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ORIGINAL ARTICLE – CANCER RESEARCH

Molecular and cellular adaptations to exercise training in skeletal muscle from cancer patients treated with chemotherapy

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Abstract

Background A growing body of evidence suggests that exercise training has beneficial effects in cancer patients. The aim of the present study was to investigate the molecular basis underlying these beneficial effects in skeletal muscle from cancer patients.

Methods We investigated expression of selected proteins involved in cellular processes known to orchestrate adaptation to exercise training by western blot. Skeletal muscle biopsies were sampled from ten cancer patients before and after 4–7 weeks of ongoing chemotherapy, and subsequently after 10 weeks of continued chemotherapy in combination with exercise training. Biopsies from ten healthy matched subjects served as reference.

Results The expression of the insulin-regulated glucose transporter, GLUT4, increased during chemotherapy and continued to increase during exercise training. A similar trend was observed for ACC, a key enzyme in the biosynthesis and oxidation of fatty acids, but we did not observe any changes in other regulators of substrate metabolism (AMPK and PDH) or mitochondrial proteins (Cyt-C, COX-IV, SDHA, and VDAC). Markers of proteasomal proteolysis (MURF1 and ATROGIN-1) decreased during chemotherapy, but did not change further during chemotherapy combined with exercise training. A similar pattern was observed for autophagy-related proteins such as ATG5, p62, and pULK1 Ser^{757}, but not ULK1 and LC3BII/LC3BI. Phosphorylation of FOXO3a at $Ser^{318/321}$ did not change during chemotherapy, but decreased during exercise training. This could suggest that FOXO3a-mediated transcriptional regulation of MURF1 and ATROGIN-1 serves as a mechanism by which exercise training maintains proteolytic systems in skeletal muscle in cancer patients. Phosphorylation of proteins that regulate protein synthesis (mTOR at Ser^{2448} and 4EBP1 at $Thr^{37/46}$) increased during chemotherapy and leveled off during exercise training. Finally, chemotherapy tended to increase the number of satellite cells in type 1 fibers, without any further change during chemotherapy and exercise training. Conversely, the number of satellite cells in type 2 fibers did not change during chemotherapy, but increased during chemotherapy combined with exercise training.

Conclusions Molecular signaling cascades involved in exercise training are disturbed during cancer and chemotherapy, and exercise training may prevent further disruption of these pathways.

Trial registration The study was approved by the local Scientific Ethics Committee of the Central Denmark Region (Project ID: M-2014-15-14; date of approval: 01/27/2014) and the Danish Data Protection Agency (case number 2007-58-0010; date of approval: 01/28/2015). The trial was registered at http//www.clinicaltrials.gov (registration number: NCT02192216; date of registration 07/17-2014).

Keywords Cancer cachexia · Endurance · Resistance · Molecular mechanisms · Rehabilitation

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Abbreviations

ACC	Acetyl-CoA carboxylase
AMPK	AMP-activated protein kinase
ATG5	Autophagy-related protein 5
Cyt-C	Cytochrome-C
COX-IV	Cytochrome-C oxidase IV
4EBP1	Eukaryotic translation initiation factor 4E binding protein 1

ATROGIN-1	F-Box protein 32
FOXO3a	Forkhead transcription factor 3a
IκB	Inhibitor of NFκB
mTOR	Mammalian target of rapamycin
LC3B	Microtubule-associated protein B
MURF1	Muscle RING-finger protein 1
NFκB	Nuclear factor κ B
PDH	Pyrovate dehydrogenase
SC	Satellite cell
p62	Sequestome 1
S6rp	Subunit 6 rbosomal protein
SDHa	Succinate dehydrogenase alpha
ULK1	UNC-51-like kinase 1
VDAC	Voltage-dependent anion channel

Background

Cancer is a leading cause of death worldwide (Collaborators GBDCoD 2017). Chemotherapy is a commonly used treatment modality that significantly improves the prognosis of the disease. Unfortunately, cancer and chemotherapy are often associated with severe complications such as muscle wasting and weakness (Senkus et al. 2015). These adverse events may be due to a combination of direct effects of chemotherapy on skeletal muscle and physical inactivity (Gilliam and St Clair 2011).

The nature of physical training involves repetitive bouts of exercise that induce metabolic and mechanical stress to the body and ultimately leads to adaptations in the involved tissues (Coffey and Hawley 2007). Many benefits of exercise training emerge from local adaptations in the exercising muscle (Egan and Zierath 2013; Hawley and Lessard 2008). Exercise stimulates a plethora of intracellular processes that underlie these adaptations, including catabolic systems, such as autophagy-lysosome, ubiquitin–proteasome, and inflammation (Moller et al. 2013, 2018; Stefanetti et al. 2015), as well as anabolic systems, such as protein synthesis (Rahbek et al. 2014; Vendelbo et al. 2014b). Activation and/or repression of specific signaling cascades regulate these processes by linking metabolic and mechanical stress to regulation of cellular enzymes (Egan and Zierath 2013; Jessen et al. 2014). Ultimately, repetitive activation of these signaling cascades leads to myocellular changes in mitochondrial function (Spina et al. 1996), metabolic function (Green et al. 1992), insulin-stimulated glucose uptake (Sjoberg et al. 2017), intracellular signaling (Coffey and Hawley 2007), and transcriptional/translational regulation (Pilegaard et al. 2003). In addition, exercise stimulates proliferation and differentiation of muscle stem cells (satellite cells) as part of the adaptive response to exercise training (Farup et al. 2014, 2015).

