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Resistance Exercise Training Attenuates Wasting of the EDL Muscle in Mice Bearing the Colon-26 Adenocarcinoma

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Resistance Exercise Training Attenuates Wasting of the Extensor Digitorum Longus Muscle in Mice Bearing the Colon-26 Adenocarcinoma

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Progressive wasting of skeletal muscle is a significant side effect of malignancy. Perturbations in protein metabolism contribute to this state of wasting. Resistance exercise increases protein synthesis and mass of healthy muscles and counteracts muscle wasting associated with several catabolic conditions. It is not known whether resistance exercise training can counteract cancer-induced muscle wasting. This study examined the effect of resistance exercise training on muscle mass and protein content in 9 mice bearing the colon-26 adenocarcinoma. The dorsiflexor (extensor digitorum longus [EDL] and tibialis anterior) and plantar flexor (soleus, plantaris, and gastrocnemius) muscles of 1 leg of the tumor-bearing and the control mice were stimulated to contract eccentrically and concentrically, respectively, using an electrical stimulation protocol consisting of 10 sets of 6 repetitions per session. The muscles were stimulated on alternate days for a total of 8 sessions. The weight and protein content of the stimulated EDL muscle in the tumor-bearing mice were significantly higher (62% and 25%, respectively) than those of the nonstimulated EDL. Training did not have significant effects on the weight or protein content of the other muscles of the tumor-bearing mice, nor did it have significant effects on the muscles of the controls. These findings demonstrated that resistance training attenuated cancer-induced muscle wasting and protein depletion in the EDL muscle. The lack of an effect of the same training protocol on the EDL muscle in the control mice suggests that the amount and intensity of exercise training that is adequate to attenuate muscle wasting may not be adequate to induce hypertrophy of healthy muscles.

Key words: *Skeletal muscle wasting, cachexia, resistance exercise, electrical stimulation, colon-26 adenocarcinoma, animal models, mice*

Approximately 50% of cancer patients suffer from a wasting syndrome known as cancer cachexia (DeWys and others 1980; Tisdale 1997). Progressive loss of skeletal muscle is a common feature of this wasting syndrome (Argiles and Lopez-Soriano 1999). Loss of muscle tissue leads to asthenia, a condition of generalized weakness, which interferes with the patient's ability to engage in activities of daily living and thereby reduces quality of life. Severe wasting also correlates with reduced efficacy of anticancer therapy (Van Eys 1982; Tisdale 2000). Advanced muscle wasting may compromise respiratory (Tisdale 2000) and cardiac (Argiles and others 1999) functions. The degree of muscle wasting is inversely correlated with

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The authors acknowledge the support of the Oncology Nursing Society, Sigma Theta Tau International, Sigma Theta Tau, Beta Eta Chapter.

BIOLOGICAL RESEARCH FOR NURSING
Vol. 2, No. 3, January 2001, 155-166

the survival time of the affected person and always implies a poor prognosis (DeWys 1985). Therefore, interventions to preserve muscle mass have important clinical implications in terms of improving the prognosis and the quality of life of cachectic cancer patients.

Although the specific mechanisms that underlie cancer-induced muscle wasting still remain to be elucidated, current evidence suggests that the activation of pro-inflammatory cytokines and various proteolytic pathways, particularly the ubiquitin proteasome pathway, within the skeletal muscle may be responsible for the problem (Argiles and Lopez-Soriano 1999; Tisdale 2000). The activation of these factors leads to perturbations in muscle protein metabolism such that muscle protein degradation exceeds muscle protein synthesis, resulting in a net negative nitrogen balance in the skeletal muscle and, hence, muscle wasting.

Interventions that specifically counteract or halt the progression of skeletal muscle wasting in the tumor-bearing host have been difficult to identify. Measures such as aggressive nutritional support (McGeer and others 1990) and the administration of appetite stimulants (Kardinal and others 1990; Loprinzi, Michalak, and others 1993) have not been shown to attenuate muscle wasting in cachectic cancer patients. Although provision of excess calories increased body weight of patients with lung cancer (Evans and others 1985), the gained weight reflected water retention as evidenced by a transient fall in the concentration of serum albumin, a decrease in hematocrit, and signs of peripheral edema. Similarly, the administration of megestrol acetate (megace), an appetite stimulant, increased fat and water content of the body (Loprinzi, Michalak, and others 1993) but did not have significant effects on skeletal muscle mass (Loprinzi, Schaid, and others 1993). Therefore, the resultant weight gain is not functionally helpful and, thus, is of questionable benefit.

An intervention that may potentially attenuate the progression of muscle wasting during malignant tumor growth is resistance exercise training. Resistance exercise training, defined as multiple repetitions of static or dynamic muscular contractions performed against high load or resistance (Evans and others 1998), increases muscle mass in healthy humans (Staron and others 1990; Jurimae and others 1996) and in animal models (Wong and Booth 1988; Yarasheski and others 1990; Tamaki and others 1992; Caiozzo and others 1996; Baar and Esser 1999). More important, resistance exercise training attenuates muscle wasting that is associated with old age (Yarasheski and others 1993; Yarasheski and others 1999; Greiwe and others 2001), prolonged bed rest (Ferrando and others 1997), human immunodeficiency virus infection (Spence and others 1990; Wagner and others 1998; Sattler and others 1999), and hind-limb suspension (Kirby and others 1992; Diffee and others 1993; Linderman and others 1994).

