Cachectic skeletal muscle response to a novel bout of low-frequency stimulation

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Puppa MJ, Murphy EA, Fayad R, Hand GA, Carson JA. Cachectic skeletal muscle response to a novel bout of low-frequency stimulation. J Appl Physiol 116: 1078–1087, 2014. First published March 7, 2014; doi:10.1152/japplphysiol.01270.2013.—While exercise benefits have been well documented in patients with chronic diseases, the mechanistic understanding of cachectic muscle’s response to contraction is essentially unknown. We previously demonstrated that treadmill exercise training attenuates the initiation of cancer cachexia and the development of metabolic syndrome symptoms (Puppa MJ, White JP, Velazquez KT, Baltgalvis KA, Sato S, Baynes JW, Carson JA. J Cachexia Sarcopenia Muscle 3: 117–137, 2012). However, cachectic muscle’s metabolic signaling response to a novel, acute bout of low-frequency contraction has not been determined. The purpose of this study was to determine whether severe cancer cachexia disrupts the acute contraction-induced response to low-frequency muscle contraction [low-frequency stimulation (LoFS)]. Metabolic gene expression and signaling was examined 3 h after a novel 30-min bout of contraction (10 Hz) in cachetic ApcMin+/Min and C57BL/6 (BL-6) mice. Pyrrolidine dithiocarbamate, a STAT/NF-kB inhibitor and free radical scavenger, was administered systemically to a subset of mice to determine whether this altered the muscle contraction response. Although glucose transporter-4 mRNA was decreased by cachexia, LoFS increased muscle glucose transporter-4 mRNA in both BL-6 and Min mice. LoFS also induced muscle peroxisome proliferator-activated receptor-γ and peroxisome proliferator-activated receptor-α coactivator-1 mRNA. However, in Min mice, LoFS was not able to induce muscle proliferator-activated receptor-α coactivator-1 targets nuclear respiratory factor-1 and mitochondrial transcription factor A mRNA. LoFS induced phosphorylated-S6 in BL-6 mice, but this induction was blocked by cachexia. Administration of pyrrolidine dithiocarbamate for 24 h rescued LoFS-induced phosphorylated-S6 in cachetic muscle. LoFS increased muscle phosphorylated-AMP-activated protein kinase and p38 in BL-6 and Min mice. These data demonstrate that cachexia alters the muscle metabolic response to acute LoFS, and combination therapies in concert with muscle contraction may be beneficial for improving muscle mass and function during cachexia.

cachexia; contraction; low-frequency stimulation; skeletal muscle; mitochondria

CACHEXIA, AN UNINTENTIONAL loss of 5% of body weight, including muscle and fat mass, given an underlying disease, is associated with many conditions, including human immunodeficiency virus-acquired immunodeficiency syndrome, renal failure, diabetes, chronic heart failure, and many cancers (13). The progression of the cachexia is associated with the degree of body weight loss and is positively correlated with mortality (14). Cachexia accounts for ~20% of cancer deaths and ~40% of colon cancer-related deaths (39, 41). Cachexia is commonly accompanied by a wide range of metabolic dysfunctions, including insulin resistance, hypertriglyceridemia, and metabolic syndrome (40, 42). Additionally, there is a loss of skeletal muscle oxidative capacity with the progression of cachexia (44, 47). While an approved treatment for cachexia remains elusive, the lack of therapeutic options may be related to the complexity of systemic inflammatory and metabolic alterations that contribute to the progression of wasting (10).

The ApcMin+ mouse is an established model of intestinal cancer that develops a slowly progressing cachexia, compared with many other cancer cachexia models, and provides physiological relevance to the human condition. A nonsense mutation in the Adenomatous polyposis coli (Apc) gene predisposes mice to intestinal adenomas (26). Cachexia is initiated around 14 wk of age, and the average lifespan of these mice is ~20 wk. Elevated circulating IL-6 levels are associated with the development of cachexia in ApcMin+ mice. Global knockout of IL-6 in ApcMin+ mice blocks cachexia development, and IL-6 overexpression accelerates cachexia progression in ApcMin+ mice (5). Exercise has been shown to be beneficial for attenuating the initiation and progression of cachexia in ApcMin+ mice. Treadmill exercise also attenuated cachexia-induced insulin resistance at the onset of wasting (34). With the progression of cachexia, there is an inverse relationship between voluntary wheel-running distance and cachexia development in ApcMin+ mice (6). However, regular treadmill exercise can reduce tumor growth in these mice (25) and also prevent the initiation of cachexia, even under conditions of chronically elevated IL-6 (34). The systemic administration of pyrrolidine dithiocarbamate (PDT) can attenuate cachexia in tumor-bearing mice and attenuate Lewis Lung Carcinoma serum-induced atrophy in C2C12 myotubes (29). PDT has anti-inflammatory and hydroxyl radical scavenger properties (18, 29, 37) and can block STAT3 and P65 signaling in tumor-bearing mice (33). While exercise training and inflammatory signaling inhibition are beneficial for muscle mass maintenance in the cachectic environment, the additive potential of these therapies has not been investigated.

