence to support the role of Notch in the impaired regulation of muscle mass with age and suggest that some of the benefits provided by resistance training may be mediated through the Notch signaling pathway.

SKELETAL muscle mass declines with age, resulting in many deleterious health consequences including frailty, increased risk of falls and associated morbidity, reduction in or prevention of ambulation, and in severe cases, mortality. Importantly, the mechanisms underlying this loss of muscle mass (or sarcopenia) with age remain incompletely understood. Recent research by Conboy and colleagues (1) has implicated the Notch signalling pathway in the repaired regenerative capacity of skeletal muscle in aged rats, although the importance of this pathway in human skeletal muscle aging or regeneration has, to our knowledge, not been investigated.

The mammalian Notch receptors (Notch1, -2, -3, and -4) are transmembrane proteins composed of an extracellular region with multiple epidermal growth factor-like repeats necessary for ligand binding. The Notch signaling pathway is initiated when Notch receptor–bearing cells interact with Notch ligands expressed on adjacent cells. Humans have at least five Notch ligands (Jagged1 and -2, and Delta-like-1, -3, and -4), which are themselves transmembrane proteins with a number of epidermal growth factor-like repeats in their extracellular domain and a unique Delta/Serrate/Lag2 (DSL)-binding domain in the amino terminus necessary for receptor interaction. Notch–ligand interaction triggers two proteolytic cleavages that release the Notch intracellular domain (Notch\textsubscript{intr}\textsuperscript{a}) from its plasma membrane tether, allowing it to translocate to the nucleus and bind to a transcriptional regulator known as CBF1/Su(H)/LAG-1. The activity of Notch\textsubscript{intr}\textsuperscript{a} can be inhibited by Numb through ubiquination, which regulates the abundance and intracellular location of the signaling molecule. The Notch\textsubscript{intr}\textsuperscript{a}–CBF1/Su(H)/LAG-1 complex recruits transcriptional coactivators that induce the gene expression of members of the Hairy-Enhancer of Split (HES) proteins. These proteins are basic helix-loop-helix (bHLH) DNA binding proteins that are thought to inhibit the expression and/or function of lineage-specifying genes such as MyoD (involved in myogenesis) (2). In skeletal muscle, Notch signaling contributes to muscle development, somitogenesis, as well as the proliferation and cell fate determination of muscle-specific satellite cells during postnatal myogenesis (3).

Mechanical loading has been shown to augment the proliferation and differentiation of satellite cells (4–7), which is thought to contribute to the repair and adaptation of the exercised muscle. Because the Notch signaling pathway has previously been implicated in the regenerative potential of rat muscle (1,8), we sought to examine whether components of this pathway were transcriptionally regulated in human skeletal muscle by exercise and during differentiation of human myoblasts in culture. We further examined whether the transcriptional regulation of Notch signaling was different between young and older human skeletal muscle both at rest and in response to a progressive 12-week heavy resistance exercise training program. It was hypothesized that: (i) the gene expression of members of the Notch signaling pathway would be increased during the differentiation of human primary cells in culture; (ii) reduced expression of these genes would be observed in the muscle of older compared to younger individuals; and (iii) increased messenger RNA (mRNA) expression would be observed following resistance training.

METHODS

Human Primary Skeletal Muscle Cell Culture

Human primary skeletal muscle cells were obtained from the vastus lateralis muscle of eight healthy volunteers (six men and two women, average age 25.1 ± 1.3 years). Informed written consent was obtained from each individual
before participation in the study, after the nature, purpose, and risks of the study were explained. All experimental procedures involved in this study were formally approved by the Deakin University Ethics Committee. Human primary skeletal muscle cells were cultured according to the technique of Gaster and colleagues (9) with modifications. Briefly, the excised muscle was immersed and extensively washed in ice-cold minimum essential medium alpha modification (a-MEM; Gibco, Invitrogen Corporation, Carlsbad, CA) before being minced and digested in 0.5% Trypsin–EDTA (Gibco). The supernatant containing the myoblasts was then collected, and the process was repeated another two times to break down any remaining tissue. Fetal bovine serum (Gibco) was subsequently added to the supernatant to a final concentration of 10%. The supernatant was filtered through a 100-μm filter to remove any connective tissue and then spun to collect the cells. The resulting cell pellet was resuspended in primary growth medium (a-MEM, 10% fetal bovine serum, with penicillin at 50 IU/mL and streptomycin at 5 μg/mL) and was then seeded onto an uncoated flask and incubated at 37°C for 25 minutes to induce fibroblast attachment, leaving myoblasts suspended in the medium. The medium was aspirated and seeded onto an extracellular matrix–coated (Sigma, St. Louis, MO) flask. The resulting primary cell cultures were maintained in primary growth medium in humidified air at 37°C and 5% CO₂.

