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Effects of strength training for prostate cancer patients during androgen deprivation therapy

DISSERTATION FROM THE NORWEGIAN SCHOOL OF SPORT SCIENCES • 2015

ISBN 978-82-502-0518-5

Summary

Androgen deprivation therapy (ADT) increases survival rates among prostate cancer (PCa) patients with locally advanced disease, but is associated with side effects that may impair daily function through negative effects on muscle tissue. Although strength training may counteract several side effects induced by ADT, additional randomized controlled trials are needed to expand this knowledge. So far no studies have investigated the effects of strength training during long-term ADT on the muscle cellular level.

Fifty-eight PCa patients on ADT were randomized to either a control group or a strength training group, which underwent three weekly training sessions for 16 weeks. The primary endpoint was change in total lean body mass (LBM). Secondary endpoints were change in regional LBM, fat mass, areal bone mineral density (aBMD), and physical function measured as maximal strength (1RM) and functional tests, in addition to muscle cellular variables in muscle biopsies obtained from *m. vastus lateralis*.

Surprisingly, we did not observe any significant increase in total LBM or in trunk LBM. However, significant effects of strength training were found on LBM in the upper and lower extremities. Previous studies have shown that the androgen sensitivity might differ between muscles in the trunk and in the extremities, and we speculate that this may have an effect on the training response during ADT. Although no effect of the intervention was observed in fat mass or aBMD, significant effects were observed in all 1RM tests and functional tests, except for only a tendency in the shuttle walk test.

The muscle fiber CSA increased with strength training, but the only significant increase was observed in type II fibers. The number of myonuclei per fiber was increased in the type I fibers, which resulted in a decreased myonuclear domain. We speculate that difference in androgen receptor content between fiber types may play a role in the strength training adaptations during ADT. No effects of strength training were observed on satellite cell numbers, which differs from reports in healthy elderly performing strength training. In addition, the content of androgen receptor, myostatin, and markers of cellular stress were unchanged through out the intervention.

In summary, strength training had beneficial effects in PCa patients during ADT on both the muscle cellular and whole muscle level, which resulted in improved muscle strength and physical function. The effects seem, however, to be lower than what is commonly reported in healthy elderly.

Sammendrag

Androgen deprivasjonsterapi (ADT) har vist seg å øke overlevelsen hos prostatakreftpasienter med lokal avansert sykdom. Behandlingen er imidlertid assosiert med bivirkninger som kan påvirke funksjon i dagliglivet. Styrketrening kan ha en positiv effekt på flere kjente bivirkninger av ADT, men det finnes få randomiserte, kontrollerte studier som dokumenterer dette. Videre foreligger det foreløpig ingen studier som har undersøkt effekten av styrketrening under langvarig ADT på muskelcellulære variabler. Femtiåtte prostatakreftpasienter ble randomisert til enten en kontroll gruppe, eller til en styrketreningsgruppe som trente tre ganger ukentlig i 16 uker. Mens det primære endepunktet var endring i total fettfri masse., var sekundære endepunkt endring i regional fettfri masse (ekstremiteter og trunkus), fettmasse, beinmineraltetthet, fysisk funksjon målt som maksimale styrketester og som funksjonelle tester, samt ulike muskelcellulære variabler analysert i biopsier tatt fra *m. vastus lateralis.*

Til vår overraskelse førte ikke styrketreningen til signifikant økning i total fettfri masse. Vi fant imidlertid signifikante effekter i både bein og armer. Tidligere studier har vist at muskulaturen i trunkus og i ekstremitetene har forskjellig sensitivitet for androgene hormon i, og vi spekulerer i om dette kan ha påvirket treningseffekten under ADT. Den gjennomsnittlige økningen i fettfri masse som ble observert i styrketreningsgruppen var mindre enn det som er rapportert hos friske eldre. Vi fant ingen effekt av styrketreningen på fettmasse eller beinmineraltetthet i denne studien. Fysisk funksjon ble imidlertid forbedret i alle testene, foruten i beep-testen hvor vi kun så en tendens til forbedring.

Styrketreningen førte til økt muskelfiberareal, og vi fant den største økningen i type II fibrene. Det var en tendens til at cellekjerneantallet økte mer i styrketreningsgruppen enn i kontrollgruppen, og her fant vi den største økningen i type I fibrene. Det er vist at flere cellekjerner i type I fibrene uttrykker androgen reseptor. Det kan tenkes at ADT derfor påvirker type I fibrene i større grad enn type II fibrene. Antall satellittceller per fiber forble uendret i begge gruppene. Vi så heller ingen intervensjonseffekt på mitokondrieproteiner eller markører for cellulært stress.

Oppsummert viser resultatene fra denne studien at styrketrening hadde positiv effekt på fettfri masse i bein og armer, samt på muskelcellenivå hos prostatakreftpasienter som ble behandlet med ADT. Dette resulterte i forbedret fysisk funksjon. Effektene ser imidlertid ut til å være noe mindre enn det som kan forventes hos friske eldre.

Preface and acknowledgements

This thesis presents research conducted in the Physical Exercise and Prostate Cancer (PEPC) trial, at the Department of Physical Performance at the Norwegian School of Sport Sciences (NSSS) in collaboration with Oslo University Hospital, from 2009 to 2015. This work was supported by the Norwegian Foundation of Health and Rehabilitation, the Norwegian Cancer Society (HF-57004/001) and co-funded by the Regional Health Authority in Southern Norway. Additional funding was also given by Eckbo's legacy, The Radium Hospital's legacy and Trivselsanlegget's legacy. A warm thank you to all the patients who participated in the study. Without you the PEPC-study would not have existed.

