Molecular Mechanisms Underlying Skeletal Muscle Weakness in Human Cancer: Reduced Myosin-Actin Cross-Bridge Formation and Kinetics

Michael J.J. Toth
Mark S. Miller, University of Massachusetts - Amherst
Damien M. Callahan
Andrew P. Sweeny
Ivette Nunez, et al.

Available at: https://works.bepress.com/mark_miller/2/
Molecular mechanisms underlying skeletal muscle weakness in human cancer: reduced myosin-actin cross-bridge formation and kinetics

Michael J. Toth, Mark S. Miller, Damien M. Callahan, Andrew P. Sweeny, Ivette Nunez, Steven M. Grunberg, Hirak Der-Torossian, Marion E. Couch, and Kim Dittus

1Department of Medicine, University of Vermont, College of Medicine, Burlington, Vermont;
2Department of Molecular Physiology and Biophysics, University of Vermont, College of Medicine, Burlington, Vermont;
3Department of Surgery, University of Vermont, College of Medicine, Burlington, Vermont

Corresponding author.
Address for correspondence: M. J. Toth, Health Science Research Facility 126B, 149 Beaumont Ave., Univ. of Vermont, Burlington, VT 05405 (e-mail: ude.mvu@htot.lean.cim).

Received December 11, 2012; Accepted February 6, 2013.

Abstract

PHYSICAL FUNCTION DETERIORATES substantially following a diagnosis of cancer (3, 48), and patients view this decline as one of the most distressing side effects of the disease, more so than classic side effects such as pain, nausea, and vomiting (13, 60). Functional disability can be the impetus for dose reduction or cessation of anticancer treatments and predicts chemotherapy toxicity and survival (12, 30, 33, 39). Our current understanding of the factors contributing to reduced functional capacity in patients with cancer is, however, severely limited.

Physiological changes that occur within the skeletal muscle of patients with cancer can contribute to functional deterioration and physical disability. The most common adaptations believed to promote functional impairment are muscle atrophy, reduced cardiorespiratory fitness, and skeletal muscle weakness (20, 32, 54). The vast majority of studies of muscle biology in cancer have focused on signal transduction mechanisms underlying skeletal muscle atrophy (20). Understanding quantitative alterations in skeletal muscle and their mechanisms is important because they have relevance for physical function (38) and clinical
outcome (18), but functional deficits persist after controlling for muscle atrophy (34, 54), and there is compelling evidence to suggest that cancer has a unique effect on the intrinsic functionality of muscle (22). In other words, a substantial proportion of the decline in physical capacity is likely explained by reductions in function per unit tissue size.

Skeletal muscle contractile dysfunction has received minimal attention as a precipitant of functional changes in patients with cancer (54, 61), with the majority of studies being focused on cardiorespiratory fitness (32). However, reduced skeletal muscle contractile function is a strong predictor of decreased physical functioning in common daily activities (49) in many studies rivaling or exceeding the contribution attributed to diminished aerobic capacity (8, 51). Additionally, at a more fundamental level, the properties of the contractile elements (i.e., myofilament proteins) determine the functional character of skeletal muscle and, correspondingly, whole-body performance (24, 28, 29). As the end effectors of muscle contraction, myofilament mechanical properties necessarily set limits for muscle functionality (10). To date, no studies have evaluated the effects of cancer on myofilament protein content, structure, or functionality in humans.

The goal of this study was to examine the effect of cancer on skeletal muscle contractile function at the molecular, cellular, whole-muscle, and whole-body levels. To accomplish this objective, we evaluated whole-body and whole-muscle performance using standard functional assessments and cellular/molecular structure and function on intact and chemically skinned fibers from the vastus lateralis muscle in patients with cancer and controls. Because cancer-related functional deficits are suggested to be more common in patients experiencing weight loss and during treatment (i.e., chemo/radiotherapy), we included both cachectic and noncachectic patients and patients undergoing cancer treatment. In this context, our cohort does not permit us to address the unique effect of cancer per se, but instead encompasses the effects of the disease, its treatment, and disease- and treatment-related sequelae such as weight loss. However, when discussing our findings, we refer to the effects of cancer for simplicity.

**METHODS**

**Subjects.**

Eleven patients with cancer (7 men, 4 women) were recruited from the multidisciplinary oncology clinics. Patient clinical characteristics are provided in Table 1. To briefly summarize, patients had diagnoses of lung (n = 8), gastrointestinal (n = 1 gastric, n = 1 pancreatic), and head-neck (n = 1 larynx) cancer (7 patients had stage III cancer; 4 patients had stage IV cancer). Five of the patients were characterized as cachectic, defined as self-reported, unintentional weight loss >5% of body weight within 6 mo prior to evaluation, whereas the other patients reported little or no weight loss. Four patients were tested shortly following diagnosis, prior to receiving any treatment, all of whom were cachectic (3 men, 1 woman), whereas the remainder were tested while receiving cancer treatment (n = 7, 5 men/2 women; 6 noncachectic, 1 cachectic). In the latter group, all testing was completed prior to cycle 4 of chemotherapy, with an average time from the start of chemotherapy to testing of 59.4 ± 9.2 days. No patients had surgical resection, but three patients had received radiation therapy (three with lung cancer, one with cancer of the larynx; two men/one woman).
Chemotherapy agents used are reported in detail in Table 1. Six of the seven patients receiving chemotherapy also received dexamethasone for 1 to 4 days following chemotherapy administration to diminish cancer-induced nausea and vomiting. Three patients on carboplatin therapy received 8 mg of dexamethasone on the day of chemotherapy administration, whereas three patients on cisplatin therapy received 36 mg total over 4 days. Nine of the patients reported having a history of smoking and five were current smokers (1.2 ± 0.5 packs/day). Neither the patient with gastric cancer nor the patient with laryngeal cancer had obstructive dysphagia as determined by the Massey Swallowing Screen (37), and none received artificial nutritional support (intravenous or nasogastric feeding). None of the patients had received any type of anabolic therapy designed to improve muscle size or function.

