Increased ceramide content and NFκB signaling may contribute to the attenuation of anabolic signaling after resistance exercise in aged males

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Rivas DA, Morris EP, Haran PH, Pasha EP, Morais dSM, Dolnikowski GG, Phillips EM, Fielding RA. Increased ceramide content and NFκB signaling may contribute to the attenuation of anabolic signaling after resistance exercise in aged males. J Appl Physiol 113: 1727–1736, 2012. First published October 4, 2012; doi:10.1152/japplphysiol.00412.2012.—One of the most fundamental adaptive physiological events is the response of skeletal muscle to high-intensity resistance exercise, resulting in increased protein synthesis and ultimately large muscle mass. However, muscle growth in response to contraction is attenuated in older humans. Impaired contractile-induced muscle growth may contribute to sarcopenia: the age-associated loss of muscle mass and function that is manifested by loss of strength, contractile capacity, and endurance. We hypothesized that the storage of ceramide would be increased in older individuals and this would be associated with increases in NFκB signaling and a decreased anabolic response to exercise. To test this hypothesis we measured ceramides at rest and anabolic and NFκB signaling after an acute bout of high-intensity resistance exercise in young and older males. Using lipidomics analysis we show there was a 156% increase in the accumulation of C16:0-ceramide (P < 0.05) and a 30% increase in C20:0-ceramide (P < 0.05) in skeletal muscle with aging, although there was no observable difference in total ceramide. C16:0-ceramide content was negatively correlated (P = 0.008) with lower leg lean mass. Aging was associated with a ~60% increase in the phosphorylation of the proinflammatory transcription factor NFκB in the total and nuclear cell fractions (P < 0.05). Furthermore, there was an attenuated activation of anabolic signaling molecules such as Akt (P < 0.05), FOXO1 (P < 0.05), and S6K1 (P < 0.05) after an acute bout of high-intensity resistance exercise in older males. We conclude that ceramide may have a significant role in the attenuation of contractile-induced skeletal muscle adaptations and atrophy that is observed with aging.

skeletal muscle growth; anabolic signaling; intramyocellular lipids; inflammation; ceramide; resistance exercise; aging

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Methods

Subjects

Nine young (mean ± SD age 22 ± 0.6 yr) and 10 older (mean ± SD age 74 ± 1.5 yr) male subjects were recruited for this study. All subjects were healthy and were not currently participating in any regular endurance or resistance exercise training during the previous 6 mo. Each subject completed a medical history questionnaire and was examined by a physician before entry into the study. Subjects were excluded if they had any acute or chronic illness or injury, had been diagnosed with neuromuscular or cardiovascular disease, had uncontrolled hypertension (>150/90 mmHg), had a body mass index (BMI) greater than 32.5 or below 19 kg/m², had experienced an upper or lower extremity fracture in the past 6 mo, or had any other unstable medical condition. The Tufts University Health Sciences Campus Institutional Review Board approved the study, and written informed consent was obtained from each participant.

Body Composition Measures

Total body mass and composition were measured at screening using dual energy x-ray absorptiometry (DXA). Whole body DXA scans were performed using a Hologic Discovery A densitometer and were analyzed locally using Hologic QDR software version 12.3 in array mode (Hologic, Bedford, MA). Scan acquisition and analysis were performed according to manufacturer guidelines, with three passes over the subject to acquire the full DXA image (right side, center, and left side). Total body measurements of fat and lean mass were reported.

Study Design

A schematic of the study design is shown in Fig. 1. On day 1 (D1), each subject was tested for bilateral leg muscle strength by measuring their 1 repetition maximum (1RM) on a knee extension and leg press machine (Cybex-VR2, Medway, MA). On day 7 (D7), each subject was admitted to the Metabolic Research Unit the day before the experimental exercise session and a baseline (BL) biopsy was performed after an overnight fast. The subjects were then provided a standard breakfast, lunch, and dinner (60% carbohydrate, 20% fat, and 20% protein) and a snack at 2200. However, caloric intake was not controlled and the subjects had ad libitum access for each meal. The morning of day 8 (D8), an acute bout of resistance exercise was performed that included bilateral leg press and bilateral knee extension consisting of three sets of 10 repetitions done at 80% of the individual subject’s 1RM. Muscle biopsies were performed immediately (OH) postexercise and 6 h (6H) postexercise as described below. All subjects were studied during the same time of day, after an overnight fast, and remained fasted until following the final muscle biopsy.