The effects of cancer and chemotherapy on these cellular and molecular processes are unclear. Previous studies suggest that chemotherapy can lead to muscle atrophy (McLoon et al. 1998), muscle weakness (Scheede-Bergdahl and Jagoe 2013), low satellite cell content (Gilliam et al. 2009), and mitochondrial dysfunction (Crouch et al. 2017). Thus, cancer and chemotherapy appear to mediate muscular adaptations opposite to those induced by exercise training. This suggests that exercise training has the potential to alleviate muscular complications associated with cancer disease and chemotherapy. A growing body of evidence has shown that exercise training improves physical performance in cancer patients (Adamsen et al. 2009; Hvid et al. 2016; Lonbro et al. 2013). Exercise training has indeed been shown to preserve skeletal muscle fiber area and mitochondrial content in cancer patients during chemotherapy (Mijwel et al. 2018). This indicates that cancer patients are responsive to training adaptations, but training adaptation on a molecular levels remains to be documented.

The aim of the present study was to investigate molecular adaptations in skeletal muscle of cancer patients during chemotherapy alone and in combination with exercise training. We hypothesized that proteins involved in adaptation to exercise training are abnormally regulated in cancer patients during chemotherapy and that exercise training counteracts this undesirable response.

Materials and methods

Study design and biopsy material

Skeletal muscle biopsies from ten female cancer patients [mean (range); age 51 years (28–62); BMI 23 kg/m^2 (17–30); cancer site: breast ($n=7$), head and neck ($n=1$), rectal ($n=1$), sarcoma ($n=1$)] and ten healthy control subjects matched on gender, age and body weight [mean (range); age 51 years (28–60); BMI 24 kg/m^2 (18–30)] were analyzed. Data on functional performance and muscle fiber cross-sectional area from these subjects have been published previously along with detailed information about the trial protocol (Lonbro et al. 2017). In short, biopsies were sampled from the cancer patients before and after 4–7 weeks of ongoing chemotherapy, and subsequently after 10 weeks of continued chemotherapy in combination with physical training. The first biopsy was sampled from all ten patients. The second biopsy was sampled from nine patients. The third biopsy was sampled from six patients, leaving a sample size of ten, nine, and six biopsies at the individual time points. The training protocol consisted of three 90-min sessions per week and included both resistance and aerobic exercise. The sessions comprised six resistance exercises (knee extension, leg press, lateral pull-down, chest-press, back extension and sit-ups). Aerobic exercise consisted of group sessions on ergometer bicycles. The intensity of both resistance and aerobic exercise increased progressively throughout the study. A single biopsy was sampled from the healthy subjects to serve as reference to the effects of chemotherapy and training in the cancer patients. All biopsies were sampled from vastus lateralis after an overnight fast using a Bergström needle after local anesthesia. Visible fat and connective tissue were removed immediately after sampling and the biopsies were frozen in liquid nitrogen and stored at − 80 °C until analysis. Approximately 75 mg muscle tissue were mounted in Tissue-Tek (OCT compound, Sakura Finetak Europe B, NL) and frozen in liquid nitrogen-cooled isopentane before storage at − 80 °C. The study was performed in accordance with the Declaration of Helsinki and approved by the local Scientific Ethics Committee of the Central Denmark Region (Project ID: M-2014-15-14; date of approval: 01/27/2014) and the Danish Data Protection Agency (case number 2007-58-0010; date of approval: 01/28/2015). The trial was registered at http://www.clinicaltrials.gov (registration number: NCT02192216; date of registration 07/17-2014). All participants gave informed consent to participate in the study.

Protein extraction and western blot analysis

Frozen muscle biopsies were freeze-dried for 48 h in a Heto Drywinner (Heto Holten A/S, Allerød, Denmark) at − 96 °C and 0.5 mmHg. Freeze-dried muscle tissue was homogenized with ceramic beads in ice-cold lysis buffer (50 mM HEPES, 137 mM NaCl, 10 mM $Na_4P_2O_7$, 10 mM NaF, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 2 mM Na_3VO_4, 1% (vol/vol) NP-40, 10% (vol/vol) glycerol, 3 mg/ml aprotinin, 5 mg/ml leupeptin, 0.5 µg/ml pepstatin, 10 µg/ml antipain, 1.5 mg/ml benzamidine, 100 µM 4-(-2-aminoethyl)-benzenesulfyl fluoride, pH 7.4) using a Precellys 24 homogenizer (Bertin Technologies, FR). Insoluble materials were removed by centrifugation at 14,000×*g* for 20 min at 4 °C. Protein concentration of the supernatant was determined using a Bradford assay (BioRad, CA, USA). Samples were adjusted to equal concentrations with milli-Q water and denatured by mixing with Laemmli's buffer and heating at 95 °C for 5 min. Equal amounts of protein were separated by SDS-PAGE using the BioRad Criterion system, and proteins were electroblotted onto PVDF membranes (BioRad). Control for equal loading was performed using the Stain-Free technology (Gurtler et al. 2013). Membranes were blocked for 2 h in a 2% bovine serum albumin solution (Sigma-Aldrich, MO, USA) and incubated overnight with primary antibodies. After incubation in primary antibodies the membranes were incubated 1 h with HRP-conjugated secondary antibodies. Proteins were visualized by chemiluminiscence (Pierce Supersignal West Dura, Thermo Scientific, IL, USA) and quantified with ChemiDoc™ MP imaging system (BioRad). Protein Plus Precision All Blue standards were used as markers of molecular weight (BioRad). The following antibodies were used: From Millipore (MA, USA); GLUT4 (cat. no. 07-1404); From Cell Signaling Technology (MA, USA); p-AMPK Thr^{172} (cat. no. 2531), AMPK (cat. no. 2532), ACC (cat. no. 3661), Cyt-C (cat. no. 11940), COX-IV (cat. no. 4850), PDH (cat. no. 3205), SDHa (cat. no. 11998), VDAC (cat. no. 4661), ULK1 (cat. no. 4773), p-ULK1 Ser^{757} (cat. no. 6888), non-p-4EBP1 $Thr^{37/46}$ (cat. no. 4923), 4EBP1 (cat. no. 9644), p-S6rp $Ser^{235/236}$ (cat. no. 4858), S6rp (cat. no. 2217), p-NFκB Ser^{536} (cat. no. 3033), mTOR (cat. no. 2972), p-mTOR Ser^{2448} (cat. no. 2971), LC3B (cat. no. 3868), ATG5 (cat. no. 12994); From Abcam (Cambridge, UK); p62 (cat. no. ab56416), ATROGIN-1 (cat. no. ab92281), MURF1 (cat. no. ab172479); From Santa Cruz Biotechnology (CA, USA); secondary goat anti-rabbit IgG HRP-conjugated (cat. no. sc-2054), secondary chicken anti-mouse IgG HRP-conjugated (cat. no. sc-2962). Full-length blots are provided in Supplemental Fig. 1.

