Regulation of caspase-3 activity by insulin in skeletal muscle cells involves both PI3-kinase and MEK-1/2

Yongmei Gao, Ronald Ordas, Janet D. Klein, and S. Russ Price

Renal Division, Emory University, Atlanta, Georgia

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Gao Y, Ordas R, Klein JD, Price SR. Regulation of caspase-3 activity by insulin in skeletal muscle cells involves both PI3-kinase and MEK-1/2. J Appl Physiol 105: 1772–1778, 2008. First published October 2, 2008; doi:10.1152/japplphysiol.90636.2008.—A hallmark of skeletal muscle atrophy is increased activities of several proteolytic systems, including caspase-3. We have previously shown that conditions involving insulin deficiency or insulin resistance increase both overall protein degradation and caspase-3-mediated actin cleavage. In the present experiments, we examined how insulin regulates caspase-3 activity in L6 myotubes. Reducing the serum concentration in the culture media from 2 to 0.5% overnight increased caspase-3 activity and actin cleavage. Addition of insulin to proteolytically active cells attenuated both responses within 4 h. Individually, inhibitors of either phosphatidylinositol 3-kinase (PI3K) or MEK1/2 partially blocked the insulin-induced reduction in caspase-3 activity; in combination, the inhibitors completely prevented insulin from attenuating caspase-3 activity. Insulin suppressed caspase-3 activity by a complex mechanism that included direct inhibition due to an increased interaction between caspase-3 and cellular inhibitor of apoptosis-1 and indirect inhibition via phosphorylation (i.e., inactivation) of the proapoptotic protein Bad, which participates in the intrinsic (i.e., mitochondrial) apoptosis activation cascade. Unlike other cell types, the phosphorylation of Bad Ser112 was mediated by the PI3K/Akt pathway rather than the MEK/ERK/ribosomal S6 protein kinase pathway. In summary, our findings indicate that insulin regulates caspase-3 activity by a multistep process that is unique to skeletal muscle, thus providing insights about the muscle-specific nature of the atrophy process.

Bad; signaling; mitogen-activated protein kinase; phosphatidylinositol 3-kinase

SKELETAL MUSCLE ATROPHY is a frequent complication of chronic diseases (e.g., cancer, kidney disease) that reduces quality of life and increases risks of morbidity and mortality in patients. Loss of lean body mass results primarily from increased activities of proteolytic systems that degrade muscle proteins, including the myofibrillar proteins (e.g., actin, myosin). Over the past decade, much attention has been focused on the ubiquitin-proteasome system because it has consistently been shown to be activated in muscles of animals and patients undergoing atrophy (10, 13, 14, 16, 26, 27). Recently, it has become apparent that other proteolytic systems also participate in the destruction of muscle proteins. For example, Sandri and colleagues (18, 34) reported that lysosomal proteolysis (i.e., autophagy) is significantly increased in muscle by starvation or denervation. Calpains appear to participate in sarcomeric protein remodeling during atrophy (4, 15, 32). Lastly, several groups have reported a link between caspase-3 activity and muscle atrophy (2, 6, 17, 19, 25, 29).

Traditionally, caspase-3 plays a key role in programmed cell death by acting as a terminal or effector protease that facilitates nuclear apoptosis. In skeletal muscle, however, some caspase-3 functions are independent of cell death, including a role in myogenesis (3, 8, 12, 23). Typically, caspase-3 is proteolytically activated by upstream initiator caspases, like caspase-9 and caspase-8, which participate in the intrinsic and extrinsic apoptosis pathways, respectively. Each of these activation pathways can independently induce mitochondrial permeability and the release of cytochrome c. Mitochondrial stability is regulated by members of the Bcl-2 protein family (24). Prosurvival family members (e.g., Bcl-xL, Bcl-2) prevent cytochrome c release from mitochondria. Proapoptotic Bcl-2 family members interact with and inhibit the function of their prosurvival counterparts (e.g., Bad) or directly affect mitochondrial membrane permeability (e.g., Bax). Upon release from the mitochondria, cytochrome c interacts with apoptotic protease-activating factor-1 and caspase-9 to form an apoptosis complex that activates caspase-9, which, in turn, activates caspase-3. In addition, the activities of caspase-3 and -9, but not caspase-8, can be finely tuned, independent of mitochondrial cytochrome c release, through interactions with members of the endogenous inhibitors of apoptosis protein (IAP) family [e.g., X chromosome-linked IAP (XIAP), cellular IAP (cIAP)-1] (20).