Muscle Biopsies

Baseline (BL), immediately postexercise (OH), and 6 h (6H) postexercise muscle biopsies were obtained from the vastus lateralis at the level of the mid thigh under local anesthesia (lidocaine 1%) with a 5-mm Duchenne biopsy needle and suction (3). Incisions were made immediately prior to the three biopsies and the procedure was alternated between each leg using three separate incision sites. After removal, the muscle samples were blotted on gauze dampened with ice-cold (4°C) normal saline to remove any excess blood and immediately placed in liquid N₂ and subsequently stored at −195°C for later analysis.

Sphingolipid Analysis

Ceramide concentrations were analyzed using high-performance liquid chromatography/tandem mass spectrometry (LC/MS²) as previously described (5), with modifications. Briefly, 20–30 mg of flash-frozen muscle was homogenized in 1 ml of a Tris/sucrose buffer, then deuterated internal standards (cat. no. 860657, 868516; Avanti Polar Lipids; Alabaster, AL) were added to the homogenate. Four hundred microliters of the homogenate was extracted using two solvent extraction solutions: 1) iPrOH:H₂O:EtOAc (30:10:60; vol/vol/vol) and 2) CHCl₃:MeOH (2:1; vol/vol). All organic layers were combined, evaporated under N₂ gas and then resuspended in 1,000 μl of mobile phase (1 mM NH₄HCO₃:0.2% H₂O;MeOH). Separation by HPLC was carried out using a C8 X-Bridge (150 mm) column (Waters; Milford, MA). Deuterated (cat. no. 860657, 868516; Avanti Polar Lipids) and nondeuterated (cat. no. 860490, 860501, 860514, 860516, 860518, 860519, 860520, 860524, 860525, 860527, 860534; Avanti Polar Lipids) standards were analyzed in a separate run to establish retention time of the compounds under the specific assay conditions. Mass spectrometry was carried out in an AB QTRAP 5500 mass spectrometer (Agilent Technologies; Lexington, MA) following the expected fragmentation pattern of each of the lipids under specific conditions.

Serum Cytokine Analysis

Serum concentrations of IL-1β and IL-6 were measured according to the manufacturer’s (Human High Sensitivity ELISA, eBiosciences, San Diego, CA) instructions, using solid-phase sandwich enzyme-linked immunosorbent assay kits with sensitivities of 0.16 and 0.08 pg/ml, respectively. Optical density was read on an automated microplate photometer (EL312e, BIO-TEK Instruments, Winooski, VT).

Western Blotting Analysis

The phosphorylation and concentration of signaling proteins were quantified by Western blot analyses as previously described (61). Muscle samples were cut and weighed frozen and homogenized in an ice-cold homogenization buffer (1:10 wt/vol) containing 50 mM Tris-HCl (pH 7.5), 5 mM Na-pyrophosphate, 50 mM NaF, 1 mM EDTA, 1 mM EGTA, 10% glycerol (vol/vol), 1% Triton-X, 1 mM DTT, 1 mM benzamidine, 1 mM PMSF, 10 μg/ml trypsin inhibitor, and 2 μg/ml aprotinin. Following

Fig. 1. Schematic of the study design. D, day; 1RM, 1 repetition maximum test; MB, muscle biopsy; BL, baseline; LP, leg press; KE, knee extension; OH, immediately postexercise; 6H, 6 h postexercise.
Table 1. Study subject characteristics

<table>
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<td>Knee extension 1RM/kg lean mass</td>
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<td>1.3*</td>
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</table>

Values are means ± SD; n = 9–10/group. BMI, body mass index; 1RM, 1 repetition maximum; ASM, appendicular lean skeletal muscle mass. Significant differences between groups (*P < 0.05 vs. younger (YNG).

centrifugation (21,000 g, 4°C) for 15 min the supernatant was collected and assayed for protein content. Nuclear fractionations were carried out using NE-PER Nuclear and Cytoplasmic Extraction Kit as per manufacturer’s instructions (Thermo Scientific, Rockford, IL). The lysates (30 μg) were solubilized in Laemmli buffer, separated by SDS-PAGE, and transferred to PVDF membranes. The membranes were then blocked (5% NFDM), and incubated overnight at 4°C with primary antibodies specific for either Akt (4685; Cell Signaling Technology, Danvers, MA), phospho-Akt Ser473 (4058; Cell Signaling), mTOR (2983; Cell Signaling), phospho-mTOR Ser2448 (2971; Cell Signaling), S6K1 (2708; Cell Signaling), phospho-S6K1 Thr389 (9234, Cell Signaling), FOXO1Thr24 (2599; Cell Signaling), IL6 (ab6672, Abcam, Cambridge, MA), IκBα (2022; Cell Signaling), phospho-NFκB Ser468 (3039; Cell Signaling), NFκB p65 (3034; Cell Signaling), phospho-IκBα (9246; Cell Signaling), IκBα (9242; Cell Signaling), PP2A (2259; Cell Signaling), PP2C (3549; Cell Signaling), GAPDH (5174; Cell Signaling), and histone H1 (N-16) (sc-34464; Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were probed with α/β-tubulin (2148; Cell Signaling) antibody to monitor protein loading. The immunoreactive proteins were detected with Supersignal Chemiluminescent Substrate (Thermo Scientific), and intensities were quantified by densitometry (Bio-Rad Chemidoc XRS+ system, San Leandro, CA).

