Subcellular responses of p53 and Id2 in fast and slow skeletal muscle in response to stretch-induced overload

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Siu, Parco M., and Stephen E. Alway. Subcellular responses of p53 and Id2 in fast and slow skeletal muscle in response to stretch-induced overload. J Appl Physiol 99: 1897–1904, 2005.—Tumor suppressor p53 and inhibitor of DNA-binding/differentiation Id2 were examined after 7 or 21 days of wing weighting in fast patagialis (PAT) and slow anterior latissimus dorsi (ALD) wing muscles of young adult and old Japanese quails. The contralateral wing served as the intra-animal control. Seven days of loading increased PAT and ALD muscle weight by 28 and 96%, respectively, in young birds. PAT and ALD muscle weight was 49 and 179% greater, respectively, than control muscles after 21 days of loading in young birds. In aged birds, no PAT or ALD hypertrophy was found after 7 days of loading; however, PAT and ALD muscle weight increased by 29 and 102%, respectively, after 21 days of loading. Id2 protein in the nuclear muscle fraction increased in both PAT and ALD muscles from young adult and old birds that were loaded for 7 days and in ALD muscles after 21 days of loading relative to contralateral control muscles. Nuclear p53 protein was greater in 7- or 21-day loaded PAT and ALD muscles relative to control muscles in both age groups. Cytosolic Id2 and p53 protein contents were not changed in loaded PAT or ALD muscles relative to control muscles at any time point. These data suggest that nuclear, but not cytosolic, Id2 and p53 are responsive to stretch-induced muscle overload. Moreover, the attenuated ability of the aged skeletal muscle to achieve hypertrophy does not appear to be explained by the subcellular changes in Id2 and p53 content with overload.

Skeletal muscle is a highly adaptable organ system that responds rapidly to overload by increasing the anabolic events via upregulation of protein synthesis and accumulation of proteins (45). As a result, muscle hypertrophy or enlargement ensues if an overload stress is applied in a repeated or sustained pattern for a period of time (41). An appropriate overload (e.g., resistance training) has been suggested to be valuable and practical in offsetting or intervening for sarcopenia or disease-associated muscle wasting (18, 21, 28, 31, 35, 52, 65). Several studies have been conducted with the aim of identifying the precise cellular and molecular mechanisms that contribute to muscle hypertrophy (22, 25). Although potential signaling pathways (e.g., IGF-I, myogenic growth factor, phosphatidylinositol 3-kinase, protein kinase B/Akt, mTOR, p70S6K, RhoA, serum response factor, and focal adhesion kinase) have been preliminarily established in having a role in the hypertrophy-associated cellular changes (20, 22, 24–26, 43, 51); the exact underlying mechanisms responsible for the hypertrophic response, especially in the aged skeletal muscle, remain to be fully resolved.

The inhibitor of DNA binding/differentiation-2 (Id2) protein is a member of the Id family, which has been demonstrated to have a dual role in regulating cell proliferation/growth and cell death under different situations (10, 11, 19, 48, 64). These opposite functions of Id2 are primarily owing to the structure of helix-loop-helix motif in the absence of basic amino acid domain as well as the Bax-mediated apoptotic property of the N-terminal region of Id2 (10, 11, 19, 48, 64). Consistent with the suggested dual role of Id2, previous studies from our laboratory have shown that Id2 is associated with both skeletal muscle hypertrophy induced by functional or stretch-induced overload and the apoptosis-related muscle atrophy (4–6, 56). Although the mechanisms coordinating the differing functions of Id2 in muscle hypertrophy and atrophy are largely unknown, our laboratory’s recent data suggest that the subcellular cytosolic protein fraction may be associated with muscle atrophy (56). Nevertheless, the hypothesis that the nuclear compartment of Id2 is responsive to muscle loading has never been tested, and this was the main purpose of the present study.

