Lean body mass, muscle fibre size and muscle function in cancer patients during chemotherapy and 10 weeks exercise

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Abstract

Background Chemotherapy can reduce muscle mass in cancer patients but the potential of exercise to ameliorate this is understudied, particularly at the myocellular level. The primary purpose was to investigate changes in lean body mass (LBM) and secondly single fibre cross-sectional area (CSA) in cancer patients during chemotherapy and in combination with 10 weeks of exercise.

Methods In a single-arm trial, patients adhered to chemotherapy for at least 4 weeks (control period) before 10 weeks of exercise adjunct to chemotherapy (exercise period). LBM (Dual Energy X-ray Absorptiometry) and single fibre CSA (muscle biopsies) were assessed at baseline, pre- and post-exercise. Muscle strength, functional performance and aerobic capacity were also assessed.

Results Ten patients were included, however only six patients completed the protocol. LBM changed over time (p=0.013), but no significant changes were observed between specific time points. Numerically, LBM decreased by 0.3 kg (p=0.41, 95% CI: -1.1;0.5) from 41.3–41.0kg, during the control period and increased by 0.7 kg (p=0.16, 95% CI: -0.6;2.0) from 40.4–41.1 kg during exercise. Muscle fibre CSA did not change significantly over time (p=0.13), but decreased numerically in the control period by 703 μm² (p=0.20, 95% CI: -1877; 470) and increased by 846 μm² (trend, p=0.08, 95% CI: -162; 1854) following exercise. Muscle strength and functional performance were unchanged during the control period but improved post-exercise.

Conclusions Despite non-significant changes in muscle mass (due to small sample size), this study adds novel information on LBM and myocellular changes in cancer patients during chemotherapy and concurrent exercise.

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Introduction

Loss of muscle mass is an undesirable and frequently occurring consequence of cancer. This either because of the disease per se (cancer cachexia) and/or due to anti-neoplastic treatment such as chemotherapy, radiotherapy or hormone treatment (1,2). Reduced muscle mass impairs muscle strength, functional performance and health-related quality of life in cancer patients (3,4). Moreover, skeletal muscle has been identified as a secretory tissue with important immunological and metabolic functions – and specifically muscle-derived cytokine secretion has recently been shown to be involved in reduced tumor growth in mice (5,6). Reduced muscle mass is also associated with tumor progression, impaired treatment tolerance and – response, and poor survival in cancer patients (7–12).

Substantial evidence shows that resistance exercise increases muscle mass, -strength and functional performance in both healthy (13–15) and sarcopenic...
individuals (16), but among cancer patients receiving anti-neoplastic treatment, only a limited number of studies have investigated the effect of exercise on muscle mass, and the results are diverging (8,17–19). Following a one-year period of combined resistance - and aerobic exercise, breast cancer patients undergoing chemotherapy increased their muscle mass and – strength (8). Similar findings have been reported in prostate cancer patients performing resistance exercise during androgen deprivation therapy (18). In contrast, others have failed to show an effect of exercise on lean body mass during treatment (19), which is why further studies are warranted.

The majority of clinical studies targeting body composition in cancer patients have focused on whole body or regional adaptations (20,21). Although these approaches provide clinically relevant information, they offer no details on the underlying cellular changes. This information is important to provide a better understanding of the mechanisms involved in changes in body composition in cancer patients and in order to facilitate the development of more effective intervention strategies. Sarcopenia in healthy individuals occurs rapidly from the age of about 50 years and is characterized by a predominant reduction in number and cross sectional area (CSA) of myofibres expressing myosin heavy chain II (MHC-II), i.e. type II fibres (22). The functional consequences of this fibre type specific atrophy are impaired fast and powerful muscle contractions, which are important during everyday activities (23,24). Research in changes in muscle fibres in cancer patients undergoing anti-neoplastic treatment or during exercise interventions are elusive, and alterations in fibre type distribution and CSA remain unresolved. So, whether anti-neoplastic treatment reduces single fibre CSA, whether it – despite an assumed faster time course than sarcopenia - follows the same pattern, and whether exercise can counteract this, remain unknown. In prostate cancer patients undergoing androgen deprivation therapy, Nilsen and colleagues showed that the CSA of type II fibres increased following a period of resistance exercise (25). Others have failed to show a CSA increase in germ cell cancer patients undergoing resistance exercise during chemotherapy (19).