To our knowledge, no previous reports have directly examined the relationship between resistance exercise training and muscle mass in the tumor-bearing host. Therefore, the purpose of this study was to investigate whether resistance exercise training would attenuate cancer-induced skeletal muscle wasting and the associated protein depletion in mice bearing the colon-26 (C-26) adenocarcinoma.

Method

Models

Tumor model. The murine C-26 adenocarcinoma, generously provided by Dr. Tanaka Yutaka, was used to induce muscle wasting in mice. This tumor cell line has been shown to induce significant cachexia and muscle wasting in mice without significant changes in food intake (Tanaka and others 1990). Accordingly, the wasting induced by this tumor is not attributed to anorexia and depression in food intake. The growth of the C-26 for 17 days has been shown to induce significant wasting of the gastrocnemius (gastroc) and extensor digitorum longus (EDL) muscles (Fujita and others 1996). Therefore, the C-26 is suitable for studying cancer-induced muscle wasting.

Resistance exercise model. Resistance exercise training of the leg muscles was performed via electrical stimulation of the motor (sciatic) nerve. This model was originally developed for the rat by Wong and Booth (1988) and later modified by Baar and Esser (1999). Electrical stimulation of the motor nerve induced simultaneous eccentric (lengthening) contractions in the dorsiflexor muscles (tibialis anterior [TA] and EDL) and concentric (shortening) contractions in the plantar flexor muscles (soleus [SO],

plantaris [PL], and gastroc). Because of this antagonistic muscle tension, the amount of resistance imposed on each muscle group was equivalent to the force produced by the opposing muscle group. Because the force produced by the larger plantar flexors was approximately 4 times as much as the force produced by the relatively smaller dorsiflexors (Wong and Booth 1988), the dorsiflexors experienced a greater loading effect than the plantar flexors, which resulted in a net plantar flexion of the ankle (Fig. 1).

Experimental Procedure

All experimental procedures were approved by the Research Animal Committee at the University of Wisconsin–Madison. Eighteen pathogen-free 7- to 8-week-old (18 to 22 g) female CD2F1 (BALB/c X DBA/2) mice (Harlan Sprague Dawley Inc., Madison, WI) were housed in individual standard cages in the Animal Care Facility of the Medical School at the University of Wisconsin–Madison. The mice were allowed free access to standard rodent chow and water throughout the study period and were maintained at 24 °C with 12:12-hour dark:light cycle. Mice were allowed a 2-day period to acclimate to their new environment before the initiation of the experimental procedures. Body weight and food intake for each animal were recorded on alternate days.

Surgical implantation of electrodes. On day 0 of the experiment, stimulating electrodes were surgically implanted in the hind limbs of each mouse following the method of Baar and Esser (1999) for electrode implantation in rats. To standardize all experimental conditions for both legs, except for the electrical stimulation, the electrodes were implanted in the experimental (right) and the nonexperimental (left) legs of the mice. In preparation for the surgery, mice were anesthetized using Fentanyl (0.03 mg/kg) subcutaneously and Etomidate (30 mg/kg) interperitoneally. A subcutaneous injection of buprenorhine (0.01 mg/kg) was given to alleviate postsurgical pain. The experimental leg was shaved free of hair and a small incision (≈1-cm) was made on the lateral aspect of the thigh. The sciatic nerve was exposed and 2 stimulating electrodes made of multistrand stainless steel Teflon-coated wire (0.009-mm diameter, Medwire, Mount Vernon, NY) were sutured using a 7.0 suture on either side of the nerve above its point of trifurcation. This position of the electrodes ensured simultaneous contractions of the dorsiflexor and plantar flexor muscles. The wire connecting the electrodes formed a loop that was tunneled subcutaneously and exited the body of the mouse at the base of the neck where it was sutured under the skin. The loop was then cut to form 2 pieces of wire, which served as the internal electrodes. Incisions in the fascia and skin were closed using a 6.0 suture. An identical surgical procedure was performed on the nonexperimental leg except that the loop connecting the 2 electrodes was cut below the fascia. Surgeries were performed under sterile conditions.

Tumor inoculation. The C-26 cells were cultured in vitro with RPMI 1640 supplemented with 10% fetal calf serum and 1% penicillin/streptomycin (Mediatech, Herndon, VA). Tumor cells were trypsinized at a subconfluent state and suspended in Hanks's balanced salt solution at a concentration of 2.5×10^6 cells/ml and were then immediately injected into the animals as described in the tumor inoculation procedure.