Insulin is a key regulator of caspase-3 activity and overall protein degradation in skeletal muscle. When plasma insulin levels were low (e.g., Type 1 diabetes) or when muscle cells become insensitive to the effects of insulin/IGF-I (e.g., chronic kidney disease, sepsis, Type 2 diabetes), protein degradation via the ubiquitin-proteasome system was accelerated, and a 14-kDa actin fragment generated by caspase-3 cleavage accumulated in muscle (1, 6, 9, 21). In L6 muscle cells, actin cleavage was induced by incubation in culture media with reduced serum (0.5 vs. 2%) (6). Adding insulin to the media during serum deprivation prevented the proteolytic response. Other than demonstrating a requirement for phosphatidylinositol 3-kinase (PI3K), this study provided few insights about how insulin achieved its effects.

To better understand how insulin regulates caspase-3 activity in muscle cells, we performed an analysis of insulin’s action on both caspase-3 activity and the cellular events that regulate this protease. Given the muscle-specific nature of the atrophy process, we hypothesized that the mechanisms regulating caspase-3 in skeletal muscle differ from those in other cell types. Our experimental strategy included incubating L6 myotubes in low (0.5%) serum overnight to activate caspase-3 and...
actin cleavage (6, 11); insulin was added the next morning to examine the temporal sequence of inhibitory events. Since insulin activates both the PI3K/Akt and MEK/ERK pathways in many cell types, we evaluated the role of both signaling systems in the regulation of caspase-3 activity. Our findings demonstrate that insulin regulates caspase-3 activity in muscle cells by a complex, multistep mechanism.

**MATERIALS AND METHODS**

**Materials.** Dulbecco’s modified Eagle’s medium (DMEM) was obtained from BioWhittaker (Walkersville, MD). Trypsin-EDTA, penicillin-streptomycin, L-glutamine, fetal bovine serum, and horse serum were from Life Technologies (Grand Island, NY). Insulin was from Novo Nordisk Pharmaceuticals (Princeton, NJ). Polyclonal antibodies directed against the COOH terminus of actin were from Sigma Chemical (St. Louis, MO). The CaspACE and Caspase-Glo assay systems were from Promega (Madison, WI). LY-294002, U-0126, and antibodies that recognize caspase-3, ERK1/2, phospho-ERK1/2 (pThr202/Tyr204), Akt, phospho-Akt (pSer473), Bad, and phospho-Bad (pSer12, pSer136, or pSer155) were from Cell Signaling Technology (Beverly, MA). Antibodies for immunoblot analysis of XIAP were from BD Biosciences Pharmingen (San Diego, CA); antibodies against cIAP-1 were from R&D Systems (Minneapolis, MN); antibodies against cIAP-2 were from Santa Cruz Biotechnology (Santa Cruz, CA). 14-3-3 Antibodies were kindly provided by Dr. Haian Fu (Emory University). Antibodies for immunoprecipitation of cIAP-1, Bcl-xl, and Bad were from Santa Cruz Biotechnology. Nitrocellulose membranes, high-performance chemiluminescence film, and enhanced chemiluminescence reagents were from American Biosciences (Piscataway, NJ). All other reagents used were of the purest grade available.

