Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans

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Bamman MM, Petrella JK, Kim JS, Mayhew DL, Cross JM. Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans. J Appl Physiol 102: 2232–2239, 2007. First published March 29, 2007; doi:10.1152/japplphysiol.00024.2007.—We applied K-means cluster analysis to test the hypothesis that muscle-specific factors known to modulate protein synthesis and satellite cell activity would be differentially expressed during progressive resistance training (PRT, 16 wk) in 66 human subjects experiencing extreme, modest, and failed myofiber hypertrophy. Muscle mRNA expression of IGF-I isoform Ea (IGF-I-Ea), mechanogrowth factor (MGF, IGF-I-Ec), myogenin, and MyoD were assessed in muscle biopsies collected at baseline (T1) and 24 h after the first (T2) and last (T3) testing bouts from previously untrained subjects clustered as extreme responders (Xtr, n = 17), modest responders (Mod, n = 32), and nonresponders (Non, n = 17) based on mean myofiber hypertrophy. Myofiber growth averaged 2.475 mm2 in Xtr, 1.111 mm2 in Mod, and −16 mm2 in Non. Main training effects revealed increases in all transcripts (46–83%, P < 0.005). For the entire cohort, IGF-I-Ea, MGF, and myogenin mRNAs were upregulated by T2 (P < 0.05), while MyoD did not increase significantly until T3 (P < 0.001). Within clusters, MGF and myogenin upregulation was robust in Xtr (126% and 65%) and Mod (73% and 41%) vs. no changes in Non. While significant in all clusters by T3, IGF-I-Ea increased most in Xtr (105%) and least in Non (44%). Although MyoD expression increased overall, no changes within clusters were detected. We reveal for the first time that MGF and myogenin transcripts are differentially expressed in subjects experiencing varying degrees of PRT-mediated myofiber hypertrophy. The data strongly suggest the load-mediated induction of these genes may initiate important actions necessary to promote myofiber growth during PRT, while the role of MyoD is less clear.

Muscle growth; myogenin; insulin-like growth factor I; muscle fiber growth; myoblasts that form multinucleated myotubes. In fact, myogenin, MyoD, myf-5, and myf-6 (28, 36). While these data are certainly valuable and progress our understanding of the powerful influence mechanical load exerts on local cellular and molecular processes, studies of these factors during long-term resistance training leading to measurable myofiber growth are rare (29, 35).

In addition to its growth hormone-dependent hepatic production, IGF-I is synthesized in peripheral tissues and acts as a powerful autocrine and/or paracrine growth factor. IGF-I stimulates protein synthesis in myogenic cells via the phosphatidylinositol 3-kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR) pathway (22, 40, 46) and serves as a potent mitogen leading to skeletal muscle satellite cell proliferation (14, 16). Further, IGF-I appears to play a role in the differentiation and fusion of satellite cells serving as nuclear donors to myofibers undergoing growth or repair (5, 11). These anabolic effects of IGF-I are perhaps best demonstrated by remarkable muscle hypertrophy in viral-mediated IGF-I gene therapy (9, 10) and localized IGF-I infusion (2), leading to a number of investigations using IGF-I to restore satellite cell function and/or muscle integrity during aging (9, 14, 15, 34). On the basis of these key growth and/or regenerative actions, the ability of mechanical load to induce muscle IGF-I expression has been a fervent area of study over the past decade (1, 3, 4, 7, 20, 21, 28).

In addition to muscle expression of the liver isoform IGF-I-Ea, mechanical load induces expression of an IGF-I splice variant unique to skeletal muscle, denoted IGF-I-Ec in humans and often called MGF. While these isoforms appear to differ only in the E-peptide extension (propeptide) rather than the active IGF-I peptide (8), we have shown that MGF appears to be more acutely responsive to mechanical load, while IGF-I-Ea upregulation may occur more slowly and last longer (35). Others have also reported more rapid expression of MGF (vs. IGF-I-Ea) in response to resistance loading (19, 23). Increased muscle mRNA expression of IGF-I isoforms has also been found after short-term (5 wk) resistance training in older men (24), although after a total of 12 wk of training, myofiber size remained unchanged and whole muscle area increased minimally (30). While there is functional redundancy between the Ea and Ec isoforms (same active IGF-I peptide), a recent report identified a unique property of the MGF E domain as a motogenic factor, promoting myogenic cell migration (33).