Five to 6 days following the surgical implantation of the electrodes, mice were weight matched and assigned into 2 groups of similar mean body weight. Mice in the tumor-bearing group ($n = 9$) were inoculated subcutaneously between the scapulae, each with a 0.2-ml cell suspension containing 5.0×10^5 cells. Mice in the control group ($n = 9$) underwent the same procedure but were inoculated with 0.2-ml phosphate-buffered saline. Mice were put back in their cages and were allowed to rest for 24 hours before the exercise training was initiated.

Electrical stimulation protocol. Before each training session, mice were lightly anesthetized using isoflurane inhalation anesthesia. The anesthetized mouse was secured in a prone position on a Plexiglas platform and the experimental leg was allowed to move freely through a hole in the platform. The internal electrodes at the base of the neck were then connected to a SD-5 stimulator, kindly provided by Dr. Karen Esser of the School of Kinesiology at the University of Illinois at Chicago. The voltage (4 to 16 V) applied to the electrodes was adjusted to produce maximal palpable contractile force. Tetanic muscle contractions were induced with 1-ms pulses at 100 Hz because at this frequency all motor units (slow and

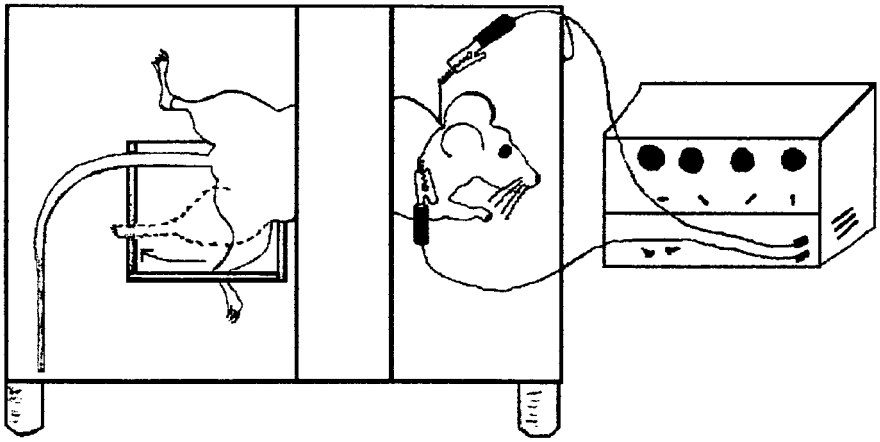

Figure 1. Plantar flexion of the ankle during muscle contractions.

fast) are recruited (Nagaraj and others 2000). Each repetition of muscle contraction lasted 3 seconds and was followed by a 10-second rest period. An additional 50-second rest period was allowed following the 6th repetition. This pattern of stimulation was repeated for a total of 10 sets of 6 repetitions per session, resulting in a total of 60 contractions with 180 seconds of actual contraction time. The stimulation protocol was repeated for a total of 8 sessions, which were performed on alternate days.

Sacrifice and muscle dissection. Forty-eight hours following the final training session, mice were anesthetized using Fentanyl (0.03 mg/kg) subcutaneously and Etomidate (30 mg/kg) interperitoneally and the stimulated and contralateral nonstimulated dorsiflexor (TA and EDL) and plantar flexor (SO, PL, and gastroc) muscles were carefully dissected out. Each muscle was trimmed of external fat and connective tissue, blotted of excess moisture, quick frozen in liquid nitrogen, and weighed on a digital scale. Muscles were stored individually in a –70 °C freezer for later analysis of protein concentration. Mice were euthanatized by cervical dislocation.

Tissue homogenization and protein assay. Muscles were homogenized in a 50-mM Tris-HCl buffer containing 0.25 mM sucrose, 5 mM EDTA, and 1% (w/v) SDS. Protease inhibitors were prepared in 1000-fold concentration cocktails and added to the homogenization buffer just before homogenization. Protease inhibitors consisted of 5 mM *N*-ethylmaleimide, 1 μM pepstatin, 2.63 μM leupeptin, 0.1 trypsin inhibitor unit/ml aprotinin, 1 mM PMSF (phenylmethylsulfonyl fluoride) AEBSF ([4-(2-Aminoethyl) benzenesulfonyl fluoride, HCL]), and 1 mM benzamidin. All reagents were obtained from Calbiochem (San Diego, CA). Muscles were individually homogenized on ice in 300-μl ice-cold homogenization buffer using a Power Gen 700D tissue homogenizer (Fisher Scientific) set at 18,000 rpm. Muscle homogenates were then centrifuged in an Alegra™ 6R centrifuge (Beckman) at 3500 rpm (2800xg) for 15 minutes at 4 °C. Supernatants were quickly aliquoted in 50-μl volumes, which were stored in a –80 °C freezer.

Muscle protein concentration. Muscle protein concentration (μg/ml of muscle supernatant) for each muscle was determined in 100-μl sample of the muscle's supernatant using the Pierce BCA protein assay (Pierce, Rockford, IL) with bovine serum albumen as a standard. Samples were read at 562-nm wavelength using a Bio Spec-1601 ultraviolet-visible spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD). Because each muscle homogenate contained the whole muscle, protein content (mg/entire muscle) was calculated as muscle protein concentration multiplied by the total amounts of muscle homogenate for each muscle.

