Eccentric contraction-induced myofiber growth in tumor-bearing mice

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Hardee JP, Mangum JE, Gao S, Sato S, Hetzler KL, Puppa MJ, Fix DK, Carson JA. Eccentric contraction-induced myofiber growth in tumor-bearing mice. J Appl Physiol 120: 29–37, 2016. First published October 22, 2015; doi:10.1152/japplphysiol.00416.2015.—Cancer cachexia is characterized by the progressive loss of skeletal muscle mass. While mouse skeletal muscle’s response to an acute bout of stimulated low-frequency concentric muscle contractions is disrupted by cachexia, gaps remain in our understanding of cachexia’s effects on eccentric contraction-induced muscle growth. The purpose of this study was to determine whether repeated bouts of stimulated high-frequency eccentric muscle contractions [high-frequency electrical muscle stimulation (HFES)] could stimulate myofiber growth during cancer cachexia progression, and whether this training disrupted muscle signaling associated with wasting. Male ApcMin/+ mice initiating cachexia (N = 9) performed seven bouts of HFES-induced eccentric contractions of the left tibialis anterior muscle over 2 wk. The right tibialis anterior served as the control, and mice were killed 48 h after the last stimulation. Age-matched C57BL/6 mice (N = 9) served as wild-type controls. ApcMin/+ mice lost body weight, muscle mass, and type IIA, IIX, and IIB myofiber cross-sectional area. HFES increased myofiber cross-sectional area of all fiber types, regardless of cachexia. Cachexia increased muscle noncontractile tissue, which was attenuated by HFES. Cachexia decreased the percentage of high succinate dehydrogenase activity myofibers, which was increased by HFES, regardless of cachexia. While cachexia activated AMPK, STAT3, and ERK1/2 signaling, HFES decreased AMPK phosphorylation, independent of the suppression of STAT3. These results demonstrate that cachectic skeletal muscle can initiate a growth response to repeated eccentric muscle contractions, despite the presence of a systemic cachectic environment.

Cancer cachexia; high-frequency electrical stimulation; muscle inflammation; myofiber growth; eccentric contractions

CANCER CACHEXIA IS A WASTING syndrome, characterized by the loss of skeletal muscle (53), and contributes to increased patient morbidity and mortality (55). Although many pharmacological therapeutics for treating cancer-induced muscle wasting have been proposed, and some tested in clinical trials, most have failed due to their singular specificity and adverse side effects (13). Exercise is a potential nonpharmacological treatment that improves indices of health related to muscle and systemic function in healthy individuals and also those with chronic disease (26, 63). While exercise has been widely discussed for therapeutic use in cancer patients, the mechanistic effect of exercise on wasting processes is currently being determined (18). Additionally, there are gaps in our understanding of how the cancer environment alters skeletal muscle’s response to contraction, and the mechanistic basis of these effects are being examined in mouse models of cancer cachexia.