Controls (four men, two women) did not participate in any exercise training or weight loss programs and were generally moderately active. On the basis of medical screening and routine clinical/laboratory tests, they were healthy and had no prior history of cancer, chronic lung or cardiovascular disease, neurological or orthopedic conditions, or other mobility-limiting ailments. All were nonsmokers. None were taking any medications that could influence skeletal muscle, with the exception of one volunteer who was on a stable regimen of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitor. Plasma creatine kinase levels were within the normative range in this volunteer and he reported no symptoms consistent with an effect of the medication on his muscles. As an aside, we have found that healthy elderly patients on stable regimens of HMG CoA reductase inhibitor do not show alterations in whole-muscle or single-fiber contractile function compared with age- and sex-matched controls not taking these medications (n = 4/group; Toth et al. unpublished observations), suggesting that inclusion of this individual would not likely influence the detection of cancer effects. Written informed consent was obtained from all volunteers prior to their participation, and protocols for controls and patients with cancer were approved by the Committees on Human Research at the University of Vermont.

Knee extensor muscle function.

Knee extensor torque production was measured under isometric conditions at 55° using a multijoint dynamometer (HUMAC/NORM; Computer Sports Medicine, Stoughton, MA), as described (58). We did not perform knee extensor function measurements on four patients with cancer who were tested shortly following diagnosis.

Six-minute walk test.

The 6-min walk test was conducted in patients with cancer as a measure of whole-body functional capacity (9). The total distance walked in the 6-min period is often used as the primary outcome of this test, but this simple metric fails to account for individual differences in body mass. To account for this variation, we calculated power production during the 6-min walk test as follows: 6-min walk power (W) = body mass (kg)·9.8 (m·s^{-2})·average gait speed (m·s^{-1}), where 9.8 represents the acceleration of gravity. As with knee extensor function, we did not perform 6-min walk tests on the four patients with cancer who were tested shortly following diagnosis.
**Total and regional body composition.**

Body mass was measured on a digital scale (ScaleTronix, Wheaton, IL). Total and regional fat mass, fat-free mass, and bone mass were measured by dual energy X-ray absorptiometry using a GE Lunar Prodigy densitometer (GE Lunar, Madison, WI), with appendicular lean tissue mass being assessed as described (26).

**Muscle tissue processing.**

Procedures for percutaneous muscle biopsy of the vastus lateralis have been described (57). Muscle tissue was partitioned for mechanical, morphological, and biochemical analysis. For single-fiber mechanical assessments, tissue was placed immediately into cold (4°C) dissecting solution [all solutions discussed herein were previously described (41), with the exception that no reducing agents were included in storage solutions]. Thereafter, muscle fiber bundles were dissected and processed for single-fiber measurements, as described (41). Muscle tissue used for morphological and biochemical analysis was processed as follows. Immediately after obtaining the biopsy, two small bundles of muscle fibers (~100 and 200 fibers, respectively) were dissected and tied to glass rods at a slightly stretched length and placed into either 2.5%/1% glutaraldehyde/paraformaldehyde (sodium cacodylate) or 4% paraformaldehyde (phosphate-buffered saline; PBS) fixative for immunohistochemical and electron microscopy (EM) studies, respectively. Processing for EM measurements has been described (41). For immunohistochemistry studies, tissue was fixed overnight at 4°C and then removed from the glass rod and paraffin-embedded. The remaining tissue that was not aliquoted for mechanical or morphological analysis was immediately frozen in liquid nitrogen and stored at −80°C until analysis. One patient with cancer did not undergo the biopsy procedure because of concomitant anticoagulant therapy.

**Myosin heavy chain (MHC) and actin protein content, and MHC isoform distribution.**

Myosin and actin protein content were evaluated via gel electrophoresis in tissue homogenates (~10 mg) as described previously (41), with minor modifications. Gels were loaded both per unit protein content and per unit wet weight of tissue, the latter to ensure that any loss of myofilament components would not skew the protein loads on the gel. Protein content of tissue homogenates was determined using the folin-phenol reagent (Bio-Rad RCDC protein assay) with bovine serum albumin (BSA) as a standard. Of note, although studies in preclinical models have suggested that cancer cachexia is associated with a preferential loss of myosin protein (1), recent studies have challenged this notion and suggested that inadequate solubilization of myosin can lead to the observation of a selective depletion of myosin protein (14). The buffer system that we employed [in mM: 300 KCl, 150 potassium phosphate, 10 sodium pyrophosphate, 5 dithiothreitol, 5 magnesium chloride; pH 6.8 with the addition of protease inhibitor cocktail (Sigma)] was similar to that used by Cosper and Leinwand (14) and should solubilize the vast majority of myosin. Tissue homogenate MHC isoform distribution was also measured by SDS-PAGE, as described (41).

**Immunohistochemical analysis.**
Average fiber cross-sectional area was determined on 10-μm-thick sections. Samples were deparaffinized by washing in xylene (96% xylene, 4% ethylbenzene), followed by progressively dilute ethanol solutions (100%, 95%, and 70%), and then distilled water. Antigen retrieval was performed in 1x DAKO Target Retrieval Solution (Dako) at ~100°C for 20 min. Samples were cooled to room temperature before being washed in PBS. Samples were then washed in 0.1% Sudan Black in 70% methanol to diminish autofluorescence, followed by a 15-min incubation in 1% BSA/0.1% Triton X-100 in PBS. Slides were blocked in fresh goat serum (10% normal goat serum/1% BSA in PBS) and then incubated overnight in a polyclonal rabbit anti-laminin antibody (1:100, ab11575; Abcam) and a mouse anti-MHC I antibody (1:250, A4.951-c; Developmental Studies Hybridoma Bank, University of Iowa) in 1% BSA in PBS. Two secondary antibodies, a goat anti-rabbit (Alexa Fluor 488, Invitrogen) and goat anti-mouse (Alexa Fluor 555, Invitrogen) were diluted 1:500 in 1% BSA in PBS and were applied for 1 h. Samples were washed with 1% BSA in PBS between each incubation, and once more with PBS before drying and covering in mounting media (Citifluor Antifadent Mounting Medium AF1; Electron Microscopy Sciences). Visualization and imaging was performed using an Olympus BX50 microscope. Cross-sectional area measurements were performed using image analysis software (Metamorph version 7.7.9.0; Molecular Devices). Immunohistochemistry was not performed on two controls; one because of limited tissue availability, and the other because of technical problems.