The muscle-specific family of basic helix-loop-helix transcription factors, or myogenic regulatory factors (MRFs), plays essential roles during muscle development by regulating myoblast differentiation and transcription of skeletal muscle-specific genes (32, 39). MyoD and myf-5 direct the commitment of somatic cells to the myogenic lineage, while myogenin and myf-6 drive the differentiation of these cells to fully committed myoblasts that form multinucleated myotubes. In fact, myoge-
nin is considered a putative marker of differentiation to fusion-competent myoblasts. The events of developmental myogenesis are recapitulated in adult muscle undergoing regeneration and growth via satellite cell activation and progression. For example, we (28) and others (13, 20, 36) have previously shown that myogenin and MyoD mRNA levels are acutely and transiently elevated following a regeneration stimulus such as resistance loading.

While repetitive bouts of resistance loading are known to induce measurable myofiber hypertrophy after several weeks of training, there is a high degree of intersubject variability in the hypertrophic response (29, 35). This may be driven largely by individual differences in the ability to upregulate key processes that drive muscle protein synthesis and myonuclear addition. We recently introduced the concept of statistical cluster analysis in showing that myonuclear addition fails in human subjects resistant to myofiber hypertrophy (35). In this report we expanded our application of cluster analysis to fully test the hypothesis that muscle-specific factors known to modulate protein synthesis and satellite cell activity would be differentially expressed during resistance training in 66 human subjects experiencing extreme, modest, and failed myofiber hypertrophy. These three “responder clusters” were evaluated for transcript levels of IGF-IEa, MGF, myogenin, and MyoD across three muscle biopsy time points during 16 wk of resistance training. With this novel approach, we report for the first time that MGF and myogenin, but not MyoD, are differentially expressed during resistance training in 66 human subjects experiencing extreme, modest, and failed myofiber hypertrophy. These findings strongly suggest the acute, transient elevations in MGF and myogenin gene expression realized with each mechanical loading bout are necessary cues that lead to human muscle hypertrophy induced by resistance training.

METHODS

Subjects. Sixty-six untrained but otherwise healthy adults were recruited from the Birmingham, AL, metropolitan area. Inclusion criteria were based on age ranges of 60–75 yr for older adults (15 women, 14 men) and 20–35 yr for younger adults (16 women, 21 men). All subjects completed health history and physical activity questionnaires. Older adults passed a comprehensive physical exam conducted by a geriatrician and a diagnostic, graded-exercise stress test with 12-lead ECG reviewed by a cardiologist. Subjects were free of any musculoskeletal or other disorders that might have affected their ability to complete resistance training and testing for the study. Subjects were not obese (body mass index < 30 kg/m²), and none had undergone knee extensor resistance training within the past 5 yr. None of the subjects were being treated with exogenous testosterone or other pharmacological interventions known to influence muscle mass. The study was approved by the Institutional Review Boards of both the University of Alabama at Birmingham (UAB) and the Birmingham Veterans Affairs Medical Center. Written informed consent was obtained before participation in the research.

Using statistical (K means) cluster analysis, we classified subjects post hoc into three clusters based on changes in vastus lateralis mean myofiber cross-sectional area (CSA, μm²) in response to 16 wk of resistance training. K-means cluster analysis is a form of partitioning clustering. It is a multivariate procedure used to identify homogeneous groups (i.e., clusters) of cases based on a common trait. The number of groups is defined by the user; however, unlike discriminant analysis, the number of cases assigned to a group is not known. Cluster analysis was used over 25 yr ago as a means of defining muscle fiber types (42). We recently introduced this form of analysis in a study of myonuclear addition (35). It seems to be an ideal model by which potential mechanisms underlying human intervention responsiveness may be revealed in an unbiased manner. For this report, the analysis resulted in the following three clusters: extreme responders (Xtr, n = 17; 3 young women, 9 young men, 3 older women, 2 older men); modest responders (Mod, n = 32; 8 young women, 11 young men, 7 older women, 6 older men); and nonresponders (Non, n = 17; 5 young women, 1 young man, 5 older women, 6 older men).