**Cell culture.** Rat L6 skeletal muscle cells (American Type Culture Collection, Manassas, VA) were grown and passaged in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C in a 5% CO2/95% O2 atmosphere. When myoblasts were 80% confluent, differentiation was induced by incubation in DMEM containing 2% horse serum for 3 days; the medium was changed daily. This protocol resulted in mature myotubes, as established by monitoring the expression of two muscle-specific proteins, myogenin and myosin heavy chain (data not shown).

**Materials and methods.** Differentiated myotubes were incubated overnight in media supplemented with 0.5% horse serum rather than normal differentiation media to activate caspase-3 activity (6). The following morning, insulin (100 nM) was added to the cells (with no media change) for various lengths of times (generally 1–8 h). All cells, treated and control (i.e., no insulin), were harvested at the same time in appropriate buffers for subsequent experiments, including measurements of caspase-3, -9, and -8 activities, Western blot analyses, and co-immunoprecipitation studies. A schematic of the experimental design is shown in Fig. 1A.

**Actin cleavage analysis.** Cells were lysed in hypotonic buffer as described (6). Lysates were incubated for 2 h at 37°C. The presence of a caspase-3-generated 14-kDa actin fragment was detected by immunoblot analysis with polyclonal antibodies that detect the carboxy terminus of actin.

**Caspase activity assays.** Caspase-3 activity was measured using the CaspACE Assay System (Promega, Madison, WI). Briefly, cells were solubilized with lysis buffer A [25 mM HEPES, pH 7.5, 5 mM MgCl2, 5 mM EDTA, 5 mM DTT, 2 mM phenylmethylsulfonyl fluoride (PMSF), 10 µg/ml pepstatin A, 10 µg/ml leupeptin]. Cell lysates (10 µl) were used in the fluorometric assay, which utilized 50 µM Ac-DEVD-AMC as a substrate; assays were conducted at 37°C for 60 min according to the recommended protocol; 7-Amino-4-methylcoumarin (AMC) release was monitored by measuring the fluorescence at 460 nm. To assess the specific contribution of caspase-3 enzyme activity in cell lysis, assays were performed in the presence and absence of a caspase-3 inhibitor, Ac-DEVD-CHO. The activity of caspase-3 was expressed as picomoles of AMC liberated per minute per micrograms of lysate protein.

Caspase-8 and caspase-9 activities were measured separately using the respective Caspase-Glo assays (Promega, Madison, WI), according to the manufacturer’s protocol; activities were measured in the presence of MG132, as suggested by the manufacturer to inhibit nonspecific activity contributed by the proteasome. Luminescence was measured in relative light units and normalized to protein content.

**Immunoblot and coimmunoprecipitation analyses.** For immunoblot analysis of phosphorylated and total ERK1/2 and Akt, cells were lysed in lysis buffer B (20 mM Tris·HCl, pH 7.4, 2.5 mM EDTA, 1% Triton, 10% glycerol, 1% deoxycholate, 0.1% SDS, 50 mM NaF, 10 mM Na2P2O7·10 H2O, 150 mM NaCl, 2 mM Na3VO4 containing 1 mM PMSF, 10 µg/ml aprotinin, 10 µg/ml leupeptin). For immunoblot analysis of activated caspase-3, cells were lysed in lysis buffer C (20...
mM Tris·HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 μg/ml leupeptin, and 1 mM PMSF). In all experiments, lysates were clarified by centrifugation at 10,000 g for 10 min at 4°C. The protein concentration in the lysates was determined using the Bio-Rad DC Protein Assay system (Hercules, CA). Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Blots were stained with Ponceau-S to verify that equal amounts of sample proteins were loaded and transferred. After blocking, the blots were incubated overnight with primary antibodies and processed for visualization by enhanced chemiluminescence, according to the manufacturer’s protocol (Amersham Biosciences, Piscataway, NJ).