Data analysis. Statistical tests were performed using the SPSS/PC+ statistical package (version 9.0).

A significance level of 0.05 was used for all analyses. Between- and within-group differences were analyzed using repeated-measures multivariate analyses of variance (RM-MANOVA). Significant interactions were decomposed using planned *t*-test comparisons.

Results

Effect of Tumor Growth on Body Weight

Changes in body weight in the tumor-bearing and the non-tumor-bearing control mice throughout the study period are depicted in Figure 2. At the time of tumor inoculation, mean (*SE*) body weights for the tumor-bearing and the control mice were 19.2 g (0.54) and 19.7 g (0.55), respectively. An RM-MANOVA using Group (tumor/no tumor) as the between-subjects variable and time as the within-subjects variable revealed significant main effects for group, $F(1, 16) = 5.23$, $P < 0.05$, and time, $F(5, 12) = 49.61$, $P < 0.001$, and a significant Group × Time interaction, $F(6, 12) = 60.29$, $P < 0.001$. Over the course of the study, weights of mice in the tumor-bearing group dropped whereas weights of the controls increased slightly. On day 15 post–tumor inoculation, mean body weight of the tumor-bearing mice was significantly lower than that of the controls ($t = –2.39$, $P < 0.05$). At sacrifice, on day 17 post–tumor inoculation, the average body weight in the tumor-bearing mice was 10% lower than that in the control mice ($t = –3.11$, $P < 0.01$). Carcass weight (body weight minus tumor weight) in the tumor-bearing mice was 20% lower than the carcass weight in the control mice ($t = –5.46$, $P < 0.001$). Mean (*SE*) tumor weight was 1.94 g (0.11), representing 10.5% of body weight at the time of sacrifice.

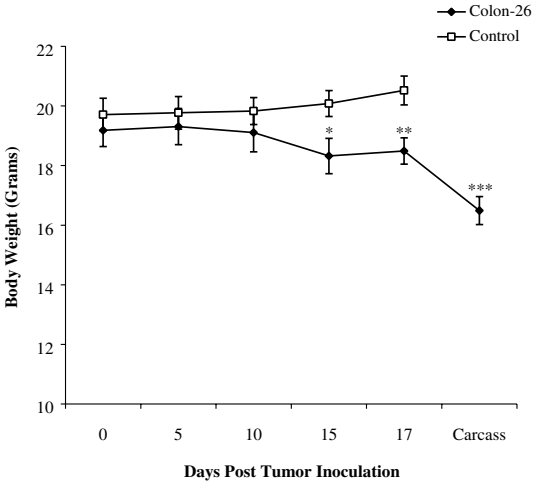

Figure 2. Changes in body and carcass weights in colon-26 (C-26) mice compared to age-matched control mice. Group means for body weight were similar on tumor inoculation (day 0). Mean body and carcass weights at sacrifice (day 17) were significantly lower in the C-26 group ($P < 0.05$). Carcass weight is body weight at sacrifice minus the weight of the tumor. $n = 9$/group. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

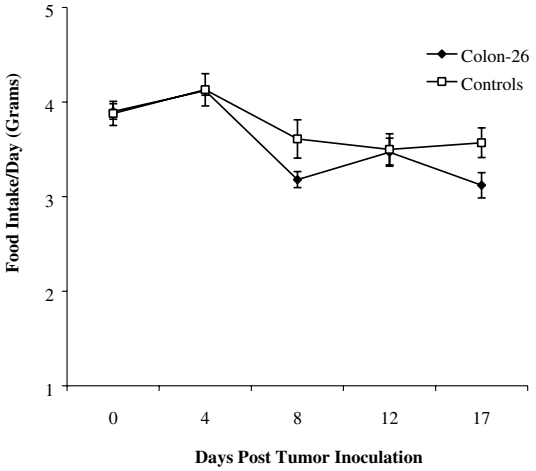

Figure 3. Food intake in colon-26 (C-26) mice compared to age-matched control mice. No significant difference in mean food intake between the C-26 and control mice at any point during the study period. $n = 9$/group.

Food Intake

Food intake for the tumor-bearing and control mice is depicted in Figure 3. There was no significant main effect for group, $F(1, 16) = 4.38$, *ns*, and no significant Group × Time interaction, $F(4, 13) = 0.785$, *ns*. Grams of food consumed per day were not significantly different between the 2 groups at any time point during tumor growth. Mean (*SE*) total food intake consumed by the tumor-bearing and control animals throughout the study were 17.85 g (0.15) and 18.69 g (0.38), respectively ($t = –2.09$, *ns*)

Table 1. Absolute Weights (mg) of Nonstimulated Muscles in Colon-26 Mice Compared to Age-Matched Control Mice

Muscle	Colon-26 (n = 9)		Controls (n = 9)		Percentage Decrease in Wet Weight
EDL	6.5	(0.68)	8.8	(1.21)	26
TA	27.0	(1.32)	33.7	(2.19)	20*
PL	10.0	(0.81)	11.2	(1.27)	11
SO	6.4	(0.78)	6.9	(0.58)	7
Gastroc	56.0	(6.02)	88.8	(4.33)	37**

NOTE: Values are expressed as mean ± *SE*. Between-group comparisons are by 2-tailed independent-samples Student's *t* test with a significance level of 0.05. EDL = extensor digitorum longus, TA = tibialis anterior, PL = plantaris, SO = soleus, gastroc = gastrocnemius.
*$P < 0.05$. **$P < 0.01$.