The primary aim of the present trial was to investigate changes in LBM and single fibre area in cancer patients during chemotherapy during a control period (chemotherapy only) followed by an exercise period (chemotherapy + exercise). Secondly, changes in muscle strength, functional performance and aerobic capacity were investigated. We hypothesized that LBM and single fibre CSA would decrease in the control period and increase in the exercise period. To study the clinical relevance of changes in muscle mass or physical performance among the patients, these were compared to the baseline levels of healthy individuals.

**Materials and Methods**

**Setting and patients**

The present study was a single center, prospective, one-armed trial. After an introductory control period in which patients adhered to their planned chemotherapy (mean duration of 5.6 weeks; range 4-7 weeks), they all underwent a 10-week physical exercise intervention period concurrently with ongoing chemotherapy (exercise period). See Figure 1 for study design. Approval was received from the local ethics committee for the Central Denmark Region (journal number: 1-10-72-15-14), the Danish Data Protection Agency (case number: 2007-58-0010) and the trial was registered at clinicaltrials.gov (identifier: NCT02192216). Body & Cancer, a multimodal exercise intervention offer for cancer patients undergoing chemotherapy in Denmark (26,27), offers a six-week intervention comprising resistance exercise, aerobic exercise, massage sessions, relaxation therapy and body awareness. All cancer patients without brain and bone metastases receiving chemotherapy are offered participation in the Body & Cancer exercise program unless physical or mental illnesses prevent participation. The inclusion criteria to the present trial were as follows: patients referred to Body & Cancer; chemotherapy given in a curative, neo-adjuvant or palliative setting; a control period of at least 4 weeks between inclusion and start of the subsequent Body & Cancer program; an anticipated continuation of chemotherapy allowing a minimum of 8 weeks concurrent exercise; age ≥18; signed consent after written and oral project information. Hematological cancer patients and patients treated with Avastin or anticoagulants were excluded. Due to an extraordinary low inclusion rate during the first six months (two patients), patients undergoing adjuvant chemotherapy were added to the inclusion criteria.
Healthy individuals

To study the clinical significance of any changes in LBM or physical performance, a group of healthy matched individuals were included. Recruitment notices were posted at Aarhus University, Aarhus University Hospital, and on a website with the purpose of including test subjects in research projects (www.forsoegsperson.dk). The healthy individuals were included to match the gender, age, body weight and height of the patients. Individuals were to be excluded in any case of previous anti-neoplastic treatment, smoking or high levels of physically activity (more than two weekly high-intensity sessions of either resistance - or aerobic exercise). Of 25 approaches from healthy subjects willing to participate, 10 subjects were chosen one-to-one with the patients for optimal matching (Table 1).

Physical exercise intervention

Following the control period, all patients initiated a 10-week, fully supervised, physical exercise period. Every week the patients met to complete three 90-minute exercise sessions of both resistance - and aerobic exercise (Monday, Wednesday and Friday). The sessions comprised six resistance exercises using conventional equipment (knee extension, leg press, lateral pull-down, chest-press, back extensions and sit-ups). The intensity progressed from 12 repetition maximum (RM, where 1RM is the maximal load you can lift with proper technique one time only) in the first week, 10 RM in the second week, 8-10 RM in week 3-6 and 8 RM in the final four weeks. Both back extensions and sit-ups were floor exercises and the intensity was kept at 15 RM throughout the training period using dumbbells if necessary. After completion of all resistance exercises, the session was interrupted by a 10-minute break in which the patients consumed a whey protein drink (provided by Arla Foods Ingredients Group P/S, Aarhus, Denmark). The protein powder was mixed with 300 ml of water and contained 30 g of whey protein, 16 g of carbohydrate and 1 g of fat providing 360 kcal per serving. Hereafter, a group session of aerobic exercise performed on ergometer bicycles (Monark, Sweden) was initiated. As described previously (27), exercise intensity was adjusted by means of the Borg Scale of perceived exertion (scored from 6-20). The exercise duration

Figure 1 Patient flow diagram and study design. #: Due to a very low inclusion rate with only two patients included in the first six months, patients undergoing adjuvant chemotherapy became part of the inclusion criteria from six months and throughout the rest of the inclusion period.
progressed from 10 to 20 minutes throughout the exercise period. Following a few minutes of warm-up an increasing number of 30 to 60 second intervals with increased load were performed, to ensure exercise intensity on the Borg Scale of 14-18. Since all included patients participated in Body & Cancer; the first six weeks of the exercise intervention was an integrated part of the Body & Cancer framework, which in addition to resistance- and aerobic exercise included four 30-minute relaxation and stretching sessions, two massage sessions and a session of body awareness previously described in detail (27). This was not included during the last four weeks of the exercise period. Throughout the entire exercise period, the patients logged the number of sets, repetitions and exercise load in exercise logs, which provided information on resistance exercise adherence and - volume.