Progressive resistance training program. Subjects underwent 16 wk of resistance training (3 days/wk) focused primarily on the knee extensors. Resistance training consisted of three exercises, including knee extension, leg press, and squats. Each exercise was performed for three sets of 8–12 repetitions to volitional fatigue using resistance exercise stations or plate-loaded stations (barbell squats and linear 45° leg press). Before each training session, subjects warmed up on a cycle ergometer or treadmill for ~5 min. All sets were separated by 90-s rest intervals. Initially, training loads were based on 80% of baseline 1 repetition maximum (1RM) strength. Loads were incremented in subsequent training sessions when a subject completed 12 repetitions for at least two of the three total sets at a given resistance while maintaining proper form. Subjects were taught and reminded to complete both concentric and eccentric phases with control (~2 s/phase). This protocol maintained a progressive resistance load throughout the training program (see RESULTS; Table 1).

Muscle biopsy and tissue preparation procedures. Muscle biopsies were performed in the Pittman General Clinical Research Center at UAB. Specimens were collected under local anesthetic (1% lidocaine) from the vastus lateralis muscle by percutaneous needle biopsy using a 5-mm Bergstrom biopsy needle under suction as previously described (17). Three biopsies were collected from each subject: baseline, 24 h after the first exercise bout, and 24 h after the final exercise bout 16 wk later. To avoid any residual effects of the initial biopsy taken from the left leg, the second biopsy was taken from the right leg, and the third, 16-wk postraining biopsy was obtained from the left leg. Samples were quickly blotted with gauze and dissected free of visible connective and adipose tissues, and a ~35 mg portion of each sample to be used for RNA isolation was immediately weighed and snap-frozen in liquid nitrogen. A separate portion for immunohistochemistry was mounted cross-sectionally on cork in OCT mounting medium mixed with tragacanth gum, and frozen in liquid nitrogen-cooled isopentane. All samples were stored at ~80 C.

Total RNA isolation. Sufficient snap-frozen muscle was available to isolate RNA and analyze the target transcripts across all three biopsy time points for 59 of the 66 subjects clustered based on myofiber hypertrophy. Transcript results are therefore reported on 15 of 17 Non, 30 of 32 Mod, and 14 of 17 Xtr. The procedures for RNA isolation have been described in detail previously (27, 28). Briefly, RNA was extracted by homogenizing muscle samples (average 35 mg portions) in a guanidinium thiocyanate solution and chloroform and precipitating the RNA by ethanol.

Table 1. Resistance training program summary by responder cluster

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Nonresponders (n = 17)</th>
<th>Modest Responders (n = 32)</th>
<th>Extreme Responders (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squat</td>
<td>Repetitions per set</td>
<td>10.5 ± 0.3</td>
<td>10.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Work intensity, %1RM</td>
<td>77.5 ± 1.7</td>
<td>73.3 ± 1.7</td>
</tr>
<tr>
<td>Leg press</td>
<td>Repetitions per set</td>
<td>10.7 ± 0.3</td>
<td>10.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Work intensity, %1RM</td>
<td>80.5 ± 2.2</td>
<td>78.0 ± 1.5</td>
</tr>
<tr>
<td>Knee extension</td>
<td>Repetitions per set</td>
<td>10.1 ± 0.3</td>
<td>10.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Work intensity, %1RM</td>
<td>68.8 ± 2.8</td>
<td>63.2 ± 1.7</td>
</tr>
<tr>
<td>Adherence, % of completed training sessions</td>
<td>93.2 ± 1.5</td>
<td>90.5 ± 1.0</td>
<td>89.3 ± 1.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. No differences on any program variable were found between clusters. RM, repetition maximum.
mg) in the TRI Reagent (Molecular Research Center, Cincinnati, OH), followed by precipitation with isopropanol, two ethanol washes, drying, and suspension in nuclease-free water at a ratio of 0.8 μg/mg muscle. Fluorometric analysis (TD-700, Turner Designs, Sunnyvale, CA) was performed to determine total RNA concentration using the RiboGreen RNA Quantitation Kit (Molecular Probes, Eugene, OR) (27, 28). RNA samples were stored at −80°C.

