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Clinical Research Article

Resistance Exercise Counteracts the Impact of Androgen Deprivation Therapy on Muscle Characteristics in Cancer Patients

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Abstract

Context: Androgen deprivation therapy (ADT) forms the cornerstone in prostate cancer (PCa) treatment. However, ADT also lowers skeletal muscle mass.

Objective: To identify the impact of ADT with and without resistance exercise training on muscle fiber characteristics in PCa patients.

Methods: Twenty-one PCa patients (72 ± 6 years) starting ADT were included. Tissue samples from the *vastus lateralis* muscle were assessed at baseline and after 20 weeks of usual care ($n = 11$) or resistance exercise training ($n = 10$). Type I and II muscle fiber distribution, fiber size, and myonuclear and capillary contents were determined by immunohistochemistry.

Results: Significant decreases in type I (from 7401 ± 1183 to 6489 ± 1293 μm^2, $P < .05$) and type II (from 6225 ± 1503 to 5014 ± 714 μm^2, $P < .05$) muscle fiber size were observed in the usual care group. In addition, type I and type II individual capillary-to-fiber ratio (C/F*i*) declined (−12% ± 12% and −20% ± 21%, respectively, $P < .05$). In contrast, significant increases in type I (from 6700 ± 1464 to 7772 ± 1319 μm^2, $P < .05$) and type II (from 5248 ± 892 to 6302 ± 1385 μm^2, $P < .05$) muscle fiber size were observed in the training group, accompanied by an increase in type I and type II muscle fiber myonuclear contents (+24% ± 33% and +21% ± 23%, respectively, $P < .05$) and type I C/F*i* (+18% ± 14%, $P < .05$).

Conclusion: The onset of ADT is followed by a decline in both type I and type II muscle fiber size and capillarization in PCa patients. Resistance exercise training offsets the negative impact of ADT and increases type I and II muscle fiber size and type I muscle fiber capillarization in these patients.

Key Words: skeletal muscle, angiogenesis, testosterone, older adults, exercise rehabilitation

Abbreviations: 1RM, 1-repetition maximum; ADT, androgen deprivation therapy; BMI, body mass index; CC, capillary contact; CD, capillary density; C/F*i*, muscle capillary-to-fiber ratio; CFPE, capillary-to-fiber perimeter exchange; CON, control; CSA, cross-sectional area; CT, computed tomography; DXA, dual-energy x-ray absorptiometry; EX, exercise training intervention; PBS, phosphate-buffered saline; PCa, prostate cancer.

Prostate cancer (PCa) is the second most common type of cancer in men, with a yearly estimated 1.4 million new cases and 375 000 deaths worldwide (1). Androgen deprivation therapy (ADT) forms the cornerstone in (locally) advanced PCa treatment. ADT suppresses serum testosterone to castration levels, resulting in an increased life expectancy (2). However, low testosterone levels also induce the loss of skeletal muscle mass in PCa patients (3). Whereas the impact of ADT on whole-body muscle mass has been well established, its impact on muscle fiber characteristics remains largely unexplored.

The age-related loss of skeletal muscle mass and strength, termed sarcopenia, has been at least partly attributed to the decline in circulating testosterone levels in older men (4). As such, it is not surprising that ADT prescription accelerates muscle loss in older adults (3, 5). This results in a further increase in the risk for falls (6), a decline in mobility (7), and a lower quality of life (8) in older PCa patients. On the muscle fiber level, age-related muscle loss is characterized by the specific decline in type II muscle fiber size (9-11). Thus far, only one study has assessed the impact of ADT on muscle fiber size in PCa patients. Nilsen et al (2016) showed no significant changes in type I or type II muscle fiber size following 16 weeks of ADT in older PCa patients. In this study, the initial muscle biopsy sample was obtained approximately 9 months

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after the onset of ADT (12). As the largest decline in lean body mass has been reported to occur during the first months following ADT initiation (3), this may explain the lack of any measurable changes in muscle fiber size and fiber composition in the study by Nilsen and colleagues. Hence, more data are required to elucidate the impact of starting ADT on muscle fiber characteristics in PCa patients.

For optimal muscle tissue function and health, adequate perfusion is of critical importance as it is responsible for the delivery of oxygen, growth factors, and nutrients, and the removal of waste products (13). Oxygen and metabolic substrates are delivered to the nuclei, located at the periphery of muscle fibers. These myonuclei are key in the transcription and translation machinery prerequisite for muscle fiber maintenance, as well as repair and growth (14). Age-related type II muscle fiber atrophy is accompanied by a fiber type–specific decline in muscle fiber myonuclei (14, 15) and capillarization (16-18). Also, low muscle fiber capillarization has been associated with impairments in cardiorespiratory fitness (19), physical function (20), and aggravation of various indices of sarcopenia (19, 21). In animal as well as human studies, testosterone levels have been implicated as a potential regulator of angiogenesis and myonuclear accretion (22, 23). However, no studies to date have assessed the potential impact of starting ADT on these various muscle fiber characteristics in PCa patients. Insight into the negative effect of ADT on specific muscle fiber characteristics could aid in designing specific intervention strategies minimizing the adverse effects of ADT on skeletal muscle mass and function. We hypothesize that the onset of ADT leads to a decline in muscle fiber size, myonuclear content, and capillarization in PCa patients.