Table 1 Baseline demography for patients and healthy individuals (all female). Adjuvant treatment (Adj.), Neo-adjuvant (N.adj.), Palliative treatment (Pall.) Epirubicin (E), cyclophosphamide(C), Doxorubicin or adramycin(dox), carboplatin (carbo), Vinorelbain or navelbine (vino), fluroacil (5-FU), Trabectedin (Tra), Gemcitabin (Gem). § denotes patients that dropped out during the exercise intervention and are not included in the exercise adherence calculation. Data presented as mean values ± Standard Error of the Mean.

<table>
<thead>
<tr>
<th>Patients</th>
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<tbody>
<tr>
<td><strong>Id</strong></td>
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<td>51</td>
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<tr>
<td>9</td>
<td>43</td>
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<tr>
<td>10</td>
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<tr>
<td><strong>Mean (SEM)</strong></td>
<td><strong>51 (3)</strong></td>
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</table>

**Assessment of primary and secondary endpoints**
All endpoints were assessed at baseline, pre- and post-exercise and for healthy control individuals at baseline only (see Figure 1). All testing and tissue sampling were dispersed over two days. Day 1 comprised a Dual Energy X-ray absorptiometry (DEXA) scan, blood- and muscle tissue sampling. Both patients and healthy controls were asked to complete the scanning and tissue sampling in an overnight fasted state. When this was not possible due to patient concerns, typically when testing in the afternoon, patients were asked to consume the same small breakfast meal before all three tests (and meet after a minimum of 4 hours of fasting). An extra DEXA scan was performed six weeks into the exercise period. Day 2 comprised all physical testing. For all patients Day 1 was completed either more than 24 hours prior to or more than 48 hours after Day 2 to minimize the risk of influence of physical activity on tissue sampling analyses. Also, the post-exercise biopsy was obtained no less than three days after the last exercise session.

**Dual Energy X-ray Absorptiometry**

Whole body LBM (primary endpoint) was evaluated using DEXA with narrow fan beam technology (Hologic QDR, Hologic Inc., Bedford, MA, USA), as is widely used to assess LBM and body composition and validated in cancer patients (28,29). Fat mass was also assessed. All analyses were performed by an experienced laboratory technician blinded in regards to the patient’s time course in the study protocol.

**Muscle biopsies**

Muscle biopsies were obtained under local anesthesia (10 ml of 10 mg/ml lidocaine) from the middle lateral part of the vastus lateralis using a Bergstrom needle (30). All sampling was done under sterile conditions leaving the risk of complications minimal (31). To further reduce the risk of infection or bleeding, due to immunosuppression or thrombocytopenia, biopsies were obtained either within a few days before or after chemotherapy administration. Biopsies were obtained alternately from the left and right mid-thigh to avoid influence on the analyses due to tissue damage from the previous biopsy. The samples were dissected free from visible fat and connective tissue and a well-aligned portion of the biopsy was immediately mounted in Tissue-Tek (Qiagen, Valencia, CA), frozen in isopentane precooled in liquid nitrogen, and stored at −80°C pending analyses. All biopsies were assigned a random unique identification number, thereby blinding the investigator to subject identity and time point. Serial transverse sections (10 μm) were cut at −20°C using a cryostat and placed onto Superfrost Plus glass slides (Menzel-Gläser, Braunschweig, Germany) mixing samples from different subjects and time points on the same slide.

**Histochemical analyses**

Muscle biopsy sections were fixed in Histofix (Histolab, Gothenborg, Sweden) followed by a 1.5 hour in blocking buffer (0.2% Triton-X, 2% BSA, 5% fetal bovine serum, 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 hour in secondary Alexa Fluor 568 goat anti-mouse IgG antibody (Molecular Probes, cat no. A11034, Invitrogen A/S, Taastrup, Denmark). Following this, the sections were incubated with primary antibodies for type I MHC (1:500; clone A4.951, Developmental Studies Hybridoma Bank, IA, USA) and laminin (1:500; cat. no. Z0097, Dako Norden) for 2 hours and secondary Alexa Fluor 488 goat anti-mouse IgG antibody and Alexa Fluor 488 goat anti-rabbit IgG antibody (Molecular Probes, cat no. A11031 and cat no. A11034, Invitrogen A/S, Taastrup, Denmark) antibodies for one hour. Finally, the section was mounted in media containing 4',6-Diamidino-2-phenylindol (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. Pax7 staining is a standard part of the procedure at our laboratory; however, data from this analysis is not presented in this paper.