RT-PCR. As described (27, 28), 1 μg of RNA was reverse transcribed using SuperScript II Reverse Transcriptase (Invitrogen, GIBCO-BRL, Carlsbad, CA) with a mix of oligo(dT) (100 ng/reaction) and random primers (200 ng/reaction). A relative RT-PCR method using 18S ribosomal RNA as an internal standard (Invitrogen, Life Technology, GIBCO-BRL, Carlsbad, CA) was used to determine expression levels of the target mRNAs as we have shown elsewhere (27–29, 35). Primers were designed using the Primer Select computer program (DNAStar, Madison, WI) and custom made by Invitrogen (GIBCO). The specific primer sets used to amplify MGF, IGF-I, myogenin, and MyoD mRNAs were published previously (27, 28). As described in detail (27), interaction tests between each target mRNA primer and the alternate 18S primers were followed by linearity tests for each PCR reaction. Each target cDNA (mRNA) was coamplified with 18S (with a 324-bp product) to express as a ratio of target mRNA/18S. The 18S primers were mixed with competitors to ensure that both 18S and each target mRNA coamplified in the linear range. The ratio of this primer:competimer mixture was optimized in preliminary experiments and ranged from 1:15 to 1:100, depending on the abundance of the target mRNA. PCR was carried out in a DNA Engine (PTC-200) Peltier Thermal Cycler (MJ Research, Waltham, MA) using 1 μl of RT product (cDNA). Specific cycle protocols were as published (27, 28). PCR products were electrophoresed (100 V constant) in a 2% agarose gel for 75–120 min varying by target transcript. Standardization procedures for the order of samples, image capture, and densitometric analyses have been detailed elsewhere (27).

Immunofluorescence microscopy. We routinely assess myofiber type distribution (I, IIA, IIX) and type-specific myofiber size via myosin heavy chain isoform immunofluorescence microscopy and have published these methods in detail elsewhere (28, 29, 35). Images were analyzed by a single technician blinded to age, sex, and time point (pre- or posttraining) of each specimen. Mean myofiber size was determined as a weighted average based on distribution and size of type I, IIA, and IIX myofibers before and after training. Myofiber distribution was determined from 1,009 ± 39 and 851 ± 38 myofibers/specimen pre- and posttraining, respectively.

Statistical analysis. Data are reported as means ± SE. K-means cluster analysis (STATISTICA 6.1) was performed to identify three responder clusters based on magnitude of change in mean myofiber CSA in response to 16 wk of resistance training regardless of age or sex. We defined the three clusters as Xtr, Mod, and Non. Levels of the target transcripts were therefore assessed via three (cluster) × three (time point) repeated-measures ANOVA. For each ANOVA model with a significant main or interaction effect, Tukey’s honestly significantly different (HSD) tests were performed post hoc to localize the effect(s). Statistical significance was accepted at P < 0.05.

RESULTS

Cluster analysis performed post hoc defined three clusters based on the magnitude of myofiber hypertrophy as shown in Fig. 1. Change in mean myofiber CSA averaged +2,475 ± 140 μm² in Xtr, +1,111 ± 46 μm² in Mod, and −16 ± 99 μm² in Non. The age-sex breakdown within each cluster was listed in METHODS for descriptive purposes only, and each cluster had representation from all four age-sex groups. The focus of this work was on differences in gene expression across responder clusters irrespective of age or sex; however, it is noteworthy that Non contained only one young man compared with 38% of older women and men. By contrast, young men clearly dominated the Xtr cluster, as 43% of young men were in Xtr compared with only 18% of the remaining subjects. Approximately 50% of each age-sex cohort was clustered as Mod. This further illustrates the highly variable nature of the resistance training-mediated hypertrophy adaptation between human subjects.

To determine whether differences in hypertrophy responsiveness were caused by differences in training intensity and/or adherence, we analyzed the training log of each subject. As shown in Table 1, the three responder clusters did not differ by training intensity, training volume, or adherence to the program. These results clearly indicate that the wide range of individual responsiveness to our resistance training program resulted from differences in factors that influence myofiber hypertrophy downstream of the mechanical loading stimulus itself.

Overall, subjects completed 91% of the training sessions. Load assignments throughout the progressive resistance training program were designed to induce volitional fatigue within 8–12 repetitions per set and resulted in an average of 10+ repetitions per set across all three movements (squat, leg press, knee extension). Training intensity for the squat and leg press averaged 74.9% and 78.5% of 1RM, respectively, throughout training. Intensity averaged 64.5% of 1RM for the knee extension movement, slightly lower than the other two movements.
most likely because of some fatigue, as knee extension was always performed last.