Satellite cell analysis

Cryosections (10 µm) were cut at − 18 °C and placed on slides. Muscle biopsy sections were fixed in Histofix (Histolab, Gothenborg, Sweden) followed by a 1.5-h incubation in blocking buffer (0.2% Triton-X, 2% BSA, 5% FBS, 2% goat serum and 0.1% sodium azide). The sections were incubated overnight at 4 °C with primary antibody for Pax7 (1:500; cat. no MO15020, Neuromics, Edina, MN, USA), followed by 1.5 h incubation in secondary Alexa Fluor 568 goat anti-mouse antibody (Molecular Probes, cat no. A11034, Invitrogen A/S, Taastrup, Denmark). Following this, the sections were incubated with primary antibodies for Type I myosin (1:500; cat. no. A4.951, Developmental Studies Hybridoma Bank (DSHB), IA, USA) and laminin (1:500; cat. no. Z0097, Dako Norden) for 2 h and secondary Alexa Fluor 488 goat anti-mouse green and Alexa Fluor 488 goat anti-rabbit green (Molecular Probes, cat no. A11031 and cat no. A11034, Invitrogen A/S, Taastrup, Denmark) antibodies for 1 h. Finally, a mounting media containing 4′,6-Diamidino-2-phenylindole (DAPI) was utilized to visualize nuclei (Molecular Probes Prolog Gold anti-fade reagent, cat. no. P36935, Invitrogen A/S) and samples were stored at − 20 °C until final analyses. Staining was verified using appropriate negative controls to ensure specificity. Images were obtained at 20 × magnification using a Leica DM2000 microscope (Leica, Stockholm, Sweden) and a Leica Hi-resolution Color DFC camera (Leica, Stockholm, Sweden). The number of Pax7 positive ($Pax7^+$) cells (SCs) associated with type I ($A4.951^+$) or type II ($A4.951^-$) fibers was quantified separately and expressed relative to the total number of type I or II fibers.

Statistical analysis

The changes during chemotherapy and chemotherapy combined with physical training were analyzed using a one-way ANOVA with repeated measurements. When a significant effect was observed, Student Newman–Keuls post hoc test was used to test differences between individual time points. A simple student's *t* test was used to test differences between cancer patients and healthy matched subjects. Normal distribution was assumed following evaluation of QQ-plots and histograms of log10-transformed data. Data are presented as geometric mean ± 95% CI (fold change from week 4–7 for cancer patients and fold difference from week 4–7 for the matched control subjects). Data were analyzed in SigmaPlot (SigmaPlot 11.0, CA, USA).

Results

Skeletal muscle fiber cross-sectional area and muscle strength

As presented in a previous paper (Lonbro et al. 2017), cross-sectional area of type II muscle fibers decreased during chemotherapy and increased during chemotherapy combined with exercise training. A similar pattern was observed in Type I muscle fibers and whole body lean body mass (LBM), but the changes did not reach statistical significance. Maximal muscle strength of the knee and elbow extensors did not change during chemotherapy, but increased during chemotherapy combined with exercise training. These data demonstrate that the chemotherapy and exercise training induced functional and morphological adaptations in skeletal muscle from the cancer patients.

Enzymes involved in substrate metabolism and mitochondrial proteins

Protein expression of GLUT4 increased during chemotherapy alone and continued to increase during chemotherapy combined with exercise training (Fig. 1a). Phosphorylation of AMPK at Thr^{172} and its substrate ACC at Ser^{79} remained unchanged during chemotherapy and training (Fig. 1b, c). Protein expression of AMPKα-pan did not change during the study (Fig. 1d), while ACC expression increased during chemotherapy alone and thereafter remained stable (Fig. 1e). Chemotherapy and exercise training did not change protein expression of the mitochondrial proteins Cyt-C, COX-IV, PDH, SDHA, and VDAC (Fig. 1f–j). None of these proteins differed in expression when comparing cancer patients with healthy subjects (Fig. 1a–j).

Autophagy-related proteins

Chemotherapy and exercise training did not change the ratio of LC3BII to LC3BI (Fig. 2a) or protein levels of ATG5 (Fig. 2b). Protein expression of p62 increased during chemotherapy alone and did not increase further during chemotherapy combined with exercise training (Fig. 2c). Chemotherapy and exercise training did not change protein levels of ULK1 (Fig. 2d), whereas phosphorylation of ULK1 at Ser^{757} increased during chemotherapy alone and leveled off during chemotherapy combined with exercise training (Fig. 2e). Protein expression and phosphorylation of autophagy-related proteins did not differ between cancer patients and healthy subjects, except from ATG5 which was elevated in those patients at baseline (Fig. 2a–e).