A second candidate gene that may have a role in muscle remodeling and aging is tumor suppressor protein p53. It has been widely recognized that p53 is an essential regulator of the cell cycle and apoptosis in mitotic cell lineages (61). However, it is unknown whether p53 has a role in mediating muscle remodeling. With an emphasis on mature skeletal muscle, several studies have attempted to investigate the response of p53 to laser irradiation, muscle disuse, muscle unloading, and ischemia (27, 30, 46, 49, 55, 56). Although a consensus on the role of p53 in muscle loss has not been achieved, there have been at least some evidence showing that p53 responds to several muscle atrophic conditions (49, 56). Although more work is needed to determine the role of p53 in muscle atrophy, there has been novel data demonstrating that p53 is upregulated after resistance exercise in skeletal muscle (16). Correspondingly, p53 has been proposed to play a role in mediating the biochemical cascades leading to muscle hypertrophy (16). In support of the proposed hypertrophic role of p53, it has also been shown that p53 is related to Ankrd2, a sarcomeric protein found in the I band, which is involved in muscle hypertrophy (32). Nonetheless, mechanisms involved in regulating p53 during muscle hypertrophy are largely unknown. For this reason, the second major purpose of this study was to deter-
mine whether p53 was altered during muscle remodeling induced by overload.

Based on the limited findings that both Id2 and p53 are responsive to muscle overload and that cytosolic but not nuclear accumulations of these proteins appear to be related to atrophy and apoptosis in muscle (56), we speculated that the nuclear fraction of these two proteins may have important roles in mediating the hypertrophic process in response to muscle loading (4, 6, 16, 32). Because aging is typically associated with lower degrees of overload-induced hypertrophy, we speculated that lower nuclear accumulations of Id2 and p53 may explain, in part, suppressed overload-induced hypertrophy with aging. Therefore, the twofold purpose of this study was to examine the subcellular response of Id2 and p53 to stretch-induced overload in young adult and aged quails. First, we hypothesized that Id2 and p53 would increase in the nuclear but not cytoplasmic subcellular protein fraction in loaded muscles. Both fast-twitch patagialis (PAT) and slow-twitch anterior latissimus dorsi (ALD) muscles hypertrophy in response to stretch-induced overload in a loading duration-dependent fashion (2, 3, 6, 7, 12–14, 34). Second, we hypothesized that nuclear and cytosolic Id2 and p53 levels would be similar in loaded adult and PAT muscles, but nuclear levels of these proteins would be lower in muscles from old animals. In the present study, the responses of Id2 and p53 were examined in young and aged quails (2 mo old) and 16 senescent aged birds (48 mo old) were provided with food and water ad libitum. Sixteen young adult birds (~2 mo old) and 16 senescent aged birds (~48 mo old) were examined in the present study. The average life span of Japanese quails is ~26–28 mo, and therefore the birds examined in this study were almost twice the age of the expected life span of these birds. Presumably, these extremely old birds represented “successful aging” in this model of aging. We anticipated that, if aging-induced changes in hypertrophic responses to Id2 or p53 occurred with aging, this would be readily detectable in these old birds. Japanese quail are both physically and sexually mature by 1.5 mo of age and do not grow thereafter (40, 50). All experimental procedures carried approval from the Institutional Animal Use and Care Committee from West Virginia University School of Medicine. The animal care standards were followed by adhering to the recommendations for the care of laboratory animals as advocated by the American Association for Accreditation of Laboratory Animal Care.

**METHODS**

**Animals.** Japanese Coturnix quails were hatched and raised in pathogen-free conditions in the central animal care center at West Virginia University School of Medicine. The birds were housed at a room temperature of 22°C with a 12:12-h light-dark cycle and were provided with food and water ad libitum. Sixteen young adult birds (~2 mo old) and 16 senescent aged birds (~48 mo old) were examined in the present study. The average life span of Japanese quails is ~26–28 mo, and therefore the birds examined in this study were almost twice the age of the expected life span of these birds. Presumably, these extremely old birds represented “successful aging” in this model of aging. We anticipated that, if aging-induced changes in hypertrophic responses to Id2 or p53 occurred with aging, this would be readily detectable in these old birds. Japanese quail are both physically and sexually mature by 1.5 mo of age and do not grow thereafter (40, 50). All experimental procedures carried approval from the Institutional Animal Use and Care Committee from West Virginia University School of Medicine. The animal care standards were followed by adhering to the recommendations for the care of laboratory animals as advocated by the American Association for Accreditation of Laboratory Animal Care.

