OXALIPLATIN INDUCES MUSCLE LOSS AND MUSCLE-SPECIFIC MOLECULAR CHANGES IN MICE

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Accepted 3 September 2017

ABSTRACT: *Introduction:* Muscle wasting is a frequent, debilitating complication of cancer. The impact of colorectal cancer chemotherapeutic oxaliplatin on the development of muscle loss and associated molecular changes is of clinical importance. *Methods:* C57BL/6J male mice were treated with oxaliplatin. Total body weights were measured and behavioral studies performed. Hindlimb muscle weights (gastrocnemius and soleus) were recorded in conjunction with gene and protein expression analysis. *Results:* Oxaliplatin-treated mice displayed reduced weight gain and behavioral deficits. Mice treated over a shorter course had significantly increased STAT3 phosphorylation in gastrocnemius muscles. Mice receiving extended oxaliplatin treatment demonstrated reduced hindlimb muscle mass with upregulation of myopathy-associated genes *Foxo3, MAFbx*, and *Bnip3*. *Discussion:* The findings suggest that oxaliplatin treatment can directly disrupt skeletal muscle homeostasis and promote muscle loss, which may be clinically relevant in the context of targeting fatigue and weakness in cancer patients.

Muscle Nerve **57**: 650–658, 2018

As cancer survival rates improve, there is an increasing focus on the patient experience[1] and the subsequent impact of life-saving cancer treatments. Fatigue and weakness are two of the most common adverse symptoms reported by cancer patients.[2,3] In advanced cancer patients, the etiology is multifactorial with both the cancer and its treatment contributing. A prominent component is cancer cachexia, a hypercatabolic, hypermetabolic wasting syndrome characterized by the involuntary depletion of lean muscle mass and adipose tissue,[4] which is largely attributed to systemic chronic inflammation resulting from complex host–tumor interactions.[5,6] The same symptoms are reported to be of equally major concern to patients undergoing treatment in the early stages of cancer. Although cancer-related fatigue has been studied extensively in recent years, relatively few studies have considered the impact that cancer treatments independently may have on skeletal muscle homeostasis and function.

Chemotherapy is associated with numerous adverse side effects, including the development of peripheral neuropathy, nausea, diarrhea, anorexia, and muscle fatigue.[7,8] Studies have indicated that chemotherapy can actually exacerbate muscle wasting, irrespective of any antineoplastic effects on tumor burden reduction.[9,10] Importantly, this muscle wasting is thought to be independent of the potential reduction in food intake resulting from chemotherapy-associated anorexia.[4,9] Furthermore, studies have also shown that cancer patients with muscle depletion are much more susceptible to developing drug-related toxicities and have significantly lower overall survival rates.[11,12]

There is preclinical evidence that chemotherapeutic agents with varying antineoplastic mechanisms can contribute to muscle wasting. It was initially found that the administration of single chemotherapy drugs such as cisplatin, cyclophosphamide, 5-fluorouracial (5-FU), or methotrexate, could induce transient body weight loss, anorexia, and a negative nitrogen balance in both healthy and tumor-bearing rats.[13] Damrauer *et al.* reported that cisplatin, CPT-11, adriamycin, and etoposide caused muscle wasting independent of tumor growth inhibition,[9] and Barreto *et al.*[4] demonstrated that Folfox combination (5-FU, leucovorin, and oxaliplatin) contributed to muscle loss and weakness in non–tumor-bearing mice. Despite various studies, the mechanisms underlying chemotherapy-associated muscle wasting remain largely unknown.

Transcription factor STAT3 (signal transducer and activator of transcription 3) is a central regulator of muscle wasting in the acute-phase response in cancer cachexia.[14–16] Activation of STAT3, via the canonical pathway involving phosphorylation of the tyrosine-705 residue, occurs in response to signaling by numerous receptor tyrosine kinases

Additional supporting information may be found in the online version of this article

Abbreviations: 5-FU, 5-fluorouracial; FoxO, forkhead box O; MAFbx, muscle atrophy F-box; MuRF1, muscle ring finger 1; PAS, photobeam activity system; PVDF, polyvinylidene fluoride; STAT3, signal transducer and activator of transcription 3; UPS, ubiquitin–proteasome system

Key words: cancer; fatigue; muscle; oxaliplatin; survivorship
Funding: Cancer Institute NSW; Translational Program Grant 14/TPG/1-05 ("Chemotherapy-Induced Peripheral Neuropathy: Assessment Strategies, Treatments and Risk Factors").

Conflicts of Interest: The authors have no conflicts of interest to disclose.

