Effects of omega-3- and omega-6-rich high-fat diets on skeletal muscle protein degradation signaling in glucocorticoid-treated mice

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Abstract. Omega-3 polyunsaturated fatty acids elicit beneficial effects in several muscle atrophy conditions; however, little is known about the effects of changing the ratio of omega-3s in high-fat diet feeding coupled with glucocorticoid treatment. The present study sought to determine whether a high-fat diet rich in n-3 is protective of glucocorticoid-induced protein degradation. For this purpose, male wild-type C57BL/6 mice were randomly divided into two groups as follows: n-6 (45% fat, 177.5 g lard) and n-3 (45% fat, 177.5 g Menhaden oil). Following a period of 4 weeks on their diets, the mice in the groups were divided to receive either daily injections of dexamethasone (Dex; 3 mg/kg/day) or sterile PBS, for 1 week while continuing the diets. Dex reduced gastrocnemius weight by 12% independently of diet. Protein degradation signaling was altered by Dex with an increased expression of atrogin-1, the decreased phosphorylation of forkhead box O3, the increased phosphorylation of glycogen synthase kinase-3β, and the increased expression of myostatin and the muscle RING-finger protein-1 gene in red, but not white gastrocnemius muscle. However, the negative effects of Dex were not prevented by an n-3 high-fat diet. On the whole, these data support the detrimental effects of Dex on muscle atrophy and report mild benefits of an n-3 high-fat diet.

Introduction

Skeletal muscle is involved in various metabolic functions contributing to whole-body metabolism and energy expenditure (1). Protein turnover, the balance between muscle protein synthesis and protein breakdown, reflects and determines the amount of skeletal muscle mass in an individual (2). Thus, maintaining muscle mass is crucial as an imbalance in protein turnover (e.g., muscle atrophy) is associated with increased morbidity and an increased risk of developing diseases (3). Muscle atrophy is associated with a number of chronic diseases and pathological conditions, such as obesity, prolonged fasting, cancer, sepsis, cachexia and AIDS, among others. In addition, treatment with synthetic glucocorticoids (GCs) also induces muscle atrophy (4). Exogenous GCs, such as dexamethasone (Dex) and prednisone, are used both acutely and chronically for the treatment of a variety of inflammatory and autoimmune diseases (e.g., cancer, asthma, rheumatoid arthritis, starvation, sepsis and metabolic acidosis). While GCs are effective at combatting inflammation, they do so at the expense of skeletal muscle mass by increasing the rate of protein degradation and suppressing protein synthesis (4-8). GCs have been shown to exacerbate metabolic derangements caused by a high-fat diet (HFD) including significant reductions in lean body mass (9). Therefore, treatments to prevent GC-induced muscle wasting are necessary in order to improve patient survival outcomes and quality of life.

The polyunsaturated fatty-acids (PUFAs), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), have emerged as key nutrients augmenting skeletal muscle protein turnover (10-18). PUFAs are essential nutrients involved in cell membrane structure, membrane fluidity, cell signaling, and the regulation of gene transcription and enzyme activity (3,19-21). Omega-3 PUFAs also exhibit anti-inflammatory, anti-cachectic, anti-catabolic and anabolic properties in skeletal muscle (14-16,19,22-25). While the PUFAs, EPA and DHA, promote an anti-inflammatory environment, the omega-6 PUFA, arachidonic acid (20:4n-6), exerts a pro-inflammatory response (26,27). Of recent interest is the manipulation of the n-6/n-3 ratio, relative to the total amount of PUFAs, as a regulator of metabolic and physiological functioning. The consumption of a HFD or 'Western' diet rich in saturated fatty-acids and linoleic acid (18:2n-6) PUFAs has been linked to adverse metabolic profiles, such as alterations in fatty acid composition, acid-base balance, glycemic load and nutrient metabolism (3,28,29). While the ideal ratio of n-6/n-3 is 2:1, the typical ratio in the Western diet is ~20:1 n-6/n-3; this relatively high intake of n-6 to n-3

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may exacerbate an already pro-inflammatory state in the context of disease (3,26,30).

Previous studies have demonstrated the potential protective effects of omega-3 PUFAs from the detrimental side-effects of GC treatment (31-33); however, when it comes to skeletal muscle, the results are mixed, with some studies demonstrating n-3 treatment to decrease muscle size and not to provide protection against GC-induced skeletal muscle atrophy (4,34). GCs are a widely prescribed class of drugs which are prescribed at a greater rate to obese individuals than non-obese individuals (35). Given the current Western diet and the increased rate of prescriptions to obese individuals, the present study examined the effects of GCs under the context of a HFD. To date, at least to the best of our knowledge, no studies have examined the effects of n-3s on skeletal muscle preservation during both GC use and the consumption of a HFD. The present study thus aimed to elucidate differences in GC-induced muscle atrophy when consuming a HFD rich in either n-6 or n-3 PUFAs, using C57BL/6 male mice.