**Ultrastructural measurements.**

EM measurements were conducted on intact (i.e., unskinned) skeletal muscle fiber bundles to assess myofibrillar area fraction, thick-to-thin filament ratio, A-band and sarcomere lengths, intermyofibrillar mitochondrial area fraction, and average area and number, as described in our prior publications (41, 57). Additionally, the Z-band width was measured as a proxy of fiber type (53) as described (57). One control volunteer did not have EM analysis because of limited tissue availability.

**Single muscle fiber mechanical measurements.**

Segments (~2.5 mm) of chemically skinned single fibers were isolated and processed for mechanical measurements on controls (n = 19 ± 1 fibers/patient) and patients with cancer (n = 15 ± 1 fibers/patient), as described in detail (40, 41). Top and side diameter measurements were made in relaxing solution (pCa 8) at three positions to calculate average cross-sectional area. The experimental apparatus for mechanical assessments, the solutions used, and the protocol have been described in detail (40), with minor modifications for the present studies. Briefly, fibers were attached to a piezoelectric motor and a strain gauge in relaxing solution, the sarcomere length set to 2.65 μm, and fiber length was measured. Each fiber underwent an initial activation (pCa 4.5) to verify the integrity of the fiber, followed by a second activation for performance of sinusoidal analysis. Sinusoidal analysis was performed under maximal Ca²⁺-activated conditions (pCa 4.5; 25°C and 5 mM P_i) to estimate myosin-actin cross-bridge mechanics and kinetics, as described (40).

Single muscle fiber tension (force/cross-sectional area) was derived from the plateau of tension following maximal Ca²⁺ activation prior to beginning sinusoidal length oscillations. We have recently shown that variation in myofilament fractional area of single fibers can
influence the interpretation of tension data (56). Thus, the fiber cross-sectional area was multiplied by the myofilament fractional area from EM measurements for each volunteer prior to calculation of single-fiber tension (i.e., force per unit cross-sectional area) to more accurately account for the amount of contractile components (i.e., myofilaments) per cross-sectional area. For one control volunteer lacking EM measurements, the average myofilament fractional area for all healthy elderly individuals evaluated in our laboratory (84.7%; n = 29) was used to adjust single-fiber tension. This adjustment represents an oversimplification because the myofilament fractional area of each fiber assayed for mechanical properties is not directly measured, but estimated from EM analysis of other fibers in that individual. We chose this approach over the use of a group average value for myofilament fractional area primarily because of the availability of EM data on nearly all subjects. Compared with the use of a single group average value for myofilament fractional area, which is a further oversimplification, variation in individual estimates of myofilament fractional area would introduce more variation into our tension data and make detection of group differences more difficult. In this context, our current approach is conservative because it would, if anything, bias our study against detecting cancer-related differences in functional parameters.

Following mechanical assessments, single fibers were placed in gel loading buffer, heated for 2 min at 65°C, and stored at −80°C until determination of MHC isoform composition by SDS-PAGE to identify fiber type, as described (41). We restricted our analysis to MHC I, IIA, and IIA/X fibers, because the numbers of IIX and other hybrid (I/IIA, I/IIA/IIX) fibers were too few to permit analysis. For MHC IIA/X fibers, the relative proportion of IIA and IIX isoforms in each fiber was determined from gels by densitometry, the migration pattern of the isoforms being based on prior studies using conditions similar to ours (4). The mechanical properties of the single fibers also serve as an indirect verification of the mobility of the various isoforms. Two patients with cancer did not have mechanical analysis performed on single muscle fibers because of limited technical resources. This was a function of time constraints related to the difficulty in recruiting, scheduling, and testing individuals with advanced cancer during their cancer treatment regimens.

To characterize myosin-actin cross-bridge mechanics and kinetics using sinusoidal analysis, complex modulus data [elastic and viscous moduli, derived as described previously (40)] at peak calcium activation were fitted to the following mathematical expression:

\[ Y(\omega) = A(\omega^k) - Bi\omega(2\pi b + i\omega) + C\omega(2\pi c + i\omega), \]

where \( \omega = 2\pi f \) in s\(^{-1}\); A, B, and C are magnitudes expressed in mN/mm\(^2\); 2\(\pi b\) and 2\(\pi c\) are characteristic frequencies expressed in s\(^{-1}\); and \( i = -1^{1/2} \); \( \alpha = 1 \) s\(^{-1}\); and \( k \) = a unitless exponent. Of note, cross-sectional area data used to normalize force measurements were adjusted for myofilament fractional area, as described above. This equation yields three characteristic processes, A, B, and C, which relate to various mechanical (A, B, C, and \( k \)) and kinetic [2\(\pi b\) and \( (2\pi c)^{-1} \)] properties of the cross-bridge cycle, as described (40). Briefly, 2\(\pi b\), the frequency portion of the B-process, is interpreted as the apparent rate of myosin force production or, in other words, the rate of myosin transition between the weakly and strongly bound states (35, 64). The inverse of the frequency portion of the C-process, or \( (2\pi c)^{-1} \),
represents the average myosin attachment time \( (t_{\text{on}}) \) to actin (44). The magnitudes of the B- and C-processes (parameters \( B \) and \( C \)) are proportional to the number of myosin heads strongly bound to actin and the cross-bridge stiffness (35). Finally, the A-process has no kinetic or enzymatic dependence (42) and, therefore, reflects the viscoelastic properties of the nonenzyromatic, passive elements in the myofilaments. Under \( \text{Ca}^{2+} \)-activated conditions, the A-process represents the underlying stiffness of the lattice structure and the attached myosin heads in series (42, 43). The parameter \( A \) indicates the magnitude of the viscoelastic modulus, and \( k \) represents the angle at which the A-process lies relative to the \( x \)-axis, which reflects the viscous-to-elastic modulus relationship of the A-process \( (k = 0 \) purely elastic vs. \( k = 1 \) purely viscous).