Changes in muscle growth factor expression within each responder cluster are shown in Fig. 2. Main training effects (P < 0.001) indicated that transcript expression for both MGF (IGF-IYa) (Fig. 2A) and IGF-IJa (Fig. 2B) increased robustly with resistance training. Tukey’s HSD post hoc testing following these main time effects indicated an overall increase in MGF mRNA after one loading bout (P < 0.001) that tended to rise further by 16 wk (P = 0.065), while, for IGF-IJa, the overall acute increase after one bout (P < 0.05) was followed by a significant, further increase by 16 wk (P < 0.001). These responses differed by hypertrophy cluster and were primarily driven by Xtr and Mod. For example, MGF mRNA levels rose abruptly after only one resistance loading bout (P < 0.05) in Mod (74%) and Xtr (71%) but failed to increase in Non, leading to a significant cluster × training interaction (P < 0.05). After the final exercise bout 16 wk later, MGF expression was upregulated to the greatest degree (126% from baseline) in Xtr, while in Mod the increase above baseline of 73% was similar to the initial acute response. MGF in Non tended to rise by 16 wk, but this was not statistically significant (56%, P = 0.052). IGF-IJa transcript expression rose by 16 wk to levels 96% above baseline and 40% above the acute response in Mod (P < 0.005), and 105% above baseline and 68% above the acute response in Xtr (P < 0.001). In Non, IGF-IJa mRNA levels at 16 wk were 61% higher than the acute response (P < 0.01).

Transcript results within each responder cluster for the myogenic transcription factors myogenin and MyoD are shown in Fig. 3. Main time effects denoted significant overall increases in both transcripts with training (P < 0.005). For myogenin (Fig. 3A), post hoc testing localized the main training effect to an acute 43% increase (P < 0.001) after the first loading bout, which did not increase further by week 16. By contrast, the overall increase in MyoD (Fig. 3B) was not realized until wk 16 (53% above baseline, P < 0.001). Myogenin upregulation was clearly driven by responders, as increases at time point 2 were noted in Mod (40%, P < 0.05) and Xtr (51%, P < 0.01) but not Non. Only Xtr tended to increase myogenin expression further by week 16 (65% above baseline, P < 0.001). For MyoD, the overall increase was not driven by any particular responder cluster; in fact, no significant increases within each cluster were noted, and Xtr showed no upward trend.

Myofiber size and type distribution results for each cluster are shown in Table 2. Type I and type II myofiber sizes after training are shown for descriptive purposes only, as cluster analysis on mean fiber hypertrophy precludes any statistical comparisons of these data pre- to posttraining. One-way ANOVAs on pretraining fiber areas confirmed no significant differences in the sizes of type I and type II myofibers among the three clusters. Myofiber type distribution data were analyzed statistically using a three (cluster) × two (time point) ANOVA. The IIx-to-Ila myofiber type shift typical of resistance training occurred in each of the three clusters (P < 0.05), indicating that the overload stimulus was sufficient to induce this shift in all three groups.

Changes in 1RM knee extension strength for each of the clusters are shown in Fig. 4. A main time effect (P < 0.001) and cluster × time interaction (P = 0.016) were found. By midtraining, all three clusters realized substantial increases in strength (Non 30.5%, Mod 25.6%, Xtr 29.4%) (P < 0.001). The interaction was driven by the failure of Non to increase knee extension strength beyond the midtraining time point. From midtraining (week 8) to posttraining (week 16), only responders continued to make strength gains (Mod 10.2%, P < 0.005; Xtr 12.5%, P < 0.001). A significant cluster × time interaction (P = 0.013) was also noted for squat 1RM strength, as only responders increased squat 1RM in the latter half of the training program (data not shown). The leg press was the only one of the three training movements that did not reveal a cluster × time interaction on 1RM testing (data not shown).

DISCUSSION

Recent advances in cellular and molecular muscle biology have markedly improved our understanding of the processes driving myogenesis (44). Human studies of contraction-mediated muscle hypertrophy have wisely capitalized on these
seminal findings in myogenic cell culture models and lower mammals, revealing common themes across systems. There now seems to be general consensus that mechanical load activates the protein synthesis and translation initiation machinery via the PI3K-Akt-mTOR signaling cascade and that the addition of donor nuclei enhances the myofiber hypertrophy adaptation. Lacking, however, is a clear understanding of the upstream mechanotransduction signals that activate these two key processes, particularly in the context of human exercise training. While a number of molecular factors responsive to mechanical load have been identified, the importance of each factor in the progression of load-mediated muscle hypertrophy has been difficult to study in healthy humans because advanced techniques such as genetic manipulation are not possible.