Statistical Analysis

Descriptive characteristics. Differences between groups were identified using a Student’s t-test performed with GraphPad Prism version 5.00 for Windows (GraphPad Software, CA, http://www.graphpad.com). Results are expressed as means ± SD, and statistical significance was accepted at P < 0.05.

Ceramide data. Baseline differences between OLD and YNG were presented as fold change compared with YNG. Differences between groups were identified using a Student’s t-test performed with GraphPad Prism version 5.00 for Windows (GraphPad Software). Results are expressed as means ± SD and statistical significance was accepted at P < 0.05.

Associations between variables of interest were performed using Pearson’s correlation and normal distribution of the data was determined using the D’Agostino-Pearson normality test with GraphPad Prism version 5.00 for Windows (GraphPad Software). Statistical significance was accepted at P < 0.05.

Signaling data. All measured proteins were normalized to the loading control, except nuclear proteins, and presented as fold change after exercise. Differences between groups were identified using a
two-way analysis of variance (ANOVA) with Bonferroni posttest performed with GraphPad Prism version 5.00 for Windows (GraphPad Software). Results are expressed as means ± SD and statistical significance was accepted at \( P < 0.05 \).

RESULTS

Study Participants, Body Composition, and Strength

The study subject’s descriptive characteristics appear in Table 1. Older participants had a higher BMI compared with young \( (P < 0.05) \). There were no differences in whole body lean mass and appendicular lean skeletal muscle mass (ASM) between age groups. However, the older subjects had significantly lower ASM normalized to height squared \( (ASM/h^2) \) compared with young \( (P < 0.05) \). The older subjects also had 7% higher whole body fat mass \( (P < 0.05) \). There was no difference in bilateral leg press strength between OLD and YNG. However, the older subjects tended to have significantly lower bilateral knee extension strength \( (P < 0.10) \).

Increased Ceramide Content in Aged Skeletal Muscle

To determine the baseline metabolic phenotype of young and aged skeletal muscle we first measured the concentration of long-chain ceramides, including C16-C24, which represent the most abundant ceramide subspecies in skeletal muscle (23). OLD had a 156% higher concentration of C16:0-ceramide compared with YNG at baseline \( (P < 0.05 \) vs. YNG; Fig. 2). C20:0-ceramide was increased 30% in OLD \( (P < 0.05 \) vs. YNG, Fig. 2). There also tended to be a higher concentration of C18:0-dihydroceramide in OLD compared with YNG \( (P = 0.07 \) vs. YNG; Fig. 2). Despite the elevation in these specific ceramide species there were no differences in total or unsaturated ceramide between age groups. However, there tended to be a greater amount of saturated ceramide in OLD \( (P = 0.09 \) vs. YNG; Fig. 2).

We observed a significant negative association between intramuscular C16:0-ceramide content from the vastus lateralis and lower leg lean mass \( (P < 0.008, \text{Fig. 3A}) \) in the younger and older subjects combined. However, there was no associa-
tion between intramuscular C16:0-ceramide concentrations and leg press (Fig. 3B), knee extensor strength (data not shown), or whole body fat mass (Fig. 3C).

No Differences in Circulating Cytokine Levels at BL, 0H, and 6H Time Points

There were no differences between young and older in circulating IL1β and IL6 levels at BL (5.82 ± 0.76 vs. 7.26 ± 1.84 and 9.63 ± 1.04 vs. 10.58 ± 0.75 pg/ml, respectively), 0H (5.48 ± 0.75 vs. 7.30 ± 1.24 and 9.29 ± 1.31 vs. 11.18 ± 0.95 pg/ml, respectively), and 6H (5.18 ± 0.84 vs. 6.74 ± 1.15 and 10.08 ± 1.07 vs. 11.10 ± 1.08 pg/ml, respectively).