For coimmunoprecipitation experiments involving cIAP-1 and caspase-3, cells were incubated overnight in medium plus 2% or 0.5% serum; the next morning, insulin (100 nM) was added to some cells (in 0.5% serum) for 8 h. Cells were lysed in caspase lysis buffer C, and the lysates were cleared by centrifugation (10,000 g for 10 min) at 4°C. Anti-caspase-3 antibodies were added to the supernatant (2 μg protein/sample), and the samples were rotated overnight at 4°C. Protein A agarose beads were added, and the samples rocked for 2 h at 4°C. Afterwards, the beads were collected by centrifugation, resuspended in Laemmli sample buffer, and the released proteins analyzed by immunoblotting techniques using anti-cIAP-1 antibodies.

BAD/Bcl-xL coimmunoprecipitation experiments were performed after cells were treated with insulin (100 nM) for 4 h. Lysates were prepared using lysis buffer D, consisting of 20 mM Tris·HCl, pH 7.4, 137 mM NaCl, 1.5 mM MgCl2, 1 mM EDTA, 0.25% Nonidet P-40, 10 mM NaF, 0.2 mM Na3VO4, 1 mM Na4MoO4, 1 μg/ml microcystin-LR, 0.25 mM PMSF, 1 μM pepstatin, 0.5 μg/ml leupeptin, and 10 μg/ml soybean trypsin inhibitor. All other aspects of the immunoprecipitation and subsequent immunoblot analysis were performed as described above.

Statistical analyses. Data are expressed as the means ± SE. Differences between two groups were analyzed by the Student’s t-test; multiple comparisons were analyzed by ANOVA with a post hoc analysis by the Student-Newman-Keuls test for multiple comparisons. Results were considered statistically significant when *P < 0.05.

RESULTS

We previously reported that serum deprivation (0.5% serum) overnight increased the amount of caspase-3-generated 14-kDa actin fragment in L6 cells, but, when cells were incubated in media with 0.5% serum and insulin (100 nM), the proteolytic response was blocked (6). To examine the early signaling events involved in suppressing caspase-3 activity, a different experimental strategy was used (Fig. 1A). Myotubes were incubated overnight in medium containing 0.5% serum to increase caspase-3 activity, as evidenced by higher levels of activated 17-kDa caspase-3 peptide and 14-kDa actin fragment (increased 321 ± 66 and 207 ± 39%, respectively; *P < 0.05 vs. 2% serum; Fig. 1B). The next morning, insulin was added for up to 8 h before evaluating caspase-3 activity and actin cleavage. Caspase-3 activity was measured directly by enzyme assay and indirectly by immunoblot analysis for the active 17-kDa caspase-3 peptide (Fig. 1, C and D). Enzyme activity remained high through the first 2 h after adding insulin but was suppressed after 4 and 8 h of treatment. The time course for the decrease in 14-kDa actin cleavage peptide coincided with that for caspase-3 activity, reaching a maximum decrease of 74 ± 11% (*P < 0.05 vs. no insulin; Fig. 1C).

Figure 2A demonstrates that insulin activates both the PI3K/Akt and MEK/ERK pathways in myotubes. Within 1 h of adding the hormone, phosphorylation of Akt and ERK was maximal (657 ± 105 and 483 ± 79%, respectively; *P < 0.05 vs. no insulin) and slowly returned to near control levels over 8 h. To examine the roles of these signaling pathways in the regulation of caspase-3 activity, the pharmacological inhibitors...
LY-294002 and U-0126 were used to block PI3K and MEK-1/2, respectively. Inhibitors were added 30 min before insulin. In the absence of insulin, LY-294002 did not change caspase-3 activity (and activated caspase-3 protein), whereas U-0126, either alone or in combination with LY-294002, significantly increased caspase-3 activity (Fig. 2B). When insulin was added, each inhibitor alone had little effect on the reduction in caspase-3 activity (and activated peptide), but adding both inhibitors simultaneously blocked the response to insulin (Fig. 2B). A similar inhibition pattern was observed with the actin fragment (Fig. 2C). In the absence of insulin, U-0126, alone or with LY-294002, increased the fragment abundance by 56 ± 11 and 78 ± 13% above the control cell level, respectively (both are P < 0.05 vs. 0.5% serum). Insulin treatment reduced the abundance of the actin fragment by 54 ± 8% (P < 0.05 vs. 0.5% serum). Neither LY-294002 nor U-0126 alone significantly prevented the reduction in actin fragment by insulin, but the combination of LY-294002 and U-0126 increased the amount of fragment by 48 ± 11% above the level in insulin-treated cells (P < 0.05 vs. 0.5% serum + insulin). The specificities of the inhibitors were confirmed by evaluating the insulin-induced phosphorylation of Akt and ERK. LY-294002 prevented the phosphorylation of Akt but not that of ERK-1/2 (Fig. 2D). Similarly, U-0126 blocked the phosphorylation of ERK-1/2 but not of Akt. Thus the inhibitors were specific for their respective pathways under the experimental conditions.