Study Design: Differentiation

Cells were initially plated in the primary growth medium until 80% confluence was attained. The plating medium was then removed, the cells were washed twice with phosphate-buffered saline, and the differentiation medium (a-MEM containing 2% horse serum) was added. Medium was changed every 48 hours throughout the 72-hour differentiation time course. Following two washes with phosphate-buffered saline, cells were extracted for RNA and protein at 12, 24, 48, and 72 hours following the addition of the differentiation medium. The control consisted of cells actively growing in the primary growth medium extracted immediately before the addition of the differentiation medium (0 hours).

RNA extraction and reverse transcription.—RNA from primary cell cultures was extracted using the RNA-Bee (Tel-Test, Friendswood, TX) reagent and protocols. RNA integrity and quantity were assessed on an Agilent 2100 Bioanalyzer with an RNA 6000 Nano LabChip Kit (Agilent Technologies, Palto Alto, CA). Reverse transcription was performed using the AMV reverse transcriptase kit (A3500; Promega, Madison, WI) protocols and reagents.

Study Design: Resistance Exercise Training

Participants.—Sixteen healthy young men (18–25 years old) and 15 healthy older men (60–75 years old) who had not participated in regular strength exercise within a year prior to commencing the study were recruited (see Table 1 for participant details). A medical history questionnaire was used to identify and exclude participants with a diagnosed condition or illness that would endanger them during strenuous exercise. Older participants were required to undergo a complete medical screening including a 12-lead electrocardiogram exercise stress test to detect any underlying cardiopulmonary conditions. All participants were informed of the nature and risks of the study before their written informed consent was obtained.

Single bout of resistance exercise.—Each participant completed a familiarization session on the Cybex NORM dynamometer (Cybex International Inc., Measham, U.K.) to become familiar with the execution of the exercise. Familiarization and strength testing was completed at least 4 days before the trial. Participants arrived at the laboratory in the fasted state for a resting muscle biopsy. For the 24 hours preceding, and the days of the trial, participants abstained from alcohol, caffeine, tobacco, and additional exercise. Following the resting biopsy, participants completed 3 sets of 12 repetitions of a maximal single-leg knee extension exercise on the Cybex Norm dynamometer with 2 minutes rest between each set. Participants were instructed to contract as hard as possible and were verbally encouraged throughout each set. Three hours after the completion of the exercise session an additional muscle sample was collected.

Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Younger Men</th>
<th>Older Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>20 ± 0.5</td>
<td>68 ± 1*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181 ± 2</td>
<td>173 ± 1*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72 ± 2</td>
<td>82 ± 2*</td>
</tr>
<tr>
<td>BMI</td>
<td>22 ± 0.6</td>
<td>27 ± 0.7*</td>
</tr>
<tr>
<td>Concentric isokinetic peak torque, Nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretraining</td>
<td>207 ± 10</td>
<td>143 ± 12*</td>
</tr>
<tr>
<td>Postraining</td>
<td>243 ± 9</td>
<td>184 ± 11*</td>
</tr>
<tr>
<td>Eccentric isokinetic peak torque, Nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretraining</td>
<td>227 ± 11</td>
<td>179 ± 12*</td>
</tr>
<tr>
<td>Postraining</td>
<td>286 ± 14</td>
<td>215 ± 15*</td>
</tr>
</tbody>
</table>

Notes: *Significantly different compared to younger participants (p < .01).  
1Significantly different compared to pretraining values (p < .01).  
BMI = body mass index.

