Exercise training during chemotherapy preserves skeletal muscle fiber area, capillarization, and mitochondrial content in patients with breast cancer

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ABSTRACT: Exercise has been suggested to ameliorate the detrimental effects of chemotherapy on skeletal muscle. The aim of this study was to compare the effects of different exercise regimens with usual care on skeletal muscle morphology and mitochondrial markers in patients being treated with chemotherapy for breast cancer. Specifically, we compared moderate-intensity aerobic training combined with high-intensity interval training (AT-HIIT) and resistance training combined with high-intensity interval training (RT-HIIT) with usual care (UC). Resting skeletal muscle biopsies were obtained pre- and postintervention from 23 randomly selected women from the OptiTrain breast cancer trial who underwent RT-HIIT, AT-HIIT, or UC for 16 wk. Over the intervention, citrate synthase activity, muscle fiber cross-sectional area, capillaries per fiber, and myosin heavy chain isoform type I were reduced in UC, whereas RT-HIIT and AT-HIIT were able to counteract these declines. AT-HIIT promoted up-regulation of the electron transport chain protein levels vs. UC. RT-HIIT favored satellite cell count vs. UC and AT-HIIT. There was a significant association between change in citrate synthase activity and self-reported fatigue. AT-HIIT and RT-HIIT maintained or improved markers of skeletal muscle function compared with the declines found in the UC group, indicating a sustained trainability in addition to the preservation of skeletal muscle structural and metabolic characteristics during chemotherapy. These findings highlight the importance of supervised exercise programs for patients with breast cancer during chemotherapy.—Mijwel, S., Cardinale, D. A., Norrbom, J., Chapman, M., Ivarsson, N., Wengström, Y., Sundberg, C. J., Rundqvist, H. Exercise training during chemotherapy preserves skeletal muscle fiber area, capillarization, and mitochondrial content in patients with breast cancer. FASEB J. 32, 5495–5505 (2018). www.fasebj.org

KEY WORDS: concurrent training · high-intensity interval training · oxidative phosphorylation · exercise training adaptation

Chemotherapy is commonly used as an adjuvant treatment strategy for primary breast cancer and provides significant survival benefits (1). The most frequently used regimens contain anthracyclines and/or taxanes. On the cellular level, anthracyclines lead to free radical generation; this effect induces DNA damage that may also affect noncancerous tissues, including skeletal muscle (2). Preclinical studies indicate that anthracyclines lead to skeletal muscle atrophy (3), muscle dysfunction, low satellite cell (SC) number, and reduced muscle motor innervation (4), likely contributing to overall muscle weakness (5). Moreover, other preclinical reports have demonstrated that anthracyclines disrupt energy metabolism by inhibiting the oxidative phosphorylation (oxphos) system in isolated heart and liver tissue and in tumors (6). Preclinical

ABBREVIATIONS: 1-RM, 1 repetition maximum; AT, aerobic training; AT-HIIT, aerobic training combined with high-intensity interval training; CS, citrate synthase; CSA, cross-sectional area; HIIT, high-intensity interval training; MHC, myosin heavy chain; oxphos, oxidative phosphorylation; RT, resistance training; RT-HIIT, resistance training combined with high-intensity interval training; SC, satellite cell; UC, usual care

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studies addressing the effects of chemotherapy on skeletal muscle mitochondria are few (7, 8), and data from humans are lacking. Taxanes reduce tumor cell growth through disruption of microtubule function; however, myofibrillogenesis has not been shown to be impaired in myocytes treated with taxanes (9). Moreover, unlike anthracycline, the effects of taxane on rodent skeletal muscle have not been shown to impair force production (10).

Chemotherapy for breast cancer is often accompanied by prolonged high-dose corticosteroid treatment, which can cause muscle atrophy and mitochondrial dysfunction (11). Furthermore, loss of skeletal muscle mass has been linked to increased toxicity (i.e., poor tolerance to chemotherapy) and leads to a worse prognosis (12). Despite the observed declines in overall muscle function, little is known regarding the effects of chemotherapy on skeletal muscle in weight-stable (noncachectic) patients with cancer. A large body of evidence has shown that, in healthy individuals, skeletal muscle adaptation to exercise training includes improvements in muscle strength, fiber area (13), markers of mitochondrial content and function, and capillarization (14). In patients with breast cancer, physical exercise counteracts the loss of muscle strength experienced after treatment (15). One training modality that induces significant muscle adaptation and health benefits in a time-efficient manner is high-intensity interval training (HIIT) (16), which has been trialed safely in patients with breast cancer (17), including in the current trial during chemotherapy (18).