Resistance exercise training is an effective intervention strategy to counteract the ADT-induced loss of muscle mass in older PCa patients (24, 25). In accordance, Nilsen et al reported significant type II muscle fiber hypertrophy following 16 weeks of resistance exercise training in PCa patients who had already been exposed to ADT over an extensive period of time. Whether resistance exercise training can offset the changes in muscle fiber characteristics following the onset of ADT remains to be determined. We hypothesize that progressive resistance exercise training counteracts the impact of ADT on muscle fiber characteristics and increases fiber size, myonuclear content, and capillarization in PCa patients starting ADT.

In the present study we assessed the changes in muscle fiber characteristics following 20 weeks of ADT with and without resistance exercise training in PCa patients.

Methods

Patient Recruitment

This study was part of a greater project investigating the impact of prolonged resistance exercise training and protein supplementation on counteracting the adverse effects of ADT on body composition, strength, aerobic capacity, and quality of life in PCa patients (26). PCa patients starting with gonadotropin-releasing hormone agonist or antagonist treatment were recruited to participate in either a 20-week resistance exercise training program, or they were recruited for the control group receiving only usual care. Exclusion criteria were any contraindications for participating in an exercise training protocol. Additional exclusion criteria were an estimated life expectancy <1 year; cognitive disorders or severe emotional instability; or the inability to speak, understand, and/or read the Dutch language. All patients were asked if they were willing to undergo an optional muscle biopsy as an additional measurement, as this was not obligatory to participate in the overall project. A total of 21 PCa patients were selected for the current study, based upon the availability of both a baseline and a 20-week muscle biopsy sample. Patients were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. The study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre+, the Netherlands (METC 16-3-040) and complied with the guidelines set out in the most recent version of the Declaration of Helsinki. This study was registered at the Dutch Trial Register (International Clinical Trial Registry Platform: NTR6432) and was independently monitored by the Clinical Trial Centre Maastricht.

Study Design

Patients were recruited at Jeroen Bosch Hospital ('s-Hertogenbosch, the Netherlands), Maastricht University Medical Centre+ (MUMC+; Maastricht, the Netherlands), and Zuyderland Medical Centre (Zuyderland M.C.; Sittard/Heerlen, the Netherlands). Potential participants were identified and referred to the investigators by their treating urologist or urology nurse. Medical history (eg, Gleason score: score for the aggressiveness of the tumor) was obtained from the electronic patient file. Baseline measurements were performed as soon as was practically feasible following the initiation of ADT. These consisted of anthropometric measurements (height and body weight), whole-body dual-energy x-ray absorptiometry (DXA) scanning, computed tomography (CT)-scanning of the dominant leg, and muscle biopsy sampling. Patients recruited for the training intervention (EX) were enrolled in a 20-week resistance exercise training protocol. Patients recruited for the control group (CON) received only usual care. In the week following the exercise intervention or the usual care period, baseline measurements were repeated (>3 days after the final training session). To avoid variation in methods and procedures, all muscle biopsies were obtained in the Maastricht University Medical Centre+ by the same medical doctor. Furthermore, all measurements were performed in a rested and overnight fasted state.

Exercise Intervention Program

Patients in the EX group performed a 20-week, twice-weekly, progressive whole-body resistance exercise training program. Following a 5-minute warm-up on a cycle ergometer patients performed 2 warm-up sets and 4 working sets in both the leg press and leg extension machines (Technogym, Rotterdam, the Netherlands). Upper body exercises were paired (chest press with lateral pulldown and shoulder press with horizontal row) and were performed in an alternating manner between exercise sessions. Four sets, including one warm-up set, were performed for each upper body exercise. Each exercise session was ended with a 5-minute cool-down on the cycle ergometer. Resting periods of 1.5 and 3 minutes were allowed between sets and exercises, respectively. During the first 3 weeks of exercise training, workload was increased from 60% single-repetition maximum (1RM) to 70% 1RM (10 repetitions). Every fourth week, workload was reduced to 60% 1RM (10 repetitions) to allow for proper recovery and minimize the risk of injury. Workload intensity was adjusted based on 1RM measurements performed before, and after 4,