Twelve-week exercise training.—Following the initial acute exercise testing session, all participants completed 12 weeks of fully supervised progressive resistance exercise training 3 days each week, with a minimum of 48 hours of rest between exercise sessions. Initially, three training sessions were conducted using light resistance to familiarize the participants with the equipment, training protocol, and correct execution of the exercises. After the familiarization sessions, strength testing was performed to determine appropriate starting weights for all participants. Repetition maximum (RM) strength was estimated from their 5RM results for all exercises. Values of 5RM were retested at Week 6 and Week 12, and the training load was adjusted accordingly to ensure that the training was progressive.

Each training session was preceded by a 5-minute warm-up on a stationary cycle followed by a full set of exercises with light weights. The exercises consisted of leg press,
Muscle Biopsy Procedure

The vastus lateralis muscle of the nondominant leg was sampled by the percutaneous needle biopsy technique (10) modified to include suction (11). Excised muscle tissue from each biopsy was immediately frozen and stored in liquid nitrogen for subsequent analysis. To minimize the potential for interference, serial biopsy samples were collected at least 2 cm from previous biopsy sites.

RNA Extraction and Reverse Transcription

RNA was extracted from ~10 mg (wet weight) of muscle using the ToTALLY RNA Kit protocol and reagents (Ambion, Austin, TX). RNA integrity and quantity were assessed on an Agilent Bioanalyzer 2100 with an RNA 6000 Nano LabChip Kit (Agilent Technologies). Reverse transcription was performed using the AMV reverse transcriptase kit (A3500; Promega) protocols and reagents.

Primer Design

To perform polymerase chain reaction (PCR), specific primers were designed for all genes using Primer Express 3.0 software (Applied Biosystems, Foster City, CA) on sequences obtained from GenBank (see Table 1 for details). Where possible, primers were designed spanning intron–exon boundaries to prevent amplification of the target region from any contaminating DNA. Primer specificity was confirmed using Basic Local Alignment Search Tool (BLAST). Primers were purchased from GeneWorks (Adelaide, South Australia). Efficiency of PCR primers was confirmed by examining the dynamic range of responses for a series of dilutions of complementary DNA. Using the slopes of the lines, the efficiency (E) of target amplification was calculated using the equation $E = (10^{-1/\text{slope}}) - 1$.

Real-Time PCR Analysis

Real-time PCR was performed using the GeneAmp 7500 Sequence Detection System (Applied Biosystems). For the PCR step, reaction volumes of 20 μL contained SYBR Green 1 Buffer (Applied Biosystems), forward and reverse primers (see Table 2), and complementary DNA template. All samples were run in duplicate. Real-time PCR was run for 1 cycle (50°C for 2 minutes, 95°C for 10 minutes) followed by 40 cycles (95°C for 15 seconds, 60°C for 60 seconds), and fluorescence was measured after each of the repetitive cycles. A melting point dissociation curve generated by the instrument was used to confirm that only a single product was amplified. Data were analyzed using a comparative critical threshold (Ct) method in which the amount of target normalized to the amount of endogenous control relative to control value is given by $2^{-\Delta\Delta\text{Ct}}$. No changes in the expression of this gene were observed (data not shown), so it was considered an appropriate endogenous control for this study.

Western Blot Analysis

Cells were resuspended in lysis buffer (50 mM Tris, pH 7.6, 250 mM NaCl, 5 mM EDTA, 0.1% IGEPSA, Complete protease inhibitor cocktail; Sigma) and were passed through a syringe. The cell extracts were centrifuged to pellet cell debris, and the supernatants were removed and analyzed for total protein content (BCA Protein Assay Kit; Pierce, Rockford, IL). Denatured total proteins (10 μg) from each sample were separated by electrophoresis on a 6% sodium dodecyl sulfate–polyacrylamide gel and transferred to nitrocellulose membranes by electroblotting. Equal loading across lanes and equal transfer were verified by staining all membranes with Ponceau-S. Membranes were blocked for 2

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Table 2. Real-Time PCR Primer Details