Images were obtained at 10x magnification using a Leica DM2000 microscope (Leica, Stockholm, Sweden) and a Leica Hi-resolution Color DFC camera (Leica, Stockholm, Sweden). Each biopsy was evaluated and only areas containing perfectly cross-sectioned fibres were included in the fibre area analyses. Distinctions between the areas of type I (type I MHC) or type II (type I MHC) fibres were made during the analyses (Figure 2). Between 171 and 407 muscle fibres (mean 286±54 fibres) were analyzed from each biopsy. Single fibre areas were measured and classified using Leica Qwin Lite v 3.5.1 software. The same blinded investigator conducted the analysis for CSA and fibre typing to secure a uniform procedure.
**Physical testing**

Maximal muscle strength was assessed using 1RM tests in leg press, knee-extension, lateral pull-down and chest-press. The 1RM test is frequently used to assess muscle strength in clinical trials including trials in cancer patients (27,32). Physical performance resembling activities of daily living was assessed using the 30s sit-to-stand test, stair-climbing test and 30s arm-curl test conducted as described previously (33). Peak oxygen consumption (VO₂peak) was assessed indirectly using the Watt-max test on an ergometer bicycle (Monark Ergomedic 839E, Sweden) following a protocol with a starting load of 30-60 W at a constant pedaling frequency of 70-80 RPM, and the load was increased progressively by 30 W every 3 minutes until exhaustion. Watt-max and VO₂peak were calculated as described previously and aerobic capacity was reported as VO₂peak (34).

**Statistics**

From a power calculation with change in LBM as the primary outcome, the trial was planned to include 16 patients. In the calculation, an expected 1.0 kg increase in LBM following the exercise period (based on results from previous studies (8,20)) was compared to unchanged LBM in the control period. Standard deviation in both periods was set to 1.3 kg (20). The calculation was based on an anticipated drop-out rate of 10%, power was 0.8 and level of significance was set at 0.05.

Given that the primary aim was not to investigate feasibility but changes in muscle mass following the intervention, all data were analyzed as per protocol excluding patients that did not complete all testing. Effects of time for all endpoints were analyzed using one-way-ANOVA analysis of variance with repeated measures and to investigate specific changes during the periods of interest — specifically baseline to pre-exercise (control period) and from pre- to post-exercise (exercise period), Students paired t-tests were performed as post hoc analyses. Linear regression analyses investigated differences between healthy individuals (omitted as reference) and the different time-points for the patients. All values are presented as mean values with 95% Confidence Intervals (95% Cl) and results are given with p-values with a 5% significance level and 95% Cl unless otherwise stated. Data were visually checked for normality using q-q-plots and histograms. All statistical analyses were performed using Stata version 11.2 (StataCorp, Texas, US).

**Results**

Patient inclusion began in February 2014 and was terminated in January 2015 when ten patients had been included in the study. Unfortunately, inclusion was
terminated prematurely because of an extraordinary low inclusion rate combined with time-constraints. The flow of patients is illustrated in Figure 1. During the inclusion period 21 patients were eligible for inclusion. Ten patients (female only) were included in the study and underwent all baseline testing. Four patients dropped out prematurely, mainly due to lack of surplus energy (Figure 1), leaving six patients to complete all exercise and testing. No data from patient medical records, pointed out any obvious differences between completers and non-completers that could explain the premature termination of the four patients. A total of 10 healthy individuals completed all baseline testing as planned. As shown in Table 1, there were no differences in demography and anthropometrics between patients and healthy individuals. No adverse events of exercise or muscle biopsy sampling were reported.