Materials and methods

Animals and experimental design. All experimental and housing protocols were approved by the Institutional Animal Care and Use Committee of the University of Memphis (Memphis, TN, USA; protocol no. 0830). A total of 32 C57BL/6 male mice, 7 weeks of age, were purchased from Envigo. All animals were kept on a 12:12-h light-dark cycle at 22±2˚C 40% humidity and had *ad libitum* access to food and water during the course of the study. Following 3 days of acclimation during which the animals received a standard chow diet, the mice were randomly divided into two groups initially to receive either a HFD rich in omega-6 (n-6; 45% fat; 177.5 g lard; 35% carbohydrate; and 20% protein; n-6:n-3 PUFA, 13:1; n=16 mice), or a HFD rich in omega-3 (n-3; 45% fat; (177.5 g Menhaden oil; 35% carbohydrate; and 20% protein; n-6:n-3 PUFA, 1:3; n=16 mice) (Research Diets, Inc). After 4 weeks on their respective diets, the mice in both groups were divided, with half of the mice receiving either a subcutaneous injection of Dex (3 mg/kg body weight) (Peoples Custom Rx) or sterile PBS, while continuing their current diet throughout the 5th and last week: 45% n-6 HFD + Dex (n-6 + Dex), n=8; and 45% n-3 HFD + Dex (n-3 + Dex), n=8 (36). This dosage is similar to what has been previously used in other studies to induce muscle wasting (4,37). Of note, 1 mouse in the n-3 + Dex group was lost during the study, likely due to a more dominant mouse. The dominant mouse was removed and single-housed to prevent further aggressive behavior, as is regularly done with aggressive mice; additionally, nesting material was provided to all cages throughout the duration of the study for enrichment and to minimize aggressive behaviors (36,38). The body weight, food intake and grooming of all the mice was closely monitored daily for the duration of the experiment. Any remaining food (from previous day consumption) and body weight were measured three times a week during the first 4 weeks and daily following the Dex injections. At the end of the study period, the animals were fasted for 5 h prior to harvesting tissue. Tissue collection was completed with the mice anesthetized using 5% isoflurane inhalation and euthanized by cervical dislocation and removal of the heart.

Tissue harvesting. At the end of the study period, all animals (14 weeks of age) were fasted for 5 h prior to harvesting the tissue. Tissue collection was completed with the mouse anesthetized by isoflurane (2-5%). The mice were euthanized by cervical dislocation while anesthetized. Hindlimb skeletal muscles (soleus, plantaris, gastrocnemius, tibialis anterior, extensor digitorum longus), epididymal fat pad, heart and spleen were excised and snap-frozen in liquid nitrogen for further analysis. The right tibialis anterior was weighed and placed in 10% neutral-buffered formalin before being processed for paraffin embedding. Prior to being frozen, the gastrocnemius was divided into red and white portions, representing portions that are high in oxidative fibers and glycolytic fibers, respectively. Tibias were also removed and measured as a correction factor for body size. There was no difference in tibia lengths across all groups (data not shown).

Protein expression. Western blot analysis was performed on harvested muscle tissue to determine differences in protein expression levels. Portions of the red and white gastrocnemius muscle were homogenized in Mueller buffer. The resulting protein homogenate protein concentrations were measured using the Bradford method (39). A total of 40 μg protein homogenates were loaded onto 10% SDS-polyacrylamide gels. Gels were run to separate proteins and were then and transferred overnight to polyvinylidene difluoride membranes. Ponceau staining (RPI Research Products) was used to stain the blots for 5 min at room temperature to visually confirm that the gel transferred and to ensure equal loading. The membranes were blocked in Tris-buffered saline with 0.1% Tween-20 (TBST) and 5% milk for 1 h at room temperature. Primary antibodies for phosphorylated (p)-forkhead box O3 (FOXO3a; cat. no. Abs554, MilliporeSigma), FOXO3a (cat. no. 12829, Cell Signaling Technology, Inc.), p-glycogen synthase kinase (GSK)-3β (cat. no. 5558, Cell Signaling Technology, Inc.) and GSK-3β (cat. no. 12456, Cell Signaling Technology, Inc.) were incubated at a ratio of 1:2,000 for 24 h in 5% TBST milk at -4˚C. Secondary rabbit conjugated antibodies (cat. no. 7074, Cell Signaling Technology, Inc.) were used at a ratio of 1:5,000 and were incubated for 1-2 h in 5% TBST milk at room temperature. Enhanced chemiluminescence (Genesee Scientific Corporation) was used to visualize the antibody-antigen interactions and developed using a Chemidoc system (Bio-Rad Laboratories, Inc.). The blots were analyzed by measuring the integrated optical density of each band using ImageJ software V1.52 (National Institutes of Health). All western blots were normalized to the non-phosphorylated control.