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Accession Number</th>
<th>Forward Primer (5’–3’)</th>
<th>Reverse Primer (5’–3’)</th>
</tr>
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<tbody>
<tr>
<td>Notch1</td>
<td>NM_017617</td>
<td>CCGTCCTACCAGTTGATGTA</td>
<td>GTTGTATTGTTGCCGACCAT</td>
</tr>
<tr>
<td>Notch3</td>
<td>NM_051435</td>
<td>TGGTCCGCGTTGATGTA</td>
<td>GCAAAAGCCTCCAGGTGTCA</td>
</tr>
<tr>
<td>Jagged1</td>
<td>NM_000214</td>
<td>TCTCAGCTCGGACATGCAA</td>
<td>CAGGTCACGGGATCTGATA</td>
</tr>
<tr>
<td>Jagged2</td>
<td>NM_002226</td>
<td>GCCCTTACCCCTTTCTGGCA</td>
<td>TTGGACCTTGTATGGCATGTGA</td>
</tr>
<tr>
<td>Hes1</td>
<td>NM_005524</td>
<td>CCGGACATTCATGCCAAATGACA</td>
<td>ACGCCACGGCCTCATCTG</td>
</tr>
<tr>
<td>Hes6</td>
<td>NM_018645</td>
<td>CCAAAAGAAGTACAGGACATCACA</td>
<td>CAAAGACTTGAACCTGCCCTACT</td>
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<tr>
<td>Delta-like 1</td>
<td>NM_005618</td>
<td>CTGCTGCTGGATTGGTGAT</td>
<td>AGACACGCCTGATAGCGCGATA</td>
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<tr>
<td>Numb</td>
<td>NM_001005743</td>
<td>CCGGACATGCCCATCAATTG</td>
<td>TCTGGCATTAGAGCAGAAAAAC</td>
</tr>
<tr>
<td>β-actin</td>
<td>NM_001101</td>
<td>AAGGCCACCCACCTCTTCTAA</td>
<td>AATGCTATCACCCTCCCGTGT</td>
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</tbody>
</table>

Notes: Primer sequences were designed using Primer Express version 2.0 software (Applied Biosystems) using sequences accessed through GenBank, and were checked for specificity using the Nucleotide-Nucleotide Blast search. PCR = Polymerase chain reaction.
hours with 5% skim milk in Tris-buffered saline (TBST) (50 mM Tris-HCl, 750 mM NaCl, 0.25% Tween), and were incubated overnight at 4°C with a polyclonal anti-MHC (MY-32; Zymed, San Francisco, CA) antibody, washed, incubated for 60 minutes with anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (1 in 10,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), then washed again. Immunoreactive bands were detected using enhanced chemiluminescence (Western Lightning Chemiluminescence Reagent; Perkin-Elmer, Boston, MA). An internal control (rat gastrocnemius muscle) was used in each gel to normalize for variation in signal observed across the membranes. A Kodak Image Station 440CF (Perkin-Elmer) was used to visualize the images, and densitometry was performed using Kodak ID 3.5 image analysis software.

Statistical Analysis
Statistical analysis was performed using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). For the differentiation study, means were compared using a one-way analysis of variance and any significant differences were analyzed using Bonferroni’s multiple comparison test. The differences in the gene expression between older and younger men were examined using an unpaired t test. The impact of age and training was compared using a two-way analysis of variance with repeated measures. Data are presented as mean ± standard error of the mean (SEM). Unless otherwise stated, a probability level of <.05 was adopted throughout to determine statistical significance.

RESULTS

Components of the Notch Signaling Pathway Are Regulated at the Transcriptional Level During Human Primary Skeletal Myoblast Differentiation

Human primary muscle cell cultures were grown to 80% confluence before being exposed to low-serum medium to induce differentiation. Differentiation was assessed by examining the expression of MHC protein by Western blot. A small degree of MHC expression was evident 12 hours after the induction of differentiation, with more marked expression detectable at 24, 48, and 72 hours postdifferentiation (Figure 2). Differentiation was accompanied by an increase in the mRNA expression of Notch3 (p < .001) by 24 hours (Figure 2), whereas no change was observed in the expression of Notch1 or Numb (data not shown). The expression of Jagged1 (2.5-fold), Hes1 (2.6-fold), and Hes6 (2-fold) transcripts all increased at 48 hours with the maximum expression of Hes6 (2.8-fold) evident at 72 hours postdifferentiation. Jagged2 and Delta-like 1 expression were not significantly altered at any time during differentiation (data not shown).