**Lean body mass**

The ANOVA analysis revealed a significant effect of time on whole body LBM (p=0.013), however no significant differences were detected between any of the time points (Figure 3). Numerically, whole body LBM decreased by 0.3 kg (n=9, p=0.41, 95% CI: -1.1;0.5) from baseline (41.3 kg) to pre-exercise (41.0 kg). Following 10 weeks of exercise, total LBM increased numerically by 0.7 kg from 40.4 to 41.1 kg (n=6, p=0.16, 95% CI: -0.6;2.0). Similar findings were observed in regional differentiation of lower and upper body LBM. Interestingly, despite the changes being non-significant, whole body LBM tended to increase by 1.8 kg (n=5, p=0.08, 95% CI: -0.4;4.1) during the first six weeks of the exercise period and decreased by 2.0 kg (n=5, p=0.11, 95% CI: -3.2;0.5) during the last four weeks of exercise. No changes were observed in fat mass.

![Figure 3](image)

**Figure 3** Mean and individual values of total lean body mass (kg) at baseline, pre-exercise, after six weeks of exercise and post-exercise. P-values from the one-way-ANOVA and T-tests are presented (n=6). P-value in the bottom denotes a significant change over time. P-value in the top of the figure denotes a trend (p=0.08) towards statistically significant difference from the previous time-point.

**Single fibre cross-sectional area (CSA) and fibre type distribution**

No significant changes over time were observed in mean muscle fibre CSA (p=0.13, Figure 4). Despite being non-significant, there was a tendency that mean fibre CSA decreased numerically by 795 μm² (n=9, p=0.18, 95%-CI -2046; 457) during the control period, whereas a significant increase by 821 μm² (n=6, p=0.04, 95% CI 29; 1613) was observed during the exercise period. No significant changes in MHC II CSA or muscle fibre type distribution (data on the latter not shown) over time were observed and no significant changes during either the control or exercise period were observed.
Maximal muscle strength

One-RM knee extension, chest press and lateral pull-down improved significantly over time (Figure 5, p<0.05). The change over time in leg press was not significant. No change occurred during the control period in either measure of muscle strength, but all measures of muscle strength increased during the exercise period; 1RM knee extension increased by 17.6 kg (33%) (n=5, p=0.01, 95% CI: 12.7;22.5), 1RM chest press by 7.0 kg (19%) (n=5, p<0.001, 95% CI: 5.6;8.0), 1RM leg press by 21.3 kg (20%) (n=5, p=0.01, 95% CI: 8.2;34.4), and lateral pull-down by 7.5 kg (20%) (n=4, p=0.03, 95% CI: 1.0;13.9).

Functional performance and aerobic capacity

The performance (Figure 6) in both the 30 s chair-rise and 30 s arm-curl test changed significantly over time (p<0.05) with no change during the control period. Chair-rise tended to improve by 4 reps from pre-exercise (25 reps) to post-exercise (29 reps) (n=5, p=0.057, 95% CI: -0.2;8.2) and arm curl performance improved by 5 reps from pre-exercise (24 reps) to post-exercise (28 reps, n=5, p<0.05, 95% CI: 0.4;8.8). Stair-climbing performance did not change significantly over time or specifically during the control and exercise period. There was a trend towards a change over time in 10-m walking performance (p=0.08), which did not change during the control period, but improved during the exercise period by 0.37 seconds (n=6; p<0.001; 95% CI: -0.25; -0.50). VO2-peak did not change significantly over time but tended to decrease by 375 ml O2 during the exercise period from 1842 to 1467 ml O2 (n=7; p=0.06; 95% CI: -776;26) with a numerical increase of 395 ml O2 during the exercise period, not reaching statistical significance (n=5; p=0.17; 95% CI: -259;1049).

Patients versus healthy individuals

Whole body LBM and myocellular CSA were not different between the healthy matched controls and the patients at any time point (Table 2). At baseline several measures of muscle strength and functional performance were significantly reduced in the patients compared to the healthy individuals (p<0.05). These differences diminished during the intervention. See Table 2 for details.

Figure 4 - top Mean (±) and individual (triangles) changes over time in single fibre area (total, MHCI and MHCIi) among the patients. Specific p-values in top of figures denote statistical change from previous time point (n=6). Figure 4 - bottom Mean curves of fibre area frequency of MHCI and MHCIi fibres at baseline, pre- and post-exercise.
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**Figure 5** Mean change in maximal muscle strength (1RM, kg) in chest press, leg press, knee extension and lateral pull-down. Data presented as means ± Standard Error of the Mean. * denotes significant difference between post-exercise and pre-exercise values (p<0.05). Specific p-values denote significant difference from previous time-point (n=6).