Despite the increasing number of studies showing the ability of exercise training to improve or maintain muscle strength and cardiorespiratory fitness in patients with cancer during chemotherapy (15), only 2 randomized, controlled exercise trials including resistance training (RT) for patients with prostate (19) and germ cell cancer (20, 21) have investigated the molecular adaptations expected to occur with exercise training. Molecular musculoskeletal responses, including mitochondrial markers to exercise training in patients with breast cancer and adaptations to different exercise modalities during chemotherapy, have not been studied.

We recently found beneficial effects on lower limb muscular strength and aerobic fitness in the 16 wk OptiTrain RCT intervention comparing moderate-intensity aerobic training (AT) combined with HIIT (AT-HIIT) and RT combined with HIIT (RT-HIIT) with usual care (UC) (18).

Based on findings from preclinical studies on the effects of chemotherapy on skeletal muscle and the established beneficial effects of exercise during chemotherapy on physiological outcomes, we hypothesized that chemotherapy alone would lead to structural and metabolic deteriorations, whereas exercise training interventions would counteract this decline and result in specific training adaptations that would be reflected in a maintained or improved muscle fiber area, SC number, markers of mitochondrial function, and capillarization.

The aim of this study was to compare the effects of AT-HIIT and RT-HIIT with the effects of UC on skeletal muscle mitochondrial markers, antioxidant capacity, muscle fiber size and type, SC presence, and capillarization in a subset of patients with breast cancer undergoing chemotherapy who were part of the OptiTrain trial (18).

MATERIALS AND METHODS

Study design

The participants in this study were from the OptiTrain trial (NCT02522260; www.clinicaltrials.gov), a 16-wk in-clinic randomized controlled exercise trial for women with breast cancer during chemotherapy (22). A flow chart of the randomization process is shown in Fig. 1. Fifty of the participants in the OptiTrain trial were randomly allocated to donate muscle biopsies and were randomized to UC, AT-HIIT, or RT-HIIT. Twenty-three participants completed this part of the study. The main reason for declining participation was unwillingness to undergo a muscle biopsy.

Participants

Participant characteristics are presented in Table 1. Participants were diagnosed with stage I–IIA breast cancer and received adjuvant chemotherapy (consisting of anthracyclines, taxanes, or a combination of the two). Exclusion criteria were: 1) advanced disease, 2) heart or lung disease, 3) cognitive dysfunction, or 4) not speaking or understanding the Swedish language. Participants completed a questionnaire about their cardiovascular health history (23) and underwent a resting echocardiogram to rule out certain cardiac pathologies. Ethical approval was obtained from the Regional Ethical Review Board in Stockholm, Sweden (Dnr 2012/1347-31/1, 2012/1347-31/2, 2013/632-32, and 2014/408-32), and all participants gave written informed consent.

Exercise training intervention

The exercise training regimens have been explained in detail elsewhere (22). In brief, the RT-HIIT and AT-HIIT groups trained in an exercise clinic twice per week for 16 wk. The sessions were supervised by an exercise physiologist or an oncology nurse. The RT-HIIT group performed resistance exercise followed by high-intensity interval exercise in each session. The participants performed 9 resistance exercises consisting of 2 to 3 sets of 8–12 repetitions at an initial intensity of 70% of their estimated 1 repetition maximum (1-RM), progressing to 80% of 1-RM when more than 12 repetitions could be performed. To ensure overload, new estimated 1-RM tests were performed when participants could lift more than 12 repetitions of their 80% 1-RM when more than 12 repetitions could be performed. To ensure overload, new estimated 1-RM tests were performed when participants could lift more than 12 repetitions of their 80% 1-RM. The RT-HIIT program concluded with 3 × 3-min bouts of high-intensity interval exercise on a cycle ergometer at a rating of perceived exertion of 16–18 on the Borg 6–20 scale (24) interspersed with 1 min low-intensity active recovery. The exercise in the AT-HIIT group began with 20 min of moderate intensity, continuous aerobic exercise at a rating of perceived exertion of 13–15 on a cycle ergometer, an elliptical ergometer or a treadmill, followed by the same HIIT as for the RT-HIIT group. All 3 groups were provided with printed written information about exercise recommendations for patients with cancer according to the American College of Sports Medicine guidelines (25) at the initiation of the intervention period.