Treadmill exercise training in ApcMin/+ mice, a model of cancer cachexia, can prevent interleukin-6 (IL-6)-induced muscle mass loss (49, 59). Interestingly, these exercise-induced improvements in muscle mass and metabolic function do not require a reduction in muscle inflammatory signaling (49). Others have reported treadmill exercise, alone or in combination with nutritional provision, can reduce tumor growth and improve muscle mass in tumor-bearing rodents (14, 45, 50). Exercise feasibility is an issue with more severe cachexia, and cachectic mice are not capable of performing vigorous treadmill exercise training. While not entirely exercise, skeletal muscle’s metabolic and growth response to contraction has been examined through the use of electrical muscle stimulation (4, 40, 48, 60). Low-frequency electrical muscle stimulation (LFES) examines acute and training adaptations to endurance-like muscle contractions (40, 44, 48). Cancer cachexia can disrupt acute LFES concentric contraction-induced metabolic signaling (48). In contrast, eccentric contractions induced by high-frequency electrical muscle stimulation (HFES) examine muscle signaling associated with hypertrophy (4, 12, 40, 60). Related to cancer cachexia, repeated bouts of eccentric contraction started at the time of C26 tumor implantation can prevent muscle extensor digitorum longus (EDL) muscle protein loss (2). The effect of cancer cachexia on eccentric contraction-induced growth signaling is not known. Additionally, it remains to be determined whether repeated eccentric contractions can induce myofiber hypertrophy after cachexia has been initiated and the cachectic environment is present. It is well established that muscle signaling pathways related to inflammation, energy status, and proteolysis are disrupted with cachexia progression and have regulatory roles in the wasting process (16, 41). Many of these same pathways can be regulated by both acute and repeated bouts of exercise (15). For example, IL-6 and subsequent muscle signal transducer and activator of transcription 3 (STAT3) signaling through the gp130 receptor is elevated during the progression of cachexia (5, 9, 10, 56), and either STAT3 inhibition (9) or gp130 loss of function (10) can attenuate muscle wasting in some mouse models of cachexia (47). Muscle IL-6 mRNA expression is transiently induced by acute exercise (28), and treadmill exercise disrupts IL-6-induced regulation of muscle mass loss in ApcMin/+ mice (49, 59). During the progression of cancer cachexia, muscle STAT3 signaling through the gp130 receptor can regulate the cellular energy-sensing enzyme 5′-adenosine monophosphate-activated protein kinase (AMPK), which is chronically activated in severely cachetic muscle (56). AMPK activation can regulate muscle protein turnover through mammalian (or the mechanistic) target of rapamycin (mTOR) inhibition (8), increased muscle-specific ubiquitin ligase expression (54), and...
autophagy processes (29). While AMPK activation can also promote mitochondrial biogenesis, chronic AMPK activation accompanies reduced mitochondrial biogenesis and content in severe cachexia (49, 56, 59). Additionally, improved metabolic function with treadmill exercise training during the initiation of cancer cachexia was accompanied by reduced AMPK activation (49). Related to muscle hypertrophy, AMPK activation can attenuate overload-induced muscle growth (39) and eccentric contraction-induced mTOR signaling (52). While multiple bouts of eccentric muscle contractions can increase muscle mass and protein content in tumor-bearing mice (2), whether repeated bouts of HFES can result in muscle hypertrophy in a microenvironment that possesses chronically elevated STAT3 and AMPK signaling is not known. Moreover, the effect of HFES-induced eccentric muscle contractions on myofiber hypertrophy in healthy or cachectic mice has yet to be established.

While initial evidence suggests repeated eccentric contractions can prevent EDL muscle protein loss in tumor-bearing mice (2), further work is needed to determine whether the development of cancer cachexia impacts muscle morphology and signaling related to myofiber growth normally induced by eccentric contractions. To our knowledge, whether cachectic muscle can initiate a growth response to repeated eccentric contractions in different fiber types after the initiation of cachexia is not known. The purpose of this study was to determine whether repeated bouts of stimulated high-frequency eccentric muscle contraction could stimulate myofiber growth during the progression of cachexia, and whether this training disrupted muscle signaling associated with the regulation of wasting processes. In addition, we determined the effect of eccentric contractions on morphological indices of muscle remodeling and oxidative metabolism during the progression of cachexia. It was hypothesized that eccentric muscle contractions would stimulate myofiber growth in all fiber types during the progression of cachexia. To test this hypothesis, ApcMin/+ mice that had initiated cachexia performed seven bouts of HFES, which elicit eccentric muscle contractions of the tibialis anterior (TA) muscle. Muscle morphology related to myofiber growth and muscle remodeling was examined in the TA muscle. In addition, muscle cachectic signaling pathways associated with wasting were examined. The results demonstrate that cachectic muscle can initiate myofiber growth in response to repeated bouts of eccentric muscle contraction, despite the presence of the cachectic environment.

MATERIALS AND METHODS

Animals. The ApcMin/+ mouse is a genetic model of colorectal cancer and cachexia (5, 36). These mice harbor a heterozygous mutation in the adenomatous polyposis coli (Apc) gene, which promotes the development of intestinal tumors, beginning as early as 4 wk of age (38). Mice develop an IL-6-dependent cancer cachexia phenotype between 3 and 6 mo of age (5, 36). Due to the slow onset and progression of body weight loss, this model is advantageous as treatments can be started after the initiation of cancer cachexia. Male ApcMin/+ mice on a C57BL/6 background were originally purchased from Jackson Laboratories (Bar Harbor, ME) and bred at the University of South Carolina’s Animal Resource Facility. All mice used in the present study were obtained from the investigator’s breeding colony within the Center for Colon Cancer Research Mouse Core. Mice were individually housed, kept on a 12:12-h light-dark cycle, and had access to standard rodent chow (no. 8604 Rodent Diet; Harlan Teklad, Madison, WI) and water ad libitum. Body weight measurements were taken weekly, and the percentage of body weight loss from peak body weight (~10-14 wk of age) was calculated. Mice lacking the ApcMin/+ mutation (C57BL/6) served as controls for all experiments. The University of South Carolina’s Institutional Animal Care and Use Committee approved all animal experimentation in this study.