RNA isolation and RT-qPCR. The following genes were analyzed for expression: Atrogin-1, muscle RING-finger protein-1 (MuRF-1), regulated in development and DNA damage response 1 (REDD-1) and myostatin. To isolate RNA from mouse red and white gastrocnemius muscle, tissue was homogenized in 3-5 ml RNA STAT-60 (Tel-Test Inc.). Total RNA was extracted from the STAT-60 solution by the addition of chloroform:isoamyl alcohol (24:1). The extracted RNA was dissolved in water, reprecipitated using sodium acetate and isopropanol, washed with 75% ethanol and quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific,

Table I. Primer sequences.

Gene	Forward (5'-3')	Reverse (5'-3')
Gapdh	GTTGTCTCCTGCGACTTCA	TGCTGTAGCCGTATTCA
Myostatin	ACCCATGAAAGACGGTACAAG	TCATCACAGTCAAGCCCAAAG
REDD-1	TGGTGCCCACCTTTCAGTTG	GTCAGGGACTGGCTGTAACC
MuRF-1	ACCTGCTGGTGGAAAACATC	AGGAGCAAGTAGGCACCTCA
Atrogin-1	GTTTTCAGCAGGCCAAGAAG	TTGCCAGAGAACACGCTATG

REDD-1, regulated in development and DNA damage response 1; MuRF-1, muscle RING-finger protein-1.

Inc.). For the qPCR analysis of RNA transcripts, 1 μg RNA was reverse transcribed into cDNA. cDNA was prepared using the High-Capacity cDNA Reverse Transcription kit as per the manufacturer's instructions (Applied Biosystems; Thermo Fisher Scientific, Inc. Cat# 4368813). The cDNA was mixed with forward and reverse primers for the intended gene target and PowerUp SYBR-Green master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). qPCR was performed on a QuantStudio 6 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The $2^{-\Delta\Delta Cq}$ method was used to determine changes in gene expression between treatment groups using *Gapdh* as the housekeeping gene (40). The primer sequences used are presented in Table I.

Histological analysis. Samples of the tibialis anterior were fixed in 10% neutral-buffered formalin after being excised and rinsed in PBS. Samples were fixed in formalin blocks and sectioned into 10-μm-thick sections using a microtome. Samples were deparaffinized and stained with hematoxylin and eosin (H&E) as previously described (41). The cross sectional area (CSA), a measure of fiber size, was analyzed using ImageJ software V1.52 (National Institutes of Health) as previously described (41). Individuals conducting the CSA analysis were blinded to the treatment groups. Myofibers comprising both deep and superficial muscle regions were manually traced; ~150-200 fibers were traced for each animal. This was determined to be an appropriate fiber number as there were no further changes in the standard deviation of myofiber areas observed.

Statistical analysis. All data are represented as the mean ± SEM. A two-way ANOVA was used to determine the effects of diet and dexamethasone treatment using GraphPad Prism 8 software (Dotmatics). Bonferroni post hoc analysis was used to examine the interactions. A value of P<0.05 was considered to indicate a statistically significant difference. Effect size was calculated using Cohen's D.

Results

Muscle mass and CSA. Muscle mass was measured at the time of sacrifice and the mean weights of the mice in each group for the gastrocnemius and the tibialis anterior muscles are presented in Fig. 1. Dex significantly decreased gastrocnemius weight by 12% in the mice regardless of diet (P=0.0089; Fig. 1A); however, there was no significant effect of diet or Dex

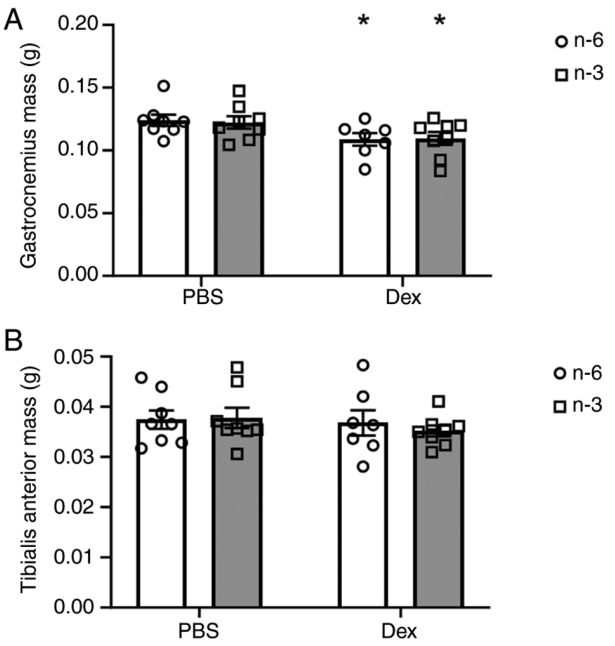

Figure 1. (A) Gastrocnemius muscle mass and (B) tibialis anterior muscle mass in mice fed either a high-fat diet rich in lard (n-6) or Menhaden oil (n-3) with Dex injection. All data are presented as the mean ± SEM. *P<0.05, significant difference vs. Dex. Dex, dexamethasone.