Finishing a PhD is by no means a one-man show, and I would like to acknowledge several contributors for their help, support and encouragement.

I would like to extend my deepest gratitude to my main supervisor Prof. Truls Raastad. I admire your knowledge and your way of encouraging students at any academic level. You have given me essential help in completing this work, and you have also been a great support when things have been rough outside the walls of the Norwegian School of Sport Sciences. Thank you for your patience, for your great advice and for caring. I owe much of my current knowledge to you, and I look forward to continuing our work together.

To my co-supervisor, Dr. Lene Thorsen, you opened the hospital door and gave me the opportunity to work with prostate cancer patients. I am grateful for the experience, and that I was allowed to bring my background from sport sciences into a hospital setting to do research. I now understand that there is more to our research than measuring more or less obscure proteins in muscle biopsies. Thank you for your critical evaluations, for watching over me closely, and for making me stick to the point. I am excited to see our future projects come to life, and proud of what we have accomplished so far.

Prof. Sophie D. Fosså, with your impressive research history of over 700 publications, I am glad that I had the opportunity to work with you. Thank you for sharing your knowledge with me.

Prof. Kerry S. Courneya, thank you for your help in designing the study, and your feedback on papers. Prof. Eva Skovlund, thank you for contributing with your expertise in statistics.

Jon Reidar Iversen, Nils K. Raabe, Kjell Magne Russnes, Gunnar Tafjord, Melanie-Birte Schulz, Siri Kjellaas and Stine Solli: thank you all for your contributions in identifying and including patients. Thanks to Arne Høiseth, Anne Gulliksen and Aud Jacobsen at Curato Røntgen for performing the DXA scans. Also, the staff at the Department of Clinical Research Support at Oslo University Hospital, and Laima Bulavaite Taylor is acknowledged for their help with data handling.

I would like to extend my gratitude to Prof. Jostein Hallén. Thank you not only for filling in for Truls and obtaining muscle biopsies every now and then, but also for starting my academic career by hiring me as a research assistant after I finished my master's thesis.

To our head engineer in the "muscle lab", Ingrid Ugelstad, thank you for your watchful eye in the lab, for assisting in obtaining muscle biopsies at short notice, and for providing our master's students with appropriate skills to do a good job in the lab. Thanks also to Dr. Kristoffer Cumming for good discussions, both lab-related and unrelated. I am also grateful to Nils Helge Kvamme, not only for your help with biopsy handling, but more importantly, for your friendship and for your great spirit. You are missed in the muscle group, and in the lunchroom.

To the masterate students contributing to the work presented in this thesis: Camilla Kirkegaard and Martin Wiig, thank you so much for all your hard work and effort. Your contributions have been valuable to the project group and to me.

Thanks to Paul Andre Solberg, Bjarne Rud, Gunnar Slettaløkken, Ola Eriksrud, Tron Krosshaug, Thomas Losnegard and Håvard Myklebust, for our in-depth discussions on an array of topics in varying surroundings and locations: hunting or fishing trips, out running, in the gym, over a coffee or over dinner. Thanks to all of you for filling my life with memories, in and outside of the NSSS.

To my fellow PhD students and colleagues in the Department of Physical Performance: thank you all for making my years at NSSS a great time. I would also like to acknowledge the staff at the NSSS library, which supplied me with paper copies of articles from journals not included in our portfolio.

Throughout my life I have experienced nothing but support and encouragement from home. Thank you, “mor” (Marit Skogstad) for all your support, for holding our family together despite everything, and for believing in me. Thanks also to Hilde and Harald Grundeland for your great support.

To my dear wife, Hege, thank you for your patience, for encouraging me whenever I was in doubt, for your care and for your unconditional support and love. I cannot promise that I will work less in the future, but I promise that I will be more present when I am around after this.

Oslo, May 2015
Tormod S. Nilsen

List of papers

Paper I

Thorsen L., Nilsen T.S., Raastad T., Courneya K.S., Skovlund E,. Fosså S.D.: **A randomized controlled trial on the effectiveness of strength training on clinical and muscle cellular outcomes in patients with prostate cancer during androgen deprivation therapy: rationale and design**. *BMC Cancer* 2012, **12**(1):123

Paper II

Nilsen T.S., Raastad T., Skovlund E., Courneya K.S., Langberg C.W., Lilleby W., Fosså S.D., Thorsen L.: **Effects of strength training on body composition, physical functioning and quality of life in prostate cancer patients during androgen deprivation therapy.** *Accepted by ACTA Oncologica.*

Paper III

Nilsen T.S., Thorsen L., Fosså S.D., Wiig M., Kirkegaard C., Skovlund E., Benestad H.B., Raastad T.: **Effect of strength training on muscle cellular variables during androgen deprivation for prostate cancer: a randomised trial.** *Accepted by Scandinavian Journal of Medicine and Science in sports.*

Paper IV

Nilsen T.S., Thorsen L., Kirkegaard C., Ugelstad I., Fosså S.D., Raastad T.: **The effect of strength training on indicators of muscle cellular stress during testosterone suppression in prostate cancer patients.** *Manuscript in preparation for Journal of Endocrinology.*