HFES. At 16 wk of age C57BL/6 and ApcMin/+ mice (N = 9/group) were subjected to multiple bouts of HFES over a period of 2 wk (Fig. 1A). HFES of the left hindlimb was performed as previously described, with slight modifications (4). During each stimulation procedure, mice were anesthetized via isoflurane (2% in O2 with 1.5% maintenance), the left leg/hip region was shaved, and two needle electrodes were placed on the left leg subcutaneously posterior to the femur to stimulate the sciatic nerve. Tetanic muscle contractions were generated using a Grass Stimulator (Grass Instruments, Quincy, MA) for 10 sets of 6 repetitions (100 Hz, 6–12 V, 1-ms duration, 3-s repetition duration). Ten seconds of rest were given between repetitions, and 50 s of rest were given between sets. The stimulation protocol recruits all motor units of the hindlimb. The maximal force production of the plantar flexors (gastrocnemius, soleus, and plantaris) are greater than the dorsiflexors (TA and EDL) (61, 62), which results in net plantar flexion of the ankle (4). Therefore, the dorsiflexors undergo lengthening eccentric muscle contractions against the shortening concentric plantar flexors. Each session lasted ~22 min in duration. Following each stimulation procedure, mice were given an intraarterial injection of warm saline and returned to cages upon complete recovery. Mice performed a total of seven stimulation sessions, with each stimulation separated by 48 h. Mice were killed 48 h after the last stimulation procedure to minimize the effects of acute muscle contraction and were fasted 5 h before death but had free access to water ad libitum.

Tissue collection. Mice were anesthetized with a subcutaneous injection of ketamine-xylazine-acepromazine cocktail (1.4 ml/kg body mass). Tissue samples were taken from the right TA muscle, and Plasma IL-6 levels were measured using a commercial ELISA kit (Invitrogen, Carlsbad, CA).

Fig. 1. Effects of multiple bouts of high-frequency electrical stimulation (HFES) on circulating interleukin (IL)-6 in C57BL/6 and ApcMin/+ mice. A: experimental design. At 16 wk of age, male C57BL/6 and ApcMin/+ mice performed multiple bouts of HFES. Each stimulation procedure was separated by 48 h, and mice were killed 48 h after the last stimulation. Mice were fasted 5 h before death, B: plasma IL-6 levels pre- and posttraining. Values are means ± SE. Statistical significance was set at P < 0.05: difference between *C57BL/6 and ApcMin/+ mice, and ‡pre- and posttraining. ND, not detectable.
samples were stored at −80°C until further analysis.

**TA morphology.** Transverse muscle sections (∼10 µm) were cut from the midbelly of the TA on a cryostat at −20°C and stored at −80°C until further analysis. Hematoxylin and eosin (H&E) staining was performed to examine muscle morphology, as previously described (7, 34, 36). H&E-stained muscle sections were digitized and analyzed using ImageJ imaging software (National Institutes of Health (NIH), Bethesda, MD). Deep and superficial regions of the TA muscle were included in all analysis performed (46). Therefore, at least 15 random non-overlapping digital images at ×25 magnification were taken from each H&E-stained muscle section per animal and were examined for centralized nuclei, as previously described (7). Centralized nuclei were defined as nuclei found equidistant from a well-defined sarcolemma and are expressed as the percentage of centralized nuclei per total number of myofibers (>900 myofibers were examined per animal). The noncontractile tissue area was examined on digital images (N = 15/animal) of H&E-stained muscle sections at ×40 magnification, as previously described (34, 36). The percentage of noncontractile tissue was determined by overlaying an 18 × 14 digital pixel grid and counting the number of pixels associated with the myofiber or extracellular matrix. Pixels that were clearly distinguishable and at least 75% in the extracellular matrix were counted. The total number of pixels associated with the extracellular matrix was divided by the total number of pixels to give the percentage noncontractile tissue. All images were coded, and analyses were performed by a researcher blinded to the treatment groups.