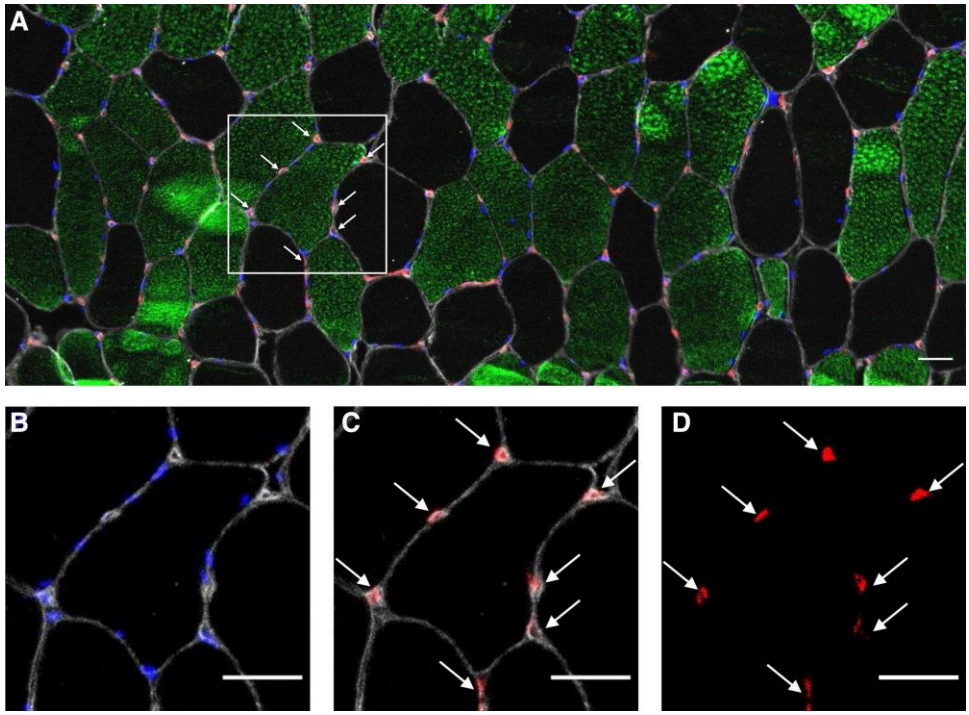

Figure 1. Representative images of the analyses for muscle fiber characteristics. Laminin (white; cell borders), DAPI (blue; myonuclei), MHC1 (green; type I muscle fibers), CD31 (red; capillaries) (A). Laminin (white), DAPI (blue) (B). Laminin (white), CD31 (red) (C). CD31 (red) only (D). White line represents 50 µm. Arrows point at the capillaries.

8, 12, and 16 weeks of the exercise training program. 1RM was determined as described previously (15).

Habitual Dietary Intake and Physical Activity

Patients were asked to report their habitual dietary intake on 2 weekdays and 1 weekend day in the week prior to the experimental test days. Average daily dietary intake was calculated using web-based software Eetmeter (Voedingscentrum, Den Haag, The Netherlands). In this same period, patients were instructed to wear a triaxial accelerometer (wGT3X-BT; ActiGraph, Pensacola, FL, USA) on their waist during wakefulness for 7 days. Data were analyzed with ActiLife (version 6.13.4; ActiGraph, Pensacola, FL, USA) and included in the analyses if patients wore the accelerometer for a minimum of 5 days and at least 10 hours per day. The assessment period for the 20-week measurements was performed during the final week (week 20) of the intervention.

Body Composition

Body weight was measured, wearing underwear and directly after voiding, using a scale to the nearest 0.5 kg. Height was measured by a fixed stadiometer to the nearest 0.5 cm. Whole-body lean mass and fat percentage were assessed by DXA (Discovery A; Hologic, Marlborough, MA and LUNAR iDXA; GE Healthcare, Chicago, IL, USA). Anatomic cross-sectional area (CSA) of the quadriceps muscle was assessed by CT scanning (SOMATOM Definition Flash; Siemens, München, Germany) as described previously (27). A single-slice image was made 15 cm proximal to the top of the patella of both legs. Quadriceps muscle CSA of the dominant leg was calculated by manual tracing using ImageJ software (version 1.52p, National Institute of Health, Bethesda, MD, USA).

Muscle Biopsy Sampling

Percutaneous needle biopsies were taken from the *vastus lateralis* muscle approximately 15 cm above the patella of the dominant leg (28). Muscle samples were prepared for analyses as previously described (29, 30).

Immunohistochemistry

From all biopsies, 5-µm-thick cryosections were cut at −20 °C using a cryostat. Samples were thaw-mounted onto uncoated precleaned glass slides. Care was taken to properly align the samples for cross-sectional orientation of the muscle fibers. Samples were stained for muscle fiber typing, capillaries, and myonuclei as described previously (31). In short, after 5 minutes of fixation in acetone, the cryosections were incubated for 1 hour with CD31 (RRID:AB_2114471; dilution 1/50; M0823; Dako, Glostrup, Denmark) in a phosphate-buffered saline (PBS) with 0.05% Tween. Slides were then washed 3 times in the Tween-PBS solution. Next, slides were incubated with horse anti-mouse (HAM) Biotine (RRID:AB_2313581; 1:500, Vector Laboratories, Burlingame, CA, USA) in Tween-PBS. Following 3 washing steps, the sections were incubated with Avidine Texas Red (RRID:AB_2336751; A2006, dilution 1/400; Vector Laboratories), and antibodies against Myosin Heavy Chain-I (RRID:AB_528384; MHC-I, A4.840, dilution 1/25; DSHB), and laminin (RRID:AB_477163; polyclonal rabbit anti-laminin, dilution 1/50; Sigma) in Tween-PBS. Following another triple-washing step in PBS, samples were finally incubated with appropriate secondary antibodies: goat anti-mouse (GAM) IgM AlexaFluor488 (RRID:AB_141357), goat anti-rabbit (GAR) IgG AlexaFluor647 (RRID:AB_2535807; Molecular Probes), and 4′,6-diamidino-2-fenylindole (RRID:AB_2629482; DAPI, Molecular Probes). After a final