An acute bout of exercise alters skeletal muscle signaling and has been shown to benefit patients with many chronic diseases (15). Contraction can regulate several signaling cascades, including fatty acid oxidation (43), glucose transport (17), mitochondrial biogenesis (7, 19), and protein synthesis (28). Many exercise responses in muscle have been related to AMP-activated protein kinase (AMPK) activation; however, chronically elevated AMPK activation, as seen during the
progression of cachexia in the Apc<sup>Min/+</sup> mouse, can suppress protein synthesis (46). After an acute bout of exercise, peroxisome proliferator-activated receptor-α coactivator-1 (PGC-1α) is rapidly increased, leading to a subsequent induction of mitochondrial-associated gene transcription and mitochondrial biogenesis (3, 32). This increased gene expression can persist for up to 4 h before returning to baseline levels (31). Additionally, S6-kinase, a target of mammalian target of rapamycin (mTOR) signaling, is suppressed in cachetic skeletal muscle (45) and has been shown to be induced 3 h after a bout of low-frequency contraction in rodent skeletal muscle (28). However, it is not known if severely cachetic skeletal muscle maintains the capacity to respond to acute contraction.

The progression of cancer cachexia disrupts skeletal muscle oxidative metabolism (44, 47). Our laboratory has previously demonstrated that treadmill exercise training attenuates the initiation of cancer cachexia-induced muscle and body weight loss (34). Low-frequency electrical stimulation (LoFS) has been shown to alter local metabolic signaling pathways in vivo, without altering the systemic environment as with whole body exercise (28). However, the metabolic signaling response to a novel, acute bout of low-frequency contraction in a muscle that is already cachetic is unknown. Muscle contraction induces several signaling pathways that are suppressed with the progression of cancer cachexia and are known metabolic regulators, such as PGC-1α, and ribosomal protein S6 (3, 28, 45, 47). Therefore, the purpose of this study was to determine whether severe cancer cachexia disrupts the acute contraction response induced by low-frequency muscle contraction. We hypothesized that an acute bout of low-frequency contraction would stimulate metabolic signaling, regulating mitochondrial biogenesis in cachetic skeletal muscle. To test this hypothesis, Apc<sup>Min/+</sup> mice were monitored until they had developed sustained weight loss. Mice then underwent an acute 30-min bout of LoFS in which one leg was stimulated and the other served as an internal control. Hindlimb muscles were harvested 3 h after the completion of the contraction, and changes in mRNA expression levels and protein expression were measured in both C57BL/6 and Apc<sup>Min/+</sup> mouse muscle. Due to PDTC’s inhibition of several cachexia-associated signaling pathways, an additional group of Apc<sup>Min/+</sup> mice received the systemic PDTC administration 24 h before contraction to determine whether this would improve the contraction-induced metabolic response of cachetic muscle.

**MATERIALS AND METHODS**

**Animals.** C57BL/6 (BL-6) and Apc<sup>Min/+</sup> (Min) mice were originally purchased from Jackson Laboratories. Mice were bred at the Animal Resource Facility at the University of South Carolina and genotyped for heterozygosity of the Apc gene. All animals were kept on a 12:12-h light-dark cycle. Animals had ad libitum access to food and water during the course of the study. All animals were fasted for 5 h before death. At 18–20 wk of age, male mice underwent an acute contraction stimulus. A subset of 18- to 20-wk-old Min mice received PDTC, 10 mg/kg body wt, a STAT3/NF-κB inhibitor, 24 h before acute LoFS. Three hours after the completion of the contraction protocol, animals were anesthetized with a ketamine-xylazine-acepromazine cocktail, and tissues were removed, weighed, and frozen in liquid nitrogen. Tissues were stored at −80°C until further analysis. All animal experimentation was approved by the University of South Carolina’s Institutional Animal Care and Use Committee.