treatment on the mass of the tibialis anterior muscle (Fig. 1B). There was a main effect of time to increase body weight (P<0.001). At week 5, the n-6 Dex-treated mice weighed significantly more than the n-3 Dex-treated mice, despite no differences in food intake throughout the study (Table II). The increased body weight was likely due to increases in fat mass, as previously reported (32).

The CSA, a marker of fiber size, measured in the tibialis anterior muscle revealed that Dex reduced the average fiber size compared to PBS in mice fed the n-6-rich diet (P<0.0001). This reduction was attenuated in the mice treated with Dex fed the n-3-rich diet, suggesting that n-3 may offer some protection against Dex-induced muscle atrophy (Fig. 2).

Protein degradation. To determine the effects of an omega-3-rich diet on GC-induced muscle atrophy, markers of muscle atrophy were measured in both the red and white gastrocnemius muscle. The phosphorylation levels of FOXO3a, a marker of both proteasomal degradation and autophagic degradation, and GSK-3β, a factor involved in

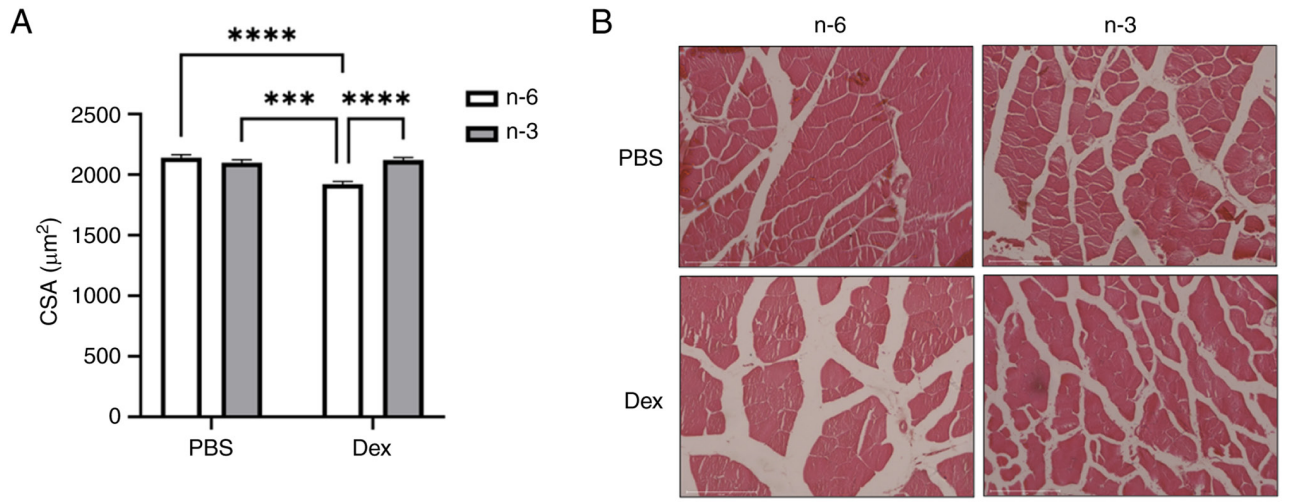

Figure 2. Tibialis anterior muscle CSA. (A) CSA was in measured in the tibialis anterior muscle of all mice; (B) representative hematoxylin and eosin-stained TA muscle. All data are presented as the mean ± SEM. ***P<0.001 and ****P<0.0001. CSA, cross sectional area; Dex, dexamethasone.