Table 1. Patient characteristics

	Control ($n = 11$)	Exercise ($n = 10$)
Age (y)	72 ± 3	73 ± 8
Height (m)	1.77 ± 0.08	1.78 ± 0.05
Weight (kg)	80.4 ± 10.4	82.6 ± 15.1
BMI (kg/m^2)	25.7 ± 2.5	26.1 ± 3.8
Fat percentage (%)	28.1 ± 7.2	28.3 ± 5.1
Step count (steps/day)	7027 ± 2373	6213 ± 3317
ADT duration (days)	39 ± 21	33 ± 22
Gleason score	8.6 ± 0.5	8.3 ± 1.2
Bone metastases, *n* (%)	6 (55)	5 (50)
Previous prostatectomy, *n* (%)	4 (36)	1 (10)
Previous radiation, *n* (%)	1 (9)	1 (10)
Previous chemotherapy, *n* (%)	0 (0)	0 (0)

Values are means ± SD. Control, usual care group; Exercise, resistance exercise training group. Abbreviations: BMI, body mass index; ADT, androgen deprivation therapy.

triple-washing with PBS, slides were mounted with Mowiol (Calbiochem). This staining procedure resulted in images with laminin in white, MHC-I in green, DAPI in blue, and CD31 in red (Fig. 1).

Slides were viewed and automatically captured using a 10× objective on a modified Olympus BX51 fluorescence microscope with a customized disk-spinning unit (DSU, Olympus, San Jose, CA, USA), computer-controlled excitation and emission filter wheels (Olympus), 3-axis high-accuracy computer-controlled stepping motor specimen stage (Grid Encoded Stage, Ludl Electronic Products, Hawthorne, NY, USA), ultra-high sensitivity monochrome electron multiplier CCD camera (C9100-02, Hamamatsu Photonics, Hamamatsu City, Japan), and controlling software (Stereo Investigator; MBF Bioscience, Williston, VT, USA). Before analyses, slides were blinded for both group and time point. All areas selected for analysis were free of "freeze fracture" artifact, and care was taken such that longitudinal fibers were not used in the analysis. Muscle fibers on the periphery of muscle cross sections were not used in the analysis. Quantitative analyses were performed using ImageJ software package (version 1.52p, National Institute of Health, MD, USA) (32). On average, 166 ± 106 muscle fibers were analyzed per muscle biopsy sample collected to determine muscle fiber type distribution, CSA, myonuclear content and domain size. The quantification of muscle fiber capillaries was performed on at least 30 type I and 30 type II muscle fibers/patient/time point, based on previous work (33). Quantification consisted of capillary contacts (CC), the capillary-to-fiber ratio (C/F*i*), capillary-to-fiber perimeter exchange (CFPE) index, and capillary density (CD).

Statistical Analysis

Data are expressed as means ± SD. Baseline characteristics between groups were compared by using a Student unpaired *t* test. Exercise training–induced changes were analyzed using repeated measures ANOVA with time (baseline vs 20 weeks) and fiber type (type I vs type II) as within-subject factors and group (CON vs EX) as the between-subject factor. In the event of significant *time* × *group* interactions, groups were analyzed separately. In the event of significant *time* × *fiber type* interactions, type I and type II muscle fibers were analyzed separately. Bonferroni correction was applied to correct for multiple testing. Significance was set at $P < .05$. All calculations were performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA).

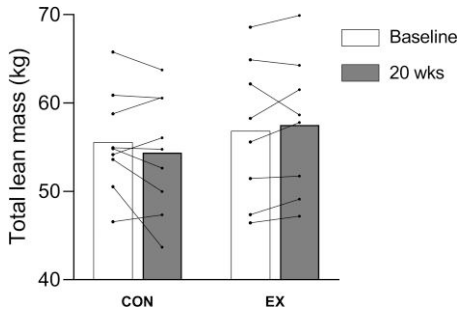

Figure 2. Total lean mass at baseline and following 20 weeks of usual care (CON, $n = 9$) or resistance exercise training (EX, $n = 8$) in prostate cancer patients on androgen deprivation therapy. Bars represent means. Dots represent individual data points, with change over time indicated with the connecting lines.