**Grip strength.** Combined hindlimb and forelimb rodent grip strength was measured before LoFS with the Grip Tester (Columbus Instruments, Columbus, OH). Mice were placed with all four limbs on a metal grid mounted at a 45° angle connected to a force transducer. Mice were pulled by the tail until they let go of the grid, and the force was recorded. Each mouse went through a series of two sets of five repetitions of force measurements, with a 2- to 3-min rest period between each set.

**Cage activity monitoring.** Two nights before stimulation, mice were single housed and placed in activity monitor cages (Opto-M3 Activity Meter, Columbus Instruments, Columbus, OH). Activity was measured for 12 h during the dark cycle (7 PM to 7 AM); the number of beams crossed in an X–Y plane was recorded for two consecutive nights. Food consumption was also recorded during this time, given that the mice were single housed.

**LoFS.** LoFS was conducted as described by Nader and Esser (28) with slight modifications. Briefly, all animals were fasted for 5 h before stimulation, and food was restricted for the remainder of the study. Animals were anesthetized with isoflurane in a chamber at 2–5% and remained anesthetized for the procedure via a nose cone that was connected to the isoflurane-oxygen. Animals were placed on a heat pad, and the left hindlimb was shaved free of hair and cleaned with alcohol followed by betadine. Electrodes were placed on both sides of the peroneal nerve and stimulated via subcutaneous needle. Proper electrode position was confirmed by observing plantar flexion at the ankle joint. This protocol elicited an overall effect of plantar flexion, resulting in tapping of the foot. The voltage was applied by a Grass S88 stimulator (Grass Technologies). Stimulation was delivered at a frequency of 10 Hz, 5 V, 10-ms duration, 90-ms delay, for a total time of 30 min. During the recovery period, animals remained on a heat pad. The right leg served as the control for each animal.

**Western blot analysis.** Western blot analysis was performed as previously described (34). Briefly, gastrocnemius muscle was homogenized, and protein concentration was determined by the Bradford method (8). Homogenates were fractionated on 8–15% SDS-polyacrylamide gels and transferred overnight to polyvinylidene difluoride membrane. Primary antibodies for phosphorylated (P)-S6, S6, P-AMPK, AMPK, mitochondrial transcription factor A (TFAM), P-P65, P-P65, STAT3, P65, STAT3 (Cell Signaling), and PCG-1α (Santa Cruz) were incubated 1:1,000 to 1:2,000 for 1 h at room temperature. Secondary antibodies were used at a concentration of 1:2,000 to 1:5,000. Enhanced chemiluminescence was used to visualize the antibody-antigen interactions and was developed by autoradiography. Blots were analyzed by measuring the integrated optical density of each band using ImageJ software. All Western blots were normalized to BL-6 controls run on the same gel, unless otherwise noted.

**RNA isolation/PCR.** RNA isolation, CDNA synthesis, and real-time PCR were performed as previously described (46) using reagents from Applied Biosystems. Glucose transporter-4 (GLUT-4), PGC-1α, PGC-1β, peroxisome proliferator-activated receptor-γ (PPAR-γ), nuclear respiratory factor (NRF)-1/2, TFAM, cytochrome b, and GAPDH primers were purchased from IDT. Real-time PCR analysis was conducted using an ABI 7300 Sequence Detection System. Data were analyzed using the cycle threshold. All gene expression data were normalized to GAPDH.

**Statistical analysis.** All data are represented as means ± SE. A Student t-test was used to determine systemic and baseline differences between BL-6 and Min mice and also between Min and PDTC-treated mice. A repeated-measures two-way ANOVA (LoFS and genotype) was used to determine the effects of contraction and cachexia. To determine the effects of PDTC on muscle mRNA expression, a repeated-measures two-way ANOVA was used (LoFS and PDTC). An intra-animal comparison of muscles from control and contracted legs from the same mouse was used as the repeated measure. Bonferroni post hoc analysis was used to examine interactions. Significance was set at P ≤ 0.05.
RESULTS

Cachexia in Min mice. Min mice lose body weight, skeletal muscle, and fat mass as cachexia progresses. As with our previous studies, these Min mice had a 13.8% loss in body weight, while BL-6 mice did not demonstrate any weight loss. Body weight loss corresponded with a 35% decrease in gastrocnemius muscle mass and a 93% decrease in epididymal fat mass compared with BL-6 mice (Table 1). Body weight loss was not associated with alterations in food intake measured during the week before death (BL-6: 1.8 ± 0.2 g/day; Min: 1.6 ± 0.4 g/day).