**Immunohistochemistry for myosin heavy chain (MHC) type IIA, IIX, and IIB.** Immunohistochemistry for myosin heavy chain (MHC) type IIA, IIX, and IIB was performed as previously described (20). Transverse muscle sections of the TA were air dried for 10 min, fixed in cold acetic acid for 1 min, and washed in PBS for 5 min. Sections were quenched in 0.3% H2O2-methanol solution for 20 min and rinsed in PBS three times for 5 min. Sections were blocked in 10% normal goat serum (Vectastain ABC kit, Vector Laboratories, Burlingame, CA) in PBS for 1 h at room temperature and then incubated overnight at 4°C with primary antibodies [mouse IgG1 monoclonal anti-type IIA MHC (clone SC-71; 1:3), mouse IgM monoclonal anti-type IIB MHC (clone BF-F3, 1:1), or mouse IgM monoclonal anti-type MHC IIX (clone 6H1, 1:1)]. All MHC antibodies were obtained from the Developmental Studies Hybridoma Bank (University of Iowa, Ames, IA). The next morning, sections were washed three times in PBS for 5 min. Secondary antibodies (biotinylated anti-mouse IgG or IgM; Vector Laboratories) were incubated with the sections for 1 h at 37°C, and sections were washed again three times for 5 min in PBS. Avidin-biotin complex (ABC) system (Vector Laboratories) was used to detect the biotinylated secondary antibody by incubating ABC solution at room temperature for 30 min. Sections were washed three times for 5 min in PBS and visualized by incubating in diaminobenzidine solution for 6 min (Vectastain DAB kit, Vector Laboratories). The sections were rinsed with distilled H2O three times, dried, and mounted by cover-slips with a mounting media. At least 10 random, non-overlapping digital images at ×40 magnification were taken, and myofiber cross-sectional area (CSA) was quantified in fibers stained positive for MHC type IIA, IIX, and IIB using imaging software (ImageJ; NIH). Images from deep and superficial regions of the TA muscle were included to exclude sampling bias due to regional fiber-type differences (46). Each fiber was traced with a handheld mouse, and the number of pixels traced was calibrated to a defined area in square micrometers. An average of 100 myofibers consisting of both deep and superficial muscle regions per animal were manually traced, which was determined to be an appropriate fiber number as there were no further changes in the standard deviation of myofiber areas observed. The analyses were performed by an investigator blinded to the treatment groups.

**Succinate dehydrogenase enzyme activity.** Succinate dehydrogenase (SDH) enzyme activity was performed as previously described to determine muscle oxidative capacity (20). Briefly, frozen cross sections were air-dried for 10 min, followed by incubation in a solution containing 0.2 M phosphate buffer (pH 7.4), 0.1 M MgCl2, 2.4 mM nitroblue tetrazolium, and 0.2 M succinic acid for 45 min at 37°C. Sections were then washed in distilled H2O for 3 min, dehydrated in 50% ethanol for 2 min, and mounted for viewing with mounting media. Digital photographs were taken from each section at ×25 magnification, and fibers were manually traced with imaging software (ImageJ; NIH). Similar to MHC analysis, whole TA muscle cross sections were examined, since fibers with high oxidative capacity are more abundant in the deep region of the muscle compared with the superficial region (46). The images were converted to 8-bit gray scale (range of gray levels 0–255) images, and an integrated optical density was created by subtracting the background intensity from each myofiber. Thresholds corresponding to high SDH enzyme activity were set manually and uniformly across all images, and myofibers were classified as having high or low SDH enzyme activities. All muscle regions were included in analysis, and the percentage of high SDH enzyme activity myofibers is expressed as the percentage per total number of myofibers. The analyses were performed by an investigator blinded to the treatment groups.