Results

Patients' Characteristics

In the overall project, patients in the EX group were randomly provided either a protein or placebo supplement throughout the 20-week exercise training program (26). As no differences were observed in muscle strength and body composition over time between the placebo and protein-supplemented group and both groups were equally represented in the current study (protein: $n = 5$, placebo: $n = 5$); all subsequent muscle biopsy analyses were performed with all patients in one EX group ($n = 10$). Patients included in the CON group ($n = 11$) did not receive any nutritional co-intervention. For 1 patient in the EX group, the muscle biopsy sample quality was insufficient to perform quantification of capillaries. All patients received treatment with a gonadotropin-releasing hormone agonist, and a total of 6 patients (EX, $n = 2$ and CON, $n = 4$) received chemotherapy (6 cycles of docetaxel) during the study period. At baseline, no differences in age, body weight, height, body mass index (BMI), fat percentage, whole-body lean mass, ADT duration, and Gleason score were observed between groups (Table 1).

Habitual Dietary Intake and Physical Activity Level

No significant differences in energy or any macronutrient (protein, carbohydrate, and fat) intake was observed between the CON and EX group at baseline (Supplementary Table S1) (34). Energy intake tended to decline (main of effect of time, $P = .050$) following 20 weeks of ADT, with no difference between the 2 groups. In contrast, protein, carbohydrate, and fat intake remained unchanged over time (Supplementary Table S1) (34).

At baseline, no significant difference in daily step count was observed between the CON and EX group (Supplementary Table S1) (34). Daily step count was significantly lower following 20 weeks of ADT compared to baseline (main effect of time, $P < .05$), with no difference between the 2 groups.

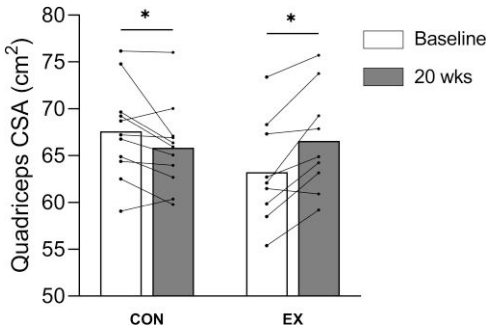

Figure 3. Quadriceps muscle cross-sectional area (CSA) at baseline and following 20 weeks of usual care (CON, $n = 11$) or resistance exercise training (EX, $n = 9$) in prostate cancer patients on androgen deprivation therapy. Bars represent means. Dots represent individual data points, with change over time indicated with the connecting lines. *Significantly different compared with baseline ($P < .05$).

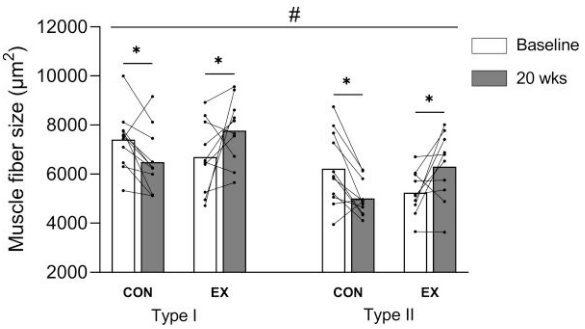

Figure 4. Type I and type II muscle fiber size at baseline and following 20 weeks of usual care (CON, $n = 11$) or resistance exercise training (EX, $n = 10$) in prostate cancer patients on androgen deprivation therapy. Bars represent means. Dots represent individual data points, with change over time indicated with the connecting lines. *Significantly different compared with baseline ($P < .05$). #: Significant main effect of fiber type ($P < .001$).

Total Lean Body Mass and Quadriceps Muscle Cross-sectional Area

No significant difference in total lean mass was observed between the CON and EX group at baseline (Fig. 2). Total lean mass remained unchanged over time in both groups. A significant *time* × *group* interaction effect ($P < .001$) was observed for quadriceps muscle CSA. Subsequent within-group analyses showed a significant decline in quadriceps muscle CSA following 20 weeks of ADT in the CON group (Fig. 3, $P < .05$). In contrast, quadriceps muscle CSA increased significantly in response to 20 weeks of resistance exercise training in the EX group (Fig. 3, $P < .05$).

Muscle Fiber Size and Distribution

No significant differences in type I and type II muscle fiber size were observed between the CON and EX group at baseline (Fig. 4). A significant *time* × *group* interaction ($P < .01$) was observed for muscle fiber size, as such, the CON and EX groups were subsequently analyzed separately. In the CON group, significant decreases in type I (from 7401 ± 1183 to 6489 ± 1293 µm^2) and type II (from 6225 ± 1503 to 5014 ± 714 µm^2) muscle fiber size were observed (main effect of time, $P < .05$, Fig. 4). In the EX group, significant increases in type I (from 6700 ± 1464 to 7772 ± 1319 µm^2) and type II (from 5248 ± 892 to 6302 ± 1385 µm^2) muscle fiber size were observed in response to the 20-week exercise training program (main effect of time, $P < .05$, Fig. 4). The proportion of type I muscle fibers, expressed as % of total fibers as well as expressed as % CSA occupied, was significantly lower at baseline in the EX compared with the CON group (both main effect of group, $P < .05$, Table 2). No changes in muscle fiber type distribution were observed over time in the CON and EX group (Table 2).