Effect of Tumor Growth on Muscle Weight

The effect of the C-26 adenocarcinoma on the absolute weight of the nonstimulated dorsiflexor and plantar flexor muscles is presented in Table 1. There was a significant main effect for group, $F(1, 16) = 16.44$, $P < 0.01$, and a significant Muscle × Group interaction, $F(4, 13) = 5.45$, $P < 0.01$. All of the 5 nonstimulated muscles in the tumor-bearing mice were smaller than the nonstimulated corresponding muscles in the control mice. However, these differences were significant for only the TA ($t = –2.63$, $P < 0.02$) and the gastroc ($t = –4.41$, $P < 0.001$) muscles. Although the EDL muscle in the tumor-bearing mice weighed 26% less than that in the controls, this difference did not reach statistical significance.

Effect of Training on Muscle Weight

The wet weights of the stimulated and nonstimulated dorsiflexor and plantar flexor muscles in the tumor-bearing and the control mice are reported in Table 2. A 3-way (Group × Muscle × Training) RM-MANOVA using the tumor-bearing and the control mice and the absolute weights of the stimulated and the nonstimulated contralateral muscles revealed a significant main effect for training, $F(1, 16) = 5.85$, $P < 0.05$, and a significant main effect for group, $F(1, 16) = 13.91$, $P < 0.005$. There was also a significant Muscle × Group interaction, $F(4, 13) = 5.18$, $P < 0.02$, as well as a significant Training × Group interaction, $F(1, 16) = 5.84$, $P < 0.05$.

The Muscle × Group interaction indicates that there were differences in muscle weights between the 2 groups of mice. The Training × Group interaction shows that the training-induced change in muscle weight differed between the tumor-bearing and the control mice. To determine what accounted for the significant interactions, separate 2-way (Muscle × Training) RM-MANOVAs were computed for each group of mice. In the tumor-bearing mice, there was a significant main effect for training, $F(1, 8) = 7.79$, $P < 0.05$, and a significant Muscle × Training interaction, $F(4, 5) = 5.27$, $P < 0.05$. All of the stimulated muscles in the tumor-bearing mice had higher average weights than their corresponding nonstimulated contralateral muscles. However, this difference was statistically significant for the EDL muscle only ($t = –2.85$, $P < 0.05$). The stimulated EDL muscle in the tumor-bearing mice was 62% heavier than the nonstimulated contralateral EDL muscle (Fig. 4). Similarly, the relative weight (muscle weight/body weight × 100) of the stimulated EDL muscle in the tumor-bearing mice was significantly higher ($t = 2.71$, $P < 0.05$) than that of the contralateral muscle (Fig. 5). Among the control mice, neither the main effect for training, $F(1, 8) = 0.038$, *ns*, nor the Training × Muscle interaction, $F(4, 5) = 0.071$, *ns*, was significant, indicating a lack of training effect on muscle weight in the control mice.

Effect of Electrical Stimulation on Muscle Protein Concentration and Content

Because the experimental EDL muscle in the tumor-bearing mice was the only muscle that resisted cancer-induced wasting, protein concentration and protein content of this muscle were considered in the analyses. Protein concentration did not differ between the exercised and the nonexercised EDL muscle. However, protein content of the exercised EDL was significantly ($t = 2.398$, $P < 0.05$) higher (25% higher) than that of the nonstimulated EDL muscle (Fig. 6).

Summary and Discussion

A mouse model of cancer cachexia was used to test the hypothesis that resistance training in the form of

Table 2. Absolute Weights (mg) of Stimulated and Contralateral Muscles in Colon-26 Mice Compared to Age-Matched Control Mice

	Colon-26 (n = 9)					Controls (n = 9)				
Muscle	Stimulated		Contralateral		Percent Δ	Stimulated		Contralateral		Percent Δ
EDL	10.5	(1.30)	6.5	(0.68)	+62*	9.4	(0.81)	8.8	(1.21)	+7
TA	29.5	(1.76)	27	(1.32)	+9	34.9	(1.70)	33.7	(2.19)	+4
PL	11.4	(0.72)	10	(0.81)	+14	11.6	(0.68)	11.2	(1.27)	+4
SO	6.6	(0.85)	6.4	(2.35)	+3	7.7	(0.64)	6.9	(0.58)	+12
Gastroc	67.5	(4.34)	56	(6.02)	+21	86.7	(4.36)	88.8	(4.33)	–2