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Published online 7 September 2017 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mus.25966

and assorted other signaling factors.[17] Upon activation, STAT3 translocates to the nucleus,[18] where it directs transcription of numerous target genes as a dimer.[19] In the context of cancer cachexia, STAT3 activation results in transcriptional upregulation of the muscle-specific E3 ubiquitin ligases MAFbx (muscle atrophy F-box)/atrogin-1 and MuRF1 (muscle ring finger 1), which are important regulators of muscle atrophy via the ubiquitin–proteasome system (UPS).[14,20,21] Furthermore, in T cells, it was shown that non-phosphorylated STAT3 localizes to the cytoplasm, where it binds and sequesters autophagy-related proteins forkhead box O (FoxO)1 and FoxO3.[22] Phosphorylation of STAT3 releases FoxO1 and FoxO3, allowing them to translocate to the nucleus and modulate target gene transcription.[22] In skeletal muscle, FoxO3 has been demonstrated to be a key regulator of atrophy via autophagy, and this process is specifically associated with increased gene expression of Bnip3.[23] Therefore, phosphorylation of STAT3 may act to regulate key genes in muscle atrophy via the UPS and autophagy signaling pathways,[24] and its involvement in chemotherapy-induced muscle wasting is of interest.

Cisplatin-induced muscle atrophy is associated with activation of the UPS and upregulation of FoxO transcription factors and autophagy genes.[7,25,26] Oxaliplatin, a third-generation platinum compound evolved from cisplatin, is routinely used in combination with other chemotherapeutic agents as a first-line treatment option for colorectal cancer.[27,28] The antineoplastic activity of oxaliplatin derives from its ability to generate platinum–DNA adducts, leading to inhibition of DNA replication, irreversible mitochondrial and cytoskeletal damage, and subsequent cellular apoptosis.[29] A recent study indicated that oxaliplatin-treated mice have reduced lean and fat tissue mass, altered muscle fiber size, calcium accumulation, collagen deposition, and reduced intramuscular protein, in association with mitotoxicity.[30] In this study, we aimed to further determine whether oxaliplatin independently causes the development of a cachexia-like response in muscle in non–tumor-bearing mice and also to identify novel contributing molecular pathways.

METHODS

Murine Models. Male 8-week old C57BL/6J mice (Australian BioResources, Moss Vale, NSW, Australia) were group-housed in sterile, well-ventilated cages at room temperature (RT), and maintained on a 12:12 hour light/dark cycle, receiving chow and water *ad libitum*. To investigate the effects of oxaliplatin treatment in non–tumor-bearing hosts, mice were administered oxaliplatin (Sigma-Aldrich, St. Louis, Missouri) or a vehicle control (0.9% saline or 5% dextrose) intraperitoneally (IP) according to predetermined dosing schedules over a short or extended regimen (see figures). Oxaliplatin was diluted in vehicle, and control mice received an equal volume of vehicle. For extended regimen studies, all mice also received 60 µl of saline IP 30 minutes before each injection and 5 days after final injection, which had no effect other than increasing hydration levels. Body weights of mice were recorded throughout the experiments. All animal experiments were conducted with approval from the animal care and ethics committee of the University of New South Wales.

Behavioral Studies. To quantify the effect of oxaliplatin on behavioral activity, open field testing was performed using a photobeam activity system (PAS; San Diego Instruments, San Diego, California) in a climate-controlled room. Mice were placed in the center of a 40 cm (width) × 40 cm (diameter) × 38 cm (height) open-top PAS chamber surrounded by a customized open-top box made of white Perspex occluding vision of the surrounding room except for the ceiling. Locomotion and rearing were recorded for 5 minutes by quantifying beams breaks. In some experiments, mice were exposed in the PAS chamber with alternative flooring containing 16 evenly spaced nosepoke holes (holeboard), which were laser activated each time the mice investigated the hole. Beam break recordings were processed using the manufacturer's software to give average speed, rearing, and nosepoke data.

Tissue Extraction. On day 13 of the short regimen and day 23 of the extended regimen of treatment, mice were euthanized and the heart, bilateral gastrocnemius, and soleus muscles were dissected, weighed, snap frozen in liquid nitrogen, and then stored at –80 °C for further studies.

Protein Expression Studies. Muscle protein extracts were prepared by homogenizing gastrocnemius muscles in ice-cold RIPA buffer (Cell Signaling Technology, Danvers, Massachusetts) supplemented with phosphatase inhibitor (1×PhosSTOP; Roche Diagnostics, Basel, Switzerland), 10 mmol/L phenylmethylsulfonylfluoride (Sigma-Aldrich), and 100 nmol/L aprotinin (Roche Diagnostics, Basel, Switzerland). Samples were rotated for 2 hours at 4 °C on a rotating wheel and supernatant lysates were collected after centrifugation at 10,000*g* for 15 minutes at 4 °C. Protein concentration was determined with a bicinchoninic assay kit (Pierce BCA Kit; ThermoFisher Scientific, Inc., Rockford, Illinois). Protein samples (25 µg) were electrophoresed through 10% polyacrylamide gels and transferred onto methanol-activated polyvinylidene fluoride membranes (EMD Millipore, Billerica, Massachusetts). Membranes were blocked with 5% bovine serum albumin (Sigma-Aldrich) in Tris-buffered saline solution with Tween 20 for 1 hour at RT. Primary antibody incubations were performed overnight at 4 °C, secondary antibody incubations (goat anti-rabbit) were performed for 1 hour at RT. Antibodies used were p-STAT3-Y705 (9131), STAT3 (9132) (Cell Signaling), and rabbit anti-human α-tubulin (ProScientific, Inc., San Diego, California). Band densitometry was performed with ImageJ software (National Institutes of Health, Bethesda, Maryland) to determine relative protein expression, which was then normalized to the reference protein α-tubulin.