VIII Abbreviations

1RM - One repetition maximum (the maximum load that can be lifted once with proper form)

6RM – Six repetitions maximum (the maximum load that can be lifted in six repetitions with proper form)

8RM – Eight repetitions maximum (the maximum load that can be lifted in eight repetitions with proper form)

10RM – Ten repetitions maximum (the maximum load that can be lifted in ten repetitions with proper form)

aBMD – Areal bone mineral density

ADT - Androgen deprivation therapy

ANCOVA - Analysis of covariance

AR – Androgen receptor

ARs – Androgen receptors

ASM - Appendicular skeletal muscle mass

BMI – Body mass index

CG – Control group

cm – centimeter

COX IV – Cytochrome c oxidase

CSA - Cross-sectional area

CT – Computer tomography

DAPI - 4',6-diamidino-2-phenylindole (binds to A-T connections in the DNA, used to visualize nucleus on muscle biopsy cross sections)

DXA - Dual x-ray absorptiometry

EDTA - Ethylenediaminetetraacetic acid

GAPDH - Glyceraldehyde 3-phospate dehydrogenase

HEM - Healthy elderly men

HRP – Horseradish peroxidase

HSP27 - Heat shock protein 27

HSP60 - Heat shock protein 60

HSP70 - Heat shock protein 70

HSPs - Heat shock proteins

kg- kilogram

LBM – Lean body mass

mg – milligram

ml – milliliter

MRi – Magnetic resonance imaging

mRNA – Messenger RNA

MVC - Maximal voluntary isometric contraction

NaCl – sodium chloride (saline)

ng – nano gram

NSSS – Norwegian School of Sport Sciences

NYHA classification – New York Heart Association, functional classification system of heart failure patients

OUH – Oslo University Hospital

PCa – Prostate cancer

PEPC – Physical Exercise and Prostate Cancer (name of the present study)

PSA – Prostate specific antigen

QoL – Quality of life

ROM – range of motion

ROS – Reactive oxygen species

RT – Room temperature

SD – Standard deviation

SSB - Statistics Norway

STG – Strength training group

UPS - Ubiquitin proteasome system

µm – micrometer

μm^2 – square micrometer

1.0 Introduction

Prostate cancer (PCa) is the most common malignancy in men [1]. In most PCa patients, testosterone stimulates tumor growth, and suppression of testosterone leads to cell cycle arrest and apoptosis [2]. Therefore, the combination of high-dose external beam radiation therapy (EBRT) and suppression of testosterone, or androgen deprivation therapy (ADT), is a life-prolonging standard therapy in patients with locally advanced PCa [3, 4]. ADT is, however, associated with negative effects on body composition [5, 6]. Testosterone is a well-known regulator of muscle mass; increased levels are associated with increased muscle mass [7, 8], and removal often leads to reduced lean body mass (LBM) (reflecting muscle mass) as reported in PCa patients during ADT [9]. Furthermore, increased fat mass and loss of bone mass, as well as reduced physical functioning and health-related quality of life (QoL) have also been reported in PCa patients during ADT [9-12]. Since approximately 50% of all PCa patients are treated with ADT during the course of the disease [13], a great number of patients are at risk of developing adverse effects induced by ADT. Therefore, it is important to identify effective strategies to counteract treatment side effects.

Strength training has been suggested as one strategy to counteract adverse effects on clinical outcomes such as LBM, fat mass, bone mass and physical function [14]. However, there are still few randomized controlled trials in the literature addressing these effects. Importantly, no studies to date have investigated the effects of strength training during ADT on muscle cellular outcomes, such as fiber area, myonuclear addition, satellite cells and indicators of cellular stress.

Therefore, the aim of the "Physical Exercise and Prostate Cancer" (PEPC) trial was to investigate the effect of strength training on changes in clinical and muscle cellular outcomes in PCa patients on ADT.

2.0 Background

2.1 Prostate cancer

In many western countries [1, 15], including Norway [16], PCa is the most common malignancy in men. According to the Cancer Registry of Norway there were 4,836 new cases of PCa in 2013, and more than 19,000 men had lived five years or longer with the disease [16]. The incidence of PCa has increased over the last decade [17], which to a certain degree can be explained by increasing age in the population, but also by increased diagnostic PCa screening, based on prostate specific antigen (PSA) (see section 2.2.1). The majority of new PCa cases are today detected at an early stage, and are associated with low mortality [18]. Between 2008 and 2013 more than 90% of all Norwegian men with a PCa diagnosis were still alive five years after their diagnosis, compared to only 57% between 1988 and 1992 [16].

2.2 Diagnosis

Since the urethra passes through the middle of the prostate gland, alterations in the prostate tissue may cause the duct to narrow. Thus, suspicion of PCa is raised by urinary problems, such as trouble in starting urination, problems with emptying the bladder, or a weak urine stream. However, many patients recently diagnosed with PCa do not experience any symptoms. In these patients the PCa suspicion is often based on elevated blood levels of PSA.

2.2.1 Prostate specific antigen (PSA)

In the 1990s PSA was introduced as a biomarker for PCa [19-21]. PSA is an androgen-regulated serine protease, produced by the epithelial cells of the prostate [22]. Since most PCa cells also express PSA, it is used as a biomarker for PCa. Today elevated serum levels of PSA provide the suspicion of PCa in most patients. However, several other conditions may also cause an increase in PSA levels, e.g. benign prostatic disease, prostatitis, prostatic infarction and acute urinary retention. Therefore, an elevated PSA is almost always followed by a prostate biopsy.