Increased NFκB Signaling in Aged Skeletal Muscle

We observed in OLD a significantly higher total protein concentration of the NFκB subunit p65 compared with YNG (P < 0.001 vs. YNG; Fig. 4A). This translated to a significant main effect of higher phosphorylation of NFκB in OLD (P = 0.01 vs. YNG; Fig. 4B). In addition, there was a significant interaction between age and time (P = 0.02; Fig. 4B) with a 30% decrease at 0H and 50% decrease at 6H in YNG and a 60% increase in OLD at 6H. There was a significantly higher total protein concentration (P < 0.02; Fig. 4C) of the NFκB p65 subunit and its phosphorylation at Ser 468 (P = 0.03; Fig. 4D) in the nuclear fraction of OLD at all time points. To confirm proper separation between cytosolic and nuclear fractions we tested and observed a difference in the amount of GAPDH (cytosolic) and histone 1 (nuclear) in each fraction (Fig. 4E). The phosphorylation of IκBα mirrored the NFκB results, where we observed an increase in IκBα phosphorylation with aging (P = 0.01; Fig. 5A) but no change in total protein expression (Fig. 5B). The protein expression of IL6 and IL1β was not different between age groups and after exercise (Fig. 5, C and D).

Protein Phosphatase Content is Unaffected by Age or Resistance Exercise

We next measured the concentration of the ceramide-modulated protein phosphatases PP2A and PP2C. There were no differences in the concentration of PP2A or PP2C between age groups or after resistance exercise (Fig. 6, A and B).

Akt/FOXO/S6K1 Signaling in Young and Aged Skeletal Muscle After Resistance Exercise

As a result of the differences in ceramide content and inflammatory signaling, we next determined the phosphoryla-

![Fig. 5. Phosphorylation and total protein content of the inhibitor of NFκB and the total protein content of cytokines at BL and after RE in YNG and OLD in skeletal muscle. Relative protein content of total IκBα (A), phospho-IκBα (B), total IL6 (C), and total IL1β (D) were quantified using Western blot analysis in skeletal muscle. (n = 8–10/group). (*P < 0.05 vs. YNG, n = 8–10/group).](http://jap.physiology.org/1731Ceramides and Exercise-Induced Muscle Growth - Rivas DA et al.)
tion of the activation site, Ser473, and the total protein concentration of the anabolic regulator, Akt. There was a significant age effect of the phosphorylation of Akt on Ser473 \((P = 0.02\) vs. YNG; Fig. 7A). In addition, there was a significant interaction between age and time \((P = 0.01;\) Fig. 7A) with a 50\% increase in YNG and a 20\% increase in OLD after resistance exercise. There was no difference in the total protein expression of Akt between YNG and OLD (Fig. 7A).

We next determined the total protein content and phosphorylation of the catabolic regulator FOXO1 on Thr24, its Akt regulated inhibitory site. There was a significant age and time effect of the phosphorylation of FOXO1 on Thr24 \((P = 0.001\) and \(P = 0.02\), respectively vs. YNG; Fig. 7B). In addition there was a significant interaction between age and time \((P = 0.001;\) Fig. 7B) with a 30\% and 90\% increase in the phosphorylation of FOXO1 on Thr24 immediately after and 6 h after resistance exercise, respectively, in YNG and a 20\% and 10\% decrease in OLD at 0H and 6H after resistance exercise in OLD. There were no differences in the total protein content of FOXO1 between YNG and OLD.

Subsequently we measured the total protein content and phosphorylation of the anabolic regulators mTOR and S6K1. There were no changes in mTOR phosphorylation on Ser2448 or total protein concentration between YNG and OLD at BL or after resistance exercise (Fig. 7C). In contrast, there was a significant age and time effect of the phosphorylation of S6K1 on Thr389 \((P = 0.045\) and \(P = 0.003\), respectively, vs. YNG; Fig. 7D) and a significant interaction between age and time \((P = 0.004;\) Fig. 7D) with a 40\% and 90\% increase in the phosphorylation of S6K1 on Thr389 immediately after and 6 h after resistance exercise, respectively, in YNG and no change in OLD at 0H and 6H after resistance exercise in OLD. There were no differences in the total protein expression of S6K1 between YNG and OLD (Fig. 7D).