**Western blotting.** Western blot analysis was performed as previously described (24). Frozen TA muscle was homogenized in ice-cold Mueller buffer, and protein concentration was determined by the Bradford method. The nonstimulated TA muscle from the control leg of C57BL/6 and ApcMin/+ mice were run on the same gel to determine differences due to cancer cachexia. TA muscles from the nonstimulated leg and HFES leg were run on the same gel to determine the effect of HFES in ApcMin/+ mice. Crude muscle homogenates were fractionated on 6–15% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes overnight. Membranes were stained with Ponceau red to verify equal loading and transfer for each gel. Membrane blots were blocked at room temperature for 1–2 h in 5% Tris-buffered saline with 0.1% Tween-20 (TBST) milk. Primary antibodies for phospho-STAT3 (S473) and phospho-Akt (Thr308) were obtained from Cell Signaling, unless otherwise stated. Membranes were incubated with IL-6 capture antibody and allowed to incubate overnight. The next morning, the plate was blocked with assay diluent buffer and washed, and IL-6 standards and plasma samples were added in duplicate to the plate. The plate was again washed and streptavidin-horseradish peroxidase reagent was added to each well. After several washes, 3,3′,5,5′-tetramethylbenzidine substrate was added, and the reaction was developed for 20 min. The reaction was stopped with sulfuric acid, and absorbance was read in a Bio-Rad iMark plate reader (Hercules, CA) at 450 nm.
Table 1. Effect of multiple bouts of HFES on body weight and muscle mass in C57BL/6 and ApcMin/+ mice

<table>
<thead>
<tr>
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<th>C57BL/6</th>
<th>ApcMin/+</th>
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<tr>
<td>Body weight, g</td>
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<tr>
<td>Peak</td>
<td>26.0 ± 0.3</td>
<td>24.8 ± 0.4</td>
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<tr>
<td>Pretraining</td>
<td>25.8 ± 0.8</td>
<td>23.0 ± 0.6 a</td>
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<tr>
<td>Posttraining</td>
<td>25.8 ± 0.7</td>
<td>22.1 ± 0.6 abc</td>
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<tr>
<td>Body weight change, % change from peak</td>
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<tr>
<td>Pretraining</td>
<td>−1 ± 1</td>
<td>−7 ± 2 b</td>
</tr>
<tr>
<td>Posttraining</td>
<td>−1 ± 1</td>
<td>−11 ± 2 b</td>
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<tr>
<td>Tibialis anterior, mg</td>
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<tr>
<td>Control</td>
<td>44 ± 2</td>
<td>33 ± 3 d</td>
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<tr>
<td>HFES</td>
<td>47 ± 1 e</td>
<td>34 ± 2 de</td>
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<tr>
<td>Gastrocnemius, mg</td>
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<tr>
<td>Control</td>
<td>120 ± 5</td>
<td>84 ± 8 d</td>
</tr>
<tr>
<td>HFES</td>
<td>119 ± 4</td>
<td>83 ± 9 d</td>
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<tr>
<td>Tibia length, mm</td>
<td>17.1 ± 0.1</td>
<td>17.0 ± 0.1</td>
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Values are means ± SE; n, no. of mice. HFES, high-frequency electrical stimulation. Statistical significance was set at \( P < 0.05 \); significantly different from “peak body weight,” “C57BL/6,” and “pretraining body weight; main effect of “ApcMin/+” and “HFES.”

Statistical analysis. Results are reported as the means ± SE. A repeated-measures two-way ANOVA was performed to determine differences between cachexia and HFES in C57BL/6 and ApcMin/+ mice using the control leg and stimulated leg from each mouse. Post hoc analyses were performed with Student-Newman-Keuls methods when appropriate. Unpaired Student’s t-test was performed to determine differences in control leg TA protein expression between C57BL/6 and ApcMin/+ mice. Paired Student’s t-test was performed to determine differences between the control leg and stimulated leg TA protein expression within ApcMin/+ mice. The accepted level of significance was set at \( P < 0.05 \) for all analysis. Statistical analysis was performed using SigmaStat version 3.5 (Systat Software, Richmond, CA).

RESULTS

Body weight, muscle mass, and plasma IL-6 levels in C57BL/6 and ApcMin/+ mice. Male C57BL/6 and ApcMin/+ mice performed seven bouts of HFES over 2 wk (Fig. 1A). There were no differences in peak body weight between C57BL/6 and ApcMin/+ before training (Table 1). ApcMin/+ mice had initiated cachexia at the beginning of HFES and continued to lose body weight during the training period (Table 1). ApcMin/+ mice had smaller TA and gastrocnemius muscle mass (−26% and −30%, respectively) at death compared with C57BL/6 mice. HFES increased the eccentrically contracted TA muscle mass, regardless of genotype, but there was no effect on the concentrically contracted gastrocnemius muscle mass (Table 1). Plasma IL-6 was elevated before training and was further increased posttraining in ApcMin/+ mice (Fig. 1B). Plasma IL-6 was below the level of detection pre- and posttraining in C57BL/6 mice. There were no differences in tibia length, a measure of body size, between C57BL/6 and ApcMin/+ mice.