Proteins related to the ubiquitin–proteasome system

Phosphorylation of FOXO3a at Ser$^{318/321}$ did not change during chemotherapy alone, but decreased during chemotherapy combined with exercise training (Fig. 2f). MURF1 decreased during chemotherapy alone and returned to baseline levels during chemotherapy combined with training (Fig. 2g). The same pattern was observed for ATROGIN-1 (Fig. 2h). Chemotherapy and exercise training did not change protein levels of IκB (Fig. 2i), whereas phosphorylation of NFκB at Ser^{536} remained stable during chemotherapy alone and tended to increase during chemotherapy combined with exercise training (Fig. 2j).

Enzymes and proteins related to protein synthesis

Phosphorylation of mTOR at Ser^{2448} increased during chemotherapy alone, but did not increase further during chemotherapy combined with exercise training (Fig. 3a). In line with this, phosphorylation of 4EBP1 at Thr$^{36/45}$ increased during chemotherapy alone, as indicated by decreased phosphorylation of non-p-4EBP1, and did not change further during training (Fig. 3b). A similar trend was observed for S6rp phosphorylation at Ser$^{235/236}$, but the change did not reach statistical significance (Fig. 3c). Chemotherapy and training did not change protein expression of mTOR, 4EBP1, and S6rp (Fig. 3d–f). We did not observe any difference in phosphorylation and expression of these proteins between cancer patients and healthy matched control subjects (Fig. 3a–f).

Satellite cells associated with type 1 and 2 muscle fibers

Chemotherapy alone tended to increase the number of satellite cells in type 1 muscle fibers, without any further change during chemotherapy and exercise training

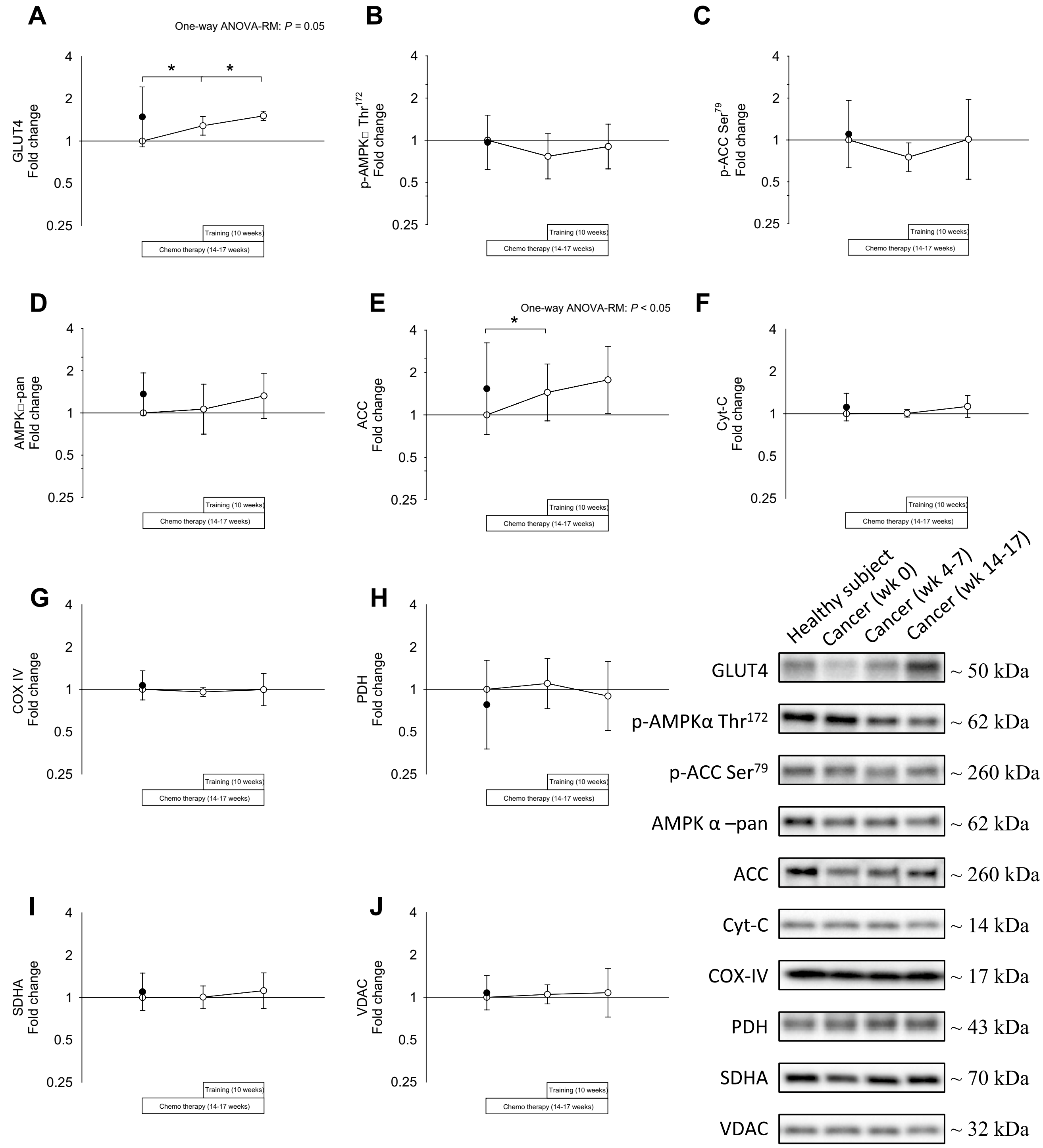

Fig. 1 Expression of enzymes involved in substrate metabolism and mitochondrial proteins. Expression and phosphorylation of selected enzymes involved in substrate metabolism and mitochondrial proteins were measured by western blot in lysates from vastus lateralis, during chemotherapy alone or in combination with exercise training in cancer patients (white dots). Healthy matched individuals served as a reference (black dots). **a** GLUT4, **b** p-AMPKα Thr^{172}, **c** p-ACC Ser^{79}, **d** AMPKα-pan, **e** ACC, **f** Cyt-C, **g** COX-IV, **h** PDH, **i** SDHA, **j** VDAC. Data are expressed as geometric mean ± 95% CI intervals. Representative western blots with approximated molecular weights based on the applied molecular marker are depicted right. *Post hoc test for effect of time showed a significant difference during chemotherapy alone or in combination with exercise training. #Depicts differences between cancer patients and healthy subjects