**Figure 6** Mean values in functional performance and aerobic capacity at baseline, pre- and post-exercise. Data presented as means ± Standard Error of the Mean. * denotes significant change over time (p≤0.05). Specific p-values denote significant difference from previous time-point (n=6).

**Discussion**

The main purpose of the study was to investigate changes in muscle mass in cancer patients during chemotherapy followed by chemotherapy with concurrent physical exercise. No significant changes in whole body LBM or single fibre CSA were reported during the control period or during the exercise period. Indeed, the statistical power was compromised with six patients completing the entire trial. Nonetheless, the patterns in changes of both LBM and single fibre CSA followed the working hypothesis with a decrease in whole body LBM and mean single fibre CSA during chemotherapy alone followed by an increase during the following period with concurrent exercise. Obviously, the lack of a group of patients not undergoing chemotherapy prevents any conclusions on specific chemotherapy-induced changes in LBM and muscle fibre size in the present study. Thus, the combined effect of both the cancer disease (cancer cachexia) as well as chemotherapy must be acknowledged both during the control period and the subsequent exercise period.
Table 2 Left part: Mean values of various endpoints at baseline, pre- and post-exercise for all patients and baseline values of healthy controls (±Standard Error of the Mean). Right part: Results from the linear regression analyses on difference between patients and healthy individuals are presented. * denotes significant difference (p<0.05); [*] denotes a trend towards statistical significant difference (p<0.1).

<table>
<thead>
<tr>
<th></th>
<th>Healthy individuals Baseline</th>
<th>Patients Baseline Pre-exercise</th>
<th>Patients Post-exercise</th>
<th>Patients baseline vs Healthy individuals baseline</th>
<th>Patient baseline Pre-exercise vs Healthy individuals baseline</th>
<th>Patient Post-exercise vs Healthy individuals baseline</th>
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<td>Whole body LBM</td>
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<td>41.3 ± 1.5</td>
<td>40.9 ± 1.5</td>
<td>41.1 ± 2.1</td>
<td>-0.7 [-4.9;3.6]</td>
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<td>-0.6 [-6.9;5.7]</td>
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<td>Lower body LBM</td>
<td>14.1 ± 0.7</td>
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<td>13.3 ± 0.6</td>
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<td>-1.1 [-2.9;0.7]</td>
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<td>Upper body LBM</td>
<td>24.7 ± 1.0</td>
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<td>24.1 ± 0.9</td>
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<td>Whole body FM</td>
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<td>22.8 ± 3.8</td>
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<td>Single fibre CSA (µm²)</td>
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<td>Mean fibre</td>
<td>3132 ± 266</td>
<td>3879 ± 398</td>
<td>3188 ± 288</td>
<td>3718 ± 382</td>
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<td>4418 ± 423</td>
<td>3619 ± 204</td>
<td>4252 ± 304</td>
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<td>100 [-910; 1111]</td>
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</tr>
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| 1 RM Muscle strength (kg) |                              |                                |                        |                                               |                                               |                                               |        |
| Chest press             | 47 ± 5                       | 35 ± 8                         | 35 ± 4                 | 44 ± 5                                       | -12 [*] [-24.0]                                  | -12 [-25; 2]                                    | -5 [-24;14]   |
| Leg press               | 118 ± 10                     | 85 ± 7                         | 94 ± 16                | 123 ± 20                                     | -33[*] [-66.0]                                  | -25 [-63; 13]                                   | 4 [-44;52]   |
| Lateral pull down       | 41 ± 3                       | 35 ± 4                         | 34 ± 3                 | 45 ± 4                                       | -6 [-15;4]                                     | -6 [-15; 4]                                    | 3 [-11;18]   |
| Knee extension          | 61 ± 6                       | 49 ± 4                         | 51 ± 5                 | 71 ± 6                                       | -12 [-28;4]                                    | -8 [-29; 9]                                    | 8 [-13;30]   |