Reduced Expression of Components of the Notch Signaling Pathway Are Evident in Older Compared to Younger Skeletal Muscle

Human skeletal muscle collected from older men (60–75 years old) demonstrated significantly (p < .05) lower mRNA expression of Notch1 (51% lower), Jagged1 (59% lower), and Delta-like 1 (41% lower) than did muscle from younger men (18–25 years old), and a strong trend toward reduced expression of Numb (58% lower, p = .05) in older muscle was also observed (Figure 3). No difference was observed in the mRNA expression of Notch3, Jagged2, or Hes6 between younger and older individuals. The expression of Hes1 in human skeletal muscle biopsies was markedly lower than that of the other genes measured, and its expression in many samples appeared to fall outside the sensitivity of the PCR technique, preventing subsequent inclusion in this analysis.

The mRNA Expression of Components of the Notch Signaling Pathway Is Altered Following a 12 Week Heavy Resistance Exercise Training Program in Both Younger and Older Individuals

Resistance exercise training significantly increased Notch1 gene expression in younger and older men by a similar magnitude both at rest (younger, 3.6-fold; older, 3.0-fold) and 2 hours (younger, 3.3-fold; older, 2.3-fold) following a single bout of exercise (Figure 3). However, the younger individuals demonstrated slightly higher absolute Notch1 gene expression at all time points. The expression of Notch3 and Jagged1 were not significantly altered by resistance exercise training in either the younger or older individuals. Conversely, a strong trend (p = .05) toward increased Jagged2 expression with training was observed in both age groups. Resistance exercise training significantly increased the expression of Hes6 in both younger (rest, 4-fold; 2 hours postexercise, 3-fold) and older individuals (rest, 3.5-fold increase; 2 hours postexercise, 3.8-fold increase). Significant reductions in Delta-like 1 were observed in the skeletal muscle of younger and older men following resistance training both at rest (younger, 3.8-fold decrease; older, 2.2-fold decrease) and 2 hours (younger, 3.4-fold decrease; older, 1.7-fold decrease) after an acute
bout of exercise. A small reduction in Numb expression following training was observed for both younger and older men, and a significant main effect for age was observed indicating that, for all time points, the older men had consistently lower Numb expression compared to the younger men. A summary of all results is presented in Table 3.

DISCUSSION

Notch Expression in Differentiating Human Primary Skeletal Muscle Cells

The expression of selected Notch signaling genes was investigated during a 72-hour differentiation time course in human primary skeletal muscle cell cultures established from the vastus lateralis of young healthy men. Interestingly, when compared to actively proliferating myoblasts, four of the seven genes (Notch3, Jagged1, Hes1, and Hes6) demonstrated significantly increased levels of expression during differentiation, no change in the expression of the Notch inhibitor Numb was observed, and Notch1 and Jagged2 also tended to increase. Our results suggest that transcriptional regulation of components of the Notch signaling pathway contribute to the progression of human primary skeletal muscle myoblast differentiation. The increased expression of these genes either preceded or occurred in concert with the increase in MHC protein expression (a marker of late differentiation). These findings are consistent with results shown previously during C2C12 differentiation (12). However, increased expression of these genes during differentiation is in contradiction to the notion that the Notch signaling pathway inhibits myoblast differentiation (13–16). Delgado and colleagues (12) suggested several possible explanations for these contrasting results. First, studies that concluded that Notch inhibited differentiation either overexpressed a constitutively active form of Notch or suppressed/activated the Notch signaling pathway (17). These methodologies may have resulted in nonphysiological regulation of Notch signaling. Notch signaling may confer both anti- and promyogenic actions depending on precise levels of expression or on timing of expression. In this instance, premature activation of Notch may have an inhibitory effect on normal myogenesis, whereas late activation may be essential for differentiation. Here we demonstrate that the expression of Notch signaling members

Figure 2. Messenger RNA expression analysis of components of Notch3 (A), Jagged1 (B), Hes1 (C), and Hes6 (D) following the induction of differentiation in human skeletal muscle primary cell cultures. The mean ± standard error of the mean of eight independent experiments is shown. *Significantly different from 0 hours (p < .05); **Significantly different from 0 hours (p < .01); ***Significantly different from 0 hours (p < .001).
with age resulting in inefficient regeneration of old muscle tissue (1). The results from our work extend these observations and indicate that reduced expression of Notch genes may contribute to the impaired regenerative capacity of older human skeletal muscle.