Gene Expression Studies. Total RNA was isolated from gastrocnemius muscles (~50 mg) by lysing tissues in TRIzol reagent (Life Technologies, Carlsbad, California) with a homogenizer (Pro200, ProScientific, Inc., San Diego,

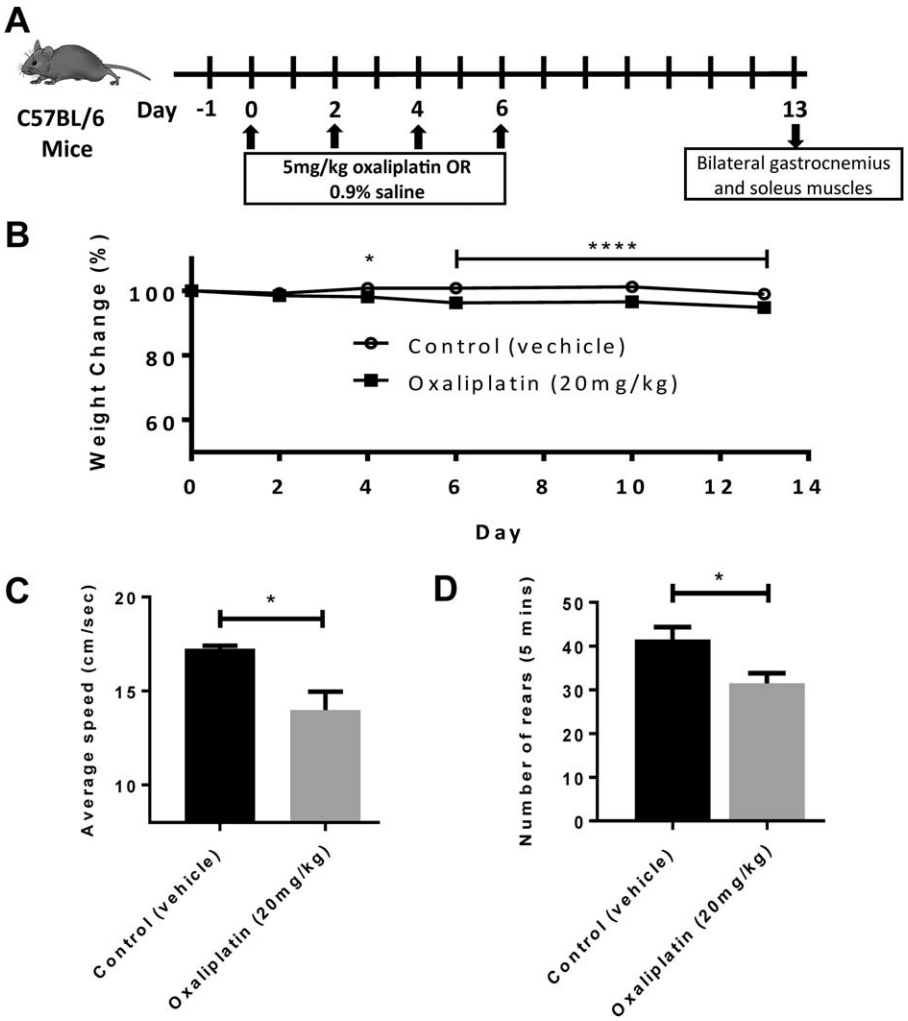

FIGURE 1. Oxaliplatin treatment results in altered weight gain and behavior. **(A)** Mice received intraperitoneal injections of 5 mg/kg oxaliplatin or 0.9% saline on days 0, 2, 4, and 6. Tissues were extracted on day 13. Arrows represent injection days. **(B)** Average percentage body weight change ($n = 8$ mice/group). **(C)** Average speed (cm/s) ($n = 4$ mice/group). **(D)** Number of rearing events over 5 minutes in an open field arena on day 6 of the regimen. Standard errors of the mean (SEM) are shown ($n = 4$ mice/group; $^{*}P < 0.05$, $^{****}P < 0.0001$).

California), according to the manufacturer's instructions (Life Technologies). Total RNA was treated with DNase (Thermo Fisher, Waltham, Massachusetts) to eliminate endogenous DNA before being reverse transcribed to cDNA using reverse transcriptase (Superscript III; Invitrogen, Carlsbad, California). Primers with high specificity to reference mRNA sequences for genes of interest were designed using NCBI Primer-Blast (National Centre for Biotechnology Information; see Table S1 in Supplementary Material, available online). Real-time quantitative polymerase chain reaction (qPCR) was performed using SYBR Green Master Mix (Invitrogen, Carlsbad, California) and forward and reverse primers (300 nmol/L) (Integrated DNA Technologies, Singapore). Reactions were performed in 384-well plates using real-time PCR (LightCycler 480, Roche Diagnostics, Basel, Switzerland). Relative mRNA levels were calculated from standard curves prepared within LightCycler software, and gene expression was normalized to *B2m* and *Gapdh*.