To better understand how insulin regulates caspase-3 activity in muscle cells, we determined whether the intrinsic or extrinsic activation cascade was activated by the low serum condition. Serum reduction produced an increase in caspase-9 activity but not that of caspase-8 (Fig. 3). Addition of insulin to proteolytically active cells decreased both caspase-8 and caspase-9 activities to below control cell levels with a time course similar to that for caspase-3 inactivation and the disappearance of the actin fragment. As a positive control, cells were treated with the relatively nonselective kinase inhibitor staurosporine in the presence or absence of insulin for 8 h. The compound increased caspase-8 activity by 122 ± 11% and caspase-9 activity by 289 ± 16% (both were P < 0.05 vs. cells in 2% serum), respectively; insulin inhibited both activities to the levels seen in cells incubated in media plus 2% serum.

We next examined the phosphorylation of the proapoptotic protein Bad because several signaling pathways have been shown to regulate its activity by modification of Ser112, Ser136, and/or Ser155 in nonmuscle cell types (5). Phosphorylation reduces Bad’s ability to interact with prosurvival proteins and enables it to interact with 14-3-3 proteins in the cytosol. Treating myotubes with insulin for 4 h increased the phosphorylation of Ser112 (1.145 ± 187%; P < 0.05 vs. 0.5% serum) and Ser155 (252 ± 68%; P < 0.05 vs. 0.5% serum), but not Ser136 (Fig. 4A). LY-294002 prevented the modification of Ser112. Notably, neither LY-294002 nor U-0126 alone affected Ser155 phosphorylation, but, in combination, they diminished the response to approximately the level in untreated cells (131 ± 21%; P = not significant vs. 0.5% serum). These modifications appear to have altered Bad activity, because insulin treatment decreased the amount of Bad that immunoprecipitated with Bcl-xL to 26 ± 11% of the control value (P < 0.05 vs. 0.5% serum) and increased the amount of Bad interacting with 14-3-3 by 57 ± 9% (P < 0.05 vs. 0.5% serum) (Fig. 4B). Cellular levels of Bad, Bcl-xL, and 14-3-3 were unchanged by the hormone.

Insulin could also act by influencing the interaction between caspase-3 and members of the IAP protein family. Incubating
myotubes in 0.5% serum overnight did not significantly change the amounts of XIAP, cIAP-1, or cIAP-2 in cells (Fig. 5A). Adding insulin for ≥2 h increased the level of cIAP-1 [181 ± 16% (2 h); 208 ± 22% (4 h); 165 ± 17% (8 h); all were \( P < 0.05 \) vs. 0.5% serum], but not of XIAP or cIAP-2 (Fig. 5B). To evaluate the interaction between cIAP-1 and caspase-3, a coimmunoprecipitation experiment was performed. Serum deprivation (i.e., 0.5% serum) reduced the amount of cIAP-1 associated with caspase-3 by 73 ± 6% \( [P < 0.05 \) vs. 2% serum] (Fig. 5C), and insulin restored the extent of interaction between these two proteins to 110 ± 8% of the level seen with control cells that were not serum starved \( (P = \) not significant vs. 2% serum). Similar results were obtained when the antibodies for immunoprecipitation and immunoblotting were reversed (data not shown). Serum reduction also decreased the interaction between cIAP-1 and caspase-9 (41 ± 6%; \( P < 0.05 \) vs. 2% serum), and insulin partially reversed the response (84 ± 8%; \( P < 0.05 \) vs. 2% serum) (Fig. 5D).