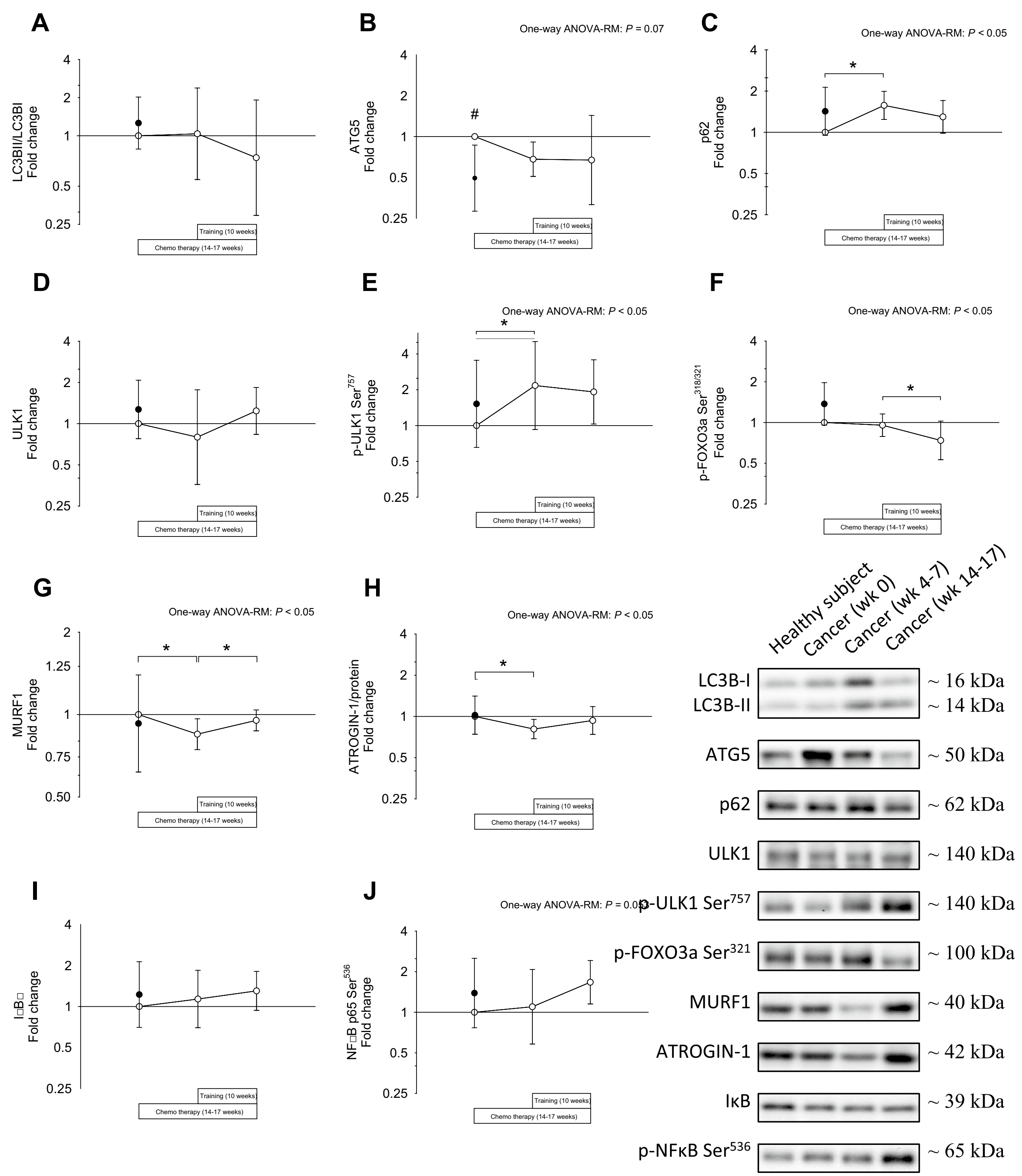

Fig. 2 Expression and phosphorylation of proteins involved in autophagy-lysosomal and proteasomal proteolytic systems. Expression and phosphorylation of selected proteins involved in regulation proteolytic pathways were measured by western blot in lysates from vastus lateralis, during chemotherapy alone or in combination with exercise training in cancer patients (white dots). Healthy matched individuals served as a reference (black dots). **a** LC3B-II/LC3B-I, **b** ATG5, **c** p62, **d** ULK1, **e** p-ULK1 Ser^{757}, **f** p-FOXO3a Thr$^{318/321}$, **g** MURF1, **h** ATROGIN-1, **i** IκBα, **j** p-NFκB p65 Ser^{536}. Data are expressed as geometric mean ± 95% CI intervals. Representative western blots with approximated molecular weights based on the applied molecular marker are depicted right. *Post hoc test for effect of time showed a significant difference during chemotherapy alone or in combination with exercise training. #Depicts differences between cancer patients and healthy subjects