Skeletal muscle biopsies

Skeletal muscle resting biopsies were obtained prior to the performance assessments at rest and pre- and postintervention. Participants were asked to refrain from strenuous activity for at least 36 h prior to the biopsy. Muscle biopsies were obtained from the vastus lateralis muscle using the Bergström needle biopsy technique (26) under local anesthesia. No medical complications
or other reported adverse events from the procedure were reported.

Protein extraction and immunoblot analysis

Muscle samples were freeze-dried and homogenized in ice-cold buffer (100 μl/mg dry weight) consisting of 2 mM HEPES (pH 7.4), 1 mM EDTA, 5 mM EGTA, 10 mM MgCl<sub>2</sub>, 1% Triton X-100, 2 mM DTT, and 1.5% phosphatase and protease inhibitor cocktail (Halt; Thermo Fisher Scientific, Waltham, MA, USA) using a BulletBlender (Next Advance, Averill Park, NY, USA) with 0.5-mm ZrO beads at 4°C. The obtained homogenates were centrifuged for 10 min at 4°C at 10,000 g, and the supernatant was collected and stored at −20°C. After determining the samples’ protein concentrations using the Pierce 660 nm protein assay (Thermo Fisher Scientific), muscle homogenates were diluted with 4× Laemmli sample buffer (Bio-Rad, Richmond, CA, USA) and homogenizing buffer to obtain the same final protein concentration among the samples. All samples were heated at 95°C for 5 min to denature proteins before being stored at −20°C until further analysis. Proteins were separated by SDS-PAGE on 26-well Criterion TGX gradient gels (4–20% acrylamide; Bio-Rad). The blots were quantified using Quantity One software (v.4.6.3.; Bio-Rad). To control for appropriate loading and transfer, target proteins were expressed relative to total protein stained at ~95 kDa obtained by staining the membranes with MemCode Reversible Protein Stain Kit (Thermo Fisher Scientific) (27). The monoclonal primary antibodies used for the detection of target total proteins were as follows: Total Oxphos Human Cocktail (ab110411, 1:1000; Abcam, Cambridge, United Kingdom), Beclin-1 (3495, 1:1000; Cell Signaling Technology, Danvers, MA, USA), LC3B (3868, 1:1000; Cell Signaling Technology), total-ULK1 (ab128859, 1:1000; Abcam), Phospho-ULK1 (Ser317) (12753, 1:1000; Cell Signaling Technology), PINK1 (ab23707, 1:1000; Abcam), Parkin (2132, 1:1000; Cell Signaling Technology), MuRF1 (sc-32920, 1:1000; Santa Cruz Biotechnology, Dallas, TX, USA), SOD2 (13141, 1:1000; Cell Signaling Technology). The secondary antibodies used were anti-rabbit (1:10,000; total-ULK1, Phospho-ULK1, Beclin-1, LC3B, Parkin, MuRF1, SOD; Cell Signaling Technology) and anti-mouse IgG antibodies conjugated with horseradish peroxidase (1:10,000; Total Oxphos Human WB Antibody Cocktail).

Citrate synthase activity

The homogenate used for protein extraction was also used for citrate synthase (CS) activity. CS activity was measured on a 96-well plate in a reagent solution (50 mM Tris-HCl, 0.2 mM DTNB, and 30 mM acetyl-CoA). The reaction was initiated by

Figure 1. CONSORT flow diagram (22).
adding oxaloacetate (10 mM), and the change in absorbance at 412 nm was measured spectrophotometrically at 25°C.