**DISCUSSION**

Muscle atrophy results from a coordinated increase in activities of several proteolytic pathways, including the ubiquitin-proteasome system and caspase-3. Although a great deal of information is known about how insulin modulates the activity of the ubiquitin-proteasome system, the present work is the first detailed analysis of the mechanism by which insulin (and IGF-I) inhibits caspase-3 activity in muscle. Our results demonstrate that caspase-3 is regulated by a complex mechanism involving multiple signaling pathways and inhibitory responses (summarized in Fig. 6).

One effect of insulin was to increase the interaction between cIAP-1 and caspase-3/caspase-9 (Fig. 6). This association directly inhibits these proteases, regardless of their activation status, and provides a way for the cell to attenuate their activities independently of the mitochondrial events that initiate the caspase-3 activation cascade. A recent report by Wang et al. (30) underscored the biological importance of this response in the context of muscle atrophy. Heterologous overexpression of XIAP in muscles of acutely diabetic mice reduced both the high level of caspase-3 activity, as well as overall proteolysis, and helped to maintain muscle mass.

A second action of insulin was to increase the phosphorylation of the proapoptotic protein Bad (Fig. 6). Bad facilitates Fig. 5. Insulin increases interactions between cellular inhibitor of apoptosis protein (cIAP)-1 and caspases-3 and -9. A: myotubes were incubated overnight in medium plus 2% or 0.5% serum. Levels of X-chromosome-linked inhibitor of apoptosis protein (XIAP), cIAP-1, and cIAP-2 in cell lysates were evaluated by immunoblot analyses. B: myotubes were serum deprived overnight, and insulin (100 nM) was added as described in Fig. 1A. The amounts of XIAP, cIAP-1, and cIAP-2 in lysates were evaluated by immunoblot analyses. C: myotubes were incubated overnight in medium plus either 2% or 0.5% HS; insulin (100 nM) was added to some 0.5% serum cells for 8 h. All cells were harvested at the same time. Anti-caspase-3 antibodies were added to equal amounts of lysate proteins (2 mg protein/sample) to immunoprecipitate caspase-3. The amount of cIAP-1 protein associated with the caspase-3 immunocomplex was evaluated by immunoblot analysis. D: the experiment in C was repeated using anti-cIAP-1 antibodies for the immunoprecipitation step, followed by immunoblot analysis using anti-caspase-9 antibodies. Each experiment was performed three times.