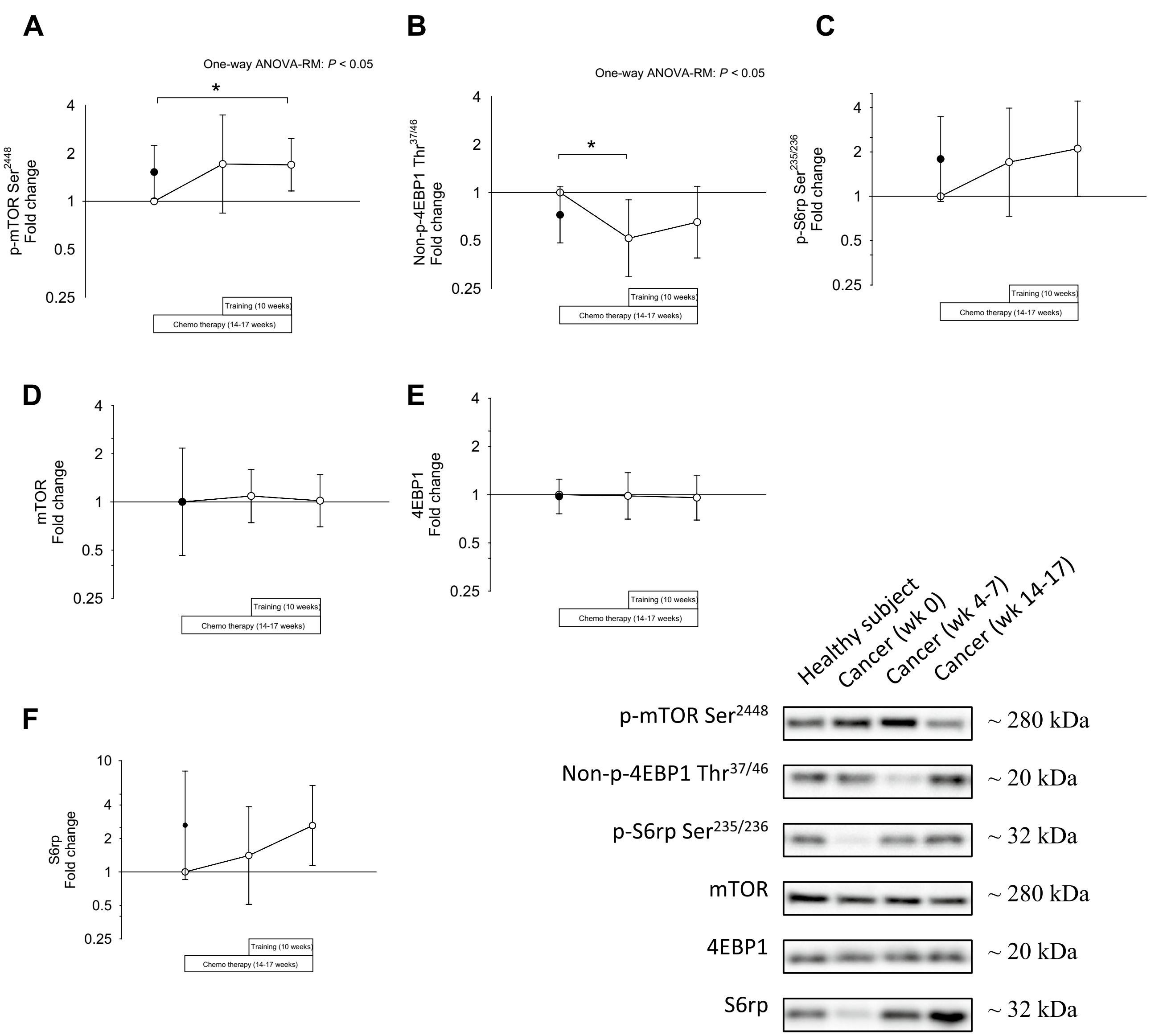

Fig. 3 Expression of enzymes and proteins involved in protein synthesis. Expression and phosphorylation of selected proteins involved in regulation of protein synthesis were measured by western blot in lysates from vastus lateralis, during chemotherapy alone or in combination with exercise training in cancer patients (white dots). Healthy matched individuals served as a reference (black dots). **a** p-mTOR Ser^{2448}, **b** non-p-4EBP1 $Thr^{37/46}$, **c** p-S6rp $Ser^{235/236}$, **d** mTOR, **e** 4EBP1, **f** S6rp. Data are expressed as geometric mean ± 95% CI intervals. Representative western blots with approximated molecular weights based on the applied molecular marker are depicted right. *Post hoc test for effect of time showed a significant difference during chemotherapy alone or in combination with exercise training. $^{\#}$Depicts differences between cancer patients and healthy subjects

(Fig. 4a). The number of satellite cells in type 1 fibers was decreased in cancer patients at baseline. The number of satellite cells in type 2 fibers did not change during chemotherapy alone, nor during chemotherapy combined with exercise training (Fig. 4b). The number of satellite cells in type 2 fibers was equal in cancer patients and healthy subjects.