NOTE: Values are expressed as mean ± *SE*. EDL = extensor digitorum longus, TA = tibialis anterior, PL = plantaris, SO = soleus, gastroc = gastrocnemius, + = increase, – = decrease.
*Significantly different from the contralateral by 2-tailed paired-samples Student's *t* test with a significance level of 0.05.

electrical stimulation of the motor nerve would attenuate cancer-related wasting in the contracting muscles. The inoculation of C-26 adenocarcinoma cells in mice resulted in significant loss of body weight by day 15 of tumor growth without significant changes in food intake. These findings are consistent with data reported by others (Tanaka and others 1990; Fujita and others 1996; Matsumoto and others 1999). At sacrifice, mice in the current study lost 10% of their body weight compared to only 6% in the study by Fujita and others (1996). This discrepancy in the magnitude of weight loss may be explained by the difference in final tumor mass between the 2 studies. Tumor mass in the current study represented 10.5% of total body weight compared to only 4% in the study by Fujita and others (1996). Based on these findings, it appears that the degree of weight loss in mice bearing the C-26 adenocarcinoma may be proportional to the size of the tumor.

At sacrifice on day 17 post–tumor inoculation, tumor-bearing mice showed significant wasting of TA and gastroc muscles only. Although the EDL muscle in the tumor-bearing mice weighed 26% less than that in the control mice, this difference did not reach statistical significance.

Resistance exercise training was performed via electrical stimulation of the motor nerve in the hind limb. The stimulation protocol resulted in simultaneous contractions of the dorsiflexor and plantar flexor muscles, which underwent eccentric and concentric contractions, respectively. Because the plantar flexors were larger than the dorsiflexors, the dorsiflexors would have contracted against higher resistance compared to the plantar flexors. Although load experienced by each muscle group was not measured in the present study, Wong and Booth (1988) reported that the force produced by the plantar flexors was approximately 4 times as much as the force produced by the relatively smaller dorsiflexors. This indicates that the dorsiflexors experienced a loading effect that was 4 times as heavy as that experienced by the plantar flexors. Muscles contracting against higher resistance (higher load) have been shown to undergo greater hypertrophy than muscles contracting against lower resistance (lighter load). For example, in rats, a significant hypertrophy was observed in the gastroc muscle that contracted against an external load but not in the gastroc muscle that underwent the same training protocol but contracted against no external load (Wong and Booth 1988). In the present study, the dorsiflexors, the TA and its synergistic EDL, would have contracted against relatively greater resistance and were expected to show the most significant training-induced attenuation of wasting. It was found, however, that the EDL in the tumor-bearing mice but not in the control mice was the only muscle that underwent significant attenuation of wasting in response to the exercise.

In contrast, protein depletion in the exercised EDL muscle was attenuated by only 25%. This attenuation of protein depletion is relatively small compared to the 62% attenuation of muscle wasting. The discrepancy between muscle weight and muscle protein content could have resulted from the fact that muscle protein concentration was determined in muscle supernatant as opposed to muscle homogenate. The centrifugation process to separate the supernatant could have resulted in a loss of most myofibrillar proteins in the pellet.

In the present study, we did not observe a change in the weight of the TA in response to the electrical stimu-

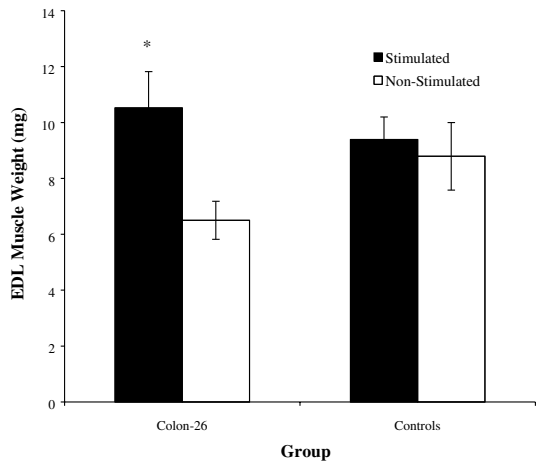

Figure 4. Absolute weight (mg) of stimulated and contralateral extensor digitorum longus (EDL) muscle in colon-26 mice compared to age-matched control mice. *n* = 9/group. *Significantly different from contralateral by 2-tailed paired-samples Student's *t* test with a significance level of < 0.005.