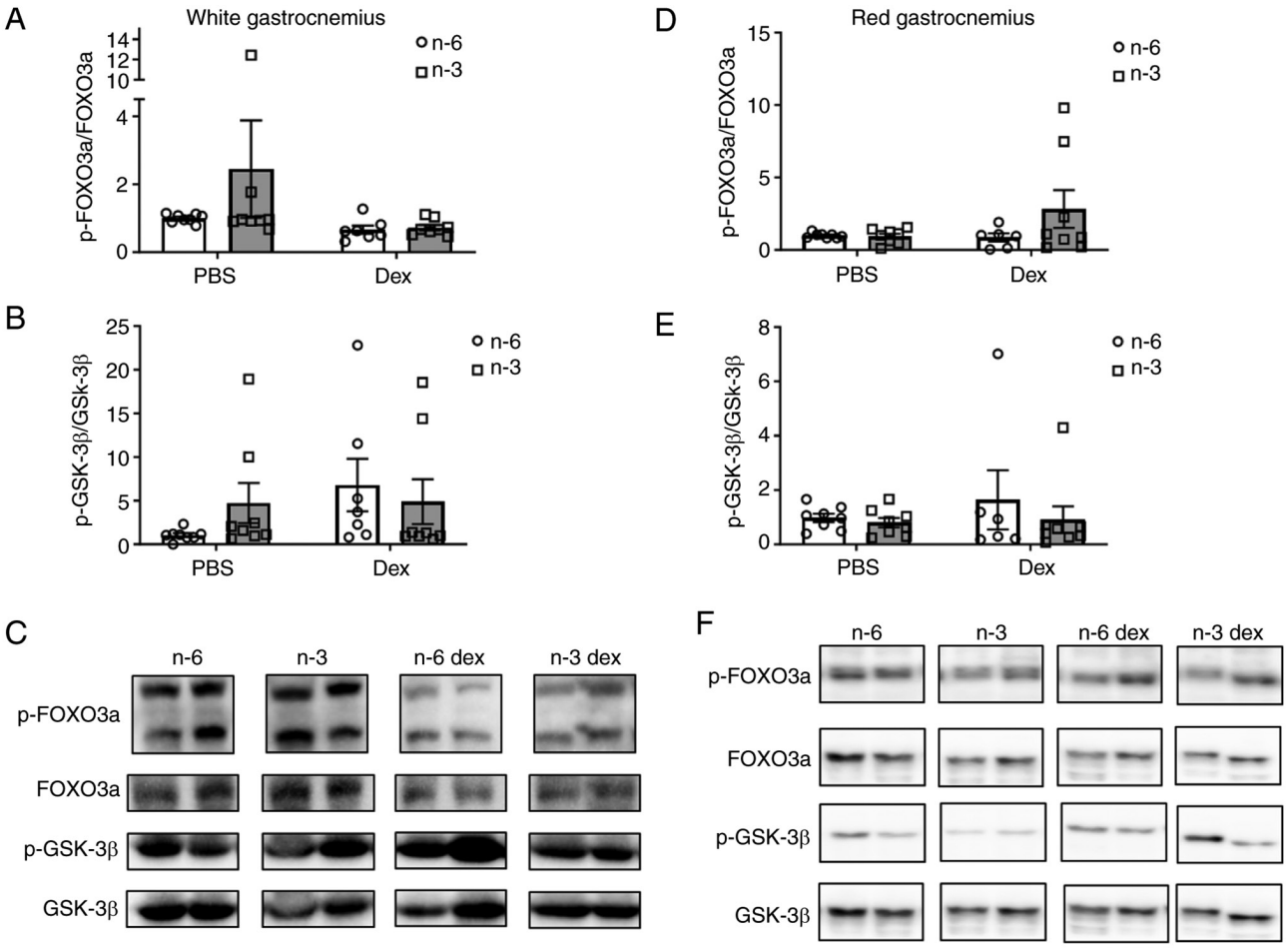

Figure 3. Protein markers of muscle atrophy in the white and red gastrocnemius muscle. Western blot analysis of (A) phosphorylated to total FOXO3a, and (B) phosphorylated to total GSK-3β. (C) Representative western blots in the white gastrocnemius. (D) Phosphorylated to total FOXO3a and (E) phosphorylated to total GSK-3β. (F) Representative western blots in the red gastrocnemius muscle in mice fed either a high-fat diet rich in lard (n-6) or Menhaden oil (n-3) with Dex injection. All data are presented as the mean ± SEM. FOXO3a, forkhead box O3; GSK-3β, glycogen synthase kinase-3β; Dex, dexamethasone.

the GC suppression of protein synthesis, were measured using western blot analysis (Fig. 3). In the white gastrocnemius, the phosphorylation of FOXO3a was not significantly altered by diet or Dex; however, there was marked effect of Dex (Cohen's d=1.42) toward a decreased phosphorylation of FOXO3a compared with the PBS group (Fig. 3A and C).