**DISCUSSION**

In the current study, we first assessed age-related differences in the accumulation of the bioactive lipid metabolite, ceramide, between age groups. Recently, it has been observed that ceramide accumulation is required for proinflammatory cytokines to induce anabolic resistance in myotubes (17, 65). Furthermore, ceramide can induce anabolic resistance through the activation of protein phosphatases such as PP2A and PP2C (11, 52, 56). We now show for the first time there was a significantly higher accumulation of specific ceramide subspecies (Fig. 2) in healthy older males compared with young, although there was no observable difference in total ceramide. In addition, C16:0-ceramide content was negatively correlated with lower leg lean mass in both age groups (Fig. 3A). Higher ceramide content was associated with an increased phosphorylation and concentration of the proinflammatory transcription factor NFκB in both the total and nuclear fraction (Fig. 4, A–D). Furthermore, there was an attenuated anabolic signaling response after an acute bout of high-intensity resistance exercise in older compared with younger males (Fig. 7).

Aging is not only associated with a loss of skeletal muscle mass but also an increase in intramuscular lipid concentrations. More specifically, we and others have previously reported an increase in intramuscular lipid triglyceride concentration with aging (15, 60). We have now, for the first time, determined the concentration of the bioactive lipid species, ceramide, in the skeletal muscle of healthy older compared with healthy younger males. Ceramide, sphingolipids that are made up of a sphingosine backbone and a fatty acid chain, are ubiquitous regulators of cellular stress. Hyde et al. (39) recently observed in cell culture that ceramide reduces protein synthesis by inhibiting Akt/mTOR signaling and amino acid transport (39). Ceramides have fatty acid chain lengths that vary from 2 to 28 carbon atoms, with longer-chain fatty acids being the most abundant and the most prevalent of these being C16:0-ceramide (40–45\% of total ceramide) (20, 23, 34). In the present study, we developed the ability to detect nine separate ceramide subspecies, or metabolites with fatty acid residues ranging from C14:0 to C24:1. We now report no observable significant differences in total or unsaturated ceramides between groups, although there tended to be higher saturated ceramides in OLD (Fig. 2). We did observe and now report significant differences in the concentration of specific ceramide carbon chains between age groups with both palmitic (C16:0) and arachidic (C20:0) ceramides being significantly higher and the ceramide metabolite stearic (18:0) dihydroceramide (dh-Cer) tending to be higher in older compared with younger males (Fig. 2). The result of higher saturated ceramide subspecies in OLD is interesting since there is a greater overall abundance of saturated ceramides within human skeletal muscle (20) and saturated fatty acids are more poorly oxidized than unsaturated, and thus are more likely to accumulate in insulin resistant tissues (28). Furthermore, saturated fatty-acids have been shown to be more associated with metabolic dysfunction than their unsaturated counterparts (22, 63).
The role of ceramide in age-associated skeletal muscle metabolic abnormalities and atrophy is still largely unexplored. Ceramide is shown to be elevated in the muscle of obese, insulin-resistant humans (1, 64) and rodents (44, 45, 68), and its accumulation affects downstream insulin signaling (63, 66). It is also well recognized that ceramide has a significant role in the induction of proinflammatory cytokines and activation of their regulators such as NFκB (27, 43). NFκB directly regulates the transcription of multiple genes, including those encoding cytokines and other mediators of immunity, regulators of redox status, cachexia and disuse atrophy, and sarcopenia (42). We now show significantly higher protein concentrations of the NFκB subunit p65 and its phosphorylation in older skeletal muscle in both total and nuclear fractions (Fig. 4, A–D). This is in agreement with Buford et al. (8) who reported higher activation of NFκB in older, sedentary individuals. However, the higher activation of NFκB in this study did not translate into higher concentrations of IL1β or IL6 (Fig. 5, C and D). These results are not unexpected since it has been previously reported that transgenic mice possessing constitutively active IkB kinase β (MIKK), thus allowing the liberation of NFkB, did not have increased cytokine production (9). This was despite a 15-fold higher NFκB activation and profound sarcopenia exhibited in this model. In agreement with the NFκB results, we also observed a significant increase in IκB phosphorylation in OLD at all time points (Fig. 5B). We further observed no difference in circulating serum cytokines with age or after resistance. Although we cannot totally discount the role of cytokines in the activation of NFκB signaling, it has been previously reported that ceramides can activate NFκB independently (17). Taken together these data show that the negative association of C16:0-ceramide concentrations with lower leg lean mass may be mediated by the effect of ceramides on NFκB signaling.