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Fig. 6. Insulin attenuates caspase-3 activity by a multistep mechanism. The schematic depicts the steps whereby insulin suppresses the activity of caspase-3. Acting through PI3K/Akt, insulin induces the phosphorylation of Bad on Ser112. This modification allows 14-3-3 to interact with Bad and sequester it in the cytosol. In the dephosphorylated state, Bad binds to and inhibits Bcl-xL, which stabilizes the mitochondria and inhibits the release of cytochrome c (Cyt C). Released Cyt C forms a complex with apoptotic protease-activating factor (APAF) and activates caspase-9, which proteolytically activates caspase-3 to cleave muscle proteins. In a second action, insulin increases the level of cIAP-1, which directly inhibits caspase-3 and caspase-9. Lastly, insulin acts by an unknown mechanism(s) that involves MEK as an intermediate, to attenuate caspase-3 activity. The overall reduction in caspase-3 activity should lead to a lower cleavage rate of skeletal muscle protein substrates.
the release of cytochrome c from mitochondria by interacting with prosurvival members of the Bcl-2 protein family (e.g., Bcl-xL). A variety of prosurvival signaling pathways, including PI3K/Akt and MEK/ERK/RSK, have been shown to inhibit Bad by phosphorylation of several different serine residues in nonmuscle cells (5). Considering that insulin activates the PI3K/Akt and MEK/ERK pathways in L6 myotubes (Fig. 2D), we predicted that both systems were involved in the inhibition of Bad. Two findings were surprising. First, insulin induced the phosphorylation of Ser112 and Ser155, but not that of Ser136. Second, the only phosphorylation event completely blocked by inhibition of either PI3K or MEK was that of Ser112 (Fig. 4A). These results were unexpected because, in many nonmuscle cells, Ser112 is typically phosphorylated by ribosomal S6 protein kinase (RSK), which is downstream of MEK/ERK (7, 22). In our experiments, however, the PI3K inhibitor LY-294002 rather than the MEK inhibitor U-0126 blocked Ser112 phosphorylation, indicating that Akt modifies this residue. Moreover, Akt typically phosphorylates Ser136, but insulin treatment did not increase its phosphorylation in myotubes. Therefore, we conclude that Akt regulates caspase-3 activity, in part by phosphorylating Bad on Ser112, whereas MEK must act by a different mechanism. Despite an inability to identify the precise step by which the MEK/ERK pathway regulates caspase-3 activity (Fig. 6), our findings are significant because they clearly demonstrate that the regulation of caspase-3 activity in skeletal muscle differs from most other cell types.

Is caspase-3 activity linked to the overall rate of protein degradation in muscle? Several lines of evidence suggest this is the case. Caspase-3 activity has generally been reported to be higher in muscles undergoing atrophy (2, 6, 25, 28). The level of caspase-3-generated 14-kDa actin fragment was higher in muscle of rats with acute diabetes or chronic kidney failure (6, 33). Incubating isolated epitropichears muscles of acutely diabetic rats with a cell-permeable caspase-3 inhibitor reduced the measured rate of proteolysis (6, 17). In rats, mechanical ventilation reduced the cross-sectional area of type I and IIA fibers of the diaphragm, and administration of a caspase-3 inhibitor prevented the atrophy (19). Lastly, infusing endothelin-treated rats with a caspase-3 inhibitor resulted in an improvement in diaphragm function (29). Coordination of caspase-3 and the ubiquitin-proteasome system could confer several advantages to muscle cells. During atrophy, caspase-3 cleaves a number of muscle proteins with different effects. Hilder et al. (12) reported that cleavage of skeletal muscle phosphorylase B resulted in a twofold increase in its activity; the increased activity helped to maintain the ATP-generating ability of muscle fibers. In the case of actin, cleavage may change the way the contractile (and cytoskeletal) protein is degraded. Myofibrils containing actomyosin filaments were reported to be resistant to direct degradation by the ubiquitin-proteasome system (26). Incubation of actomyosin directly with caspase-3 generated fragments that were shown to be degraded by the proteasome (6). Lastly, caspase-3 has been reported to increase the activity of the 26S proteasome complex in muscle cells by cleaving several regulatory subunits of the 19S regulatory particle at the end of the 20S proteasome core (31). Thus increased caspase-3 activity may facilitate the destruction of muscle proteins by producing substrates (e.g., protein fragments, ATP) for the ubiquitin-proteasome system, as well as increase the system’s degradation capacity.

In conclusion, conditions associated with insulin resistance or insulin deficiency induce muscle atrophy through both transcriptional and posttranscriptional responses that activate multiple proteolytic systems, including caspase-3. In the present study, we examined how insulin (and presumably IGF-I) regulates caspase-3 activity in differentiated myotubes. The regulation of caspase-3 activity in skeletal muscle cells is complex and involves multiple signaling pathways. The unique mechanisms we identified may help to explain why, under identical systemic conditions, skeletal muscle undergoes atrophy when other organs (e.g., liver, kidneys, heart) are either unaffected or undergo hypertrophy.

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