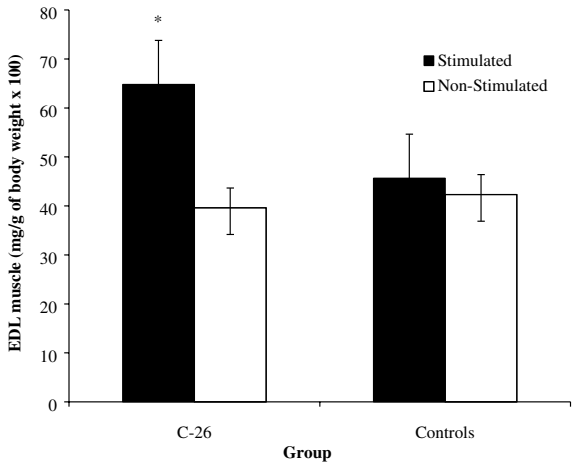

Figure 5. Percentage difference in the relative weight (muscle weight/body weight × 100) between stimulated and nonstimulated extensor digitorum longus (EDL) muscle relative to body weight. Relative weight of stimulated EDL muscle from the colon-26 (C-26) mice was 64% higher than the contralateral EDL by paired-samples Student's *t* test. *n* = 9/group. *$P < 0.03$.

lation protocol. Others, using an electrical stimulation protocol with rats similar to the one used in the present study with mice, have reported a 14% to 17% increase in the weight of the TA of healthy rats (Wong and Booth 1988; Baar and Esser 1999). This lack of training effect on the TA muscle may be due to the use of mice as opposed to rats. The mice have relatively smaller motor neurons, which could have been easily damaged by the repeated stimulation. If such damage occurred, not all muscle fibers would have been recruited to contract. Second, mice in this study were trained for only 8 sessions as opposed to at least 12 in previous research. Training was terminated following the 8th session because mice in the tumor-bearing group had lost a significant amount of weight by the day of the 8th session, which increased the risk of animal loss due to increased morbidity. Evidence suggests that training-induced muscle hypertrophy is greater with higher numbers of training sessions. For example, cross-sectional area of elbow flexors in humans increased by 8% following 8 weeks and by 23% after 14 weeks of resistance training (Ikai and Funkunaga 1970). Similarly, the weight of the rat TA muscle increased by 17% (Wong and Booth 1988) and 14% (Baar and Esser 1999) following 32 and 12 sessions of resistance training, respectively.

Finally, the stimulation protocol employed in this study allowed 1 day of rest between training sessions as opposed to 2 or 3 days in previous research. It is generally accepted that rest periods of 2 to 3 days between training sessions provide the muscle with the necessary time for regeneration and growth (Wong and Booth 1988; Booth and Thomason 1991) and, therefore, are presumably more efficient in terms of enhancing the effectiveness of exercise on muscle mass. Wong and Booth (1988) reported a significant increase of 16% in the weight of the stimulated over the nonstimulated TA muscle in rats that had 2 to 3 days of rest between training sessions. The lack of significant changes in the absolute or the relative weights of the plantar flexor muscles in response to training is consistent with previous research on healthy rats (Wong and Booth 1988; Baar and Esser 1999). As explained earlier, the plantar flexors (SO, gastroc, and PL) contracted against lower resistance compared to the dorsiflexors. This difference in the amount of resistance might have contributed to the different responses between the 2 muscle groups. Wong and Booth (1988) reported a hypertrophy that ranged between 13% and 18% in the plantar flexor muscles that contracted against greater resistance (external load) compared to no change in the weights of the plantar flexors that underwent the same training protocol but contracted

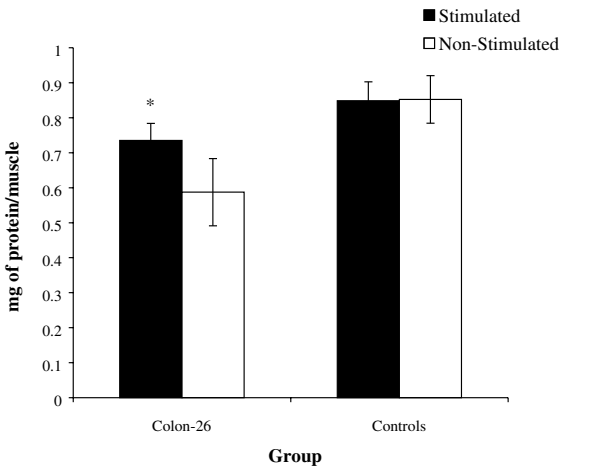

Figure 6. Protein content (mg/entire muscle) of stimulated and contralateral extensor digitorum longus muscle in colon-26 mice compared to age-matched control mice. *n* = 9/group. *Significantly different from contralateral by 2-tailed paired-samples Student's *t* test with a significance level of < 0.05.

against lower resistance (no external load). This clearly indicates that the amount of resistance imposed on the muscles is critical to muscle hypertrophy. This very same notion may be used to explain the increase in the mass of the EDL muscle but not that of the TA muscle in this study. The EDL is a relatively smaller muscle compared to the TA. Accordingly, it could be argued that the EDL muscle contracted against larger resistance relative to its weight compared to the TA muscle. This potential difference in the amount of resistance against which the 2 muscles contracted could have contributed to the different response by the 2 muscles.