**Stretch-induced overload.** Fast-twitch α-fiber-predominated PAT and slow-twitch β-fiber-predominated ALD muscles are flexed with the wing on the birds’ back at rest, but they are both stretched when the wing is extended. In our experimental stretch-overload model, we placed a tube containing ~12% of the bird’s body weight over the left humeral-ulnar joint (7). This maintains the joint in extension throughout the period of stretch and induces stretch at the origin of the PAT and ALD muscles. Previous studies have consistently shown that this stretch-overloading protocol results in muscle hypertrophy of the PAT and ALD muscles and that the magnitude of increased muscle mass is dependent on the duration of the overload (2, 3, 6, 12–14). The unstretched right PAT and ALD muscles served as the intra-animal control muscle for each bird. Consistent with the fact that Japanese quails show no maturational changes in body weight and carcass composition beyond ~1.5–2 mo after hatching (38, 39, 63), it has been demonstrated that the body weights of Japanese quails do not change throughout stretch overloading and do not differ between adult and aged quails (1, 7–9, 14, 36). Therefore, the responses to the same absolute and relative loads could be compared in muscles from young adult and aged quails.

The left wing of eight young and eight aged birds was loaded for 7 days, and then the birds were killed by an overdose of pentobarbital sodium. The remaining young and aged quails were loaded for 21 days and then killed. The whole PAT and ALD muscles were dissected from the surrounding connective tissue, removed, weighed, and frozen in isopentane cooled to the temperature of liquid nitrogen and then stored at ~80°C until used for analyses.

**Subcellular protein fractionation.** The fractionation method described by Rothermel et al. (53) was adopted with minor modification to extract the cytosolic and nuclear protein fractions from the PAT and ALD muscles. We have previously obtained the fractionated cytosolic and nuclear proteins from skeletal or heart muscles using this modified protocol (56–59). In brief, after removal of connective tissues, muscle was homogenized on ice in lysis buffer (10 mM NaCl, 1.5 mM MgCl2, 20 mM HEPES, pH 7.4, 20% glycerol, 0.1% Triton X-100, 1 mM DTT) supplemented with a protease inhibitor cocktail containing (in mM) 104 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, 0.08 aprotinin, 2 leupeptin, 4 bestatin, 1.5 pepstatin A, and 1.4 E-64 (Sigma-Aldrich, St. Louis, MO). After centrifugation at 2,400 g for 15 min at 4°C to pellet the nuclei and cell debris, the supernatants were collected, and these supernatants were further centrifuged three times at 3,500 g for 5 min at 4°C to remove residual nuclei. The final collected supernatants were stored as nuclei-free total cytosolic protein fraction. The remaining nuclear pellets were washed three times with ice-cold lysis buffer, resuspended in 360 μl of lysis buffer in the presence of 49.8 μl of 5 M NaCl and protease inhibitor cocktail, and rotated for 2 h at 4°C to lyse the nuclei. After a spin at 21,900 g for 15 min at 4°C, the supernatants were collected and stored as cytosol-nuclear protein fraction. This protein fractionation procedure has been routinely used in our laboratory to obtain high-purity protein fractions as assessed by immunoblotting the fractions with an antibody H2B (a nuclear protein) and an anti-CuZnSOD (a cytosolic isoform of superoxide dismutase) antibody (56, 57, 59).

The protein contents of the protein extracts were quantified in duplicate by direct current protein assay (Bio-Rad, Hercules, CA) based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent, which was similar to Lowry assay (37). As a further means to confirm the protein contents, all the protein samples were measured in duplicate on a different occasion by bichinchoninic acid protein assay (Pierce, Rockford, IL) based on the biuret reaction and the bichinchoninic acid detection of cuprous cation (60).