Human cancer patients exhibit decreased physical activity and increased fatigue with cachexia (23, 27). We have previously reported that Min mice also demonstrate decrements in functional status and physical activity with the initiation and progression of cachexia (6). We extend these observations to demonstrate that Min mice have dramatically reduced cage activity with severe cachexia. There was a significant reduction in XY plane cage activity (Fig. 1A) and a decrease in rearing activity (Fig. 1B) during the active dark cycle. Volitional grip strength was decreased 27% in severely cachectic Min mice; however, the decrease in force may be attributed to an overall loss in body mass, as no differences were seen when values are normalized with body weight (Table 2). These data demonstrate that, with severe cachexia, the loss in body mass is accompanied by a dramatic decrease in activity level and voluntary force production; however, the capacity to contract skeletal muscle remains intact.

LoFS regulation of metabolic genes in cachectic mice. To examine if cachexia altered the capacity of LoFS to induce muscle expression of metabolic genes, mRNA expression was examined 3 h after a novel bout of contraction (Fig. 2). Contraction induced a threefold increase in GLUT-4 mRNA levels in BL-6 mice (Fig. 2A). Similarly, contraction induced a 4.7-fold increase in GLUT-4 mRNA in cachectic skeletal muscle, despite having basal expression suppressed by cachexia (Fig. 2A). PPAR-γ, a regulator of lipid metabolism, was increased threefold by contraction in BL-6, and a similar increase was seen in cachectic muscle (Fig. 2B). Contraction did not induce PGC-1α mRNA expression in BL-6; however, it induced a sixfold increase in PGC-1α mRNA in cachetic muscle, despite suppressed basal expression (Fig. 2C). Contraction suppressed PGC-1β mRNA expression 45% in wild-type mice. Cachexia inhibited muscle PGC-1β expression 78%, and contraction did not change PGC-1β in cachetic muscle (Fig. 2D).

Table 1. Cachexia in Min mice is associated with muscle mass loss

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<tr>
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<th>BL-6</th>
<th>Min</th>
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<tr>
<td>n</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Maximum BW, g</td>
<td>28.0 ± 0.4</td>
<td>25.2 ± 0.9</td>
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<tr>
<td>BW at death, g</td>
<td>28.0 ± 0.4</td>
<td>21.7 ± 0.09*</td>
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<tr>
<td>%Change in BW</td>
<td>0.0 ± 0.0</td>
<td>−13.8 ± 2.9*</td>
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<tr>
<td>Gastrocnemius, mg</td>
<td>137.0 ± 3.0</td>
<td>88.3 ± 5.4</td>
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<tr>
<td>Epididymal fat, mg</td>
<td>468.5 ± 40.9</td>
<td>29.6 ± 12.2*</td>
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<tr>
<td>Spleen, mg</td>
<td>85.8 ± 3.0</td>
<td>437.8 ± 54.0</td>
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<tr>
<td>Tibia length, mm</td>
<td>16.9 ± 0.1</td>
<td>16.9 ± 0.1</td>
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Values are means ± SE; n, no. of mice. BL-6, C57BL/6 mice; Min, ApcMin/+ mice. There was no difference between the stimulated and nonstimulated legs 3 h postcontraction. Body weight (BW) was monitored throughout the course of the study. Mass of gastrocnemius, epididymal fat, and spleen was weighed at the time of death. *Significantly different from BL-6, P < 0.05.