Immunohistochemistry

Muscle tissue samples for immunohistochemical analysis were frozen in cooled isopentane immediately after collecting the biopsy. Sections (10 μm thick) were cut in a cryostat (Microm HM 560; Thermo Fisher Scientific) and air dried for 1 h. For Paired box protein (Pax7), sections were fixed in Baker’s fixative (Bio-Optica, Milan, Italy) for 15 min. Pax7 sections were permeabilized with 0.01% Triton X-100 for 1 h, blocked in bovine serum albumin (1%) and dry milk (1%), and incubated overnight at 4°C in anti-Pax7 (A-11008; Thermo Fisher Scientific) for 30 min. Endothelial cells were identified using an FITC CD31 antibody (1:100, 561813; BD Biosciences, San Jose, CA, USA). Slides were fixed in ice-cold acetone and incubated overnight at 4°C. Coverslips were placed on Pax7 and CD31 stained slides using Vectashield mounting medium containing DAPI (H-1200; Vector Laboratories, Burlingame, CA, USA). For fiber type–specific fiber size analysis, muscle sections were blocked in bovine serum albumin (2%) and stained at 4°C overnight with primary antibodies for laminin (1-1000, L9393; MilliporeSigma, St. Louis, MO, USA), myosin type I (1:100, BA-F8; Developmental Studies Hybridoma Bank, Iowa City, IA, USA), and myosin type IIA (1:100, SC-71; Developmental Studies Hybridoma Bank). The following appropriate fluorescent secondary antibodies were then added to the tissue sections: goat anti-mouse IgG1 568 (A-1300, A-21124; Thermo Fisher Scientific), goat anti-mouse IgG2b 488 (A-1300, A-21141; Thermo Fisher Scientific), and donkey anti-rabbit 633 (1:200, A-21070; Thermo Fisher Scientific). Images were captured at ×10, 40, and 63 magnification. Image J Software (28) was used for analysis. SCs were counted as the mean number of SCs per muscle fiber. For the muscle cross-sectional area (CSA), the outlined type I and IIA muscle fibers were reported in squared micrometers. Fibers that had a circularity below 0.5 (I = perfect circle) were excluded from the analysis to ensure that no longitudinal fibers were included in the analysis. Circularity was calculated as \( 4\pi \times \text{CSA}/(\text{perimeter})^2 \) (29). Capillary density was calculated as the mean number of capillaries per muscle fiber. For all analyses, a minimum of 50 muscle fibers were counted.

Myosin heavy chain typing

The protocol for high-resolution separation of myosin heavy chain (MHC) isoforms in a mini-gel electrophoresis system was adapted from a published method (30). Briefly, a portion of the same homogenates obtained for immunoblot analysis was assessed for protein quantification, and then a portion was diluted to a final concentration of 10 ng/μl in loading buffer consisting of 0.08 M Tris-HCl (pH 6.8), 2% glycerol, 2% SDS, 50 mM DTT, and 0.1% bromophenol blue and heated to 90°C for 3 min. Subsequently, 100 ng of protein was loaded onto a gel that consisted of 8.5% acrylamide/N,N,N’,N’-tetramethylethylenediamine with a 1-cm stacking part that consisted of 4% acrylamide/N,N,N’,N’-methylenebis (99:1), 35% glycerol, 0.2 M Tris-HCl (pH 8.8), 0.1 M glycine, 0.4% SDS, 0.1% ammonium persulfate, and 0.05% N,N,N’,N’-tetramethylmethylenediamine with a 1-cm stacking part that consisted of 8.5% acrylamide/N,N,N’,N’-methylenebis (99:1), 35% glycerol, 0.2 M Tris-HCl (pH 8.8), 0.1 M glycine, 0.4% SDS, 0.1% ammonium persulfate, and 0.05% N,N,N’,N’-tetramethylmethylenediamine. The upper chamber was filled with running buffer, which consisted of 0.3 M Tris Base, 0.450 M glycine, and 0.3% SDS. The lower chamber was filled with a running buffer, which was diluted 1:6 in distilled water. Gels were run at a constant 140 V for 22 h at 4°C, except for the first 40 min, which were limited to 10 mA. Gels were stained with SilverXpress Silver Staining Kit (LC6100; Thermo Fisher Scientific) following the manufacturer’s instructions and photographed in white light on a white background. Relative band density was assessed using ImageJ (28).