**Western immunoblot analyses.** Id2 and p53 were examined in both nuclear and cytosolic fractions. Fixed micrograms of nuclear or cytosolic protein was boiled for 5 min at 95°C in Laemmli buffer and was loaded on each lane of a 12% polyacrylamide gel and separated by SDS-PAGE. The gels were blotted to nitrocellulose membranes (VWR, West Chester, PA) and stained with Ponceau S red (Sigma, St. Louis, MO) to verify equal loading and transferring of proteins to the membrane in each lane. As another approach to validate similar loading between the lanes, gels were loaded in duplicate with one gel stained with Coomassie blue. The membranes were then blocked in 5% nonfat milk in PBS with 0.05% Tween 20 at room temperature for 1 h and probed with anti-p53 mouse monoclonal antibody (1:200 dilution, sc-99, Santa Cruz Biotechnology, Santa Cruz, CA) or anti-Id2 rabbit polyclonal antibody (1:200 dilution, sc-489, Santa Cruz Biotechnology) diluted in PBS with 0.05% Tween 20 with 2% BSA incubated at 4°C for overnight. Whole cell lysate of actively dividing human 293T cells was included as a positive control for probing Id2 and p53. Secondary antibodies were conjugated to horseradish peroxidase (Chemicon International, Temecula, CA), and signals were...
defined by enhanced chemiluminescence detection kit (Amersham Biosciences, Piscataway, NJ). The signals were then visualized by exposing the membranes to X-ray films (BioMax MS-I, Eastman Kodak, Rochester, NY), and digital records of the films were captured with a Kodak 290 camera. Resulting bands were quantified as optical density × band area by a one-dimensional image analysis system (Eastman Kodak) and recorded in arbitrary units. The molecular sizes of the immunodetected proteins were verified by using prestained standard (LC5925, Invitrogen Life Technologies, Bethesda, MD).

**Statistical analyses.** Statistical analyses were performed using the SPSS 10.0 software package. ANOVA followed by Tukey’s honestly significant difference post hoc analysis was used to examine differences between groups. The main effects of age, time, overload, subcellular fraction, and muscle group were examined by a five-way ANOVA. Statistical significance was accepted at $P < 0.05$. All data are given as means ± SE.

**RESULTS**

**Change in PAT muscle mass after stretch overload.** The extent of muscle hypertrophy was estimated by examining the percent difference in the whole PAT or ALD muscle wet weight between the loaded and the intra-animal contralateral control wings. After 7 days of loading in PAT muscle, there was a 28% hypertrophy in young adult birds, whereas no significant extent of hypertrophy was found in aged birds (Fig. 1A). After 21 days of loading, there was a 49 and 29% hypertrophy in PAT muscles of young and aged birds, respectively (Fig. 1A). The percentages of hypertrophy in young PAT muscles after 7 and 21 days of loading were both significantly greater than the aged muscles (Fig. 1B).

**Change in ALD muscle mass after stretch overload.** After 7 days of loading in ALD muscle, there was a 96% hypertrophy in young adult birds, whereas no significant extent of hypertrophy was found in aged birds (Fig. 2A). After 21 days of loading, there was a 179 and 102% hypertrophy in ALD muscles of young and aged birds, respectively (Fig. 2A). The percentages of hypertrophy in young ALD muscles after 7 and 21 days of loading were both significantly greater than the aged muscles (Fig. 2B). Consistent with the previous reports demonstrating that the degree of hypertrophy is attenuated in the muscle from aged birds compared with young adults (2, 3, 6, 12–14), our results confirmed that the extent of hypertrophy, as estimated by increase in whole PAT or ALD muscle wet weight, in response to stretch overload was attenuated in aged muscle compared with the muscle from young adult birds.

**Nuclear and cytosolic Id2 protein content in PAT muscle.** According to our immunoblot analysis, the nuclear Id2 protein content of the loaded PAT muscle was 103 and 85% ($P < 0.05$) higher than the contralateral control muscle after 7 days of loading in young and aged birds, respectively (Fig. 3A). After 21 days of loading, there was no difference between the nuclear Id2 protein content between the loaded and control PAT muscles in the young and aged birds ($P > 0.05$; Fig. 3A). In contrast, cytosolic Id2 protein content was not different between the loaded and control PAT muscles after 7 and 21 days of stretch overload in both young adult and aged birds (Fig. 3B).

**Nuclear and cytosolic Id2 protein content in ALD muscle.** The nuclear Id2 protein content of the loaded ALD muscle was 227 and 201% ($P < 0.05$) higher than the contralateral control muscle after 7 days of loading in young and aged birds, respectively (Fig. 4A). After 21 days of loading, the nuclear Id2 protein content in the loaded ALD muscle was 186 and 188% greater than the control muscles in the young and aged birds (Fig. 4A). On the contrary, cytosolic Id2 protein content was not different between the loaded and control ALD muscles after 7 and 21 days of stretch overloading in both young adult and aged birds (Fig. 4B).