Table 2. Grip strength in the Min mouse is decreased during severe cachexia

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<th>BL-6</th>
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<tr>
<td>n</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Average grip strength, N</td>
<td>2.27 ± 0.07</td>
<td>1.66 ± 0.19*</td>
</tr>
<tr>
<td>Maximum grip strength, N</td>
<td>2.80 ± 0.08</td>
<td>2.16 ± 0.09*</td>
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<tr>
<td>Average grip strength/BW, N/g</td>
<td>0.079 ± 0.002</td>
<td>0.068 ± 0.006</td>
</tr>
<tr>
<td>Maximum grip strength/BW, N/g</td>
<td>0.097 ± 0.003</td>
<td>0.090 ± 0.005</td>
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Values are means ± SE; n, no. of mice. Volitional grip strength was measured in mice before stimulation. Grip strength was normalized to BW taken at the time of testing. *Significantly different from BL-6, P < 0.05.
to NRF-1, TFAM expression was induced 90% by contraction in BL-6 mice, whereas contraction was unable to induce TFAM expression in cachectic muscle (Fig. 3B). We also measured the expression of a mitochondrial encoded gene, cytochrome b mRNA was measured in the gastrocnemius. Values are means ± SE. †Main effect genotype; ‡significantly different from all other comparisons: P < 0.05.

LoFS regulation of protein expression in cachectic mice. We examined if cachexia altered the capacity for LoFS contraction to increase signaling regulating protein synthesis. We measured the phosphorylation of S6 ribosomal protein as an indicator of protein synthesis signaling (Fig. 4). Contraction induced an eightfold increase in BL-6 muscle S6 phosphorylation; however, there was no contraction-induced phosphorylation of S6 in cachectic muscle (Fig. 4A). We next examined if cachexia altered contraction-induced expression of
mitochondrial proteins (Fig. 4). TFAM protein expression was not altered by cachexia (Fig. 4B); however, cachexia suppressed the expression of muscle cytochrome c and PGC-1α protein expression (Fig. 4, C and D). Contraction induced a 30% increase in TFAM expression in BL-6 mice (Fig. 4B), but there was no contraction-induced increase in cachectic muscle. Similarly, contraction increased cytochrome c expression 40% in BL-6 mice, but cachexia blocked the contraction-induction of cytochrome c protein (Fig. 4C). PGC-1α protein expression was suppressed by cachexia (Fig. 4D). Unlike TFAM and cytochrome c, there was a main effect of contraction to increase muscle PGC-1α protein expression in both BL-6 and cachectic muscle (Fig. 4D). These data demonstrate that cachectic muscle lacks contraction induction of mTOR signaling. Although contraction did not induce mitochondrial protein expression in cachectic muscle, it did induce protein expression of the upstream regulator, PGC-1α.

**Effect of PDTC on cachectic muscle response to LoFS.** We next examined if the acute suppression of systemic inflammation altered cachectic muscle’s response to contraction. Inflammation was suppressed by administration of PDTC, a STAT3/ NF-κB inhibitor, 24 h before contraction. The phosphorylation
of STAT3 and AMPK was elevated in cachectic muscle (Fig. 5A). There was a main effect of contraction to induce muscle STAT3 and AMPK phosphorylation in all groups, regardless of cachexia and PDTC treatment. Contraction increased p65 phosphorylation in BL-6 mice, but not cachectic mice. However, the phosphorylation of p65 was increased by cachexia. PDTC administration attenuated STAT3, P65, and AMPK phosphorylation in cachetic muscle. However, contraction-induced STAT3, P65, and AMPK phosphorylation was not altered by PDTC (Fig. 5A). The administration of PDTC increased basal levels of PGC-1α target mRNAs NRF-1, TFAM, and cytochrome b in cachetic muscle; however, con-

![Graphs and images showing phosphorylation levels and mRNA expression changes.](image-url)
traction did not increase PGC-1α target mRNAs (Fig. 5B). The inability of contraction to induce P-S6 in cachectic muscle was rescued by the PTDC treatment (Fig. 5C). Interestingly, PTDC treatment increased protein expression of TFAM and cytochrome c in cachectic muscle; however, the contraction-induced increase in S6 phosphorylation was not associated with contraction-induced increases in protein levels of cytochrome c or TFAM with PTDC (Fig. 5C). These data demonstrate that inhibition of inflammatory signaling in cachectic muscle rescues the suppression of contraction-induced protein synthesis signaling and reverses the cachexia-induced suppression of mitochondrial-associated genes.