Protein phosphatases are signal transduction enzymes that dephosphorylate cellular phosphoproteins (47). Altered expression and activation levels of protein phosphatases have been observed in models of obesity, insulin resistance, diabetes, and increased lipid concentration (e.g., ceramide, free fatty acids, etc.) and are believed to have a role in decreased Akt and S6K1

![Image of Western blot analysis](https://example.com/image.png)

Fig. 7. Phosphorylation and total protein content of anabolic and catabolic regulators at BL and after RE in YNG and OLD. Relative protein levels of phospho- and total Akt (A), phospho- and total FOXO1 (B), phospho- and total mammalian target of rapamycin (mTOR) (C), and phospho- and total S6K1 (D) were quantified using Western blot analysis in skeletal muscle. Significant differences between groups (*P < 0.05 vs. BL, #P < 0.05 vs. YNG, ^P < 0.05 vs. OLD 0H, 6H; n = 8–10/group).
activation (35, 51, 56). However, we observed no differences in the concentration of the protein phosphatases PP2A (Fig. 6A) or PP2C (Fig. 6B) between groups or after exercise. This is likely a result of the concentration of the phosphatases not being as important as their activation which has been reported previously (11). Further work should be attempted to determine the role in the activation of the protein phosphatases during aging.

In healthy, young humans high-intensity resistance exercise is a robust activator of anabolic pathways such as Akt/mTOR (10, 18). In contrast, previous studies have shown a significant attenuation of anabolic stimulation with aging (21, 26, 31). We now report a significant stepwise increase in the phosphorylation of Akt on Ser473 (Fig. 7A) and S6K1 on Thr189 (Fig. 7D) in young at 0H and 6H after acute exercise; however, this response is attenuated in older males (Fig. 7, A and D). This is in line with previous studies that have observed a blunted effect of anabolic signaling after an acute bout of resistance exercise in elderly humans (26). The Akt/mTOR pathway plays a central role in the regulation of translation initiation and subsequent increase in muscle hypertrophy. In agreement with the present study, there is a known inability to activate this pathway after resistance exercise in aging skeletal muscle. A limitation of this study is that we only had the ability to measure signaling in a limited number of time points and have extrapolated these signaling measures with changes in skeletal muscle protein synthesis. Future studies are needed to determine fractional synthetic rates using isotopic tracers, a more robust measure of protein synthesis, to compare older and younger humans after exercise.

One potential mechanism for the inhibition of anabolic signaling is an increase in ceramide accumulation. Ceramide has been shown to inhibit both Akt and S6K1 activation in vitro (39) and the inhibition of its synthesis in vivo has been revealed to reverse these effects (37). Of interest, Ferreira et al. (23) recently observed that increasing ceramide concentrations with a sphingomyelinase enzyme repressed skeletal muscle contractile force in vitro. We now show that decreases in contraction-induced anabolic signaling in older humans may be associated with increased ceramide content in skeletal muscle.

The Akt target and catabolic regulator FOXO1 has a recognized role in the transcriptional control of ubiquitin ligases (29). We demonstrate for the first time that the phosphorylation and subsequent inhibition of FOXO1 on its Thr24 site is significantly increased after resistance exercise at 0H and 6H in young but remains unchanged in aged males (Fig. 7B). This is important because FOXO1 has an essential function regulating components of the ubiquitin proteasomal pathway such as muscle-RING-finger protein 1 (MuRF1) and Atrogin1/MAFbx (29, 48, 71). These muscle-specific (E3) ubiquitin ligases are responsible for the majority of intracellular protein degradation associated with muscle atrophy (24). Recently it has been shown that elevated gene expression of these two E3 ligases is highly associated with aging and multiple atrophy models, supporting the possibility of a common proteolytic program (2, 24). Raue et al. (57) recently reported a divergence in the regulation of ubiquitin proteasome-related genes (MuRF and MAFbx) at rest and after resistance exercise in younger and older humans. Therefore, the impairment of aging skeletal muscle to inhibit FOXO1 and the E3 ligases after resistance exercise may explain the decreased ability of skeletal muscle to hypertrophy in older humans.

In summary, the present results demonstrate that anabolic signaling after an acute bout of resistance exercise is blunted in aging skeletal muscle. This appears to be associated with an increased concentration of specific ceramide subspecies and the subsequent activation of the NFκB kinase. We were able to quantify nine different ceramide metabolites and observed three saturated fatty acid subspecies higher in older males. We conclude that ceramide may play a significant role in the attenuation of contractile-induced skeletal muscle adaptations and atrophy that is observed with aging.

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DISCLAIMER
Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

DISCLOSURES
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AUTHOR CONTRIBUTIONS

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