In the absence of molecular manipulations or pharmacological treatment or blockade experiments, we propose that statistical K-means cluster analysis as performed herein provides a valuable and powerful means of revealing key steps in human muscle growth. In this cohort of 66 volunteers, we have identified 17 subjects with a remarkable ability to hypertrophy myofibers and an equal number of individuals incapable of muscle growth, at least not within the constraints of a 4-mo resistance training program. Despite the extraordinarily different propensities for muscle hypertrophy between Xtr and Non,

### Table 2. Myofiber CSA and type distribution by responder cluster

<table>
<thead>
<tr>
<th></th>
<th>Nonresponders (n = 17)</th>
<th>Modest Responders (n = 32)</th>
<th>Extreme Responders (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myofiber CSA, μm²</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4,905 ± 267</td>
<td>4,225 ± 198</td>
<td>4,131 ± 263</td>
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<tr>
<td>16 wk</td>
<td>4,896 ± 256</td>
<td>4,896 ± 171</td>
<td>5,969 ± 321</td>
</tr>
<tr>
<td>Type II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4,296 ± 367</td>
<td>3,839 ± 201</td>
<td>4,273 ± 342</td>
</tr>
<tr>
<td>16 wk</td>
<td>4,390 ± 289</td>
<td>5,130 ± 243</td>
<td>7,096 ± 415</td>
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<tr>
<td><strong>Myofiber type distribution, %</strong></td>
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<td></td>
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<tr>
<td>Type I</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>35.0 ± 2.7</td>
<td>36.6 ± 2.3</td>
<td>35.8 ± 3.0</td>
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<tr>
<td>16 wk</td>
<td>37.4 ± 3.0</td>
<td>35.6 ± 2.3</td>
<td>34.1 ± 2.4</td>
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<tr>
<td>Type IIα</td>
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</tr>
<tr>
<td>Baseline</td>
<td>52.2 ± 3.0</td>
<td>47.0 ± 1.7</td>
<td>48.3 ± 1.3</td>
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<tr>
<td>16 wk</td>
<td>61.8 ± 3.1*</td>
<td>62.6 ± 2.3*</td>
<td>63.5 ± 2.1*</td>
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<tr>
<td>Type IIx</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.9 ± 2.0</td>
<td>16.4 ± 1.7</td>
<td>15.8 ± 2.4</td>
</tr>
<tr>
<td>16 wk</td>
<td>0.9 ± 0.5*</td>
<td>1.8 ± 0.5*</td>
<td>2.3 ± 1.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Myofiber size results by cluster are shown for descriptive purposes only. CSA, cross-sectional area. *Within-groups training effect, P < 0.05. †Main training effect, P < 0.05.
both groups realized a robust increase in muscle expression of IGF-IeA. Enhanced expression of a gene previously shown to have myogenic properties may lead to the presumption that this factor plays an integral role in the myogenic process. While the cluster analysis does not afford the power to argue against IGF-IeA being necessary for resistance training-mediated hypertrophy of the vastus lateralis, the data suggest upregulation of its transcript is not sufficient to induce hypertrophy. We cannot rule out the possibility that the Xtr and Non clusters may have differed in translational efficiency (i.e., muscle IGF-I protein concentrations were not determined). In a similar fashion, while MyoD may play an integral role during hypertrophy, the results in Xtr strongly suggest that upregulated expression of MyoD mRNA is not necessary for resistance training-mediated myofiber hypertrophy: MyoD transcript levels increased 53% with training across the entire cohort of 66 subjects, but the Xtr cluster contributed little to this main effect. It should be pointed out that the interpretation of these findings is based strictly on load-mediated changes in transcript levels.

The utility of cluster analysis is also demonstrated by the results for MGF mRNA. Unlike the IGF-IeA and MyoD findings, MGF was differentially expressed across clusters. Xtr experienced the most robust increase, while Non saw only a nonsignificant upward trend in MGF mRNA. Of course further study would be required to be definitive, but these findings lead to the attractive possibility that the capacity to induce MGF gene expression above some threshold is necessary to activate hypertrophy process(es) during resistance training. Likewise, the starkly contrasting myogenin gene expression results between Xtr and Non strongly suggest some minimum level of myogenin mRNA induction is also necessary for hypertrophy to occur. Whether the induction of MGF and/or myogenin gene expression is sufficient to promote hypertrophy cannot be determined in this model.