LoFS-mediated regulation of protein synthesis signaling. Finally, we examined if the induction of AMPK, p38, and Akt phosphorylation by contraction was altered in skeletal muscle from cachectic mice. These signaling pathways are known regulators of mTOR signaling. Cachexia increased muscle AMPK phosphorylation (Fig. 6A), which has been previously reported (46). The ability for contraction to increase muscle AMPK phosphorylation was not altered by cachexia. Contraction induced an 80% increase in muscle AMPK phosphorylation in BL-6 mice and a 50% increase in cachectic mice (Fig. 6A). Neither basal nor contraction-induced muscle p38 phosphorylation was changed by cachexia (Fig. 6B). Akt phosphorylation was elevated fourfold in cachectic muscle (Fig. 6C). Increases in Akt phosphorylation with cachexia have been previously reported (45). Contraction elicited a 60% increase in Akt phosphorylation in BL-6 mice; however, contraction did not alter the phosphorylation in cachectic muscle (Fig. 6C). These data demonstrate that the interaction of cachexia and contraction differentially regulate several signaling pathways that have the potential to control the muscle’s metabolic response.

**DISCUSSION**

A current reality is that many cancer patients are not diagnosed until there has been significant muscle and body mass loss (40). Therefore, in addition to cachexia prevention, it is imperative that we understand how cachectic muscle responds to therapeutic interventions to develop viable treatment options. Muscle contraction may have therapeutic value for the treatment and management of cachexia. Several studies have suggested the use of exercise training to resolve muscle wasting in conditions of cachexia (51). While it is well established that cachexia diminishes the metabolic capacity of skeletal muscle, early therapeutic interventions could improve overall muscle mass and function.
muscle, few studies have examined if severely cachectic skeletal muscle retains the metabolic plasticity to respond to a bout of exercise. We have reported that treadmill exercise training can attenuate the initiation of cachexia in mice. Our present study extends these findings by presenting evidence that severely cachectic skeletal muscle has an altered metabolic response to a novel bout of contraction. Cachexia caused deficiencies in the contraction-induced activation of mitochondrial biogenesis and protein synthesis signaling. The contraction induction of muscle PGC-1α gene targets was also inhibited by cachexia, even though PGC-1α mRNA and protein expression was induced by contraction. Interestingly, the cachexia-induced alteration in contraction-induced gene expression and signaling was partially reversible. The administration of PDTC improved contraction-induced metabolic signaling and restored protein synthesis signaling in cachetic muscle.

Skeletal muscle contraction is widely considered advantageous for offsetting metabolic dysfunction in a variety of disease states, as it stimulates signaling pathways involved with glucose uptake, intracellular energy status, and calcium signaling (19). Activation of these signaling pathways can result in enhanced mitochondrial biogenesis and function, ultimately leading to healthier skeletal muscle. AMPK and p38 are examples of signals activated by contraction that can regulate muscle metabolism, and we report that these pathways maintain the ability to be activated in severely cachetic muscle. Although AMPK and p38 are acutely regulated by contractile activity (16), chronic activation of these pathways has been reported with muscle wasting (24, 46, 48–50). Contraction-induced activation of AMPK is associated with increased PGC-1α protein expression (2). Contraction-induced metabolic homeostasis also includes the regulation of GLUT-4 for increased muscle glucose uptake in an insulin independent manner (9, 21). We report that cachexia suppressed basal muscle GLUT-4 mRNA expression. Translocation of AMPK into the nucleus is associated with increased GLUT-4 mRNA expression, and this is enhanced under conditions of low glycogen, as seen after exercise (38). Similar to type 2 diabetes (20), we demonstrate that acute contraction maintains the ability to increase GLUT-4 mRNA expression, despite the presence of severe cachexia; however, increases in mRNA expression are not sufficient to indicate improved muscle glucose uptake. Future research is necessary to determine whether contraction-induced AMPK and GLUT-4 protein translocation is altered with cachexia.