Statistical Analysis. All results are expressed as mean and standard error (mean +/− SEM). Western blots show independent samples. Two-tailed unpaired *t*-tests were performed to test the significance of differences between 2 groups; multiple unpaired *t*-tests with Holm–Sidak *post-hoc* correction were used for STAT3 phosphorylation and extended regimen behavior comparisons. Two-way repeated-measures analysis of variance with Sidak *post-hoc* correction was used to determine differences in percentage weight change. $P < 0.05$ was considered a statistically significant difference.

RESULTS

Oxaliplatin Treatment Results in Weight Loss and Altered Behavior. Adult (8-week-old) male C57BL/6J mice treated with 4 injections (5 mg/kg) of oxaliplatin over 6 days (cumulative dose of 20 mg/kg; see schematic diagram in Fig. 1A) exhibited significant weight loss compared with vehicle-treated control mice, beginning 4 days after the first injection (Fig. 1B). This reduction in body weight continued until day 13, while the weights of control vehicle-treated mice remained consistent over the

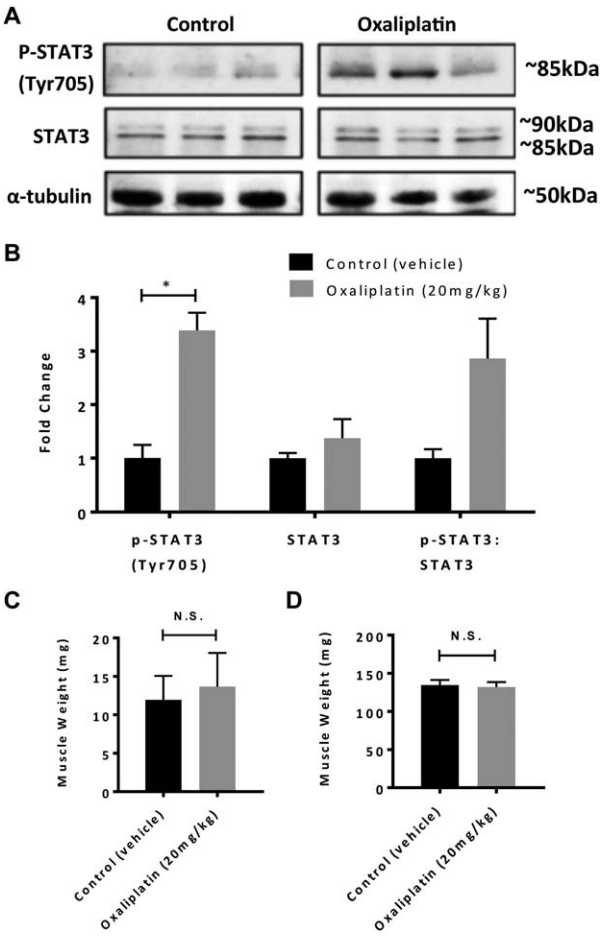

FIGURE 2. Oxaliplatin treatment leads to STAT3 phosphorylation in skeletal muscle. **(A)** Western blot of p-STAT3 and STAT3 protein expression determined by densitometry and normalized to α-tubulin. **(B)** The p-STAT3:STAT3 ratio was also calculated as an indicator of overall protein phosphorylation. Data are presented as the mean fold change relative to controls with SEM ($n = 3$; $^*P < 0.05$). Weights of hindlimb **(C)** soleus and **(D)** gastrocnemius muscles from day 13 are shown ($n = 8$).

duration of the model (Fig. 1B). From PAS open field testing, mice treated with oxaliplatin demonstrated significantly reduced average speed (Fig. 1C) and rearing (Fig. 1D) events on day 6, coinciding with their final injection of oxaliplatin.

Oxaliplatin Treatment Leads to STAT3 Phosphorylation in Skeletal Muscle. To determine if the observed reduction in weight, combined with decreased locomotive and investigative behavioral measurements, was associated with skeletal muscle atrophy, we then investigated STAT3 phosphorylation and hindlimb muscle wasting. We observed a significant increase in STAT3 phosphorylation at the tyrosine 705 residue (pSTAT3-Tyr705) in the gastrocnemius muscles of mice treated with oxaliplatin (Fig. 2A and B). No significant differences in the gross weights of soleus (Fig. 2C) and gastrocnemius (Fig. 2D) muscles were detected in oxaliplatin-treated mice when compared with controls.