2.2.2 Gleason score

The Gleason score describe the tumor cells and their growth pattern (Table 1), and contributes to the risk categorization of the individual tumor [23]. In 1966, Donald

Gleason and colleagues developed a tumor grading system [24]. Based on pathological patterns in the tissue obtained by a biopsy, the two most prominent patterns are determined, each ranging from 1 to 5. The sum of these primary and secondary grades is summarized, and are reported as the Gleason score [25] (e.g. 3+4= Gleason score of 7). The Gleason usually ranges from 6 to 10, with higher values indicating increasing risk of tumor progression.

Table 1. Gleason grading system (adopted from [25]).

Gleason score	Description
1	Consist of small uniform glands
2	Consists of small glands with more space between the glands
3	Consists of small glands that have not fused together
4	Consists of small glands with fusion
5	Consists of sheets, cords (groups of cells), or single cells, without discernable glands

2.2.3 Clinical staging of the tumor

Clinical staging by rectal examination of the prostate and by the use of a range of imaging techniques (e.g. ultrasound, MRi or CT) provides information on the extent of the malignant disease, which is correlated with prognosis. Staging is based on the TNM-system (Table 2), which is reviewed in about four-year intervals. “T” grades the extent of the tumor burden (within or outside the prostate gland), “N” (nodes) indicates presence of cancer cells in draining lymph nodes and/or regional lymph nodes, and “M” (metastases) indicates evidence of spreading of cancer cells elsewhere [26].

Table 2. Staging of the tumor by the TNM-system. Also, within each stage the letters a-c describes grades of severity, before progressing to the next stage.

Stage	Indicators
T0	No evident tumor
T1	No indication of tumor by palpation, ultrasound or Mri, but cancer is evident by prostate biopsies
T2	Tumor evident by palpation or by imaging techniques, and restricted to the prostate gland
T3	Tumor growth outside the prostate gland, without spreading to surrounding tissues
T4	Tumor growth from the prostate gland to the surrounding tissues (bladder, rectum, muscles or bone)
N0	No spreading to the lymph nodes
N1	Detection of cancer cells in regional lymph nodes
M0	No metastasis
M1	Metastasis

2.2.4 Risk profile groups

After PSA determination, analysis of the tumor biopsy and clinical staging, patients are placed into risk groups depending on the estimated risk of recurrence or cancer

progression [27, 28]. Patients without metastases are categorized into low, intermediate or high risk group according to their T-category, Gleason score and serum PSA level [29].

2.3 Treatment options for prostate cancer

The appropriate choice of treatment for a non-metastatic patient is based on his risk group, his age and general health, and his own preferences. Treatment guidelines may differ between countries, and the following section is based on the Norwegian treatment guidelines for prostate cancer [29].

2.3.1 Radical prostatectomy

Radical prostatectomy involves surgical removal of the prostate gland and the seminal vesicles. The procedure can be performed as an open surgery, as laparoscopic surgery or as robot-assisted laparoscopic surgery. Radical prostatectomy is traditionally recommended if the tumor is believed to be inside the prostate, but has also recently been used for more advanced tumors [29].

2.3.2 Radiation therapy

Radiation therapy with curative intention involves the delivery of high-energy ionizing radiation to the prostate area by EBRT, and/or by a radioactive source inserted into the prostate gland, termed brachytherapy [30]. Radiation is an effective measure to damage the tumor DNA, to some degree via direct ionization and more commonly indirect via reactive oxygen species (ROS) as a by-product of the hydrolysis of water [31]. EBRT is an option for PCa patients in all risk groups, but the radiation dose and radiation target volume differ between the individual risk groups [29]. One Norwegian hospital offers EBRT in combination with brachytherapy [29].

2.3.3 Androgen deprivation therapy

Androgen receptors (ARs) are extensively expressed in the prostate, and in most PCa cells [32]. At the cellular level, withdrawal of testosterone and inhibition of AR function is associated with cell cycle arrest and tumor cell death in androgen-dependent tumors [2]. Therefore, testosterone suppression is an effective treatment to counteract growth and proliferation of PCa cells [33]. An alternative treatment to bilateral orchiectomy is medical castration, achieved by inhibition of the pituitary stimulation of testicular testosterone production [34]. Medical castration for one to two years is applied together

with radiation therapy [29]. The clinical benefit of this (neo-)adjuvant treatment in terms of increased survival in PCa patients has been documented in several reviews [4, 35-37]. In patients with distant metastases, lifelong castration is applied. ADT is, however, associated with a number of adverse effects, some of which are discussed below.

2.3.3.1 Adverse effects of ADT on body composition

Testosterone is involved in several bodily processes, and suppression of testosterone can lead to severe side effects. In addition to reduced libido and erectile function, prospective clinical studies have shown that PCa patients treated with ADT experience changes in body composition and bone health [33, 38, 39]. ADT may also impair cognitive and psychological function, increase fatigue and reduce quality of life [40]. This thesis, however, will focus on changes in body composition, and especially on skeletal muscle.

2.3.3.1.1 Lean body mass

Removal of testosterone leads to increased rates of muscle loss, reflected by the reduction in LBM that has been reported during ADT [5, 6, 41-47] (Table 3). In one prospective, controlled study no change in LBM was observed in the healthy, age-matched control group, whereas LBM of ADT-treated PCa patients decreased by 3.2% over two years [6].