| Functional performance  |                              |                                |                        |                                               |                                               |                                               |        |
| 10 m gang (s)           | 4.88 ± 0.34                  | 4.67 ± 0.24                    | 4.78±0.35              | 4.12 ± 0.37                                  | -0.21 [-1.1;0.68]                                | -0.18 [-1.14; 0.79]                               | -0.4 [-1.31;0.44] |
| Stair climbing (s)      | 8.05 ± 0.17                  | 9.28 ± 0.77                    | 9.33 ± 1.02            | 7.93 ± 0.54                                  | 1.23 [-0.54;3.00]                                | 1.27 [-0.80; 3.35]                                | 0.11 [-1.53;1.75] |
| 30 s chair rise (reps)  | 28 ± 1                       | 22 ± 2                         | 23±2                   | 29 ± 2                                       | -7* [-11;-2]                                    | -5 [-11; 0.17]                                   | 0 [-6;5]     |
| 30 s Arm curl (reps)    | 24 ± 1                       | 22 ± 2                         | 22 ± 2                 | 29 ± 2                                       | -3 [-7.1]                                      | -2 [-6; 3]                                      | 5[*] [0;11]  |
| VO2peak (ml O2 x min⁻¹) | 2284 ± 147                   | 1777 ± 167                     | 1467 ± 164             | 2114 ± 280                                   | -507 [-985;28]                                  | -800 [-1354;245]                                 | -170 [-854;518] |

Control period

The fact that whole body LBM and single fibre CSA did not change significantly during the control period, may be interpreted as a positive clinical finding, since chemotherapy-induced muscle atrophy has been reported previously (10,35–37). The mean 5.6-week control period of the present study may have been too short for a potential catabolic effect of chemotherapy to induce a measurable change in whole body LBM or single fibre CSA. This is supported by the lack of changes in muscle strength or functional performance during the control period. We speculate that a longer control period or a larger sample size, as originally intended, would...
have provided a statistically significant reduction in LBM and single fibre CSA. Within the present data, additional analyses found no correlation between individual changes in LBM or single fibre CSA and the duration of the control period ranging from 4-7 weeks among the patients.

Despite not reaching statistical significance, both LBM and single fibre CSA changes followed the working hypothesis and decreased during the control period. This is in line with previous reports with larger populations and longer duration of the chemotherapy period (10,19,37). The present study is one of few few studies to obtain muscle biopsies from cancer patients during anti-neoplastic treatment. Interestingly, the patterns from the present trial support the results from Christensen et al. (19) and Nilsen et al. (25) who report adverse effects from systemic anti-neoplastic treatment (whether being chemotherapy or androgen deprivation therapy) on the myocellular level in terms of decreased single fibre CSA.

**Exercise period**

No significant increases in neither whole body LBM nor single fibre CSA were observed during the 10-week exercise period; however, a trend towards an increase in mean single fibre CSA was revealed (p=0.08). This was primarily related to changes in MHC I CSA (p=0.04) although a similar non-significant pattern was observed for CSA of MHC II fibres. The lack of significant changes may partly be explained by a large variation primarily due to the small sample population. Several additional factors could explain why data did not reveal a positive response to exercise. For instance, the exercise adherence declined to an average of two weekly sessions during the last four weeks of the exercise period (due to illness, patients resuming work etc.) potentially limiting the exercise response (see Supplementary Material). However, in healthy individuals, two weekly exercise sessions is regarded as a sufficient hypertrophic exercise stimulus (38). The limited and diverging scientific evidence cannot conclude whether this is sufficient in cancer patients during chemotherapy (8,19). In addition to exercise adherence, the character of the exercise intervention comprising both resistance- and aerobic exercise may impede the hypertrophic response to exercise. As suggested previously (39) the addition of aerobic exercise to resistance exercise may initiate myocellular signaling pathways that interfere with the expected hypertrophic response to resistance exercise. Thus, the character of the exercise intervention may actually impede LBM increases during chemotherapy. This would infer that the exercise modality should specifically target either muscle hypertrophy (resistance exercise) or improvement in aerobic capacity (aerobic exercise) – not both. In contrast, others have failed to support an inhibitory effect of concurrent exercise on muscle growth (40).

In their randomized trial Christensen and colleagues (19) found no significant differences in mean single fibre CSA between the group doing resistance exercise compared to a non-exercising control group of germ cell cancer patients during chemotherapy treatment. In contrast, in a matched group of healthy individuals performing the same resistance exercise regime an increase in single fibre CSA was observed. These results indicate an inhibitory effect of chemotherapy on muscle growth, which is supported by the non-significant decrease in fibre CSA during the initial chemotherapy period in the present trial. In opposition, Nilsen et al. found that prostate cancer patients undergoing androgen deprivation therapy, increased mean single fibre CSA significantly during resistance exercise compared to a control group, with a larger increase in the MHC II fibre areas (25). Collectively, the variations in patient characteristics, treatment and exercise interventions prevents a proper comparison of results from these few studies.