The degree of myofiber hypertrophy found within the Mod cluster is characteristic of average myofiber growth rates reported in resistance training studies of similar duration (25, 31, 43), indicating the progressive training program employed was not unusual but rather typical. Individuals on the extreme ends of the “responsiveness” continuum, however, are not revealed by standard statistical models, and human training studies do not generally have a sample size with sufficient statistical power to explore responsiveness post hoc. The cluster analysis presented herein clearly shows that there is a large degree of interindividual variability in the ability to hypertrophy myofibers as an adaptive response to resistance training. The underlying causes of this high degree of variation remain elusive, but in the present study we can rule out a few obvious possible sources. First, one might expect subjects with unusually large myofibers and/or a large proportion of type I myofibers to be less capable of resistance training-mediated hypertrophy (preferential to type II myofibers). However, the histological phenotype of the three clusters did not differ on the basis of pretraining sizes of type I and type II myofibers or on the distribution of the primary myofiber types (I, IIa, IIX). Additionally, within the cohort were 15 subjects with large pretraining myofibers (mean fiber size > 5,000 µm²), and 10 of these 15 subjects experienced robust hypertrophy (5 Xtr, 5 Mod), indicating no apparent bias based on pretraining myofiber size. Second, age and sex (primarily in young adults) influence the ability to hypertrophy myofibers, as we have shown previously (29, 35). However, as confirmed by the subject composition of these clusters, not all elderly were unresponsive and not all young men were extreme responders; thus factors other than the independent variables of age and/or sex must play a role in responsiveness. Third, and perhaps most obviously, individual training intensity and dedication profoundly influence the efficacy of any exercise treatment. However, the clusters did not differ on the basis of resistance training intensity or adherence to the program. Certainly the training intensity sufficiently overloaded the vastus lateralis in all three clusters, as the IIX-to-IIa myofiber shift typical of resistance training was seen equally across the clusters, and 1RM strength gains, at least through the first 8 wk of training, were quite similar across clusters. The failure of Non to increase knee extension and squat strength beyond the first 8 wk appears to affirm the prevailing view that gains in strength during the initial phase of resistance training (i.e., first few weeks) are driven mainly by neural and/or other nonmuscle mass adaptations. Continued increments in strength in the later stages of a program, such as those realized by Mod and Xtr in the second 8 wk, are thought to become increasingly dependent on muscle hypertrophy.

Genotypic differences across apparently “normal” humans have been shown to influence muscle performance (38), as well as one’s propensity for resistance training-mediated muscle hypertrophy (26). We do not know whether any polymorphic variants existed in either Xtr or Non to promote or prevent hypertrophy, respectively. It is noteworthy, however, that the three clusters did not differ in basal expression of the four myogenic genes studied. Targeted gene arrays and/or genotyping studies may prove very useful in the future toward unraveling differences between Xtr and Non.

With no clear phenotypic or behavioral differences among the three clusters, and no known genotypic differences, we postulate that there may be differences across subjects in the sensitivity and/or signaling efficacy of mechanotransduction pathways. The immediate effectors of local muscle growth factor and/or MRF expression in response to mechanical load are not known. Our findings suggest a failure point in this upstream, load-sensitive signaling among subjects in the Non cluster, as basal myogenic factor expression was normal but load-mediated expression was blunted. The dystrophin-associated transmembrane protein complex is a likely candidate for mechanotransduction signaling and has been implicated in such processes as hepatocyte growth factor release from the extracellular matrix and satellite cell activation (6, 45, 48). There is also evidence that the expression and/or activation of focal adhesion complex proteins involved in integrin signaling (such as focal adhesion kinase and paxillin) are highly sensitive to the loading state of skeletal muscle (18). Further, as shown in cardiac muscle, integrin-costameric protein complexes responsible for transducing forces bidirectionally between the contractile apparatus and the extracellular matrix contain a growing list of proteins that demonstrate signaling kinase activity in a load-sensitive manner and communicate with growth factor receptors (41). Future studies should pursue the precise mechanism(s) by which mechanical stress signals through this transmembrane complex to activate myogenesis. Therein may lay a key difference in individual responsiveness to load-mediated hypertrophy stimuli.
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