Statistics.

Differences between patients with cancer and controls were determined using unpaired \( t \)-tests. When cancer-related differences were found, subanalysis was conducted to determine whether these differences were due to differential effects in cachectic or noncachectic patients or patients tested following diagnosis vs. those tested during cancer treatment using ANOVA (SPSS version 19; IBM SPSS Statistics, Armonk, NY). For those variables in which multiple observations were performed within the same individual (e.g., single-fiber structural, morphological, and mechanical indices), a linear mixed model (SAS Version 9.3; SAS Institute, Cary, NC) was used, with group assignment being the between-subject factor (i.e., control vs. cancer for most comparisons, with the cancer group being further divided into cachectic vs. noncachectic, or with vs. without treatment, to investigate the effect of variation within the cancer population on the phenotype). In this model, a group effect is included as the between-subject factor, along with a repeated effect to account for the clustering of observations within individuals. Inclusion of this repeated effect is necessary because the standard general linear model assumes that each measurement is independent, which is not the case for fibers evaluated from different volunteers (i.e., fibers from the same subject are related). In other words, each fiber cannot be considered a single observation. Finally, relationships between variables were determined by Pearson correlation coefficients, with the normality of dependent and independent variables confirmed by Shapiro-Wilk test (SPSS version 19). All data are reported as mean ± SEM.

RESULTS

Disease and physical characteristics.

Disease characteristics of the patients with cancer are summarized in Table 1, with details provided in the Subjects subsection of the METHODS section. Body composition and physical characteristics of patients with cancer and controls are shown in Table 2. No differences in age, height, body mass, or composition were found between patients with cancer and controls. When patients with cancer were divided into cachectic and noncachectic groups, the lack of differences persisted, except for fat mass \( (P < 0.05 \) group effect), where cachectic patients were lower than noncachectic patients \( (P < 0.05) \) and showed a trend toward being lower than controls \( (P = 0.09) \).

Whole-muscle function.
Knee extensor contractile function and its relationship to walking performance are shown in Fig. 1. Patients with cancer showed reduced knee extensor isometric torque ($P < 0.05$; data not shown in Fig.), which persisted after adjustment for variation in leg fat-free mass ($P = 0.016$; Fig. 1 top). The functional relevance of reduced knee extensor torque in patients with cancer was suggested by its correlation to decreased power output during the 6-min walk test in patients with cancer ($P < 0.01$).

**Single muscle fiber size and myofilament content/ultrastructure.**

Single muscle fiber morphometry and myofilament ultrastructure are shown in Fig. 2. Single fiber cross-sectional areas in MHC I and II fibers were lower (−19% and −16%, respectively) in patients with cancer vs. controls, but not significantly (Fig. 2A). The fractional area occupied by MHC I and II fibers did not differ between patients and controls (data not shown). The average number of fibers evaluated for immunohistochemical analysis per volunteer was similar between groups (cancer, 163 ± 21 vs. control, 153 ± 29). Finally, no differences were noted between patients and controls for myofilament fractional area (Fig. 2B), thick-to-thin filament ratio (Fig. 2C), or A-band length (Fig. 2D). Moreover, the sarcomere length of EM preparations did not differ between groups, suggesting that the absence of differences between groups in structural parameters was not related to differences in the degree of stretch of the preparation.

**Myofilament protein expression.**

The expression of myosin protein and the relative expression of myosin isoforms were measured by gel electrophoresis and are shown in Fig. 3. No differences in myosin protein content were found, and this result was similar whether gels were loaded and results normalized per unit wet weight of tissue or per unit protein content of the tissue homogenate (latter not shown). No difference in myosin protein content was found when comparing cachectic vs. noncachectic patients (data not shown). Moreover, the expression of MHC isoforms did not differ between patients and controls. Finally, no differences in actin protein content were found (data not shown).

**Single-fiber contractile function.**

Single-fiber maximal $Ca^{2+}$-activated tension data for MHC I and IIA fibers are shown in Fig. 4. Single-fiber tension was 14% lower in MHC IIA fibers from patients with cancer vs. controls ($P = 0.05$) but did not differ in MHC I fibers. There was no difference in MHC IIA tension between cachectic and noncachectic patients, indicating that group differences were not due to weight loss. Similarly, there was no difference in MHC IIA tension between patients tested following diagnosis and those tested during cancer treatment.