Table 3. An overview of studies that have investigated the effect of ADT on LBM. Data are presented as relative changes from baseline (onset of ADT).

				Relative change in total lean mass from baseline					
Authour	**Year**	**Method**	***n*=**	**3 months**	**6 months**	**9 months**	**12 months**	**18 months**	**24 months**
Smith et al.[41]	2001	BIA	22	-2.4					
Berruti et al.[42]	2002	DXA	35		-1.9		-1.9		
Smith et al.[43]	2004	DXA	79				-2.4		
Boxer et al.[5]	2005	DXA	30		-2.1				
Lee et al.[44]	2005	DXA	65				-2.0		
Gãlvao et al.[45]	2008	DXA	68			-2.4			
Levy et al.[46]	2008	DXA	23						-1.7
Van London et al.[6]	2008	DXA	70		-0.3		-1.7	-2.7	-3.2
Torimoto et al.[47]	2011	BIA	39	-0.3	-0.5	-0.6	-0.6		
Relative mean change from baseline				**-1.4**	**-1.2**		**-1.7**		**-2.4**

BIA; Bioeletrical impedance analysis, DXA;Dual X-ray absorptiometry

There seems to be great variation in the reported loss of LBM. At six months the reported mean loss ranges from 0.6 % to 2.1 %, and from 0.6 to 2.4 at 12 months. The reason for this is unclear, but one review concludes that the LBM loss has been shown to

be more pronounced during the initial phase of the treatment [12]. However, based on Table 3, this assumption seems to be generated on three studies [5, 42, 48].

Also, some of the variation may be attributed to differences in the assessment method: whereas most studies have used DXA to estimate LBM [5, 6, 42-44], two studies used bioelectric impedance analysis (BIA) [41, 47]. Nevertheless, the mean LBM loss from 12 months on ADT, calculated from the studies included in Table 3 (1.7%), seems to exceed the annual loss during normal ageing (1% per year after the age of 50) [49, 50].

In line with the decline in LBM, there are indications in the literature that ADT may also impact physical performance in PCa patients. In a cross-sectional study Basaria et al. (2002) showed that PCa patients on ADT for more than 12 months showed less upper body muscle strength in the bench press exercise compared to non-ADT treated PCa patients and healthy controls [51]. No differences were observed in the leg press exercise. However, longitudinal studies evaluating the effects of ADT on muscle function are needed.

2.3.3.1.2 Fat mass

It has been shown that testosterone supplementation reduces fat mass in a dose-dependent manner in young men [7]. Testosterone has also been shown to increase adipocyte lipolysis [52]. On the other hand, castration has the opposite effect by decreasing basal lipolysis [53]. Consequently, the percent body fat is higher in hypogonadal men compare to men with normal testosterone levels [54]. Increases in fat mass have also been reported during ADT [5, 6, 41-47].

Table 4. An overview of studies that have investigated the effect of ADT on fat mass. Data are presented as relative changes from baseline (onset of ADT).

				Relative change in total fat mass from baseline					
Authour	**Year**	**Method**	***n*=**	**3 months**	**6 months**	**9 months**	**12 months**	**18 months**	**24 months**
Smith et al.[41]	2001	BIA	22	8.4					
Berruti et al.[42]	2002	DXA	35		14.4		19.3		
Smith et al.[43]	2004	DXA	79				11.4		
Boxer et al.[5]	2005	DXA	30		9.5				
Lee et al.[44]	2005	DXA	65				6.6		
Gãlvao et al.[45]	2008	DXA	70		5.4		5.8	6.3	7.5
Levy et al.[46]	2008	DXA	68			13.8			
Van London et al.[6]	2008	DXA	23						5.1
Torimoto et al.[47]	2011	BIA	39	0.6	1.3	1.7	2.1		
Relative mean change from baseline				**4.5**	**7.6**		**9.0**		**6.3**

BIA; Bioeletrical impedance analysis, DXA;Dual X-ray absorptiometry

Based on studies included in Table 4, the average change in fat mass seems to be 9% (range 2-19%) during the first year on ADT, which typically converts into an absolute increase of 1.5-2.0 kg. Most of the studies suffer from a lack of control groups [41-44, 47], thus making it difficult to reach conclusions on the relative impact of ADT on fat mass gain, since age is a confounding factor. However, one study included a healthy control group, where no change in fat mass was observed from baseline during the 24-month assessment, compared to a 7.5% increase in the ADT-treated PCa patients [6].

2.3.3.1.3 Bone mass

In addition to suppression of testosterone, ADT is known to lower estrogen levels, since the substrate for aromatases, namely testosterone, is decreased [55]. Loss of estrogen has been shown to play a crucial role in loss of bone mass, measured as area bone mineral density (aBMD), in men [56]. The effect of ADT on aBMD in PCa patients has been described in some studies (Table 5) [41, 42, 45, 57-61].

Table 5. An overview of studies that have investigated the effect of ADT on lumbar spine aBMD. Data are presented as relative changes from baseline (onset of ADT).

