Muscle satellite cell content and mRNA signaling in germ cell cancer patients – effects of chemotherapy and resistance training

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To the Editor,

Cisplatin-based chemotherapy has significantly improved treatment outcomes in testicular germ cell cancer (GCC) patients, but is associated with elevated risk of serious long-term complications and preventive measures are an unmet need [1]. Physical exercise is known to mitigate chemotherapy toxicities [2], and current cancer-specific guidelines emphasize the capacity of exercise to ameliorate muscle dysfunction by retaining muscle mass and improving physical function in patients undergoing toxic anti-cancer therapies [3].

Skeletal muscle is regulated through complex molecular mechanisms, and chemotherapy is known to target intracellular pathways involved in muscular protein turnover and function. Particularly, muscle progenitor cells, known as satellite cells, have recently gained attention due to their essential role in repair of damaged muscle [4], as well as a source of new myonuclei for muscle fibers undergoing hypertrophy in response resistance exercise. In the oncology setting, concerns are raised as to whether satellite cells entering the cell cycle in response to resistance exercise would be susceptible to chemotherapy damage potentially resulting in a loss of satellite cells [5], which could have implications for muscle repair and/or the progression of sarcopenia. Moreover, acute intramuscular dysregulation of protein turnover pathways could moderate muscle adaptations to resistance training. With current cancer-specific exercise guidelines supporting the addition of muscle preserving resistance exercise during chemotherapy, it is important to assess whether this combination negatively affects the muscle satellite cell pool [5], and whether chemotherapy is associated with dysregulated pathways involved in myofibrillar protein turnover.

In a randomized controlled trial, our group recently found that GCC patients undergoing standard care lost approximately 2.5 kg of lean mass over nine weeks of cisplatin-based chemotherapy, while resistance exercise tended to attenuate the loss of lean mass and improved muscle strength [6]. Here, we extend on our previous findings with the objective to describe intramuscular adaptations induced by chemotherapy with or without concurrent resistance exercise. Specifically, we present an explorative substudy in subjects who had repeated muscle biopsies taken before and after chemotherapy and/or resistance exercise, in order to evaluate changes in muscle satellite cell content and gene expression in GCC patients and healthy individuals.

Material and methods

The study was a randomized controlled trial (ISRCTN 32132990), described in detail elsewhere [7]. Reports on the overall safety and efficacy of resistance training [6], as well as changes in systemic inflammatory profile [8] from the same study have been previously published.

Subjects

Patients with disseminated GCC belonging to the ‘good prognostic group’ according to international guidelines [9] were eligible. Exclusion criteria were: (1) age <18 or >50 years; (2) evidence of cardiovascular disease (cardiomyopathy, coronary heart disease etc.); (3) chronic disease (diabetes mellitus, chronic obstructive pulmonary disease etc.); (4) inability to read and understand Danish.

All patients received standard bleomycin-etoposide-cisplatin (BEP) chemotherapy (cisplatin 20 mg/m² and etoposide 100 mg/m² daily for five days, and bleomycin (15,000 IE/m² weekly, administered in a three-week schedule for a total of nine weeks) as well as standard antiemetic treatment with prednisolone (50 mg daily), 5HT3-antagonists and metopimazine during the initial five days of each cycle.

A reference group of age- and body mass index (BMI)-matched healthy male individuals was recruited and screened for the same criteria.
Before administration of first dose of chemotherapy, patients had muscle biopsies taken from m. vastus lateralis using the Bergstrom technique with added suction. Samples were immediately mounted with Tissue-Tek, frozen in isopentane cooled with liquid nitrogen, and stored at −80 °C. After baseline assessments, GCC patients were randomly allocated 1:1 to either a resistance training intervention (INT) or usual care control (CON). Subjects in the healthy reference group (REF) had a muscle biopsy taken using same procedure, and were allocated without randomization to resistance training. Post-treatment biopsies were collected from INT- and CON-groups 48 hours after administration of the last dose of chemotherapy. Post-training biopsies in the REF-group were collected 48 hours after the final training session. All post-biopsies were collected ~2 cm proximal to the pre-biopsy incision site from the same muscle.

INT and REF were allocated to resistance training and performed three weekly supervised sessions for nine weeks comprising 3–4 sets of 10 repetitions at 10–12 RM load in four exercises: leg press, knee extension, chest press and lateral pull down, using stationary equipment (Technogym, Gambettola, Italy).

Muscle biopsy analyses
Study personnel conducting the assessment and analyses of muscle biopsies were blinded to group assignment and time point.