We also evaluated group differences in maximal $Ca^{2+}$-activated tension in MHC IIA/X fibers (Fig. 5). MHC IIA/X fiber tension was 20% lower in patients with cancer compared with controls, although this difference was not significant. Variation in the relative admixture of MHC IIA and IIX in single fibers could influence tension. The relative distribution of IIA and IIX isoforms showed a strong trend ($P = 0.065$) toward differing between groups, with a lower proportion of IIA and a higher proportion of IIX isoform in patients with cancer. However, we found no linear relationship of MHC IIA/X distribution to single-fiber tension...
in the whole group \((r = \pm 0.144);\) note that the \(\pm\) symbol prior to the correlation coefficient designates the reciprocal relationships depending on whether MHC IIA or IIX distribution is related to tension or kinetic variables), which is in agreement with recent studies showing minimal differences in force generating capacity among the fast-twitch human MHC isoforms (36). The relative distribution of MHC IIA and X isoforms expressed in hybrid fibers, however, was correlated to myosin attachment time \((r = \pm 0.490; P < 0.02)\) and rate of force production \((r = \pm 0.43; P < 0.04)\). This complicates the interpretation of differences in MHC IIA/X cross-bridge kinetics between groups because functional differences may be due to differences in the admixture of isoforms in each fiber. Therefore, we did not perform these comparisons.

**Myosin-actin cross-bridge mechanics and kinetics.**

To explore the effects of cancer on muscle function at the molecular level, we employed sinusoidal analysis to estimate myosin-actin cross-bridge mechanics and kinetics (Fig. 6). In MHC I fibers, there was a slowing of cross-bridge kinetics, as evidenced by a 25% increase in myosin attachment time in patients with cancer compared with controls \((P < 0.05)\). Other cross-bridge kinetic and mechanic parameters, however, did not differ between groups in MHC I fibers. Cachectic and noncachectic patients with cancer were found to have similar myosin attachment times in MHC I fibers, suggesting that cancer-related differences were not associated with prior weight loss. Moreover, myosin attachment times in MHC I fibers were similar in patients tested following diagnosis and those tested during cancer treatment. Finally, to explore the functional significance of reduced myosin-actin cross-bridge kinetics in MHC I fibers, we assessed the relationship of cross-bridge kinetic parameters to knee extensor isometric tension. We found that an index of reduced cross-bridge kinetics, decreased myosin rate of force production, was correlated to decreased knee extensor torque \((r = 0.689; P < 0.05)\).

In MHC IIA fibers, \(B\) and \(C\), parameters that reflect the number of strongly bound cross-bridges and cross-bridge stiffness, were reduced \((21\% \text{ and } 22\%, \text{ respectively}; \text{ both } P < 0.05)\) in patients with cancer compared with controls. In contrast, other cross-bridge kinetic and mechanic parameters did not differ between patients and controls. Comparisons of \(B\) and \(C\) between cachectic and noncachectic patients showed no differences between groups, suggesting that aforementioned effects of cancer are not likely explained by prior weight loss. Similarly, there was no difference in \(B\) and \(C\) between patients tested following diagnosis and those tested during cancer treatment.

**Mitochondrial content and structure.**

Cancer and its treatment have been associated with mitochondrial dysfunction, mitochondria loss, and increased oxidative stress in skeletal muscle (23, 62). Alterations in energetic homeostasis may contribute to impaired skeletal muscle contractile function (50). To begin to evaluate this possibility, we measured mitochondrial content and structure (Fig. 7). Mitochondria fractional area was 50% less \((P < 0.001)\) in patients with cancer than in controls. This reduction was due to decreases in the average size \((-37\%; P < 0.01)\), with no difference in the number of mitochondria \((-26\%)\), although the latter showed a strong trend toward significance \((P = 0.06)\). There were no differences between cachectic and
noncachectic patients for mitochondrial fractional area, average area or number, nor when comparing patients who were tested following diagnosis vs. those tested during cancer treatment \( (P = 0.45, 0.51, \text{ and } 0.37, \text{ respectively}) \). We found that the Z-band width was shorter in patients with cancer compared with controls \( (73.2 \pm 3.1 \text{ vs. } 82.8 \pm 3.1 \text{ nm, respectively}; \ P < 0.03) \), suggesting the possibility that the EMs from patients with cancer contained a greater proportion of MHC II fibers \( (53) \). However, Z-band width was not correlated to mitochondrial fractional area or average area, and statistical control for Z-band width did not diminish the lower mitochondria fractional content \( (−50%; \ P < 0.01) \) or average area \( (−44%; \ P < 0.01) \) in patients with cancer. To evaluate whether these alterations in mitochondrial content/structure are related to the myofilament contractile properties found to differ between patients with cancer and controls, correlations were examined. An increase in myosin attachment time in MHC I fibers was correlated with a reduction in mitochondria fractional area \( (r = −0.754; \ P < 0.01) \) and with a reduction in average area \( (r = −0.737; \ P < 0.01) \). Mitochondrial variables did not, however, correlate with variation in single-fiber tension or \( B \) or \( C \) in MHC IIA fibers, although correlations were trending toward significance between the average area of mitochondria and both \( B \) \( (r = 0.472; \ P = 0.10) \) and \( C \) \( (r = 0.472; \ P = 0.10) \). To further explore the functional relevance of mitochondrial rarefaction, we examined the relationships with whole-muscle and whole-body physical performance. Mitochondria fractional area showed a trend \( (P = 0.08) \) toward being related to power output during the 6-min walk test \( (r = 0.693) \), but was not related to knee extensor isometric torque.

**DISCUSSION**

This is the first study to evaluate the effects of cancer on skeletal muscle structure and function in humans at the cellular and molecular levels. In fact, aside from a case report of a single patient with atypical, rapidly progressive lower extremity atrophy and paralysis \( (5) \), to our knowledge, only two studies have systematically evaluated muscle contractile function and size in patients with cancer. Although both studies showed reduced knee extensor strength with cancer \( (54, 61) \), only one showed weakness per unit muscle size \( (54) \). Because we found a 25% reduction in knee extensor isometric torque after statistical adjustment for leg muscle mass, our results support the conclusion that cancer is associated with intrinsic contractile dysfunction (i.e., diminished function per unit muscle size). Perhaps as important, the relationship between reduced knee extensor isometric torque and decreased power output during the 6-min walk test demonstrates a link between diminished lower extremity muscle contractile performance and functional limitations in patients with cancer in an important activity of daily living. Other studies have observed correlations between muscle weakness and diminished walking performance \( (9, 51) \), but ours is the first to demonstrate the relevance of muscle contractile dysfunction to physical disability in patients with cancer, where functional limitation has been primarily related to reduced aerobic fitness \( (32) \).