			Relative change in lumbar spine BMD from baseline							
Authour	**Year**	***n*=**	**3 months**	**6 months**	**9 months**	**10 months**	**12 months**	**18 months**	**20 months**	**24 months**
Smith et al.[41]	2001	22		-1.2			-3.3			
Berruti et al.[42]	2002	35		-1.4			-2.3			
Mittan et al.[58]	2002	15		-1.5			-2.8			
Preston et al.[59]	2002	39		-0.1			-0.5	0.0		0.0
Greenspan et al.[57]	2005	30		-3.0			-3.7			
Ryan et al.60	2007	13	0.9	2.3	0.9		-3.1			
Gãlvao et al.[45]	2008	69			-3.9					
Ziaran et al.[61]	2011	89				-4.2			-13.3	
Relative mean change form baseline				**-0.8**			**-2.6**			

One of the most commonly reported measuring sites for aBMD in the literature is the lumbar spine aBMD, therefore articles that report on this site are included in Table 5. Most studies report a loss of aBMD during ADT [42, 45, 48, 57-59, 61]. As a clinical implication, some studies show that ADT is associated with an increased risk of bone fractures [62, 63]. However, one study reported an increased aBMD in the lumbar spine during the first nine months of ADT [60]. The study population in this study is, however, quite small (n=16) and one patient dropped out between the baseline and the 3-month evaluation. This patient could have been an outlier in the dataset, as the standard deviation is reduced at 3 months compared to baseline.

2.3.3.2 Potential adverse effects of ADT on muscle cellular outcomes

No studies to date have investigated the effect of ADT on muscle cellular outcomes in PCa patients.

2.3.3.2.1 Muscle fiber cross-sectional area

The loss of LBM during ADT indicates that the muscle fiber cross-sectional area (CSA) is reduced during ADT. The muscle fiber CSA is regulated by the balance between muscle protein synthesis and muscle protein breakdown [64]. Through the AR, testosterone has been shown to increase transcription [65], and also directly stimulate protein synthesis [66]. In rodent models, removal of testosterone has been shown to result in decreased CSA [67] through increased atrophy signaling [68], which results in increased muscle protein breakdown. Therefore, it is likely that ADT results in decreased CSA of muscle fibers in PCa patients.

2.3.3.5.2 Number of myonuclei per fiber

According to the myonuclear domain theory, the cytosolic volume that one myonucleus is able to serve is limited [69]. Therefore, it is a traditional belief that additional nuclei are needed for comprehensive expansion of the fiber CSA. Although it has been shown that testosterone supplementation results in increased numbers of myonuclei in a dose-dependent manner [8, 70], apoptosis (or loss) of myonuclei are rarely seen [71]. Therefore it is unlikely that PCa patients will experience loss of myonuclei during ADT.

2.3.3.5.3 Number of satellite cells per fiber

Myonuclei are unable to undergo mitosis. Therefore, muscle cells are dependent upon nuclear donation from satellite cells to increase the number of myonuclei and thus increase transcription capacity [72, 73]. Satellite cells are muscle progenitor cells located between the basal lamina and the sarcolemma [74], and are able to donate their nuclei to the growing muscle fiber [72]. In adult muscle, satellite cells account for 3-6% of all muscle-related nuclei (nuclei within the basal lamina), and are normally fond in a quiescent state [75]. ARs are present in satellite cells, and testosterone stimulates both satellite cell proliferation and nuclear donation [76]. Consequently, testosterone supplementation results in increased numbers of satellite cells in a dose-dependent

manner [8, 70]. The effect of castration (ADT) on the satellite cell pool is, however, not known.

2.3.3.5.4 Content of androgen receptors in muscle

Testosterone acts on skeletal muscle via the AR and promotes cell growth, both through classical AR action and non-genomic pathways [66]. Castration does not change the mRNA expression of AR in bovine muscles [77], nor the protein levels of AR in the plantaris muscle of rats [78]. The effect of ADT on AR in muscles has not been investigated in PCa patients, but based on animal studies it is unlikely that ADT would change AR content in muscles.

2.3.3.5.5 Content of myostatin in muscle

Myostatin is a member of the TGF-β superfamily of cytokines, and provides an inhibitory effect on muscle growth by repressing protein synthesis signaling [79]. Consequently, myostatin plays a crucial role in muscle atrophy [80], and inhibition of myostatin has shown promising results in preventing LBM loss in PCa patients during ADT [81]. The effect of ADT on myostatin levels in muscle is, however, not known.

2.3.3.5.6 Content of mitochondrial proteins in muscle

Since ADT leads to LBM loss, we would expect the muscle cells to be under some sort of stress during ADT, leading to muscle protein breakdown. The effect of ADT on markers of cellular stress has not been investigated in PCa patients. However, it has been shown that mitochondrial dysfunction induces muscle protein degradation [82]. Testosterone supplementation has been shown to increase mitochondrial proteins (COXIV), along with increasing mRNA levels of genes encoding mitochondrial biogenesis, which in turn are downregulated in AR-deficient mice [83]. Therefore, since testosterone seems to have an effect on the regulation of mitochondrial function, mitochondrial dysfunction may play a role in ADT-induced loss of muscle mass.

2.3.3.5.7 Content of heat shock proteins in muscle

Heat shock proteins (HSPs) may prevent excessive loss of muscle mass during cellular stress. HSPs are molecular chaperones, which refold misfolded or denatured proteins [84, 85]. The small HSPs (HSP27) may translocate from the cytosol to the cytoskeleton [86] to reinforce the cellular structure, and thus prevent cell damage [87, 88]. Therefore,

HSPs play an important role in preserving muscle cell following exposure to various cellular stresses [89, 90]. It has been showed that the elderly have higher baseline levels of HSPs than younger subjects [91], and it can be speculated that ADT may increase this level further. However, the effects of ADT on HSPs in skeletal muscle have not been investigated in any study to date.