Effect of cachexia and HFES on TA MHC type IIA, IIX, and IIB myofiber CSA in C57BL/6 and ApcMin/+ mice. The CSA and size distribution of MHC type IIA, IIX, and IIB myofibers were examined in the TA muscles of C57BL/6 and ApcMin/+ mice. Cachexia decreased the mean CSA of all fiber types compared with C57BL/6 mice (Fig. 2). HFES increased the mean CSA of all fiber types, regardless of genotype (Fig. 2).

When myofiber size distribution is examined, which can demonstrate remodeling related to small and large myofibers, HFES increased the percentage of large-diameter myofibers and decreased the percentage of small-diameter myofibers in all fiber types, regardless of genotype (data not shown). These data demonstrate that all fiber types can hypertrophy after HFES, regardless of cachexia severity.

Effect of cachexia and HFES on TA muscle extracellular matrix remodeling, regeneration, and myofiber oxidative capacity in C57BL/6 and ApcMin/+ mice. TA muscle extracellular matrix remodeling and regeneration were examined in C57BL/6 and ApcMin/+ mice. While there was an increase in the percentage of noncontractile tissue in ApcMin/+ mice compared with C57BL/6 mice (Fig. 3, A and B), HFES attenuated the increase in noncontractile tissue in ApcMin/+ mice (Fig. 3, C).
A and B). The percentage of myofibers containing centralized nuclei was not different between ApcMin/+ and C57BL/6 mice (Fig. 3C). HFES did not affect the percentage of myofibers containing centralized nuclei in C57BL/6 and ApcMin/+ mice (Fig. 3C). SDH activity, an index of myofiber oxidative metabolism (20, 56), was examined in the TA muscle of C57BL/6 and ApcMin/+ mice. There was a decrease in the percentage of high SDH activity myofibers in ApcMin/+ mice compared with C57BL/6 mice (Fig. 3D). HFES increased the percentage of high SDH activity myofibers, regardless of genotype (Fig. 3D). Cachectic muscle had a lower percentage of high SDH activity myofibers and an increased extracellular matrix area, but there was not evidence for myofiber regeneration, as indicated by centrally located nuclei. Coinciding with the induction of myofiber hypertrophy, HFES suppressed extracellular matrix remodeling and induced SDH activity in cachectic muscle without inducing myofiber regeneration.

Effect of cachexia and HFES on TA muscle cachectic signaling in ApcMin/+ mice. Cachectic signaling related to muscle wasting was examined in the control TA muscle of C57BL/6 and ApcMin/+ mice. Cachexia increased control muscle STAT3 (Y705) phosphorylation ninefold, AMPK (T172) phosphorylation sixfold, ERK1/2 (T202/Y204) phosphorylation eightfold, and atrogin-1 protein expression ninefold compared with C57BL/6 mice (Fig. 4A). The phosphorylation of NF-κB p65 (S468) was highly variable in ApcMin/+ mice and did not reach statistical significance compared with C57BL/6 mice (Fig. 4A). The effect of HFES on cachectic muscle signaling was examined in ApcMin/+ mice. While NF-κB p65 (S468) phosphorylation was highly variable in the control muscle of ApcMin/+ mice, HFES decreased NF-κB p65 (S468) phosphorylation in ApcMin/+ mice (Fig. 4B). Furthermore, AMPK (T172) phosphorylation was decreased by HFES in ApcMin/+ mice (Fig. 4B). STAT3 (Y705) phosphorylation, ERK1/2 (T202/Y204) phosphorylation, and atrogin-1 protein expression were not altered by HFES in ApcMin/+ mice (Fig. 4B). In summary, while cachexia activated several signaling pathways associated with skeletal muscle wasting, repeated eccentric muscle contractions induced by HFES attenuated muscle signaling related to inflammation and energy status in cachetic muscle.