PGC-1α is involved in the regulation of mitochondrial biogenesis. Both acute contraction and exercise training stimulate transcriptional coactivator PGC-1α expression (3). Whereas PGC-1α gene expression can be increased after acute exercise in humans, the duration and intensity of the exercise may play a role in its induction (36). We found that LoFS contraction increased PGC-1α mRNA expression in cachetic mice, whereas there was only a trend for a change in wild-type mice. However, contraction was able to increase PGC-1α protein expression independent of cachexia. The relative exercise intensity, the muscle’s intrinsic oxidative capacity, and the fatiguability of the muscle may be responsible for the large contraction induction of PGC-1α mRNA in cachetic mice. Interestingly, despite the contraction increase in PGC-1α mRNA and protein in cachetic muscle, there was not a corresponding induction of PGC-1α transcriptional targets, TFAM and NRF-1 mRNA, or mitochondrial-encoded cytochrome b mRNA. It is possible that the induction of PGC-1α expression without any changes in its target genes is related to mRNA stability; many important regulators of mitochondrial biogenesis, such as PGC-1α and TFAM, have relatively short mRNA half-lives (12). Lai et al. (22) have demonstrated accelerated mRNA turnover of PGC-1α and TFAM after chronic contraction. Recently D’Souza et al. (12) demonstrated that inherent muscle oxidative capacity could affect mRNA stability, resulting in changes in mRNA turnover. Additionally, the phosphorylation of PGC-1α by p38 has been shown to increase PGC-1 protein half-life (16). A decrease in the half-life of PGC-1α protein during cachexia could explain why we did not observe any increases in PGC-1α targets. Further work is necessary to identify why cachexia had a selective regulation of contraction-sensitive gene expression related to regulators of mitochondria biogenesis and oxidative metabolism.

The process of mRNA translation related to protein synthesis is a necessary component of muscle metabolic plasticity. Muscle protein synthesis is regulated by many stimuli, including hormonal, nutrient, mechanical, and inflammatory inputs. The regulation of skeletal muscle mitochondria and oxidative metabolism through PGC-1α and NRF-1/2 has been shown to involve protein synthesis signaling through mTOR (11, 35). Dysregulation of protein synthesis signaling has been identified in humans and in animal models during cancer cachexia (40, 42, 46, 47). Inflammation may be a potent protein synthesis suppressor, as IL-6 overexpression accelerates muscle atrophy and decreases protein synthesis signaling in Min mice and C57BL/6J myotubes (46). We demonstrate that cachexia suppresses the ability of contraction to induce protein synthesis signaling. Interestingly, cachexia did not suppress the contraction induction of PGC-1α or mechanical signaling through p38 phosphorylation, which have the potential to regulate muscle protein synthesis, and metabolic signaling. Acute suppression of systemic inflammatory signaling in Min mice by PDTC administration can rescue contraction-induced S6 phosphorylation. Inhibition of systemic IL-6, skeletal muscle gp130 signaling, and STAT3 signaling is not sufficient to rescue protein synthesis signaling (33, 45). PDTC is an inhibitor of both STAT3 and NF-κB (18, 29). NF-κB may have a role in suppression of mTOR, as IL-6 overexpression in Min mice and the progression of cachexia activate NF-κB signaling (34). Although the induction of NF-κB signaling with cachexia is commonly associated with protein catabolism, it is also induced by muscle contraction and has a role in the regulation of muscle metabolism (1, 4, 30). Further work is needed to identify the mechanisms by which inflammation may be mediating contraction-induced metabolic regulation in cachetic muscle.

To our knowledge, we are the first to report that severe cancer cachexia can alter the metabolic plasticity of skeletal muscle to an acute bout of contraction. We report that acute LoFS contraction was able to induce PGC-1α and PPAR-γ mRNA expression in cachetic muscle. However, cachexia blocked contraction-induced NRF-1 and TFAM mRNA expression, which are PGC-1α transcriptional targets. Coinciding with these findings, contraction-induced S6 phosphorylation, a target of protein synthesis signaling, was inhibited by cachexia. Inhibition of systemic inflammation related to NF-κB and STAT3 signaling was sufficient to rescue contraction-stimu-
lated S6 phosphorylation in cachectic muscle, but not TFAM or cytochrome c protein expression. These data suggest that the response of cachectic muscle to a novel, acute bout of low-frequency contraction is disrupted, and combinatorial therapeutic approaches involving exercise may prove beneficial for cachectic patients. Additionally, further research is needed to determine whether multiple bouts of exercise or contraction improve the metabolic response of cachectic muscle to an acute bout of contraction.

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GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: M.J.P. and J.A.C. conception and design of research; M.J.P. performed experiments; M.J.P. analyzed data; M.J.P. and J.A.C. interpreted results of experiments; M.J.P. prepared figures; M.J.P. and J.A.C. drafted manuscript; M.J.P., E.A.M., R.F., G.A.H., and J.A.C. edited and revised manuscript; M.J.P., E.A.M., R.F., G.A.H., and J.A.C. approved final version of manuscript.

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