Other measures

Participants were asked to report their current activity level at baseline and at 16 wk as either sedentary (not meeting the exercise recommendations of at least 150 min moderate intensity exercise/week or at least 75 min vigorous intensity exercise/wk) or regular exercisers (meeting exercise recommendations). Exercise session attendance was calculated as the mean of the individual percent-ages (attended exercise sessions divided by the total number of sessions). Muscle strength was measured by isometric mid-thigh pull as described previously (18). Cancer-related fatigue was self-assessed at baseline and at 16 wk using the EORTC QLQ-C30 questionnaire (version 3.0) (31). Muscle strength and EORTC-QLQ-C30 questionnaire results including all patients of the

### TABLE 1. Participant characteristics at baseline

| Characteristic              | UC (n = 10) | AT-HIIT (n = 6) | RT-HIIT (n = 7) | P         |
|-----------------------------|------------|----------------|----------------|-----------|
| Age (yr)                    | 51.0 ± 13.1| 51.5 ± 7.0     | 54.3 ± 11.0    | 0.33      |
| Body mass (kg)              | 68.4 ± 5.9 | 66.3 ± 13.7    | 70.1 ± 11.8    | 0.66      |
| Height (cm)                 | 165.3 ± 8.5| 162.2 ± 7.9    | 167.0 ± 5.0    | 0.68      |
| Activity level [%]          |            |                |                |           |
| Inactive/low                | 5 (50.0)   | 3 (50.0)       | 5 (71.4)       |           |
| Moderate/high               | 5 (50.0)   | 3 (50.0)       | 2 (28.6)       |           |
| Tumor profile [%]           |            |                |                |           |
| Triple negative             | 3 (30.0)   | 0 (0.0)        | 1 (14.3)       | 0.31      |
| HER2+, ER+, PR+             | 1 (10.0)   | 0 (0.0)        | 1 (14.3)       |           |
| HER2+, ER+, PR              | 2 (20.0)   | 5 (83.3)       | 3 (42.7)       |           |
| HER2+, ER, PR               | 3 (30.0)   | 0 (0.0)        | 1 (14.3)       |           |
| HER2+, ER, PR+              | 1 (10.0)   | 1 (16.7)       | 0 (0.0)        |           |
| Chemotherapy regimen [%]    |            |                |                | 0.27      |
| Anthracycline therapy       | 2 (20.0)   | 3 (50.0)       | 4 (57.2)       |           |
| Anthracyline + taxane therapy | 8 (80.0) | 2 (33.3)       | 3 (42.8)       |           |
| Taxane therapy              | 0 (0.0)    | 1 (16.7)       | 0 (0.0)        |           |

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.
OptiTrain trial have been published previously (32). Higher scores on the EORTC-QLQ-C30 scale indicate higher levels of fatigue.

**Statistical analysis**

Normal distribution of the data was visually checked through QQ plots and by assessing skewness. Baseline medical and demographic characteristics of each group were summarized using descriptive statistics. Exact $\chi^2$ tests and Fisher’s exact test were used to evaluate if differences existed between groups for categorical variables at baseline. For between-group analyses, ANCOVA was used, with the baseline test result as a covariate and with the posttest as the dependent variable. A 2-tailed value of $P < 0.05$ was considered significant. Data that were not normally distributed were log transformed prior to performing ANCOVA analyses. Paired samples Student’s $t$ test was used to assess within-group differences pre- to postmeasurement. Pearson’s coefficient of correlation ($r$) was used to evaluate the association between changes in lower limb muscle strength and muscle CSA and SC count, changes in CS activity and capillaries per fiber, and between cancer-related fatigue and CS activity and muscle CSA. All analyses were performed using SPSS version 24 (IBM, Armonk, NY, USA) statistical package for Windows.

**RESULTS**

Participant characteristics for all groups were balanced at baseline (Table 1). From pre- to postintervention, a lower proportion of participants in the UC group met exercise recommendations (50–20%), compared with increases in the AT-HIIT group (50–100%) and the RT-HIIT group (29–86%). Attendance to the exercise sessions was 80% in the AT-HIIT group and 57% in the RT-HIIT group.

**CS activity and oxphos complexes**

CS activity and oxphos complexes were measured as a proxy of mitochondrial content and function (33). Over the intervention, the UC group exhibited a significantly reduced CS activity ($P = 0.017$) that was significantly different compared with the exercise groups (UC vs. AT-HIIT, $P = 0.005$; UC vs. RT-HIIT, $P = 0.027$) (Fig. 2). The AT-HIIT group displayed significantly elevated levels of complex IV ($P = 0.04$) pre- to postintervention and was superior to UC for all the protein levels of measured oxphos complexes (complex I: $P = 0.003$; complex II: $P = 0.007$; complex IV: $P = 0.004$). A significant difference was detected between AT-HIIT and RT-HIIT (complex I: $P = 0.011$; complex II: $P = 0.005$; complex IV: $P = 0.002$). No significant alterations were found for protein levels of complex V within or between any groups (Fig. 3A–D).