We next sought to determine if heavy resistance exercise training was capable of attenuating the decline in Notch gene expression evident with advancing age. Both younger and older men completed a single bout of resistance exercise both before and after a progressive 12-week heavy resistance exercise training program. No changes were observed in any of the genes examined 2 hours following an acute bout of exercise in the untrained state. Following training, however, the differences in expression patterns between the two age groups were no longer evident, suggesting that exercise training may have a beneficial effect on the expression of Notch genes in older individuals. Resistance exercise has been shown to have a positive effect on muscle mass, strength, and functional capacity in even very elderly individuals (18). Previous research has indicated that artificial activation of Notch signaling may “rescue” the impaired regenerative capacity of skeletal muscle in older

### Table 3. Summary of Results

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Observed Changes in Gene Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Differentiation of Human Primary Skeletal Muscle Cells</td>
</tr>
<tr>
<td>Notch1</td>
<td>Transmembrane receptor protein–interacts with ligands to initiate signaling</td>
<td>No change</td>
</tr>
<tr>
<td>Notch3</td>
<td>Transmembrane receptor protein–interacts with ligands to initiate signaling</td>
<td>Increased expression at 24, 48, and 72 h</td>
</tr>
<tr>
<td>Jagged1</td>
<td>Transmembrane Notch ligand</td>
<td>Increased expression at 48 and 72 h</td>
</tr>
<tr>
<td>Jagged2</td>
<td>Transmembrane Notch ligand</td>
<td>No change</td>
</tr>
<tr>
<td>Delta-like 1</td>
<td>Transmembrane Notch ligand</td>
<td>No change</td>
</tr>
<tr>
<td>Numb</td>
<td>Inhibitor of Notch protein</td>
<td>No change</td>
</tr>
<tr>
<td>Hes1</td>
<td>Downstream transcriptional target of Notch signaling</td>
<td>Increased expression at 48 h</td>
</tr>
<tr>
<td>Hes6</td>
<td>Downstream transcriptional target of Notch signaling</td>
<td>Increased expression at 48 and 72 h</td>
</tr>
</tbody>
</table>

is only increased 24 hours after the induction of differentiation. This increased expression corresponds with the increased expression of myogenin (12) and the beginning of differentiation. Thus the temporal regulation of Notch signaling may be such that the expression of these genes is low during early myogenesis allowing for the progression of myoblasts toward terminal differentiation. The subsequent increase in the expression of the components of Notch signaling may relate to an as yet unidentified role in the latter stages of myogenesis.

### Effect of Age and Training Status on Notch Gene Expression

We next sought to determine whether human skeletal muscle from older individuals demonstrated reduced expression of Notch signaling genes. Interestingly, three of the genes measured (Notch1, Jagged1, and Delta-like 1) showed reduced expression in older skeletal muscle when compared to younger muscle at rest. To our knowledge, this is the first study to describe the effect of age on Notch expression in human skeletal muscle. Research in rats has described impaired injury-induced activation of the Notch ligand Delta

Figure 3. The effects of heavy resistance exercise training on the expression of Notch1 (A), Notch3 (B), Jagged1 (C), Jagged2 (D), Delta-like 1 (E), Numb (F), and Hes6 (G) in young (white bars) and old (black bars) men. The men completed a single bout of extension exercise consisting of 3 sets of 12 repetitions of maximal leg extension exercise before and after 12 weeks of resistance exercise training. Vastus lateralis muscle samples were collected before the exercise (rest) as well as 2 hours postexercise. The mean ± standard error of the mean is presented. # Significantly different compared to young men (p < .05). mRNA = Messenger RNA.
rats (1). The data presented here seem to suggest that at least one mechanism by which exercise may exert these benefits is through the regulation of the expression of Notch signaling genes.