Myonuclear Content and Domain Size

No significant differences in type I and type II myonuclear contents were observed between the CON and EX group at baseline (Table 2). A significant *time* × *group* interaction ($P < .05$) was observed for myonuclear content; as such, the CON and EX groups were subsequently analyzed separately. In the CON group, no significant changes in type I and type II myonuclear content were observed in response to 20 weeks of ADT (Table 2). In the EX group, significant increases in type I and type II myonuclear content were observed in response to 20 weeks of resistance exercise training (main effect of time, $P < .05$; Table 2). At baseline, no significant differences in type I and type II myonuclear domain size were observed between the CON and EX groups (Table 2). Type I and type II myonuclear domain size remained unchanged over time in both the CON and EX groups (Table 2).

Muscle Fiber Capillarization

At baseline, no significant differences were observed in type I and type II muscle fiber capillarization, expressed as CC, C/F*i*, CFPE-index, or CD, between the CON and EX group (Table 2). A significant *time* × *group* interaction ($P < .05$) was observed for CC; consequently, the CON and EX groups were analyzed separately. Significant decreases over time were observed in type I, type II, and mixed CC in the CON group (main effect of time, $P < .05$, Table 2). Type I, type II, and mixed CC tended (main effect of time, $P = .055$) to increase in response to 20 weeks of resistance exercise training in the EX group (Table 2). Based on a *time* × *fiber type* interaction ($P < .05$) for C/F*i*, type I and type II muscle fibers were analyzed separately. Subsequently, a *time* × *group* interaction was observed for type I and type II C/F*i* (both $P < .05$). In the CON group, significant decreases in type I (from 1.81 ± 0.30 to 1.58 ± 0.25) and type II (from 1.37 ± 0.30 to 1.07 ± 0.25) C/F*i* were observed (main effect of time, $P < .05$). Whereas no changes were observed in type II C/F*i*, type I muscle fiber C/F*i* increased significantly over time (from 1.59 ± 0.31 to 1.85 ± 0.31, $P < .05$) in response to 20 weeks resistance exercise training in the EX group (Fig. 4). For CFPE-index a significant *time* × *group* interaction ($P < .05$) was observed. However, post hoc analyses revealed no significant changes over time in both the CON and EX group separately (Table 2). Type I and type II CD remained unchanged over time in both the CON and EX group (Table 2).

Discussion

The present study is the first to show a significant decrease in type I and II muscle fiber size and capillarization 20 weeks after the onset of ADT in PCa patients. In response to progressive resistance exercise training, these ADT-induced adverse effects were fully prevented, with muscle fiber size and

Table 2. Type I and type II muscle fiber characteristics at baseline and following either 20 weeks of usual care (Control) or resistance exercise training (Exercise) in prostate cancer patients on androgen deprivation therapy

	Control ($n = 11$)		Exercise ($n = 10$)	
	Baseline	20 weeks	Baseline	20 weeks
Fiber type distribution (fiber %)				
Type I	52 ± 11	50 ± 13	39 ± 15**	41 ± 12
Type II	48 ± 11	50 ± 13	61 ± 15	59 ± 12
Fiber type distribution (CSA %)				
Type I	56 ± 12	57 ± 11	44 ± 15**	46 ± 9
Type II	44 ± 12	43 ± 11	56 ± 15	54 ± 9
Myonuclei (*n*/fiber)				
Type I	3.50 ± 0.84	3.11 ± 0.63	3.12 ± 1.16	3.66 ± 0.92*
Type II	3.25 ± 1.01	3.02 ± 0.61	2.66 ± 0.71	3.12 ± 0.69*
Myonuclear domain (μm^2)				
Type I	2202 ± 549	2226 ± 749	2319 ± 715	2191 ± 376
Type II	2006 ± 539	1759 ± 549	2117 ± 728	2063 ± 484
Capillary contacts				
Type I	4.15 ± 0.67	3.69 ± 0.43*	3.62 ± 0.80	4.20 ± 0.65
Type II	3.73 ± 0.80	3.09 ± 0.61*	3.52 ± 0.98	3.85 ± 0.78
Mixed	2.48 ± 0.39	2.08 ± 0.32*	2.20 ± 0.45	2.45 ± 0.41
CFPE-index (capillaries/1000 μm)				
Type I	4.99 ± 1.11	4.53 ± 0.85	4.45 ± 0.80	4.97 ± 1.12
Type II	3.88 ± 0.63	3.45 ± 0.86	3.91 ± 0.67	4.15 ± 1.11
Capillary density (capillaries/mm^2)				
Type I	271 ± 79	256 ± 61	266 ± 63	274 ± 89
Type II	227 ± 46	233 ± 71	260 ± 60	268 ± 98

Values are means ± SD. Abbreviations: CSA, cross-sectional area; CFPE, capillary-to-fiber perimeter exchange. *Significantly different compared with baseline ($P < .05$). **Significant baseline difference between groups.

capillarization being increased throughout the 20 weeks of resistance exercise training.