Buttressing our whole-muscle data suggesting intrinsic contractile dysfunction, we found reduced isometric tension in single MHC IIA fibers from patients with cancer. When we evaluated MHC IIA/X hybrid fibers, the next most prevalent fast-twitch fiber phenotype, we found a similar relative reduction in isometric tension, although this difference did not reach statistical significance. In contrast, tension was maintained in MHC I fibers. Nonetheless, the
The magnitude of the reduction in tension in MHC IIA fibers (14%) suggests that a substantial proportion of the decrease in whole-muscle strength per unit muscle size in patients with cancer (25%) could be explained by impaired myofilament protein function.

What is the molecular mechanism underlying reduced tension in MHC II fibers? Results from sinusoidal analysis suggest a reduction in the number of strongly bound cross-bridges during maximal Ca\(^{2+}\) activation as a potential explanation. This is because single-fiber tension is proportional to the number of strongly bound cross-bridges and the force per cross-bridge (11, 27). More specifically, we found that the cross-bridge mechanical parameters \(B\) and \(C\), which reflect the number of strongly bound cross-bridges and cross-bridge stiffness (35), were reduced in MHC IIA fibers in patients with cancer compared with controls. Parameters that reflect the nonenzymatic, viscoelastic properties of the myofilaments (\(A\) and \(k\)), including cross-bridge stiffness, did not differ between groups, suggesting alterations in \(B\) and \(C\) were due to a reduction in the number of strongly bound myosin heads. A reduction in strongly bound cross-bridges could be explained by a reduction in myosin duty ratio (i.e., the relative amount of total myosin-actin cross-bridge cycle time that myosin is strongly bound to actin) secondary to: 1) a decrease in myosin attachment time; or 2) an increase in myosin detachment time; or 3) a combination of these. Regarding the former, myosin attachment time was unaltered in MHC IIA fibers in patients with cancer, indicating that alterations in the duty cycle would have to derive from changes in myosin detachment time. Because we did not assess myosin detachment time, we cannot formally test this possibility. However, there was a decrease (~11%), albeit nonsignificant, in the rate of myosin force production in MHC IIA fibers from patients with cancer, an index of cross-bridge kinetics that reflects the sum of the forward and reverse rate constants for the transition of myosin-actin cross-bridges from the weak to the strongly bound state (35, 64). Assuming that the reduction in this parameter reflects a decrease in the forward rate constant, myosin would spend less time in the strongly bound, force-producing state and, thus, more time in the weakly bound or detached state.

Another explanation for the reduced number of strongly bound cross-bridges is alterations in myofilament protein content; specifically, a loss of myosin, which has been suggested by results from preclinical models (1) and patients with cancer (19, 52). However, we found no evidence for myosin or actin protein loss in patients with cancer. It could be argued that we failed to observe a reduction in myosin protein content because we included noncachectic patients in our cohort, considering that prior studies evaluated cachectic animals/patients (1, 19, 52). However, we found no difference in myosin protein content between cachetic and noncachetic patients. A more likely explanation for our differing results is an incomplete solubilization of myosin during muscle tissue homogenization in prior studies, which recent reports have shown can lead to the spurious observation of myosin protein loss because only a small fraction of the myosin pool is sampled (14). In the present study, we utilized a buffer system that solubilizes the majority of myosin (14). Thus, our results argue against selective myosin loss as a characteristic of skeletal muscle in cachectic or noncachectic human patients with cancer. Parenthetically, because controls were slightly, albeit nonsignificantly, older than patients, one could argue that an age-related loss of myosin (15) might mask myosin depletion in the patients with cancer. However, we do not...
believe this is the case, because we have consistently not observed age-related changes in myosin content using methodology similar to that employed in the present study (55) (Miller et al. unpublished results).

Loss of MHC IIA tension was not the only myofilament functional alteration noted in patients with cancer. Cross-bridge kinetics were reduced in MHC I fibers, as evidenced by an increase in myosin attachment time. This result is in keeping with prior data from our laboratory showing increased myosin attachment time in human heart failure in both MHC I and IIA fibers (40), and in human aging in MHC IIA fibers (Miller et al. unpublished observations). The reason for the fiber type-specific nature of cross-bridge kinetic alterations in these different conditions is not clear, but data collectively suggest that reduced myosin-actin cross-bridge kinetics is a common phenotype in aging and acute/chronic disease. From a functional perspective, in the absence of changes in myosin detachment time, an increase in attachment time would increase the myosin duty ratio and, correspondingly, single-fiber tension. However, because we found no group differences in tension in MHC I fibers and no alterations in myosin protein content, there must be an equivalent percent increase in myosin detachment time and, in turn, total cycle time, leading to no change in the duty cycle or tension—a scenario consistent with circumstantial evidence supporting increased myosin detachment time in MHC IIA fibers detailed above. Parenthetically, although tension was unaltered, the increase in myosin attachment time may not be functionally benign. This is because we would predict that increased attachment time would decrease single fiber contractile velocity (46) and, accordingly, power output.