**Skeletal muscle morphology**

Over the 16-wk period, the UC group displayed a significant reduction in muscle CSA for both type I ($P = 0.01$) and type IIA muscle fibers ($P = 0.026$), whereas RT-HIIT resulted in a significant increase in type I muscle fiber CSA ($P = 0.049$). Between groups, a significant difference was found between UC and both exercise groups for type I muscle fiber CSA (UC vs. AT-HIIT, $P = 0.02$; UC vs. RT-HIIT, $P < 0.001$). For type IIA muscle fiber CSA, a significant difference was found between UC and AT-HIIT ($P < 0.001$) and between AT-HIIT and RT-HIIT ($P = 0.006$) (Fig. 4). For SC count per fiber, RT-HIIT was superior to both UC ($P = 0.007$) and AT-HIIT ($P = 0.038$) (Fig. 5A and Supplemental Fig. 2). Over the intervention, within-group analysis showed that the UC group had significantly lower capillaries per muscle fiber ($P = 0.009$), whereas AT-HIIT displayed a significant increase in capillaries per muscle fiber ($P < 0.001$). Correspondingly, both AT-HIIT ($P < 0.001$) and RT-HIIT ($P = 0.002$) were superior to UC for capillaries per muscle fiber (Fig. 5B).

**MHC isoforms**

Over the intervention period, there was a lower proportion of MHC isoform type I for UC ($P = 0.006$), which was significantly different compared to the RT-HIIT group ($P = 0.016$). Neither the AT-HIIT nor the RT-HIIT group showed within-group differences for MHC isoforms (Fig. 6A–C).

**Autophagy/mitophagy**

Over the intervention period, the UC group showed significantly lower levels of the mitophagy marker PINK1 ($P = 0.031$), whereas the levels in the AT-HIIT and RT-HIIT groups remained stable. Correspondingly, there was a significant difference between UC and AT-HIIT ($P = 0.012$) (Fig. 7A). No within- or between-group differences were found for other measured protein levels (i.e., Parkin-1, Beclin-1, MuRF1, T-ULK, P-ULK, the ratio between P-ULK/T-ULK, LCB3-I, LCB3-II, and the ratio between LCB3-II/LCB3-I).

**Antioxidant defense**

Over the intervention period, the UC group displayed significant increases in protein levels of SOD2 ($P = 0.005$). However, no between-group differences in SOD2 protein levels were detected (Fig. 7B).

**Figure 2.** CS activity (nM/min/mg) pre- to postintervention in UC, AT-HIIT, and RT-HIIT groups. *$P < 0.05$ at post vs. premeasurement, † $P < 0.05$ compared with UC.
Muscle strength and associations with muscle CSA and SC count and mitochondrial content and associations with capillary density

We found significant associations between changes in lower limb muscle strength and changes in both muscle CSA ($r = 0.5; P = 0.033$) and SC count ($r = 0.6; P = 0.003$). Moreover, there was a significant association between muscle CSA and SC count ($r = 0.5; P = 0.023$) as well as between changes in CS activity and capillaries per fiber ($r = 0.6; P = 0.004$) (Supplemental Fig. 3A–D).

Cancer-related fatigue and associations with mitochondrial content and muscle CSA

We found a significant inverse correlation between changes in cancer-related fatigue and changes in muscle CSA ($r = -0.4; P = 0.086$) (Supplemental Fig. 3E, 3F).

DISCUSSION

This study provides insights into the effects of chemotherapy and chemotherapy in conjunction with exercise on skeletal muscle function and morphology in patients with breast cancer. Here we show that AT-HIIT and RT-HIIT are potent stimuli in counteracting the decay in mitochondrial content (as represented by CS activity) displayed in patients with breast cancer undergoing UC. Furthermore, AT-HIIT was superior to the UC and RT-HIIT groups for protein levels of the electron transport chain. This difference between the exercise groups was likely the result of the extra 20 min of moderate-intensity AT performed by the AT-HIIT group, indicating the importance of training volume in improving mitochondrial content, which has been
demonstrated in healthy individuals (34). The exercise interventions also counteracted the decrease in muscle fiber CSA and capillarization found in the UC group. Indeed, the RT-HIIT group exhibited a significant increase in muscle fiber CSA, whereas AT-HIIT resulted in an increased number of capillaries per fiber postintervention. In agreement with our hypothesis, these findings indicate the specificity of training stimuli and that exercise training adaptations on the musculoskeletal level can occur in conjunction with chemotherapy.