DISCUSSION

Physical activity and exercise interventions have clear therapeutic implications for treating and preventing muscle wasting associated with cancer cachexia. Whole body treadmill exercise can prevent muscle mass loss in tumor-bearing mice (14, 45, 50) and also rescue suppressed muscle oxidative metabolism at the initiation of cachexia (49, 59). However, the inability of severely cachetic mice to perform voluntary exercise has limited our understanding of how cachexia alters the muscle response to exercise. Stimulated low-frequency concentric muscle contractions have demonstrated that cachetic muscle’s metabolic response is disrupted compared with healthy muscle (48); however, the growth response to stimulated high-frequency eccentric muscle contractions is not well understood. While repeated bouts of eccentric muscle contraction performed during the duration of tumor development in

![Fig. 3. Effects of HFES on TA muscle extracellular matrix remodeling, regeneration, and myofiber oxidative capacity in C57BL/6 and ApcMin/+ mice. A: representative images of hematoxylin- and eosin-stained TA muscle cross sections. Scale bar, 50 μm. The percentage of noncontractile tissue (B), centralized nuclei (C), and high succinate dehydrogenase (SDH) enzyme activity myofibers (D) in C57BL/6 and ApcMin/+ mice is shown. All analysis was performed in the TA muscle. Values are means ± SE. Statistical significance was set at P < 0.05: †difference between all groups; ‡difference between all groups; &main effect of HFES; and #main effect of ApcMin/+ mice.]
mice can prevent muscle protein loss (2), the capacity of cancer cachexia to disrupt the muscle’s growth response to eccentric contractions has not been determined. We report the novel findings that repeated eccentric contractions after the initiation of cachexia can induce myofiber growth in the cachectic TA muscle. This growth occurred in the presence of the cancer environment that induced severe wasting in the contralateral noncontracted muscle. Collectively, these results add to the field by demonstrating that cachetic muscle has the capacity to initiate myofiber growth in response to repeated bouts of eccentric muscle contraction, despite the presence of the cachetic environment.

HFES of the sciatic nerve produces eccentric contractions of the rodent TA and EDL muscles and has been used to prevent muscle protein loss in tumor-bearing mice (2). However, cachectic skeletal muscle’s capacity for myofiber growth induced by HFES after the initiation of cachexia is not currently understood. The ApcMmi+/− mice in our present study demonstrated characteristics consistent with the initiation of cachexia at the start of HFES, and cachexia symptoms progressed during the 2-wk treatment period. While body weight was reduced and plasma IL-6 levels were elevated before the first bout of HFES, ApcMmi+/− mice continued to lose body weight, and circulating IL-6 levels continued to increase during the treatment period. There was also significant muscle wasting as myofiber CSA was decreased in all fiber types of ApcMmi+/− mice. Although fiber type has been reported to affect wasting susceptibility (32), we did not find preferential wasting due to fiber type, which is consistent with our laboratory’s previous studies in ApcMmi+/− mice (6). While functional overload-induced muscle growth involves the hypertrophy of all fiber types (17), specific fiber-type hypertrophy after HFES has not been reported in either healthy or cachetic muscle. We report that HFES induced myofiber growth in all fiber types examined in mice, regardless of cachexia. These results demonstrate that repeated bouts of HFES can induce a muscle growth response in the presence of the tumor-induced cachetic environment and appreciably extend previous observations related to overload-induced muscle growth in tumor-bearing rats (42, 43). While there is a growing body of research demonstrating that overload can induce growth in tumor-bearing rodents, there is currently an extremely limited understanding of the cachectic environment-induced signaling mechanisms that muscle contraction could counteract. Whether HFES could override ca-
cachexia-induced suppression of anabolic signaling or suppress catabolic signaling in muscle, despite the presence of the cachectic systemic environment, remains to be investigated.