Table II. body weight and food intake.

	n-6, PBS			n-3, PBS			n-6, Dex			n-3, Dex		
Week	Body weight (g)	Food intake (g/mouse/day)	No. of mice	Body weight (g)	Food intake (g/mouse/day)	No. of mice	Body weight (g)	Food intake (g/mouse/day)	No. of mice	Body weight (g)	Food intake (g/mouse/ day)	No. of Mice
1	22.15±0.54	2.85±0.05	8	23.28±0.33	2.86±0.05	8	22.93±0.31	2.97±0.07	8	23.05±0.63	3.09±0.39	8
4	27.39±0.82	2.73±0.20	8	26.30±0.42	2.39±0.26	8	27.78±0.55	2.78±0.10	8	26.34±0.99	3.48±0.33	8
5	26.83±0.59	2.42±0.26	8	26.21±0.30	2.56±0.15	8	27.69±0.67[a]	3.02±0.52	7	25.41±0.77	3.19±0.00	8

[a]Significant difference (P<0.05) from n-3 Dex within time point. Dex, dexamethasone.

Although the phosphorylation of GSK-3β was not significantly (P=0.19) altered by diet or Dex, Dex exhibited a large effect size (Cohen's d=1.42) toward the increased phosphorylation of GSK-3β (Fig. 3B and C). In the red gastrocnemius, there was no effect of either diet or GCs to alter the phosphorylation of FOXO3a or GSK-3 β (Fig. 3D and F).

The present study then examined the gene expression of REDD-1, myostatin, MuRF-1 and atrogin-1. There was no marked difference in REDD-1 expression with diet or Dex treatment in the white gastrocnemius (Fig. 4A). The level of myostatin, a negative regulator of muscle mass, was not significantly altered by diet or Dex treatment; however, there was a notable effect of Dex (Cohen's d=2.1) towards the upregulation of myostatin expression (Fig. 4B). As markers of the proteasomal degradation pathway, the E3 ligases MuRF-1 and atrogin-1 were measured. There was no significant effect of either diet or dexamethasone (P=0.15) on MuRF-1 expression. However, Dex (Cohen's d=6.47) increased MuRF-1 expression regardless of diet in the white gastrocnemius (Fig. 4C). Atrogin-1 expression was significantly higher with Dex (P=0.0035) in the mice fed either diet when compared to the control. Additionally, within the n-6-fed group, Dex significantly increased atrogin-1 expression (P=0.04; Fig. 4D). In the red gastrocnemius, Dex decreased REDD-1 expression (Fig. 4E). Dex also increased myostatin (Fig. 4F), MuRF-1 (Fig. 4G) and atrogin-1 (Fig. 4H), regardless of diet. These data suggest that Dex induces muscle atrophy via the upregulation of the ubiquitin pathway, independently of HFD composition. Additionally, Dex may target more oxidative fibers.

Discussion

Supplementation with omega-3 PUFAs has well-known benefits on skeletal muscle protein turnover (25,42). In various models of muscle atrophy (i.e., sepsis, arthritis, starvation, cancer and cachexia), studies investigating n-3s have demonstrated that supplementation with either n-3s or altering the n-6/n-3 ratio maintains or increases protein synthesis and inhibits muscle atrophy. Exogenous GCs are administered as a drug therapy in a variety of muscle wasting and inflammatory conditions; however, chronic GC usage is linked to increased muscle atrophy. Recent studies investigating both n-3 supplementation and GC-induced muscle atrophy have found conflicting results compared to the aforementioned benefits observed in other atrophy conditions (4,34). Additionally, studies have shown that a HFD contributes to muscle wasting by altering protein synthesis and increasing markers of ubiquitination-proteasome system (UPS) degradation (43,44). Altering the n-6/n-3 ratio could alleviate the deleterious effects of consuming a high n-6 to n-3 diet. However, little is known of the effects of n-3s on GC-induced muscle atrophy while consuming a HFD. Therefore, the present study sought to induce muscle atrophy by Dex and observe the effects of an omega-3-rich vs. an omega-6-rich HFD on markers of protein degradation. It was observed that Dex administration increased muscle atrophy by significantly reducing muscle weight and upregulating several atrogenes; however, a HFD rich in n-3s did not attenuate these alterations. These findings were not surprising, as it is well-established that GC-induced atrophy is fiber-type specific, namely affecting glycolytic fibers, such as the gastrocnemius (45). Notably, in the present study, there