**Nuclear and cytosolic p53 protein content in PAT muscle.** The nuclear p53 protein content of the loaded PAT muscle was significantly higher than the contralateral control muscle in all groups of birds (Fig. 5A). After 7 days of loading, nuclear p53 protein content was 182 and 150% greater in the loaded side when compared with the control side in young adult and aged birds, respectively (Fig. 5A). The nuclear p53 protein content in the 21 days-loaded PAT muscle was still elevated by 113 and 152% relative to the intra-animal control muscle in young and aged birds, respectively (Fig. 5A). No difference was found
in the cytosolic p53 protein content between the loaded and the contralateral control PAT muscles in all groups of birds ($P > 0.05$; Fig. 5B).

**Nuclear and cytosolic p53 protein content in ALD muscle.** The nuclear p53 protein content of the loaded ALD muscle was significantly higher than the contralateral control muscle in all groups of birds (Fig. 6A). After 7 days of loading, nuclear p53 protein content was 206 and 194% greater in the loaded side compared with the control side in young adult and aged birds, respectively (Fig. 6A). The nuclear p53 protein content in the 21 days-loaded ALD muscle was still elevated by 140 and 154% relative to the intra-animal control muscle in young and aged birds, respectively (Fig. 6A). In contrast, we did not find any difference in the cytosolic p53 protein content between the loaded and the contralateral control ALD muscles in all groups of birds ($P > 0.05$; Fig. 6B).

**Main effects of age, time, overload, subcellular fraction, and muscle group.** For Id2, the outcomes of the ANOVA indicated that there were significant main effects of age [$F(1,224) = 8.27$, $P < 0.01$] and interaction of overload $\times$ subcellular fraction [$F(1,224) = 59.25$, $P < 0.001$]. Moreover, we found that a significant main effect of interaction of overload $\times$ subcellular fraction existed in explaining the variance of p53 [$F(1,224) = 7.20$, $P < 0.01$]. The existence of significant main interaction effect (overload $\times$ subcellular fraction) in Id2 and p53 suggested that the response of these two proteins to stretch-induced overload was in a subcellular compartmentalized manner (nuclear vs. cytosolic).

**DISCUSSION**

In this study, we have shown that Id2 and p53 respond to stretch-induced muscle overload in both quail fast- and slow-
twitch skeletal muscles in a specific subcellular compartmentalized fashion. We demonstrate that nuclear, but not cytosolic, protein contents of Id2 and p53 are elevated in muscle following stretch-induced overload in both young adult and aged birds. Both of these proteins increased in the nuclear fraction of loaded fast PAT and slow ALD muscles relative to the intra-animal contralateral control muscles following 7 or 21 days of stretch-induced overload. Moreover, we observed that the subcellular changes of Id2 and p53 occur in a similar extent in the muscles from young adult and aged birds, suggesting that the subcellular responses of Id2 and p53 do not account for the age-related difference in the capability of muscle hypertrophy. Collectively, our findings are consistent with the hypothesis that nuclear but not cytosolic Id2 and p53 are responsive to stretch-induced overload, and it is possible that these proteins may be associated with muscle gain as a result of stretch overload.

Response of Id2 to muscle overload. Id gene expression has been exhibited to be augmented in response to mitogenic stimuli and associated with the stimulation of DNA synthesis (10, 17, 47). Accordingly, it has been suggested that Id2 may have an important role in the regulation of cell growth/proliferation (10, 17, 47). Id2 may also have a regulatory role in muscle cell lineage, including growth/proliferation. This proposition is primarily based on the findings demonstrating that overexpression of Id prolongs proliferation and inhibits differentiation in myogenic lineage cells (17, 29, 33) and that Id2 gene-deficient mice do not survive at birth with a noticeable lack of muscle tissues (33). Consistent with these observations in Id2 in vitro and knockout animals, our laboratory has previously provided in vivo data suggesting that Id2 may be involved in the mediation of fiber hypertrophy during overload.