The muscle’s microenvironment includes the local levels of secreted cytokines and growth factors, which regulate the activity of many cell types found in muscle (11). This regulatory mix can alter the muscle’s extracellular matrix and affect the capacity for muscle regeneration and growth (33, 51). There are currently gaps in our understanding of how cancer cachexia affects the muscle microenvironment, and whether cancer-induced alterations to extracellular matrix remodeling could impair growth-related processes. Increased muscle regeneration has been reported in human pancreatic cancer and C26 tumor-bearing mice (1, 3, 23). We report the novel finding that cachexia progression increased TA muscle noncontractile tissue, and this induction of noncontractile tissue was not associated with histological evidence of muscle degeneration and regeneration. This is in contrast to reported findings in the cachectic oxidative soleus muscle, which demonstrated muscle regeneration without extracellular matrix expansion (36). These differences between the glycolytic TA muscle and oxidative soleus muscle may represent the differential effect of muscle phenotype on the cachectic response. Interestingly, we demonstrate in the present study that the dysregulation of the extracellular matrix did not disrupt HFES-induced myofiber growth, and HFES resulted in the attenuation of noncontractile tissue expansion in cachectic skeletal muscle. Further research is needed to determine the implications of disrupted extracellular remodeling during the progression of cancer cachexia and the role of muscle contraction on extracellular matrix regulation in cachectic skeletal muscle. Unaccustomed eccentric muscle contractions promote greater muscle damage compared with concentric muscle contractions, and muscle injury and inflammatory cell infiltration have been reported in rat TA muscle following an acute bout of HFES (35). Interestingly, we did not find evidence of overt muscle damage 48 h following the last stimulation procedure in either wild-type or $Apc^{Min/+}$ mice. It is reasonable that early eccentric contraction-induced muscle damage was resolved before the end of the training study, or the initial contraction stimulus elicited protection from subsequent eccentric contraction-induced damage. Additional studies are needed to clearly establish how cachexia severity influences the susceptibility to eccentric contraction-induced muscle damage. Nonetheless, progressive eccentric exercise training can increase strength and mobility in cancer survivors (30, 31), and these same benefits can be achieved in prostate cancer survivors on androgen deprivation therapy (19). While these studies provide initial evidence for the use of eccentric muscle contractions in cancer patients, the therapeutic potential of resistance exercise to improve muscle mass and function in cachectic cancer patients remains to be determined.

AMPK is a potent regulator of skeletal muscle metabolism (27), which can be activated by cellular energy status, calcium levels, and IL-6/STAT3 signaling (21). Related to muscle wasting, AMPK has a role in the regulation of mitochondrial biogenesis, autophagy, and protein turnover (37). While several mouse models of cancer cachexia demonstrate chronically elevated AMPK activity in skeletal muscle (47, 48, 57, 58), the direct signals activating AMPK during the progression of cachexia have not been established. In the present study, cachetic $Apc^{Min/+}$ mice had elevated AMPK signaling in the nonstimulated control muscle, which is consistent with previous studies (48, 57, 58). The physiological consequences of this activation are also not fully understood. In healthy muscle, exercise can stimulate AMPK activity and subsequent induction of autophagy and mitochondrial biogenesis (22, 25). Interestingly, mitochondrial biogenesis was not associated with the induction of AMPK in cachetic muscle, where muscle oxidative metabolism was suppressed (56, 57). Consistent with prior studies, we report that the induction of muscle AMPK phosphorylation was associated with chronically elevated STAT3. The direct relationship between AMPK activation and STAT3 signaling in cachectic muscle has not been firmly established. Our laboratory has previously found that IL-6-induced STAT3 and AMPK activity coincide with mTOR suppression in vivo and in vitro (58). However, AMPK inhibition, but not STAT3 inhibition, can rescue IL-6-induced suppression of mTOR signaling in myotubes (58). Additionally, treadmill exercise at the initiation of cachexia can attenuate AMPK phosphorylation, independent of reductions in elevated muscle STAT3 signaling (49). We have extended these observations by demonstrating that repeated HFES suppressed chronic activation of AMPK, although STAT3 signaling remained significantly increased in severely cachectic skeletal muscle. While we provide initial evidence that eccentric contractions attenuated AMPK signaling in cachectic muscle, mechanistic studies are needed to determine whether these changes were associated with altered regulation of mitochondrial biogenesis, autophagy, and protein turnover. Additionally, further work is needed to determine the relationship between AMPK and STAT3 signaling for the suppression of anabolic signaling and muscle oxidative metabolism in cachectic skeletal muscle.

In summary, we demonstrate that cachetic skeletal muscle retains the anabolic plasticity to initiate growth in response to eccentric muscle contractions. We report that repeated eccentric contractions after the initiation of cachexia can induce myofiber growth, regardless of fiber type in cachectic muscle. Coinciding with myofiber growth were reduced extracellular matrix remodeling and the suppression of chronically activated AMPK signaling. These data suggest that cachetic skeletal muscle can initiate growth in response to repeated bouts of eccentric muscle contractions, despite the presence of a systemic cachectic environment. Further research is needed to determine whether the initial improvements in myofiber growth can be sustained over time with the progression of cancer cachexia. Additionally, whether exercise training enhances skeletal muscle’s response to an anabolic stimulus, such as feeding, will improve our efforts for treating the cachectic cancer patient.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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