Extended Regimen of Oxaliplatin Treatment Results in Impaired Weight Gain, Altered Behavior, and Reduced Hindlimb Skeletal Muscle Mass. Based on results from the shorter regimen of oxaliplatin treatment, it was hypothesized that the early physiological and molecular signs observed may precede gross muscle wasting. Therefore, an extended course of oxaliplatin treatment was assessed to determine whether any additional changes in muscle homeostasis were evident. In this model, mice were treated with 12 injections (2.5 mg/kg) of oxaliplatin over 17 days (30-mg/kg cumulative dose; see schematic diagram in Fig. 3A) and assessed until the endpoint at day 23. Oxaliplatin-treated mice exhibited reduced total body weights during the extended treatment course, whereas control mice gained weight. As a result, there were significant differences in the weights of oxaliplatin-treated mice compared with control mice (days 4–23) (Fig. 3B). Mice also demonstrated decreased average speed in the open field arena, which was nonsignificantly reduced at day 16 and significantly impaired at day 22 (Fig. 3C). Investigative behavior in the form of rearing was also impaired, reaching statistical significance on both day 16 and day 22 (Fig. 3D). Furthermore, for this regimen, nosepoke flooring was assessed and we demonstrated that the mice exhibited significantly decreased nosepoke activity (Fig. 3E). Crucially, at the experimental endpoint of day 23, mice treated with oxaliplatin displayed markedly lower muscle weights of both soleus (Fig. 3F) and gastrocnemius (Fig. 3G) muscles compared with controls (–12% and –19% compared with controls, respectively).

Oxaliplatin Treatment Induces Cachexia-Like Gene Expression Changes in Skeletal Muscle. Following the detection of reduced hindlimb muscle weights resulting from an extended regimen of oxaliplatin, the expression of several key regulatory genes in hindlimb gastrocnemius muscles was investigated. Expression of *Foxo1* was unchanged (Fig. 4A), but *Foxo3* gene expression was significantly upregulated in oxaliplatin-treated mice (Fig. 4B). We observed a corresponding significant increase in *Bnip3* expression in oxaliplatin-treated mice (Fig. 4C). In gastrocnemius muscles of the oxaliplatin-treated mice, there was no significant increase in *MuRF1* gene expression (Fig. 4D), but there was a statistically significant increase in *MAFbx* gene expression (Fig. 4D).

DISCUSSION

Our study findings suggest that administration of the platinum-based antineoplastic compound