Patients with cancer were characterized by profound reductions in mitochondrial fractional density and average size, consistent with results from preclinical models (62). To our knowledge, however, our study is the first to demonstrate this phenomenon in human patients. More importantly, these decrements in mitochondrial fractional area correlated with increased myosin attachment time in MHC I fibers (Fig. 7), suggesting a link between mitochondrial and myofilament dysregulation. Although we recognize that cause-effect cannot be drawn from correlations, the novelty of this observation invites speculation. We acknowledge that because of the widely disparate clinical states of the two groups, these correlations may simply reflect the fact that deficits in aerobic metabolism and myofilament function develop/developed concurrently with cancer. Alternatively, reductions in mitochondria content/size may be a biomarker and/or an instigator of an intracellular milieu conducive to the development of myofilament contractile dysfunction. Prior studies have shown that increased oxidative stress, which has been documented in cancer and in response to cancer treatment (6, 23, 47), leads to mitochondrial fission and autophagy (16, 31). This may explain the mitochondrial rarefaction we observed in our patients. If an environment of increased oxidant activity exists in the skeletal muscle of patients with cancer, modification of myofilament proteins could occur, leading to contractile dysfunction (50). In support of such a scenario, studies have shown that exposure of skinned muscle fibers to reactive oxygen species reduced tension, cross-bridge kinetics, and the number of strongly bound cross-bridges, and increased cross-bridge cycle time (25, 45), effects that are generally consistent with our findings. In this context, oxidative modification of myofilament proteins is a tenable explanation for contractile adaptations in both fiber types in patients with cancer.
Together with the finding of reduced single fiber MHC IIA tension, our data support the hypothesis that diminished skeletal muscle myofilament protein function may contribute to decreased whole-muscle function and, in turn, physical disability in patients with cancer.

Several limitations to our study deserve discussion. First, our cancer population was heterogeneous with respect to cancer site. However, most patients had lung cancer \( (n = 8) \) and seven out of eight volunteers assessed for single-fiber function and cross-bridge kinetics were patients with lung cancer. Thus, our single-fiber and cross-bridge level measurements largely reflect the effects of lung cancer. Second, building on this last point, most patients with lung cancer are smokers and have some degree of lung pathology. Although none of the patients with lung cancer had a diagnosis of chronic pulmonary disease, we cannot exclude the possibility that some portion of the observed cancer phenotype is related to smoking, lung disease, or a combination of these factors. In severe lung disease, such as chronic obstructive pulmonary disease, knee extensor function is reduced, although it is controversial whether this reduction persists after adjustment for muscle size \( (59) \). Indeed, ex vivo analysis of skeletal muscle contractility found preserved muscle function in patients with chronic obstructive pulmonary disease \( (17) \). Similarly, reductions in muscle strength in smokers are generally believed to be minimal \( (7, 63) \). Nonetheless, further studies utilizing comorbidity-matched volunteers as controls will be required to address this issue. Third, we cannot discount the possibility that muscle disuse pursuant to the development of cancer contributed to the myofilament phenotype. Of note, all patients were ambulatory, but may have lowered their physical activity in response to cancer, treatments, or both. Although muscle disuse is well-known to cause contractile dysfunction, most studies have focused on complete disuse \( [\text{e.g., bedrest, immobilization (2)}] \). The effect of a reduction in weight-bearing activity on contractile function in ambulatory individuals is not known. Some studies have shown that imposition of catabolic stimuli worsens contractile dysfunction with bedrest \( (21) \). Thus, it is possible that the combination of reduced physical activity and the catabolic milieu present in patients with cancer may promote contractile dysfunction. Here again, use of a morbidity-matched control population and careful assessment of historical and current physical activity are needed to resolve this issue. Fourth, building on this last point, several of the patients received short courses of dexamethasone concurrent with their chemotherapy \( (1 \text{ to } 4 \text{ days for each cycle; average cycle length, } 19.8 \pm 2.8 \text{ d}) \) as antiemetic prophylaxis \( (n = 6) \). Studies suggest that \(~1 \text{ mo of glucocorticoids exacerbate the detrimental effects of muscle disuse on single-fiber function in humans (21), but the nature of the changes with respect to the functional characteristics affected and their fiber type specificity differed from the current results, arguing against an effect of dexamethasone on single-fiber function. Fifth, because all of our patients had advanced, stage III or IV cancer, results may not be generalizable to patients with less severe forms of cancer. However, because this was the first attempt to characterize the effects of cancer on skeletal muscle myofilament protein expression, structure, and function, we felt it prudent to study patients most likely to exhibit functional deficits. Further work will be needed to extend our observations to different points along the disease severity continuum. Finally, we are unable to define the unique effects of cancer vs. cancer treatment vs. cachexia on the myofilament functional phenotype. However, the phenotype did not differ by cachectic or treatment status, suggesting that our results are
likely a generalizable phenomenon of those factors associated with the clinical syndrome of cancer, rather than an effect of any one of these factors specifically.

In summary, our results show skeletal muscle contractile deficits in patients with cancer at the molecular, cellular, and tissue levels. At the molecular level, this is reflected in reduced myosin-actin cross-bridge formation and kinetics. Considering that contractile dysfunction scales up through various anatomical levels and that there are relationships among these deficits, our results suggest myofilament contractile dysfunction as a potential molecular mechanism underlying muscle weakness and increased rates of physical disability in human cancer. Delineation of the exact cause of the molecular functional deficits in patients with cancer, however, will require further study.

GRANTS

This study was funded by a grant from the University of Vermont College of Medicine. M.S.M. is funded by Mentored Research Scientist Development Award AG-031303, and D.M.C. is funded by Institutional National Research Service Award HL-007647.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: M.J.T., S.M.G., H.D.-T., M.E.C., and K.D. conceived and designed research; M.J.T., M.S.M., D.M.C., A.P.S., and I.N. performed experiments; M.J.T., M.S.M., and A.P.S. analyzed data; M.J.T., M.S.M., and D.M.C. interpreted results of experiments; M.J.T. prepared figures; M.J.T. drafted manuscript; M.J.T., M.S.M., D.M.C., A.P.S., I.N., S.M.G., H.D.-T., M.E.C., and K.D. edited and revised manuscript; M.J.T., M.S.M., D.M.C., A.P.S., I.N., S.M.G., H.D.-T., M.E.C., and K.D. approved final version of manuscript.