In addition to the greater resistance against which the dorsiflexors contracted, these muscles underwent eccentric compared to concentric contractions in the plantar flexors. It is generally accepted that the tension generated by muscles during eccentric contractions is greater than that generated during concentric contractions (Hawkins and others 1999). If the amount of tension were a key variable for increasing muscle protein synthesis, then muscles that perform eccentric contractions would undergo greater hypertrophy compared to muscles that perform concentric contractions, provided that muscle protein degradation rate is similar for the 2 types of contractions. Wong and Booth (1990) found that relative to nonexercised controls, myofibrillar protein synthesis rate in the TA muscle (54%-56%) that underwent eccentric contractions was significantly higher than that in the gastroc muscle (38%) that underwent concentric contractions. Additionally, protein synthesis rate in the eccentrically contracted TA but not in the concentrically contracted gastrocnemius muscle continued to be higher than that in the nonstimulated control muscles at 36 to 41 hours following an acute stimulation bout (Wong and Booth 1990). Consistent with these findings, Baar and Esser (1999) reported a significant increase in the phosphorylation of $p70^{S6k}$, a regulatory protein involved in protein synthesis, in the eccentrically contracted TA and EDL but not in the concentrically contracted SO. Although the phosphorylation of $p70^{S6k}$ was significant in the concentrically contracted PL muscle, this phosphorylation was less potent than that of the EDL and TA. Taken together, these findings suggest that compared to concentric contractions, eccentric contractions result in greater and more prolonged increases in muscle protein synthesis rates. This elevated protein synthesis rate may explain the hypertrophy observed in the eccentrically contracted but not the concentrically contracted muscles in the same set of animals.

Although the training protocol used in this study significantly attenuated the wasting of the EDL muscle in the tumor-bearing mice, it did not have a significant effect on the EDL muscle of the control mice. This finding suggests that the amount and intensity of exercise that was adequate to attenuate muscle wasting may not have been adequate to cause hypertrophy of healthy muscles. There is data to suggest that wasted muscles respond differently than nonwasted muscles to exercise training. For example, patients with HIV who have more wasting tend to gain more lean muscle mass in response to resistance training compared to patients with less wasting (Roubenoff and others 1999). Also, as few as 5 sessions of resistance training attenuated wasting of the SO muscle by 74% in hindlimb suspended rats (Kirby and others 1992). Five sessions of resistance training is unlikely to cause hypertrophy of nonsuspended, nonwasted muscles. Similarly, Farrell and others (1999) reported significant attenuation of the wasting of the SO and gastroc muscles in diabetic rats in response to a resistance exercise protocol consisting of 50 repetitions per session, 3 sessions per week, for a total of 24 sessions. The same ex-

ercise protocol did not cause significant hypertrophy of the same muscles in the control nondiabetic rats. In addition, endurance training that does not necessarily increase the mass of healthy muscles (Holloszy and Booth 1976) attenuates muscle wasting in hind-limb suspended rats (Thomason and others 1987; Hauschka and others 1988; Graham and others 1989; Norman and others 2000). Accordingly, it appears that attenuation of muscle wasting may be easier to achieve than inducing hypertrophy of healthy nonwasted muscles, which suggests that persons with wasted muscle may not need to exercise as vigorously in order to attenuate wasting of their muscles.

In conclusion, to the best of our knowledge, this is the first study to directly examine the effect of resistance training on cancer-related muscle wasting. Results of this study demonstrate that 8 sessions of resistance training significantly attenuate wasting of the EDL muscle in tumor-bearing mice. This attenuation of wasting was paralleled with an increase in muscle protein content, suggesting that the increase in muscle weight was due to an increase in the actual mass of the muscle and not merely due to edema. Therefore, our hypothesis that resistance exercise training would attenuate muscle wasting during malignant tumor growth was supported. Findings of this study also suggest that the dose of training that attenuates the wasting of the EDL muscle in the tumor-bearing mice is not sufficient to induce hypertrophy in the EDL muscle in the control non-tumor-bearing mice.

In addition to replicating this study on a larger scale, future research should attempt to examine the potential mechanisms that might have contributed to the training-induced attenuation of muscle wasting during the tumor-bearing state. For muscle weight to increase, muscle protein synthesis rate must exceed the muscle protein degradation rate. Whether the exercise-induced attenuation of muscle wasting was a result of an increase in muscle protein synthesis or a decrease in muscle protein breakdown needs to be explored in future research. There is increasing evidence to support the hypothesis that the accelerated muscle proteolysis during catabolic states (Mitch and Goldberg 1996) including cancer (Llovera and others 1994; Llovera and others 1995) is mediated via the activation of the ubiquitin proteasome pathway within skeletal muscle. In addition, tumor necrosis factor α has also been shown to be involved in the induction of cancer-induced muscle wasting. It would be important to examine whether the training-induced muscle-sparing effect was related to changes in the activities of the ubiquitin proteasome pathway and/or tumor necrosis factor α within the contracting muscles.

Future research should examine the functional benefit of training-induced attenuation of cancer-related muscle wasting. A major problem associated with muscle wasting is muscle weakness, which increases the overall morbidity from cancer. It would be particularly important to examine whether the muscle-sparing effect of training is paralleled with an increase in muscle strength. Findings from such research may provide further evidence to support a beneficial role of resistance exercise training for cancer-related muscle wasting.

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