We detected a significantly higher SC count per fiber for RT-HIIT compared with UC and AT-HIIT. The changes in SC count were significantly associated with changes in muscle CSA and with muscle strength, indicating that the increase in Pax7-positive cells in the RT-HIIT group reflects an activation of skeletal muscle regeneration processes. The increase in SC count in our study is in contrast to findings from an RT intervention that included patients with prostate cancer receiving androgen deprivation therapy (19) and from a trial that included patients with germ cell cancer receiving cisplatin-based chemotherapy (20). Furthermore, in a trial by Christensen et al. (21), no improvements in muscle CSA after a 9 wk RT intervention were shown. Nilsen et al. (19) showed improved CSA for type II muscle fibers, whereas in the present study the improvements were shown for type I muscle fibers. Sex and/or treatment differences and the differences in type and duration of the exercise regimens may explain the discrepant findings between the studies.

The magnitude of improvement (∼30%) in muscle fiber CSA for the RT-HIIT group was similar to what has been found in healthy elderly subjects after 16 wk of RT (35), indicating that patients with breast cancer respond similarly to healthy elderly individuals.

A significantly reduced proportion of MHC isoform type I was found in the UC group from pre- to postintervention. The reported shift from slow to fast MHC isoforms indicates muscle disuse due to loss of mechanical

Figure 4. A–C) Immunohistochemical labeling in skeletal muscle for type I muscle fiber CSA (A), type IIA muscle fiber CSA (B), and muscle fiber CSA (C) for all fibers in UC, AT-HIIT, and RT-HIIT groups. Biopsy samples from 2 individuals were of poor quality and were excluded from fiber size analysis (UC, n = 1; AT-HIIT, n = 1). D) Images show immunofluorescent staining of myosin type I stained in green, myosin type IIA stained in gray, and basement membrane (laminin) stained in red. *P < 0.05 at post vs. premeasurement, †P < 0.05 compared with UC, §P < 0.05 between RT-HIIT and AT-HIIT.
loading and/or neuronal loss and is usually accompanied by preferential atrophy of type 1 fibers (36, 37). These outcomes, together with the finding that 80% of the participants in the UC group reported being inactive, leads us to speculate that the loss in muscle CSA may be caused by muscle disuse rather than by chemotherapy. The loss of type I fibers is in line with recent findings assessing human skeletal muscle from both cachectic and weight-stable patients receiving cancer treatment, showing a slow to fast shift in MHC isoforms and a reduction in muscle CSA

Figure 5. Immunohistochemical labeling in skeletal muscle for SCs per fiber (A) and capillaries per fiber (B), pre- to postintervention in UC, AT-HIIT, and RT-HIIT groups. Images show immunofluorescent staining of Pax7 stained in green for SC analysis (A) and of CD31 stained in green for capillary analysis (B) with myonuclei stained in blue. *P < 0.05 at post vs. premeasurement, †P < 0.05 compared with UC, ‡P < 0.05 between RT-HIIT and AT-HIIT.

Figure 6. MHC distribution (%) of type I (A), type IIa (B), and type IIx (C) muscle fibers pre- to posttraining intervention in UC (A, D), AT-HIIT (B, D), and RT-HIIT (C, D) groups. *P < 0.05 at post vs. premeasurement, †P < 0.05 compared with UC, ‡P < 0.05 between RT-HIIT and AT-HIIT.
from the vastus lateralis muscle (38). In contrast, an RT trial (21) for patients with germ cell cancer found no significant changes after cisplatin-based chemotherapy in the UC group, although the direction of change in MHC proportion was similar to our findings. Moreover, the same trial found no differences between exercise and control groups. Alternatively, it may be speculated that the lower proportion of MHC isoform type I in the UC group from pre- to postintervention was caused by mitochondrial DNA damage (39) induced by anthracyclines given that mitochondrion-rich type I fibers may be more susceptible to the toxicity of anthracyclines.

MuRF1, which is involved in the ubiquitin proteasome pathway, is an early marker of muscle atrophy (40). We found no significant alterations in MuRF1 protein levels in the UC group despite reductions in muscle CSA. According to Moriscot et al. (41), MuRF1 is fiber-type dependent and is preferentially induced in type II muscle fiber atrophy. Our data did not support this finding since we found declines in both type I and type II muscle fiber CSA in the UC group.