It is generally accepted that MHC II fibres are more responsive to heavy resistance exercise compared to MHC I fibres regardless of age (41). One explanation for the lack of hypertrophy in MHC II fibres in the present trial is that the statistical power is reduced due to the reduced sample size. Thus, the contrasting response of two patients (reduced MHC II CSA) largely affects the group mean changes. Additional within-group analysis in the patients showed that mean CSA in MHC I fibres were significantly larger than MHC II CSA throughout the intervention (p=0.01, Figure 3 and 4). This is consistent with the findings in the group of healthy individuals (p≤0.01) and in healthy elderly in general (42) suggesting a selective MHC II fibre atrophy more related to age (43) than the cancer disease or treatment per se. Nonetheless, others have failed to show this difference in CSA between fibre types in cancer patients (44).

Unexpectedly, a trend towards a transitory change in whole body LBM during the exercise period was observed. LBM increased by 1.8 kg (p=0.08) from pre-exercise to six weeks into the exercise, where after it decreased by 2.0 kg again at week 10 (p=0.11). The lower adherence during the last four weeks is unlikely to explain this decrease over four weeks, which is usually only observed during immobilization or under severe catabolic conditions (45). We speculate if the validity of the DEXA scan may have been compromised by variations in oedema caused by chemotherapy or other uncontrolled factors affecting patient hydration. This is a recognized source of overestimation when assessing body composition using DEXA scans as shown in peritoneal dialysis patients with oedema (46). Specifically, the side-effects of Docetaxel, which was administered to certain patients in the present trial, include the formation of oedema caused by a capillary
protein leakage (47,48); however no data from patient medical records revealed signs of oedema, and we can only speculate if this was in fact a source of variation in our data.

During the exercise period, maximal muscle strength increased significantly up to 33%, which is in line with the findings of the original Body & Cancer studies (27), reporting similar increases after six weeks of exercise in both male and female patients.

**Differences between patients and healthy individuals**

No significant differences in whole body LBM or myofibre CSA were observed between patients and the healthy individuals (Table 2). Several measures of muscle strength and functional performance were either significantly lower or trending to be lower in patients at baseline compared to the healthy individuals. These findings emphasize the reduced physical condition of the patients when entering the trial. Despite the fact that patient performance did not change significantly during the control period, the differences were mostly evened out already pre-exercise. This is likely due to the observed drop in sample size and missing values in each statistical test reducing the power of the analyses.

**Strengths and limitations**

This is one of few investigations of changes in muscle mass before and after exercise in cancer patients undergoing chemotherapy, and we provide valuable information on changes at the myocellular level. No adverse events from the muscle biopsy sampling were reported. *Body & Cancer* is an established offer for cancer patients during chemotherapy in Denmark. For this reason, it was not ethically acceptable to conduct the present study in a randomized controlled setting. This prevented the inclusion of a non-exercise control group. Instead a prospective design was used wherein the patients served as their own controls and in addition a healthy non-exercise control group was included. However, the duration of the control period may have been too short for the expected changes in muscle mass to occur. Moreover, the fact that the trial did not include a group of patients not undergoing chemotherapy, prevents any conclusions on the specific effect of chemotherapy on muscle mass, since any changes could also be induced by the cancer disease per se (cancer cachexia). Four of the ten included patients dropped out prematurely reducing the statistical power and increasing the risk of type 2 errors. For a few of the functional performance tests and the 1RM the sample sizes were further reduced because of a few instances where patients were unable to be tested primarily due to minor injuries. Furthermore, the ability to interpret the changes in muscle mass in relation to individual variations in patient age, cancer diagnosis, treatment modality, and timing of treatment was also limited. Ultimately, interpretation of the data should also be done with caution.

**Conclusion**

Despite a reduced statistical impact, important information on whole body LBM and muscle cellular changes during chemotherapy and concurrent exercise in cancer patients are provided. Obviously, further prospective controlled studies are warranted, however together with existing knowledge these results cautiously suggest that chemotherapy unfavorably affects muscle mass (single fibre CSA), and that this may be counteracted by concurrent physical exercise. Larger studies are needed to further elucidate muscle mass and single fibre responses to exercise in cancer patients during chemotherapy. To distinguish the specific effects of chemotherapy and cancer cachexia, the challenge of conducting research in cancer patients with active disease but not undergoing chemotherapy must be taken on.

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**Declaration of interest**

The authors report no conflicts of interest.

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