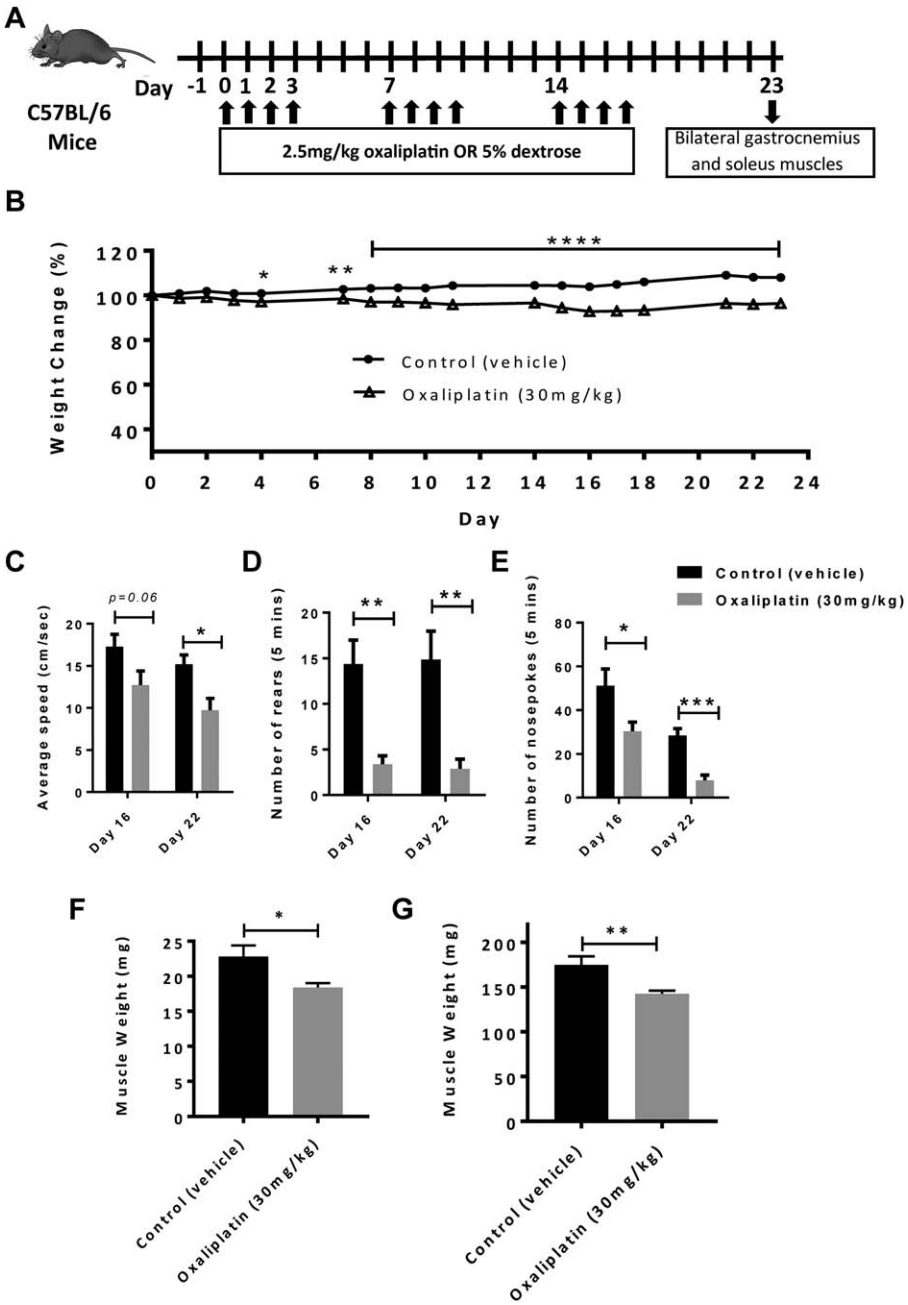

FIGURE 3. An extended regimen of oxaliplatin treatment results in prolonged impairment of weight gain, altered behavior, and reduced hindlimb skeletal muscle mass. **(A)** Mice received intraperitoneal injections of 2.5 mg/kg oxaliplatin or 5% dextrose on 4 consecutive days over 3 cycles, with 3 days between each cycle. **(B)** Average percent body weight change. **(C)** Average speed (cm/s), **(D)** number of rearing, and **(E)** nosepoke events over 5 minutes in an open field arena on days 16 and 22 of the extended regimen. Weights of hindlimb **(F)** soleus and **(G)** gastrocnemius muscles. Data are presented as mean SEM ($n = 8$ mice/group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$).

oxaliplatin, routinely used in the treatment of colorectal cancer, to non–tumor-bearing mice results in a series of progressive behavioral, physical, and molecular alterations associated with skeletal muscle dysfunction. Together, these observations are consistent with previous studies demonstrating that chemotherapeutic agents, including the platinum compound cisplatin, can contribute to a cachexia-like phenotype, and even exacerbation of cancer cachexia-associated muscle wasting in tumor-bearing mice.[7,9]

We observed small, but significantly reduced body weights in oxaliplatin-treated mice in accordance with previous studies demonstrating that platinum-based chemotherapeutics, including cisplatin and oxaliplatin, cause weight loss or reduced weight gain in rodents when compared with age-matched vehicle-treated controls.[13,30,31] Mice treated with oxaliplatin displayed significant behavioral changes in average speed, rearing, and nosepoke events while in the PAS open field arena, which are