ADT forms the cornerstone in PCa treatment, but it has a negative impact on muscle mass (3). In accordance, we show a decline in quadriceps muscle CSA following 20 weeks of ADT in PCa patients. The ADT-induced loss of leg muscle mass was accompanied by a decline in both type I and type II muscle fiber size. These results differ from previous reports by Nilsen et al, who did not find significant changes in muscle fiber size following 16 weeks of ADT in older PCa patients (12). The apparent discrepancy may be explained by differences in patient inclusion characteristics with regard to the onset of ADT. In the present study, the baseline muscle biopsy was taken within the initial weeks (5.0 ± 3.2 weeks) following the onset of ADT. In contrast, Nilsen and colleagues recruited patients approximately 9 months following onset of ADT. As the loss of lean tissue mass occurs mainly in the first months upon treatment initiation (3), it is possible that Nilsen et al may have missed much of the atrophy that had occurred prior to the patients' enrollment in the study. In contrast, we clearly show fiber atrophy in PCa patients during the weeks following onset of ADT. Interestingly, this decline in muscle fiber size was not accompanied by a decline in myonuclear number. As myonuclei are key in the transcription and translation of proteins, they are of critical importance in muscle tissue homeostasis, repair, and growth (14). The fact that myonuclear content remained unchanged during the initial months of ADT in these patients suggests that this will most likely not form a limiting factor in the response readiness of muscle tissue to quickly upregulate synthesis rates following resistance exercise. However, whether the myonuclear efficiency is hampered as a result of ADT remains to be further investigated.

In order to maintain skeletal muscle function and health, adequate amounts of oxygen, nutrients, and growth factors delivered by the muscle fiber capillary network are of critical importance (13). Low muscle fiber capillarization has been reported to be a limiting factor in muscle tissue repair, maintenance, and growth following exercise training, which may be of even greater importance in older adults (35, 36). The current study is the first to examine capillarization in PCa patients and shows a substantial decline in type I (−12% ± 12%) and type II (−20% ± 21%) muscle fiber capillarization after 20 weeks of ADT. To our knowledge, only 2 previous studies also evaluated changes in muscle fiber capillarization in cancer patients. Christensen and colleagues (2014) found no significant changes in muscle fiber capillary density in germ cell cancer patients undergoing 9 weeks of chemotherapy (37). In contrast, in a recent study by Mijwel et al (2018) a significant decline in muscle fiber capillary density (~17%) was observed in breast cancer patients following 16 weeks of treatment (38). However, treatment in this study population consisted of adjuvant chemotherapy for all patients. Animal studies clearly show that chemotherapeutic agents have a major negative influence on various muscle fiber characteristics (39), although

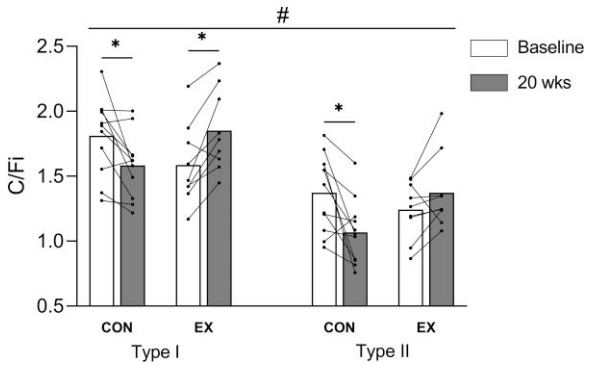

Figure 5. Type I and type II muscle fiber capillary-to-fiber ratio (C/F*i*) at baseline and following 20 weeks of usual care (CON, $n = 11$) or resistance exercise training (EX, $n = 9$) in prostate cancer patients on androgen deprivation therapy. Bars represent means. Dots represent individual data points, with change over time indicated with the connecting lines. *Significantly different compared with baseline ($P < .05$). #Significant main effect of fiber type ($P < .001$).

data on the impact on capillarization is lacking. In our CON group 4 PCa patients received additional chemotherapy (docetaxel, once every 3 weeks for 6 cycles) during the study period. Interestingly, additional post hoc analyses showed a significantly greater reduction in type I and type II CFPE-index and CD in patients who received both adjuvant chemotherapy combined with ADT, compared with patients who only received ADT (Supplementary Table S2) (34). This suggests that chemotherapy may exacerbate the negative impact of ADT on muscle fiber capillarization in older PCa patients. However, additional research including larger group sizes is warranted to more firmly establish these results. Apart from the possible direct adverse effects, adjuvant chemotherapy as well as ADT itself, may indirectly affect skeletal muscle tissue by inducing changes in lifestyle factors such as habitual dietary intake (for example due to loss in appetite) and physical activity level (for example due to increased fatigue). In the present study, habitual dietary intake was assessed at baseline and in week 20 of the ADT intervention period. Whereas energy intake tended to decline by a small degree, we observed no significant changes in protein, carbohydrate, or fat intake during the intervention period in both groups. In contrast, we do report a significant decline in the number of daily steps in response to the 20-week intervention, with no difference between the 2 groups. As such, we cannot exclude the possibility that the atrophy observed in the CON group may, in part, be explained by a decline in habitual physical activity level. However, larger cohort studies will be required to elucidate the impact of changes in lifestyle factors on skeletal muscle mass and function during ADT in PCa patients.