Mitophagy is a central process that is critical for maintaining functional and healthy mitochondria. Mitophagy includes a selective degradation of damaged mitochondria (42) and at the final stage requires the activation of the PINK1/Parkin pathway. Pre- to postintervention, protein levels of PINK1 were reduced in the UC group, indicating a possible down-regulation of mitophagy and the mitochondrial quality control. This is consistent with findings from a preclinical study by Gouspillou et al. (7) that demonstrated reductions in mitophagy-related proteins in skeletal muscle after anthracycline treatment. AT-HIIT counteracted the decline in PINK1 levels, as shown in the UC group. Findings from studies on healthy mice show an increased mitochondrial turnover as an adaptation to AT (43, 44), whereas sedentary behavior is associated with down-regulation of the mitophagy signaling pathways (45). We saw no significant mitophagy response in the exercise groups, which is in line with a report investigating mice treated with doxorubicin after exercise training (46). None of the other measured protein levels involved in the mitophagy or autophagy pathway was altered in any group, possibly due to the time point of biopsy collection in the present study. Assuming the skeletal muscle mitochondrial quality control was impaired in the UC group, we cannot determine whether the reduction in mitophagy was induced by chemotherapy or by physical inactivity.

SOD2 is an important scavenger of reactive oxygen species. In vitro, an overexpression of SOD2 has been shown to have a negative impact on mitochondrial function (47). We found a significant increase in protein levels of SOD2 in the UC group, indicating a chronic oxidative stress response, whereas SOD2 remained stable in the exercise groups. The effects of chemotherapy on SOD2 levels in human skeletal muscle have not been reported previously. However, preclinical studies did not show exacerbated levels of SOD2 in skeletal muscle after anthracycline treatment (8, 48, 49). These findings remain inconclusive since we did not detect between-group differences.

It has been suggested that alterations in energy metabolism may contribute to fatigue (50). We found a significant inverse correlation ($r = -0.7$) between changes in self-reported fatigue and changes in CS activity. This correlation was still present ($r = -0.6$) when removing the training groups from the analysis. Our data are supported by findings in individuals with chronic fatigue syndrome showing an impaired oxidative phosphorylation compared with healthy individuals (50). Given that cancer-related fatigue is a chronic problem in over two thirds of patients with cancer and that close to 40% describe it as severe for at least 6 mo after treatment (51), these results highlight the importance of AT in order to preserve good mitochondrial function during chemotherapy.

A strength of this study is that it is an in-clinic randomized controlled trial with muscle biopsies obtained before and after the intervention from all groups, including 2 exercise modalities comprising the emerging exercise modality HIIT. Limitations include the dropout rate by the UC group, which introduces a potential...
selection bias; therefore, the findings need to be interpreted cautiously. An additional limitation is that the current approach does not allow for separating the effects of anthracyclines from those of taxanes. However, a subgroup analysis in the general trial showed similar effects on muscle strength and cardiorespiratory fitness regardless of chemotherapy regimen (18). The low attendance rate in the RT-HIIT group may have underestimated the potential effects of an RT-HIIT regimen in patients with breast cancer. However, this is the first study on the effects of chemotherapy alone and in conjunction with exercise training on skeletal muscle morphological and mitochondrial markers in patients with breast cancer. Here we show that participants performing AT-HIIT as well as participants performing RT-HIIT had maintained or even improved markers of skeletal muscle function and morphology opposite to the decline shown in patients with breast cancer undergoing UC. These findings illustrate the importance of implementing exercise programs for patients with breast cancer during chemotherapy to prevent the negative side effects of chemotherapy and inactivity through preserving skeletal muscle mass and function. This could also reduce treatment toxicity and ultimately lead to improved survival.

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AUTHOR CONTRIBUTIONS

S. Mijwel, D. A. Cardinale, J. Norrbom, Y. Wengström, C. J. Sundberg, and H. Rundqvist, designed the study; C. J. Sundberg collected the muscle biopsies; S. Mijwel, D. A. Cardinale, M. Chapman, and N. Ivarsson performed molecular analyses; S. Mijwel, D. A. Cardinale, and H. Rundqvist analyzed the data and drafted the manuscript; and all authors reviewed and approved the final manuscript.

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