![Graphs showing the changes in Id2 and p53 protein content in ALD and PAT muscles](http://jap.physiology.org/)

**Fig. 4.** A: nuclear Id2 protein content in ALD muscle. Data are expressed as OD × resulting band area and expressed in arbitrary units. Insets: representative blots for Id2 in control and loaded muscles isolated from young adult and aged animals. *P < 0.05, data are significantly different from the corresponding intra-animal control muscles. B: cytosolic Id2 protein content in ALD muscle. Data are expressed as OD × resulting band area and expressed in arbitrary units. Insets: representative blots for Id2 in control and loaded muscles isolated from young adult and aged animals. Data are means ± SE.

**Fig. 5.** A: nuclear p53 protein content in PAT muscle. Data are expressed as OD × resulting band area and expressed in arbitrary units. Insets: representative blots for p53 in control and loaded muscles isolated from young adult and aged animals. *P < 0.05, data are significantly different from the corresponding intra-animal control muscles. B: cytosolic p53 protein content in PAT muscle. Data are expressed as OD × resulting band area and expressed in arbitrary units. Insets: representative blots for p53 in control and loaded muscles isolated from young adult and aged animals. Data are means ± SE.
Id2 protein content is increased in the loaded PAT or ALD muscle compared with the contralateral control muscle in both young adult and aged quails after 7 or 21 days of stretch-induced overload. However, these changes were not observed for the cytosolic Id2 protein content in all groups of birds. Together with our laboratory’s previous data demonstrating that cytosolic but not nuclear Id2 protein content is elevated during unloading-induced muscle atrophy (56), these findings are consistent with the overall hypothesis that Id2 is involved in regulating both hypertrophic and atrophic processes in skeletal muscle (4–6). Our results support the hypothesis that nuclear Id2 is responsive to muscle overload, whereas cytosolic Id2 is related to atrophy. Nonetheless, it warrants further research in exploring other possible regulatory mechanisms (e.g., phosphorylation of Id2) that might be comprised in Id2 and muscle remodeling.

p53 and muscle hypertrophy. p53 protein has been implicated in the pathogenesis of severe diseases and aberrant regulation of tumor suppressor (e.g., carcinogenesis). This finding has prompted extensive research examining the cellular functions of p53 in cell division and apoptotic cell death primarily in mitotic cell lineages (61). Conversely, the role of p53 in postmitotic muscle remodeling has not been well studied. Indeed, there has been preliminary data suggesting that elevation of p53 may be involved in muscle wasting under certain atrophic situations (49, 56). Ohnishi and coworkers (49) have shown that accumulation of p53 protein is evident in atrophic rat muscle after spaceflight. In addition, our laboratory has also demonstrated both the nuclear and cytosolic p53 protein contents are increased during unloading in previously hypertrophied quail PAT muscles (56). Nevertheless, additional research is required to verify the role of p53 in muscle wasting because there is still a lack of consensus on the role of p53 in other atrophic conditions (27, 30, 46, 55).

In contrast to muscle atrophy, there have been novel data suggesting that p53 is involved in overload-induced muscle hypertrophy (16). By using the microarray technique, Chen et al. (16) found that three p53-associated growth inhibitory genes, including GADD45, TRPM-2, and PC3, are all upregulated in rat skeletal muscle after an acute bout of maximally activated eccentric contraction, and furthermore, they reported an increase in p53 protein content, as estimated by Western immunoblotting, in the nuclear fraction extracted from exercised muscle. The potential role of p53 in muscle hypertrophy has been further indicated by the findings illustrating that p53 is associated with a hypertrophy-related sarcromeric protein called Ankrd2 (32). Consistent with these previous findings, we reported that the elevation of nuclear, but not cytosolic, p53 protein content is evident in quail muscle after stretch-induced overload. Our data support the hypothesis that p53 is involved in mediating the cellular cascades resulting in muscle hypertrophy or enlargement during muscle overloading. Moreover, our results indicate that the hypertrophic role of p53 is limited to the change of p53 content in the nuclei but not the cytosol during overload. Correspondingly, these observations lead to us to speculate that the role of p53 in muscle hypertrophy may be associated with the p53-mediated transcriptional events that occur in the nuclei. However, it is apparent that the proposed hypertrophy-mediating role of p53 during muscle overload is contradictory to the identified traits of p53 in inhibiting cell growth and/or promoting apoptosis (54, 61). Extensive data from mitotic cell lineages show that p53 has a crucial role in deciding the fate of cells by responding to DNA damage,
incorporating subcutaneous bromodeoxyuridine implantation to initiate widespread activation of myogenic precursor cells aged animals and humans. It explains in part reduced hypertrophic adaptation in muscles of old age (12), which limit stretch-induced muscle hypertrophy (13, 14). p53, perhaps in satellite cell activation and/or differentiation sensitive signaling pathways downstream from nuclear Id2 and p53AIP1 (42, 54, 61). It is unknown whether the observed elevation of nuclear p53 protein abundance in the present study was stimulated by the moderate cellular stress (e.g., microtrauma) that might have been incident with stretch overload, and this presumably repairing/regenerating process may be part of the episode mediating the anabolic and hypertrophic processes in skeletal muscle during overload. Nonetheless, there is a need to have more investigations to confirm whether p53 is directly and/or indirectly involved in the mediation of hypertrophy in overloading skeletal muscle of young animals or whether it is only responsive to loading but not directly responsible for mediating hypertrophy.