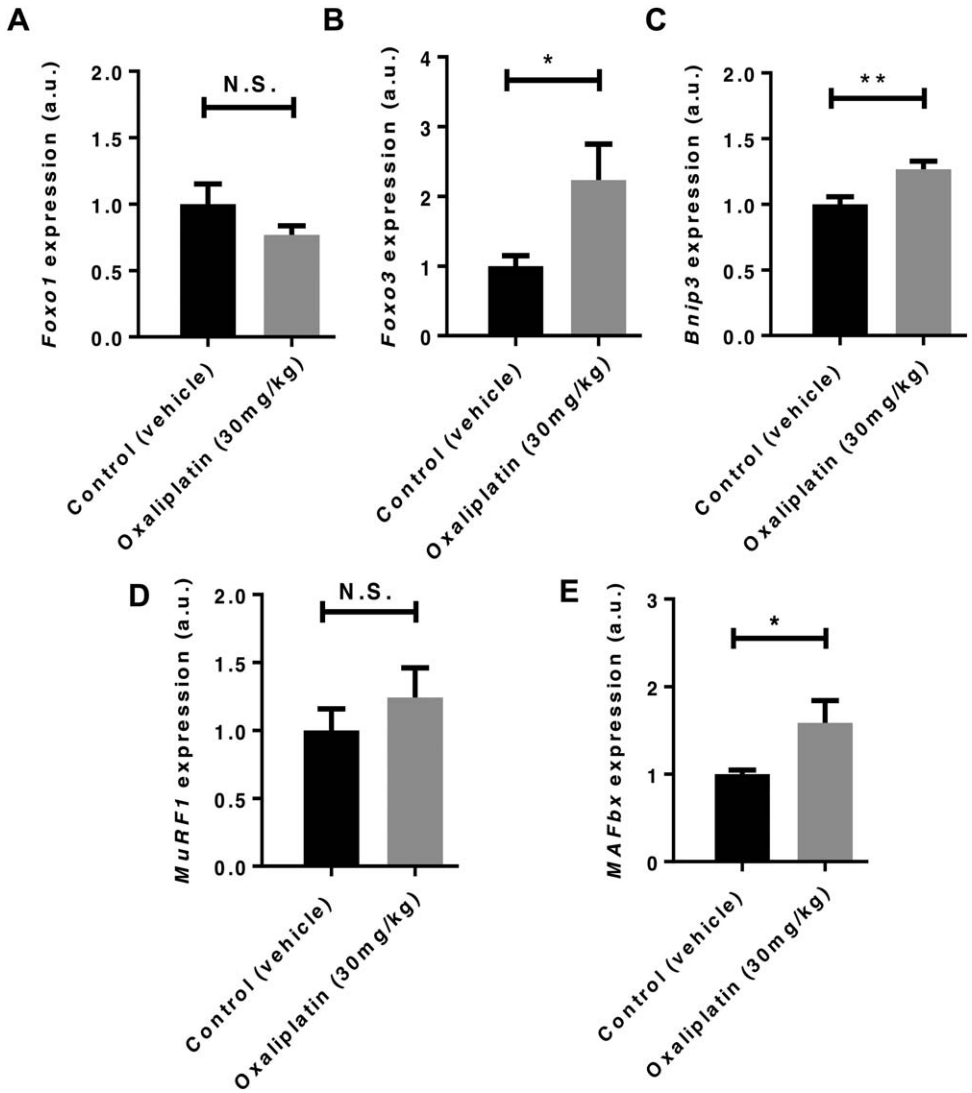

FIGURE 4. Molecular expression of selected muscle atrophy–associated molecules in response to oxaliplatin treatment. Gene expression of muscle atrophy–associated genes including members foxo transcription factor family **(A)** *Foxo1* and **(B)** *Foxo3*, and muscle specific markers of autophagy **(C)** *Bnip3* and the ubiquitin proteasome system (UPS) **(D)** *MuRF1* and **(E)** *MAFbx*. Data are presented as mean SEM ($n = 8$; $^{*}P < 0.05$, $^{**}P < 0.01$).

representative of locomotive, investigative, and exploratory/escape behavior,[32] respectively. One study, using a similar apparatus, showed that oxaliplatin reduces the total distance traveled in mice (representing locomotive behavior).[33] Other studies suggested that nosepoke (exploratory/escape behavior) was non-significantly reduced in oxaliplatin-treated rats[34] and voluntary exploratory behavior in oxaliplatin-treated mice was unaltered when measured over a 24-hour period.[30] The voluntary exploratory behavior included interaction with food and water, which was independently significantly increased in oxaliplatin-treated mice, and this may have confounded aspects of these results. Regardless, it is difficult to compare our data, considering the great difference in time frame of measurement, with our findings demonstrating robust differences in behavioral activity over a standard 5-minute testing period. Although our behavioral measures are not definitively related to fatigue, they are suggestive of a fatigued profile in the absence of considerable motoneuron dysfunction, which is not reported to be a common outcome after oxaliplatin treatment.[35–37] Although oxaliplatin can induce peripheral neuropathy due to neurotoxicity, particularly in sensory neurons, it very rarely produces features of motor neuropathy, which are generally limited to muscle cramps and fasciculations.[35] Furthermore, given the comorbidities of muscle atrophy and reduced weight gain, there is reasonable evidence to suggest these behavioral deficits are reflective of fatigue. Importantly, cancer-related fatigue is a complex condition with an unknown pathophysiology, which is purported to include contributions of both the central nervous system, as well as peripheral neuromuscular components, including skeletal muscle damage.[38–40]

A recent randomized study,[41] along with other reports,[42,43] strongly supported the physical and psychological benefits of exercise for patients with post-cancer fatigue. Furthermore, another novel study showed that biomarkers of muscle degradation were upregulated in cancer patients after chemotherapy treatment and that exercise was protective against muscle damage.[39] Consequently, peripheral mechanisms, such as muscle weakness and degradation in response to cancer treatment, may represent critical mediators of cancer-related fatigue.

Gross skeletal muscle weights remained unaltered in response to oxaliplatin treatment in the short treatment regimen, despite findings suggestive of muscle involvement, including reduced body weight gain, altered locomotive behavior, and induction of STAT3 protein phosphorylation in gastrocnemius muscles. However, given the dose concentration and short time frame of this treatment regimen, it is perhaps not surprising that extreme muscle wasting was not detectable. Indeed, another study showed that mice treated with 18-mg/kg cumulative dose of oxaliplatin had no significant skeletal muscle mass loss at day 15 after first injection.[30] Interestingly, however, in a model of cisplatin-induced muscle atrophy, mice treated with cisplatin for 4 consecutive days with a cumulative dose concentration of 12 mg/kg had significant hindlimb and quadriceps muscle wasting and reduced myofiber diameters.[7] The increased dosing frequency and shorter model timeline from initial treatment to dissection may explain the significant skeletal muscle toxicity of cisplatin observed in that study. Alternatively, it is also possible that cisplatin contributes to muscle wasting over a shorter time frame due to its subtly different composition and toxicity profile.[28]