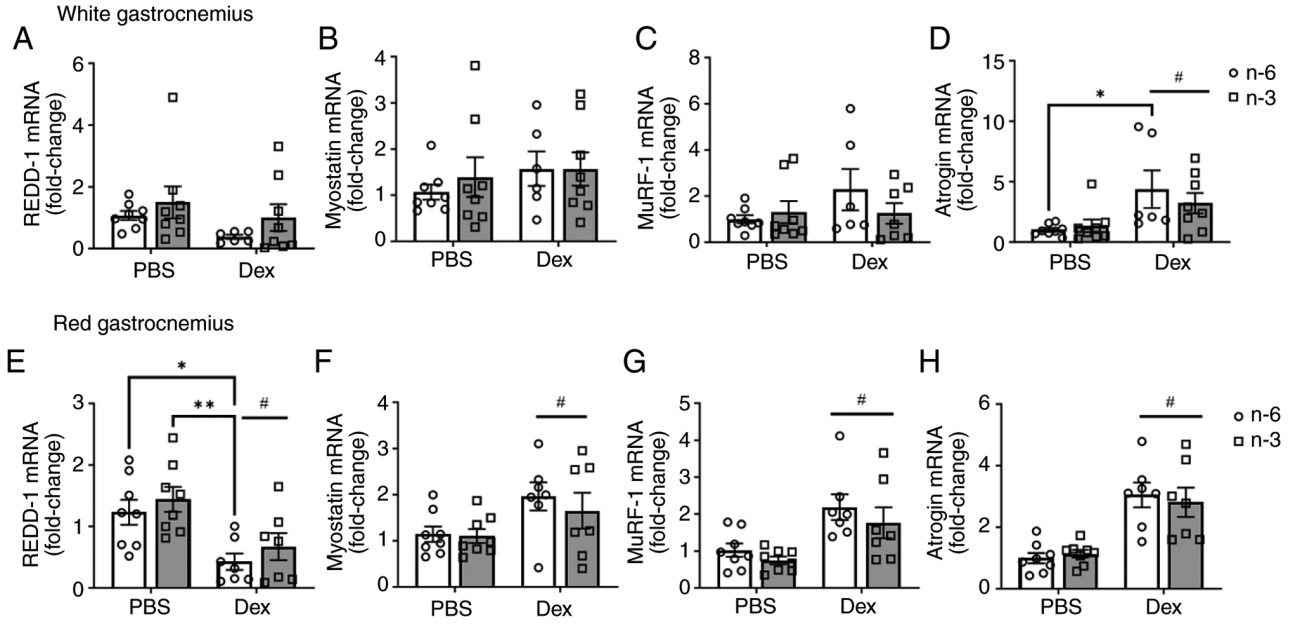

Figure 4. Transcriptional markers of protein degradation pathways. The mRNA expression of (A) REDD-1, (B) myostatin, (C) MuRF-1, and (D) atrogin in white gastrocnemius muscle and (E) REDD-1, (F) myostatin, (G) MuRF-1, and (H) atrogin in red gastrocnemius muscle was measured in mice fed either a high-fat diet rich in lard (n-6) or Menhaden oil (n-3) with Dex injection. All data are presented as the mean ± SEM. *P<0.05 and **P<0.01; $^{\#}$P<0.05, significant effect of Dex,. REDD-1, regulated in development and DNA damage response 1; MuRF-1, muscle RING-finger protein-1; Dex, dexamethasone.

was no change in muscle weight of the primarily glycolytic tibialis anterior muscle with Dex treatments, although there were minimal changes in CSA. While not fully established in mice, Bass *et al* (46) demonstrated that in humans some muscles, such as the tibialis anterior appear to be more atrophy-resistant compared to the gastrocnemius. This could suggest that a larger stimulus or a longer atrophy stimulus may be required to fully induce atrophy in the tibialis anterior compared to the gastrocnemius.

Several studies have found n-3 PUFAs to have a protective, anabolic and anti-catabolic effect on skeletal muscle mass in healthy and diseased conditions (10,14-17,47,48). By contrast, Fappi *et al* (4) investigated the effects of n-3s on GC-induced muscle atrophy and reported that while Dex decreased gastrocnemius muscle weight, concomitant n-3 supplementation did not alter these reductions. The results obtained in the present study are consistent with those of the study by Fappi *et al* (4); indeed, Dex significantly reduced gastrocnemius mass; however, a HFD rich in n-3s did not attenuate muscle loss (Fig. 2). A HFD rich in saturated fat and n-6 PUFAs has been shown to reduce muscle mass by decreasing myofibrillar proteins via the overactivation of the UPS, myonuclear apoptosis and oxidative stress (44,49). It was hypothesized that n-3 supplementation alone is not sufficient to negate the effects of both GCs and a HFD acting on the UPS and other cell signaling mechanisms regulating protein turnover. There is increasing evidence to indicate that n-3 supplementation may amplify anabolic stimulation, such as post-prandial and post-exercise, and it may help with muscle recovery (50-52). However, research on the effects of n-3 on protein synthesis is conflicting when coupling n-3 with atrophic stimuli, and may be the result of a myriad of differentially regulated signaling pathways depending on the atrophic stimulus. REDD-1, a known GC target (53,54) and negative regulator of protein synthesis, is also required for the proper functioning of mitochondrial capacity and insulin sensitivity, both of which influence protein turnover (55,56). Moreover, REDD-1 deletion has been reported to prevent Dex-induced muscle loss (57). The results of the present study suggest a homeostatic role for REDD-1 and demonstrate that Dex-induced muscle atrophy occurs independently of REDD-1 upregulation with no effect of fatty acid composition.