In this study, we have provided evidence showing that subcellular inhibitor of DNA-binding/differentiation protein Id2 and tumor suppressor protein p53 respond to the hypertrophic stimulus initiated from stretch-induced overload in both fast and slow skeletal muscles of young adult and aged quails. We have demonstrated that the nuclear but not the cytosolic protein contents of Id2 and p53 significantly increase in loaded muscles compared with contralateral control muscles after 7 or 21 days of loading. It is noted that the muscle tissue homogenates (i.e., cytosolic and nuclear fractions) being used in the present study to examine Id2 and p53 contained some non-myogenic cell populations (e.g., fibroblasts and endothelia). Noticeably, the nuclear changes in Id2 and p53 content have not been measured by others or us on nonmyogenic cell types in loaded muscles of young or aged animals, so we cannot rule out some contribution of these cell types to our results.

Although nuclear Id2 and p53 appear to be involved in pathways leading to stretch overload-induced muscle hypertrophy, our findings suggest that the attenuation of muscle hypertrophy in old age is not explained by the nuclear responses of Id2 and p53. Furthermore, both nuclear Id2 and p53 protein content increased in the muscles of aged birds that were loaded for 7 days, but these loaded aged muscles did not show any sign of significant muscle hypertrophy (i.e., muscle weight gain). These findings are consistent with the hypothesis that nuclear Id2 and p53 are responsive to muscle loading but may not be directly responsible for hypertrophic adaptations. We speculate that aged muscles may have deficits in loading-sensitive signaling pathways downstream from nuclear Id2 and p53, perhaps in satellite cell activation and/or differentiation (12), which limit stretch-induced muscle hypertrophy (13, 14). Furthermore, other factors, including increases in an apoptotic environment (4, 56, 59), inappropriate alterations in gene expression (23, 31), or the hormonal milieu (15, 26), may explain in part reduced hypertrophic adaptation in muscles of aged animals and humans.

The stretch-induced muscle overload model has been shown to initiate widespread activation of myogenic precursor cells (e.g., satellite cells) in avian muscles (6, 12, 44, 62). By incorporating subcutaneous bromodeoxyuridine implantation during overload and subsequent immunocytochemical staining on muscle sections, it has been shown that 14 days of stretch resulted in ~7 and ~15% bromodeoxyuridine positively labeled muscle-related nuclei (relative to the total number of muscle-related nuclei) in PAT and ALD muscles, respectively (6, 12). Nevertheless, nonmitotic skeletal myonuclei are the major component and the largest portion of the nuclear cell population in the loaded skeletal muscle. We anticipate that most of the present findings are attributed to the changes that have occurred in the skeletal myonuclei rather than the activated myogenic precursor cell population, which constitutes a relatively small portion compared with the total myonuclei population in myocytes. Our data do not allow us to determine the precise contribution of skeletal myonuclei and activated myogenic precursor cells to the changes of Id2 and p53 in response to stretch-induced muscle overload. Thus the results reported here primarily reflect alterations in nonmitotic myonuclei, but we cannot rule out the possibility that activated myogenic precursor cells may also have contributed in part to the changes in Id2 and p53.

GRANTS
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REFERENCES