Importantly, we have demonstrated that oxaliplatin does cause reduced body weight gain and skeletal muscle mass loss in an extended treatment model. Given that mice 8 weeks of age are still growing, reduced weight gain in this population may persist in older mice, and we have observed that body weight gain remains significantly reduced for >60 days in mice treated with the extended regimen (30-mg/kg oxaliplatin cumulative dose). Although food intake was not measured in this model, a recent publication found that oxaliplatin treatment caused weight loss without a significant reduction in food or water intake in mice, suggesting that muscle loss was independent of chemotherapy-associated anorexia.[30] Interestingly, Barreto *et al.*[4] found that mice exposed to Folfox, which contains oxaliplatin as a major constituent, did not have severe cachexia when compared with mice treated with Folfiri (5-FU, leucovorin, and irinotecan). This may be explained by the dosing schedule used in this model; Folfiri was administered twice a week, whereas Folfox was only administered once a week. Furthermore, the dose of oxaliplatin used in the model (6 mg/kg/week) was lower than in our extended regimen (approximately 10 mg/kg/week), which may explain the lack of a clear cachectic phenotype with Folfox treatment. Even so, Barreto *et al.*[4] found that mice treated with Folfox still had marked quadriceps muscle wasting when compared with control mice, potentially suggesting an early muscle-specific response to Folfox treatment.

Increased STAT3 tyrosine phosphorylation (tyrosine 705) is a pertinent indicator of canonical STAT3 protein activation, and is representative of an initial upstream inflammatory mediator of muscle wasting.[15] Importantly, STAT3 is a major contributor to muscle atrophy in cancer cachexia and various other muscle pathologies, with blockade of STAT3 signaling leading to amelioration of muscle loss.[15,44] Alternatively, some studies have demonstrated that oxaliplatin downregulates STAT3 phosphorylation (Tyr705) and activation in cancer cells.[45,46] Our results therefore suggest novel systemic effects in skeletal muscle and a tissue-specific molecular response to oxaliplatin. Furthermore, *Foxo3*, *Bnip3*, *MAFbx*, found to be upregulated in response to extended oxaliplatin treatment, are all pivotally associated with processes of muscle wasting.[23,24] The gene expression changes detected in skeletal muscle in the extended model suggest that oxaliplatin may activate similar pathways in regulating muscle loss to those activated during cancer cachexia. Indeed, in a recent study, Barreto *et al.* used a proteomics-based approach to compare the protein signatures of two different models of cachexia: the colon-26 (C26) model of cancer cachexia and a Folfiri combination treatment modeling chemotherapy-associated cachexia.[10] Notably, common signaling pathways were activated in both conditions, suggesting that combination treatment strategies may be desirable to concurrently target the side effects of chemotherapy-associated and tumor-associated cachexia.

A limitation of the present study, like others previously, is that the model does not take into account the small but potentially important role of micrometastatic disease in a subset of all patients who receive adjuvant chemotherapy. In such patients, both the low-level tumor burden and chemotherapy treatment may contribute equally to muscle dysfunction and fatigue and the complex interaction between tumor-associated and chemotherapy-associated molecular alterations cannot be appreciated. Importantly, the doses delivered in our models were representative of clinically

relevant concentrations, and therefore provide a reasonable representation of possible systemic side effects of chemotherapy in the majority of adjuvant patients.

Although only gastrocnemius muscles were analyzed in this study, there may be different degrees of wasting, and varying molecular expression patterns seen across other muscle groups. Of particular interest is whether oxaliplatin may induce selective atrophy of a subtype of muscle fibers based on metabolic profile, as in the C26 model of cancer cachexia, where glycolytic muscle fibers are affected preferentially.[14] Interestingly, in the extended regimen, differences in gross heart weight were also detected, with the hearts of oxaliplatin-treated mice weighing significantly less than those of controls (see Fig. S1 in Supplementary Material). Although cardiotoxicity associated with use of the chemotherapy agent doxorubicin is well characterized,[47] the impact of oxaliplatin on cardiac muscle remains unclear, with no long-term cardiac complications having been reported. Consideration of differences across muscle groups and potential cardiac changes therefore represent possible directions for future study.

In conclusion, our study has provided novel evidence that oxaliplatin treatment contributes to skeletal muscle mass loss and associated molecular and functional changes suggestive of fatigue and atrophy. Fatigue is a common side effect of cancer and chemotherapy, affecting patients before, during, and after treatment.[39] However, there is a significant minority of patients in whom the fatigue is persistent and disabling, for months or years after likely curative cancer treatment.[44,48,49] The symptom complex of this posttreatment fatigue is very similar to that of chronic fatigue syndrome, including the characteristic fatigue, neurocognitive difficulties, disturbances in sleep–wake cycle and mood, and reduced physical function.[39] Given that the impact of posttreatment fatigue in the adjuvant setting is a key issue affecting cancer survivors, our work has implications for examining the role of interventions, such as graduated exercise[50–52] and pharmacological interventions. The lack of a clear pathophysiology model has been one of the issues hampering efforts to intervene effectively,[51] so given the complex, multifactorial nature of fatigue,[38] consideration of peripheral mechanisms, including muscle dysfunction, remains imperative. In this study we have demonstrated that administration of oxaliplatin in non–tumor-bearing mice appears to have notable effects on skeletal muscle homeostasis, which is clinically relevant as drug-associated muscle loss may contribute to increased morbidity in cancer patients after chemotherapy, preventing them from resuming their normal lives.

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