Prolonged resistance exercise training is an effective intervention strategy to counteract ADT-induced loss of muscle mass in older PCa patients (12, 24, 25). As such, we show a significant increase in quadriceps muscle CSA following 20 weeks of resistance exercise training in PCa patients. On the muscle fiber level, resistance exercise training resulted in significant type I and type II muscle fiber hypertrophy (Fig. 4), which was accompanied by myonuclear accretion in both muscle fiber types. These results are in line with Nilsen et al, who also showed significant muscle fiber hypertrophy following 16 weeks of resistance exercise training in PCa patients who had been on ADT for an extended period. Together, these studies clearly show that even though serum testosterone levels are reduced to castration level, skeletal muscle growth in response to prolonged resistance exercise training is viable in PCa patients. For muscle fiber capillarization, previous studies have reported mixed results on the impact of prolonged resistance exercise training in (healthy) older adults. Whereas some do (40), others do not (35) show an increase in muscle fiber capillarization following whole-body resistance exercise training in older adults. It has been suggested that, apart from duration of the training period, exercise intensity and/or volume of the resistance exercise training program may explain some of these discrepant results. Therefore, it is quite astonishing that in the present study we observed a substantial (~20%) increase in muscle fiber capillarization following a resistance exercise training program performed merely twice weekly in this compromised patient population (Fig. 5). This shows that next to the considerable hypertrophic response, testosterone does not seem to be essential to obtain a significant angiogenic response.

The loss of skeletal muscle mass is one of the hallmarks of initiating ADT, and clearly accelerates the age-related muscle loss in older PCa patients. Age-related muscle loss is mainly characterized by the loss of type II muscle fiber size, myonuclear content, and capillarization (9, 11, 16-18). The current study is the first to show that ADT initiation in PCa patients results in a decline in both type I and type II muscle fiber size, as well as a decline in capillarization. Although the gradual age-related lowering in testosterone levels may contribute to the age-related muscle loss (4), the severe decline in testosterone levels following ADT in PCa patients clearly has a larger and more acute impact on muscle tissue health. This is evident from the sheer magnitude in muscle fiber atrophy (type I: $-12\% \pm 14\%$, type II: $-17\% \pm 17\%$) and loss in muscle fiber capillarization (type I: $-12\% \pm 12\%$, type II: $-20\% \pm 21\%$) in both muscle fiber types observed over such a short period of time in our patients. More importantly, the present study demonstrates that merely bi-weekly resistance exercise training does not only prevent the decline in muscle mass, it actually increases muscle fiber size and capillarization in PCa patients who recently started ADT. In addition, these improvements even negate the potential negative impact of the reduced physical activity level (expressed as steps per day) observed in the CON as well as the EX group during ADT. This underlines the clinical relevance of including resistance exercise training in the weekly routine and overall treatment strategy of PCa patients to effectively preserve and even improve muscle health. With a training session adherence of ~80% in our complete study population and relatively low side effect–induced training disturbances, resistance exercise training seems feasible. However, because of the long duration of ADT treatment (sometimes lifelong), maintaining adherence could be a challenge. Therefore, it will be important to develop an exercise routine that can be implemented effectively in the daily routine of PCa patients. Implementation of the exercise regimen within a social context with fellow patients might maximize adherence and compliance (41), and further contribute to the interventional strategy to maintain function and quality of life in this growing patient population.

It is important to note that the present study utilized a nonrandomized design—that is, participants were included at 2 different hospital sites within the south of the Netherlands. At one hospital site, patients were enrolled in the EX group, whereas at the second site they were allocated to the control

condition. There were, however, no baseline differences between groups on any disease or other outcome measure. This nonrandomized approach was chosen to avoid selection bias by patients preferring the exercise or control condition. Moreover, patients recruited in a separate control group are less likely to start exercising themselves or dropping out when they are not informed about a second exercise group (42).

In conclusion, androgen deprivation therapy reduces both type I and II muscle fiber size and capillarization in PCa patients. Supervised resistance exercise training prevents this decline and effectively increases muscle fiber size and capillarization in PCa patients following the onset of androgen deprivation therapy.

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Disclosures

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Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Clinical Trial Information

Dutch Trial Register (International Clinical Trial Registry Platform) No. NTR6432.

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