2.3.3.5.8 Content of free ubiquitin and ubiquitinated proteins in muscle

It is well established that the majority of proteolysis in skeletal muscle occurs through the ubiquitin proteasome system (UPS) [92]. Here muscle proteins are ubiquitinated by muscle specific ubiquitin ligases (e.g. muscle atrophy F-box containing protein/Atrogin1 (MAFbx/Atrogin-1) and muscle RING finger-containing ligase (MuRF-1)), and broken down to amino acids by the proteasome. Castration-induced atrophy in male rats has been shown to result in an increased gene expression of atrogin-1 and MuRF-1, which returned to baseline after testosterone supplementation [93]. Similarly, testosterone supplementation has been shown to attenuate glucocorticoid-induced muscle protein breakdown in rat skeletal muscle [94], possibly through inhibition of the myostatin signaling pathway, which induces muscle loss [95]. Therefore, the loss of LBM during ADT may be a result of increased ubiquitination of muscle protein, leading to muscle protein breakdown.

2.4 Exercise in cancer patients

Cancer diagnosis and its treatment often impact the lives of patients to a great extent, and much effort and research are directed to improving daily life in cancer survivors. Exercise interventions have shown promising results in this field of research [96].

The first exercise oncology papers were published in the late 1980s. A randomized controlled trial involving 45 women undergoing adjuvant chemotherapy for breast cancer showed that 10 weeks of interval-based aerobic endurance training was not only safe, feasible and increased physical performance [97], but also decreased treatment toxicity such as nausea [98]. Since then evidence of the beneficial effects of exercise interventions in cancer care has emerged from numerous studies including animal models, observational studies and randomized controlled trials, to influence an array of clinically important outcomes [99]. In the 2010 review by Speck et al., a total of 102 articles representing 82 studies met the inclusion criteria [96]. While breast cancer

patients were included in 83% of all publications, PCa patients were included in 10% of the publications. Importantly, there has been a shift in the recommendations given in the literature for cancer patients, from bed rest and inactivity to being physically active within the patient's capacity [100].

2.4.1 Exercise in PCa patients

Since loss of muscle mass is one of the great concerns in PCa patients on ADT, the majority of studies involve some kind of strength training, either alone [101-104], in combination with endurance training [105-111] or in combination with jump training [112]. Strength training is the main focus of the present thesis, and therefore studies that have included strength training in a gym setting [102-104, 106, 109, 111-113] or similar [101] are included, but not home-based resistance training [105, 107, 108, 110]. Details of the timing and intervention of all studies on the effect of strength training in PC patients are listed in Table 6.

Table 6. Overview of studies investigating the effect of strength training in PCa patients on ADT

First author	Year	n	Duration of ADT at baseline (weeks)	Intervention duration (weeks)	Sessions per week	Training intensity/ training load	Sets	Exercises	Weekly training volume*
Segal et al.[111]	2003	135	56	12	3	Starting at 8-12 reps at 60% of estimated 1RM, then increase by 5lb when 12 reps were successfully completed	1 to 2	10 exercises (3 leg, 7 UB)	Leg: 9 to 18 UB: 21 to 42
Galvão et al.[102]	2006	10	162	20	2	6-12 RM	2 to 4	12 exercises (4 leg, 8 UB). The first 10 weeks concentric phase only, last 10 weeks concentric and eccentric phase.	Leg: 16 to 32 UB: 32 to 64
Segal et al.[104]	2009	121	15	24	3	Started at 8-12 reps at 60% of estimated 1RM, then increased by 5lb when 12 reps were successfully completed	1 to 2	10 exercises (3 leg, 7 UB)	Leg: 9 to 18 UB: 21 to 42
Hansen et al.[101]	2009	26	69	12	3	Increased negative work at same perceived level of exertion	Self selected 15 to 25 rounds on the leg press machine	1 exercise (eccentric leg press)	Leg: 15 to 25
Galvão et al.[109]	2010	37	60	12	2	6-12 RM. An aerobic component was also included: 15 to 20 minutes walking or cycling at 65 to 85 % of HR_{max}	2 to 4	8 exercises (3 leg, 5 UB)	Leg: 12 to 24 UB: 20 to 40
Alberga et al.[113]	2011	23	15	24	3	Started at 8-12 reps at 60% of estimated 1RM, then increased by 5lb when 12 reps were successfully completed	1 to 2	10 exercises (3 leg, 7 UB)	Leg: 9 to 18 UB: 21 to 42
Hanson et al.[103]	2013	17	186	12	3	Each set started at 5 RM, then the load was decreased so 3 more reps could be completed. This was repeated until 15 reps were accomplished	1	6 exercises (3 leg, 3 UB)	Leg: 9 UB: 9
Winters-Stone et al.[112]	2014	51	156	52	3 (2xgym, 1xhome based**)	Ranging from 6-8 reps at 8-10 RM, to 12-14 reps at 13-15 RM. The Jump training consisted of two-footed jumps from the ground to a target height. Training progression was secured by weight wests	1 to 2	8 exercises (4 leg, 4 UB). Weights were replaced with body weight exercises and rubber bands at the home based sessions.	Leg: 8 to 16 UB: 8 to 16
Cormie et al.[106]	2015	63	1	12	2	6-12 RM. An aerobic component was also included: 20 to 30 minutes walking or cycling at 70 to 85 % of HR_{max}. The aerobic training was performed prior to the strength training	1 to 4	8 exercises (4 leg, 4 UB)	Leg: 8 to 32 UB: 8 to 32