Discussion

Physical training has been shown to ameliorate the detrimental effects of cancer and chemotherapy on human skeletal muscle (Mijwel et al. 2018). In the present study, we provide insight into molecular mechanisms that mediate

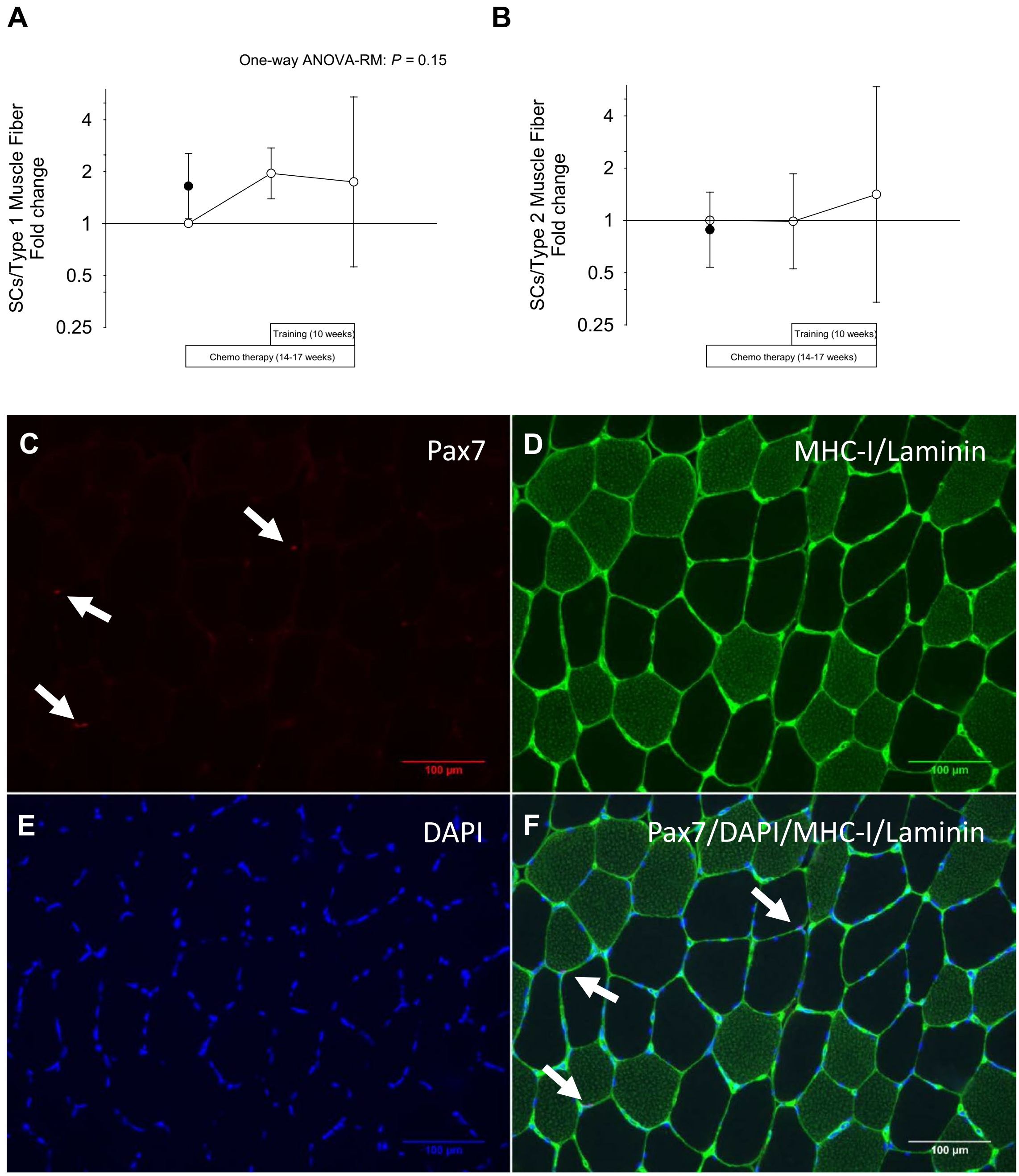

Fig. 4 Satellite cell number in type 1 and type 2 fibers. The number of Pax7$^+$ cells (satellite cells) associated with type 1 and 2 fibers were quantified by immunofluorescence analysis of muscle cross-sections. **a** Number of satellite cells associated with type 1 fibers, **b** number of satellite cells associated with type 2 fibers. Data are expressed at geometric mean ± 95% CI intervals. *Post hoc test for effect of time showed a significant difference during chemotherapy alone or in combination with exercise training. #Depicts differences between cancer patients and healthy subjects. Representative muscle cross-section stained for Pax7 (**c**), MHC-I and Laminin (**d**), nuclei (**e**), and merged (**f**). Scale bar 100 µm

these beneficial effects. We demonstrate that chemotherapy alone disrupts molecular signaling pathways involved in adaptation to exercise training and that adding exercise to chemotherapy may prevent further disruption. These findings indicate that the molecular basis of training adaptation remains intact in cancer patients during chemotherapy and emphasize the therapeutic potential of exercise training as a strategy to combat muscular complications to cancer and its treatment.

Skeletal muscle mass is regulated by the balance between protein synthesis and degradation (Wackerhage and Rennie 2006). At the cellular level, protein synthesis proceeds on the ribosomes, whereas the two major systems for protein degradation are the ubiquitin–proteasome system and the autophagy–lysosome system (Schiaffino et al. 2013). Exercise training has previously been shown to prevent loss of muscle mass in cancer patients during concomitant chemotherapy (Mijwel et al. 2018). We hypothesized that these effects would be reflected at the molecular level by altered expression of proteins involved in regulation of protein balance. Accordingly, protein expression of MURF1 and ATROGIN-1, which target proteins for degradation in the proteasomes, decreased during chemotherapy alone. Same pattern was observed for the expression and phosphorylation of autophagy-related proteins such as ATG5, p62, and p-ULK1 Ser^{757} that were either elevated or decreased during chemotherapy alone. Phosphorylation of mTOR Ser^{2448} and its downstream substrates 4EBP1 $Thr^{37/46}$ and S6rp $Ser^{235/236}$ were elevated during chemotherapy alone, and although only 4EBP1 $Thr^{37/46}$ reached statistical significance, the consistency of the data suggests that regulation of protein synthesis is altered. These findings demonstrate that expression and phosphorylation of proteins involved in regulation of protein synthesis and degradation are disrupted in skeletal muscle from cancer patients during chemotherapy. Interestingly, the expression and phosphorylation of all of the above mentioned proteins leveled off after 10 weeks of chemotherapy combined with exercise training. This suggests that exercise training prevents further disruption of these systems and emphasize the therapeutic potential of exercise training in cancer patients.