The UPS and autophagic systems are activated in catabolism and function synergistically to promote degradation. In any condition inducing muscle atrophy, atrogenes such as MuRF-1 and atrogin-1 are modulated by GCs, insulin/IGF-1 and myostatin-SMAD2/3 pathways, and FOXO and NF-κb transcription factors (5,37,58). Although a HFD is one factor involved in disrupting protein turnover processes, PUFAs have been shown to positively affect Akt/FOXO signaling. In some muscle wasting conditions, n-3 supplementation increases the phosphorylation of FOXO, thus decreasing atrogin-1 and MuRF-1 expression (13,18,19). By contrast, GCs induce muscle atrophy by activating FOXO to upregulate the transcription of MuRF-1 and atrogin-1. In the present study, there was an effect of Dex to decrease the phosphorylation of FOXO3a and increase myostatin, regardless of diet, increasing atrogin-1 expression. Other studies have shown n-3 supplementation to aggravate Dex-induced atrophy by further increasing the activation of atrogin-1 and MuRF-1 compared to Dex alone (4,34); however, the present study found no notable differences in mice fed a HFD high in n-3s vs. a HFD high in n-6s with concomitant dexamethasone administration; however additional markers of muscle atrophy need to be examined in the future.

To the best of our knowledge, the present study is the first to investigate the effects of a HFD rich in omega-3 vs. omega-6 on GC-induced muscle atrophy. It was demonstrated that Dex administration reduced gastrocnemius weight; however a HFD rich in n-3s did not elicit any protective effects on muscle mass

compared with a HFD rich in n-6s. There was an effect of Dex to decrease the phosphorylation of FOXO3a; however, a HFD rich in n-3s did not attenuate this. Dex administration significantly increased atrogin-1 expression, regardless of HFD composition or muscle type. There was a marked effect of Dex to increase MuRF-1 and myostatin gene expression, with no attenuation by a HFD rich in n-3s. The present study corroborates the potentially harmful effects of GCs on skeletal muscle. As GCs are used as a therapeutic treatment for a variety of inflammatory, autoimmune and cancerous conditions, any nutritional approach to negate their negative effects would be beneficial to the affected population. However, the present study demonstrates that omega-3 supplementation in a HFD does not protect skeletal muscle from the detrimental effects of GC-induced atrophy. Further studies are warranted to examine the interaction with other metabolically active tissues. It is possible that even in the context of a HFD, omega-3 PUFAs provide metabolic protection to active tissues, which could still confer some protective effects from the development of metabolic syndrome that is associated with long term treatment with GCs.

One limitation of the present study may be the use of a mouse model; generally, rats are more sensitive to GC-induced muscle atrophy compared to mice. Markedly lower doses of Dex (<0.5 mg/kg) will cause notable atrophy in a rat over a period of 7 days vs. a higher dose of 1-3 mg/kg required to observe the same effects in a mouse (45). This may be the reason why Fappi *et al* (4) demonstrated more robust effects of Dex on markers of protein degradation than was observed herein. Additionally, the sensitivity of humans to GCs needs to be examined. Another limitation of the present study is that there is no standard dosage for Dex among studies. Dex was administered at 3 mg/kg over a 7-day period; perhaps given a longer duration, greater effects may have been observed. Further studies are required to consider the effect of omega-3 supplementation in a HFD with other nutritional supplements to combat GC-induced muscle atrophy. The present study analyzed FOXO3a as a regulator involved in the activation of atrogenes, as well as the subsequent activation of genes regulated by FOXO, such as MuRF-1 and atrogin. Additional genes that are known to regulate muscle mass, such as REDD-1 and myostatin were also included, which have GC response elements. However, the authors were only able to examine these transcriptionally. There is the possibility that the genes and protein levels would differ. The inflammatory environment may play a role in a number of the pathways examined; thus, further studies are required to examine the inflammatory cytokine and fatty acid profile changes in comparison to a low-fat diet control group.

In conclusion, the present study demonstrates that the negative effects of Dex are not prevented by an n-3 high-fat diet. The data presented herein support the detrimental effects of Dex on muscle atrophy and report mild benefits of an n-3 high-fat diet.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MP and KB conceptualized the present study. KB, APe, APr, JL, DC, NW and MP were involved in data investigation and data curation. KB was involved in the writing and preparation of the original draft of the manuscript. NW and MP were involved in the writing, reviewing and editing of the manuscript. MP, KB, APe and NW were involved in visualization. All authors have read and agreed to the published version of the manuscript. MP and KB confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The animal study protocol was approved by the Institutional Review Board of The University of Memphis (Memphis, TN, USA; protocol no. 0830; date of approval: October 25, 2018).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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