Resistance exercise training altered the expression of several Notch genes in the younger as well as the older individuals. Increased expression was observed in Notch1 and Hes6, a nonsignificant increase was observed in Jagged2, and Delta-like 1 and (to a lesser extent Notch inhibitor Numb) expression was reduced. Increased expression of Notch1 might enhance the capacity for Notch signaling and, subsequently, increased expression of its downstream targets, such as Hes6. The possible reasons for the marked reduction in Delta-like 1 are somewhat less clear. There are, however, numerous other Notch receptors (such as Delta-like 3 and 4, and Jagged 1 and 2). The specific role these receptors play in Notch signaling is currently not known. Each of these proteins may have unique cellular functions, and thus regulation (either up or down) of their expression may contribute to the development of specialized phenotypes. The similar changes in Notch gene expression observed between the two age groups with training indicate that the capacity of skeletal muscle to regulate these genes is not diminished with age. These findings may indicate that the regulation of Notch by exercise is not age-related and may in fact be a common signaling mechanism integral to exercise-induced skeletal muscle adaptation. The one notable exception to the increased expression of Notch genes with exercise training was observed with Numb. Numb expression was consistently lower in aged muscle, and this pattern was not altered at any time point following exercise training. Increased Numb expression has been found to attenuate Notch signaling and lead to the commitment of progenitor cells to the myoblast cell fate. Furthermore, asymmetric localization of Numb expression in actively proliferating myoblasts has suggested that Numb may be involved in cell fate determination and the maintenance of the satellite cell pool in mature muscle (8). As such, impaired expression of Numb in aged human skeletal muscle may be a potential mechanism, independent of resistance exercise, which contributes to the impaired regenerative capacity observed in this age group. The specific functions of Notch signaling in skeletal muscle postexercise remain undefined and provide an exciting avenue for further investigations.

Taken together, the results of the in vitro and in vivo experiments presented herein support a role for the Notch signaling pathway in the adaptive and/or regenerative capacity of human skeletal muscle. The increased expression of Notch genes during differentiation described here and elsewhere (12) as well as the increased expression following resistance exercise training, however, point toward an additional role in the regulation of later stages of myogenesis. However, much of the previous research presented to date suggests a role for Notch in the inhibition of myogenic differentiation (13–16). Although the proliferating myoblasts investigated in the present study demonstrated lower expression of Notch genes, we cannot discount a role for Notch in early proliferative events. Following injury, increased expression of Notch components are observed within satellite cells and promote the rapid expansion of the satellite cell progeny (8). The increased expression of Notch genes following exercise training may therefore reflect the residual activity of satellite cells within the muscle. The data presented here, however, do not distinguish the specific stage of myogenesis during which Notch genes are transcriptionally regulated, nor can we determine if the expression is predominantly associated with satellite cells or mature myofibers. It is, however, highly likely that Notch signaling expression and activation in skeletal muscle is essential at multiple stages of adult myogenesis, and its temporal and spatial regulation is tightly controlled (3).

The results of this study demonstrate for the first time a reduced expression of Notch signaling genes in aged human skeletal muscle. Furthermore, heavy resistance exercise training was able to alter the transcriptional profile of the genes investigated such that the differences observed between younger and older muscle pretraining were no longer evident. These results provide further evidence to support the role of Notch signaling in the impaired regulation of muscle mass with age, and suggest that some of the benefits provided by resistance exercise training may be mediated through the Notch signaling pathway. Further analysis of the impact of the Notch signaling pathway, including overexpression or knockout models, are required to further elucidate the contribution exerted by this pathway on muscle form and function.

Acknowledgments

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References


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Editor Nominations
Journal of Gerontology: Psychological Sciences

The Gerontological Society of America’s Publications Committee is seeking nominations for the position of Editor of the Journal of Gerontology: Psychological Sciences, the Society’s journal on the psychological science of aging.

The position will become effective January 1, 2008. The Editor makes appointments to the journal’s editorial board and develops policies in accordance with the scope statement prepared by the Publications Committee and approved by Council (see the journal’s General Information and Instructions to Authors page). The Editor works with reviewers and has the final responsibility for the acceptance of articles for the journal. The editorship is a voluntary position. Candidates must be dedicated to developing a premier scientific journal.

Nominations and applications may be made by self or others, but must be accompanied by the candidate’s curriculum vitae and a statement of willingness to accept the position. All nominations and applications must be received by April 2, 2007. Nominations and applications should be sent to the Publications Committee, Attn: Patricia Walker, The Gerontological Society of America, 1030 15th Street, NW, Suite 250, Washington, DC 20005-1503.