Despite the evident changes in proteins involved in regulation of protein synthesis and degradation, we did not observe any changes in expression of other proteins regulating the same systems. This could indicate that these proteins play a minor role in regulation of these systems. However, it could also be a consequence of the modest changes in muscle fiber cross-sectional area observed in these patients as reported previously (Lonbro et al. 2017). Thus, it is likely that the effects observed in the present study would be even more evident in cancer patients with profoundly affected muscle mass.

Phosphorylation of mTOR Ser^{2448} and its downstream substrates 4EBP1 $Thr^{37/46}$ and S6rp Ser^{32} increased during chemotherapy alone, whereas MURF1 and ATROGIN-1 decreased. As presented previously, this was associated with a trend toward reduced cross-sectional area of the muscle fibers. These findings might seem counterintuitive, as increased mTOR signaling usually is associated with increased protein synthesis and increased protein expression of MURF1 and ATROGIN-1 are associated with increased protein degradation in human skeletal muscle (Bak et al. 2016; Vendelbo et al. 2014a). It can be speculated that these systems are regulated to compensate for the detrimental effects of chemotherapy and disuse on skeletal muscle from cancer patients.

Phosphorylation of FOXO3a at $Ser^{318/321}$ and NFκB Ser^{536} increased only during exercise training and chemotherapy. Evidence from studies in cultured cells have demonstrated that phosphorylation at these specific residues cause translocation to the nucleus where FOXO3a and NFκB control transcription of genes, including those encoding MURF1 and ATROGIN-1 (Bu et al. 2016; Milan et al. 2015; Tong et al. 2009). In line with this, protein expression of MURF1 and ATROGIN-1 increased during exercise training and chemotherapy. This could indicate that recruitment of proteins to the proteasomes is elevated during chemotherapy combined with exercise training. This observation is in agreement with previous findings demonstrating that ATROGIN-1 expression is required for training-induced adaptation in mice (Baehr et al. 2014). Thus, increased protein expression of MURF1 and ATROGIN1 after exercise training may promote proper adaptation in skeletal muscle from cancer patients.

Protein expression of GLUT4 and ACC increased during chemotherapy alone, and while GLUT4 continued to increase during chemotherapy combined with exercise training ACC levelled off. We did not observe any changes in other regulators of substrate metabolism or mitochondrial proteins. Thus, we only observed minor changes in metabolic enzymes and mitochondrial proteins. These findings are in contrast to previous studies that demonstrate increased expression of metabolic regulators and mitochondrial proteins in human skeletal muscle after exercise training (Frosig et al. 2004; Zampieri et al. 2016). This could indicate that cancer patients who receive chemotherapy are resistant to training adaptation in skeletal muscle. However, we cannot exclude that the training stimulus imposed to the patients was too low to induce metabolic adaptations, as the patients completed only 75% of the training sessions (Lonbro et al. 2017). Moreover, the majority of the training intervention consisted of resistance exercise, which is supposed to induce hypertrophic rather than metabolic adaptations. On the other hand, the training intervention itself could have encouraged the patients to increase their habitual activity level. We did not collect data on physical activity level in the present study, but this is something that should be considered in future studies.

The number of satellite cells did not increase during chemotherapy alone, nor during chemotherapy combined with exercise training in type 1 and 2 muscle fibers. Expansion of the satellite cell pool associated with type 2 fibers has been suggested to serve as a regenerative response to physical exercise in human skeletal muscle (Farup et al. 2014). In our data, the number of satellite cells associated with type 2 muscle fibers increased in four patients and decreased dramatically in one patient, and consequently, the statistical analysis remained insignificant. Thus, based on the present study, we cannot exclude that satellite cells remain sensitive to exercise training in cancer patients despite ongoing treatment.

The present study is associated with some limitations. First, the patients represent a small heterogeneous group of cancer patients, and although the majority had a breast cancer diagnosis, patients with head and neck, rectal, and sarcoma cancer were also included. Second, the patients were treated with different chemotherapies with expectedly distinct effects on skeletal muscle tissue. Finally, the study design does not allow us to distinguish the effects of training from the effects of chemotherapy, as no non-exercising group was included due to ethical aspects as described previously (Lonbro et al. 2017). To deal with this issue we included skeletal muscle biopsies from healthy subjects to serve as a reference. Although these limitations should lead to cautious interpretation of the data, they do contain valuable information from tissues not easily accessible.

Conclusions

Cancer and chemotherapy disturb molecular signaling cascades involved in adaptation to exercise training, and exercise training may prevent further disruption of these signaling cascades. This indicates that the molecular basis of training adaptation remains intact in cancer patients during chemotherapy and emphasizes the therapeutic potential of exercise training as a strategy to combat muscular complications to cancer and its treatment.

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Author contributions ABM, SL, URM, IH, NJ conception and design of research; ABM, SL, TV, NR, URM, JW, JF performed experiments and collected data; ABM, JF, JW, SL, URM, and NJ analyzed and/or interpreted data; ABM prepared figures and first draft of manuscript; all authors revised the draft and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest No conflicts of interest are declared by the authors.

Ethics approval and consent to participate The study was approved by the local Scientific Ethics Committee of the Central Denmark Region (journal number: 1-10-72-15-14) and the Danish Data Protection Agency (case number: 2007-58-0010). The trial was registered at http//www.clinicaltrials.gov (Clinical trial registration number: NCT02192216).

Consent for publication Consent for publication has been obtained by the participants.

Availability of data and material All data generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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