Satellite cells
From biopsy samples, serial sections (10 μm) were cut in a cryostat (−20 °C) and stored at −80 °C. Satellite cells were identified on sections stained with antibodies against Pax7, myosin Type I and laminin, as previously described [10]. The number of Pax7 cells associated with all fibers, and Type I and II fibers separately, was determined and expressed relative to the of number fibers included in the assessment.

mRNA analysis
Different mRNA targets were quantified by real time RT-PCR exactly as previously described [11]. Briefly, total RNA was purified from 10 to 20 mg muscle using TriReagent and converted into cDNA using OmniScript Reverse Transcriptase. The cDNA was then quantified with specific primers (Supplementary Table 1, available online at http://www.informahealthcare.com) using Quantitect SYBR Green Master Mix and an MX3005P real time PCR machine. Ct values were related to a standard curve to get copy numbers and RPLP0 was used for normalization.

We were unable to control for the time from the last exercise session to the post-biopsy sampling, and changes in mRNA responses from pre- to post-biopsies are consequently not comparable in the two exercise groups, thus only pre-to-post change in CON is presented.

Statistical analyses
Baseline characteristics were compared using unpaired students t-test and one-way ANOVA for parametric data and χ²-test for categorical data. Satellite cell data is presented as mean ± SEM and changes from pre- and post-biopsies were analyzed by two-way repeated measures ANOVA with group (CON, INT, REF) and time (pre, post) as the two factors. mRNA data were log-transformed before statistical analysis and compared with students t-test, unpaired or paired as appropriate. mRNA data are presented as geometric mean ± back-transformed SEM. All tests were two-tailed and significance level set at 0.05.
Results

At baseline, 30 GCC patients were included and randomized to INT \( (n = 15) \) or CON \( (n = 15) \), and 19 healthy subjects were allocated to REF \( (n = 19) \) (Supplementary Table 2, available online at http://www.informahealthcare.com). For the pre-to-post treatment/training analyses we obtained biopsies for useful analyses in a total of 17 patients (eight from the INT-group and nine from the CON-group) and 13 healthy subjects. For these completers, mean adherence to resistance training was 22.6 sessions (84%) in the INT-group, and 21.2 sessions (78%) in the REF-group.

**Satellite cells**

At baseline, we found no significant differences in satellite cell number for GCC patients versus healthy controls for

![Figure 2](https://example.com/figure2.png)

*(Caption: mRNA expression. Relative difference in mRNA expression of pathways involved in myofibrillar protein turnover in biopsies obtained at baseline and after 9 weeks of chemotherapy. mRNA data is presented as geometric mean ± back-transformed SEM, at baseline (A) for germ cell cancer patients \( (n = 26) \), right bar ± back-transformed SEM) relative to healthy individuals \( (n = 15) \), left; set to 1 ± back-transformed SEM), and change (B) at week 9 (POST, bar ± back-transformed SEM) in fold differences relative to baseline (PRE, set to 1) in the CON-group \( (n = 9) \). * = \( p < 0.05 \).)
all fibers (0.079 ± 0.004 vs. 0.090 ± 0.010, \( p = 0.299 \)), or for Type I fibers (0.071 ± 0.005 vs. 0.069 ± 0.008, \( p = 0.867 \)) or Type II fibers (0.087 ± 0.006 vs. 0.095 ± 0.014, \( p = 0.536 \), Figure 1). Over nine weeks from pre- to post-treatment/train-
ing, we found no significant group x time interaction in satellite cell content for all fibers (\( p = 0.366 \)), Type I fibers (\( p = 0.300 \)) or Type II fibers (\( p = 0.132 \)) and no individual effect of either factor was seen (Figure 1).

**mRNA expression**

Profiling mRNA markers at baseline in GCC patients and healthy subjects showed no differences, except for increased IGF-1Ec (MGF, Figure 2A). Nine weeks of BEP chemotherapy showed limited chronic effects in the CON-group, except for a slight increase in IL-6 and myostatin, but no changes in TNF-\( \alpha \)-related mRNA or other cachexia-related markers were seen (Figure 2B).

**Discussion**

Attention to cancer patients’ muscular profile has traditionally been confined to the wasting syndrome of cancer cachexia [12], with several reports showing molecular changes associated with tumor-induced subclinical myopathy in muscle biopsies sampled during cancer surgery [13,14]. However, pre-
treated with tumor-induced subclinical myopathy in muscle [12], with several reports showing molecular changes associ-
ated with significant intra-muscular dysregulation of path-
ways involved in myofibrillar protein turnover. Although these findings are based on a small-scaled, explorative substudy, we believe this information is reassuring for oncologists and therapists involved in the prescription of exercise, including muscle preserving resistance training, to cancer patients during chemotherapy. However, we emphasize that the present data should be interpreted with care due to the explorative nature of the study. Indeed, we believe our findings should be confirmed in larger scaled trials with sufficient power to detect significant differences in these outcomes in order to improve the limited mechanistic understanding of chemother-
apy-induced muscle degradation and the capacity of resist-
ance training to ameliorate muscle dysfunction during therapy.

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**Disclosure statement**

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**References**

5. Clarkson PM, Kaufman SA. Should resistance exercise be recom-
10. Molsted S, Andersen JL, Harrison AP, Eidesvik I, Mackey AL. Fiber type-specific response of skeletal muscle satellite cells to high-