ACKNOWLEDGMENTS

We thank all the volunteers who dedicated their valuable time to these studies, and Alan Howard for his assistance with statistical analysis.

REFERENCES


**Figures and Tables**

**Table 1.**

Clinical characteristics of patients with cancer

<table>
<thead>
<tr>
<th>Cancer site (n)</th>
<th>All</th>
<th>Cachectic</th>
<th>Nonecachectic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Gastric</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
## Patients with cancer

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Cachectic</th>
<th>Noncachectic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head/Neck</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Stage (III/IV)</td>
<td>7/4</td>
<td>2/3</td>
<td>5/1</td>
</tr>
<tr>
<td>Histology (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Squamous-cell carcinoma</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Radiotherapy (n)</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Chemotherapy (n)</td>
<td>7</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>
Patients with cancer

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Cachectic</th>
<th>Noncachectic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinum-based</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Pemetrexed</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ECF</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Etoposide</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Dexamethasone (n)</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Weight loss (kg)</td>
<td>6.9 ± 2.2</td>
<td>14.9 ± 1.2</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>History of smoking (n)</td>
<td>9</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 2.

Physical characteristics in controls and patients with cancer

Data are mean ± SE. Platinum-based therapies include carboplatin \((n = 3)\) and cisplatin \((n = 3)\). ECF, epirubicin/cisplatin/5-fluorouracil. Doses for dexamethasone are provided in the *Subjects* section of the *METHODS*.
## Patients with cancer

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>All</th>
<th>Cachectic</th>
<th>Noncachectic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>74.5 ± 6.1</td>
<td>70.3 ± 5.4</td>
<td>60.1 ± 7.3</td>
<td>78.8 ± 6.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.3 ± 2.7</td>
<td>170.5 ± 3.1</td>
<td>168.3 ± 6.2</td>
<td>172.4 ± 2.9</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>21.9 ± 3.0</td>
<td>19.9 ± 3.7</td>
<td>11.6 ± 2.4</td>
<td>26.9 ± 5.1*</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>50.5 ± 3.9</td>
<td>48.9 ± 3.1</td>
<td>46.0 ± 5.9</td>
<td>51.3 ± 3.3</td>
</tr>
<tr>
<td>Leg fat-free mass (kg)</td>
<td>15.1 ± 1.5</td>
<td>14.8 ± 1.1</td>
<td>14.1 ± 2.1</td>
<td>15.4 ± 1.1</td>
</tr>
</tbody>
</table>

Data are mean ± SE.

*P < 0.05 group effect, where cachectic were less than noncachectic (*P < 0.05) and tended toward being less than controls (*P = 0.09).

Fig. 1.
Knee extensor isometric torque in controls and patients with cancer and its relationship to power output during a walking endurance test in patients with cancer. For group comparisons, knee extensor isometric torque data were adjusted for leg fat-free mass. Note that $n = 7$ for patients with cancer. Data are mean ± SE. *$P < 0.05$.

Fig. 2.
Skeletal muscle fiber morphology and myofilament ultrastructure in controls and patients with cancer. Representative images and average data are shown for muscle fiber cross-sectional area (A), myofilament fractional area (B), thick-to-thin filament ratio (C), and A-band length (D). Scale bars are 75 μm (A), 1 μm (B and D), and 100 nm (C). Sample sizes are n = 5 for controls and n = 10 for patients with cancer for all measures, except in A, where n = 4 for controls. Data are mean ± SE.

**Fig. 3.**
Skeletal muscle tissue homogenate myosin protein content and isoform distribution in controls ($n = 6$) and patients with cancer ($n = 10$). Representative gel images of bands for controls (C) and patients with cancer (Ca) are shown, with black lines demarcating where intervening lanes have been spliced out. Data are mean ± SE.

**Fig. 4.**

![Bar chart showing single skeletal muscle fiber maximal Ca$^{2+}$-activated (pCa 4.5) isometric tension in MHC I and MHC IIA fibers (25°C) in controls ($n = 6$; open bars) and patients with cancer ($n = 8$; closed bars). The number of fibers evaluated is at the base of each bar. Data are mean ± SE. *$P = 0.05$.](image)

**Fig. 5.**
Single skeletal muscle fiber maximal Ca\(^{2+}\)-activated (pCa 4.5) isometric tension in MHC IIA/X fibers (25°C) and relative proportion of MHC IIA and IIX isoform expressed in each fiber in controls \((n = 5)\) and patients with cancer \((n = 5)\). MHC IIA/X hybrids were not found in all volunteers, so the number of volunteers in each group and the total number of fibers (base of each bar, top) is reduced compared with MHC I and IIA fibers. Data are mean ± SE.

**Fig. 6.**
Sinusoidal analysis model parameters for maximal Ca\(^{2+}\)-activated (pCa 4.5) MHC I and IIA fibers in controls (\(n = 6\)) and patients with cancer (\(n = 8\)). The number of fibers studied is shown at the base of each bar on the panel showing myosin attachment time. Definitions for each variable are provided in METHODS. *\(P < 0.05\).

**Fig. 7.**
Skeletal muscle mitochondrial density and structure in controls ($n = 5$) and patients with cancer ($n = 10$), and the relationship of mitochondrial fractional area to cross-bridge kinetics. Representative images for controls and patients with cancer are shown for intermyofibrillar mitochondria (scale bar = 1 μm), along with group average data. Data are mean ± SE. *$P < 0.01$; **$P < 0.001$. Note that group differences in mitochondria number showed a strong trend ($P = 0.06$) toward differing between groups and that the correlation reflects $n = 8$ patients with cancer and $n = 5$ controls who were assessed for both single-fiber contractile function and mitochondrial density.

Articles from Journal of Applied Physiology are provided here courtesy of American Physiological Society