*Training volume was calculated as (number of exercises x sets x sessions per week)
**The home based session was not included in calculation of the weekly training volume, since it was not performed in a gym setting
RM; Repetition maximum, UB; Upper body,

2.4.1.1 Effect of strength training on body composition in PCa patients on ADT

2.4.1.1.1 Lean body mass

The effect of strength training on LBM in healthy elderly men is well known. The latest meta-analysis summarizes the effect of strength training on LBM in the elderly (mean

age 65) to be 1 kg increase, from two to three sessions per week, for an average duration of 20 weeks [114]. This results in an average increase of 0.05 kg per week.

The effect of strength training on LBM in PCa patients during ADT has been investigated in five studies [102, 103, 106, 109, 113] (Table 7). Three studies reported significant effects from the interventions [103, 109, 113], and one study reported a strong tendency towards a positive effect [106]. No significant effect was reported in PCa patients on ADT in one study [102]. Importantly the intervention effect reported in two studies seems to be the result of large LBM decreases in the control groups [106, 113], which is of equal clinical importance.

Table 7. Effect of strength training on LBM (kg) in PCa patients on ADT.

First author	Year	n	Group	Duration (weeks)	Within group change in LBM (kg)	Change per week
Galvão et al.[102]	2006	10	STG	20	-0.2	-0.01
Galvão et al.[109]	2010	29	STG	12	0.7	0.06
		28	CG	12	0.0	
Alberga et al.[113]	2011	23	STG	24	-0.1	0.00
		26	CG	24	-1.4	
Hanson et al.[103]	2013	17	STG	12	1.7	0.14
Cormie et al.[106]	2015	32	STG	12	-0.6	-0.05
		31	CG	12	-1.4	
Change within the STGs					**0.3**	**0.03**

The interventions that reported significant effects of strength training on LBM [103, 106, 109, 113] included full body strength training programs, with several exercises targeting the same muscle, with a training frequency of two or three times a week. The training sessions were conducted in small groups under the direct supervision of a qualified instructor. The interventions lasted for at least 12 weeks, and patients completed 94-96% [106, 109] of all planned sessions (adherence rates were not reported by Hanson et al. [103]).

In addition to DXA, one study used ultrasound to measure muscle thickness, and reported statistically significant effects on *m. quadriceps femoris* [102]. No effect on muscle thickness was observed in the upper arm or other measuring sites on the thigh. Hansen et al. (2009) used MRi to evaluate the effect of eccentric strength training on the

volume of *m. quadriceps femoris*, but no statistically significant effect of the intervention was reported [101].

At the time the PEPC trial was planned, no strength training study had shown statistically significant changes in LBM [102, 111]. Therefore, to increase the likelihood of detecting increases in the present study, the training volume was increased compared to that used by Segal et al. [111], and the intervention duration was increased compared to Galvão et al. [102]. Also, the response to strength training in the absence of testosterone may differ between the extremities and the trunk, as the response to testosterone supplementation differs between upper and lower body muscles [115]. A higher number of myonuclei expressing ARs in *m. trapezius* than *m. vastus lateralis* [116] may explain the difference in testosterone sensitivity in trunk muscles. Thus, the effect of strength training on LBM in PCa patients on ADT might differ between appendicular and trunk body muscles, but this has not been addressed in any of the studies conducted so far.

2.4.1.1.2 Fat mass

Strength training may not be the appropriate intervention to reduce in fat mass, even in the healthy elderly [117]. However, by increasing lean mass the percentage of body fat will be reduced. The effect of strength training on fat mass in PCa patients on ADT has been reported in four studies [102, 103, 106, 109] (Table 8). In addition, one study report on the percentage of body fat, but the subgroup analysis of PCa patients receiving ADT complicates conversion to absolute fat mass [113].

Table 8. Effect of strength training on fat mass (kg) in PCa patients on ADT.

First author	Year	n	Group	Duration (weeks)	Within group change in fat mass (kg)	Change per week
Galvão et al.[102]	2006	10	STG	20	-0.8	-0.04
Galvão et al.[109]	2010	29	STG	12	-0.2	-0.02
		28	CG	12	0.3	
Hanson et al.[103]	2013	17	STG	12	-0.1	-0.01
Cormie et al.[106]	2015	32	STG	12	0.6	0.05
		31	CG	12	0.9	
Change within the STGs					**-0.1**	**0.00**

Whereas most studies reported no effect [102, 103, 109], one study showed that the gain in fat mass observed in the control group was prevented in the strength training group [106]. One study reported no change in fat mass, but reduced fat percentage [103]. However, no change in fat percentage was reported were reported in one other study [113]. Strength training showed no statistically significant effect on waist circumference or sum of skinfolds in one study that did not include DXA [111].

2.4.1.1.3 Bone mass

A recent meta-analysis showed that exercise in general had positive effects on aBMD in the lumbar spine and femoral neck in the healthy elderly [118]. However, 15 of the 19 studies in the meta-analysis were performed in female populations, where loss of bone mass is more pronounced. The effect of strength training on aBMD in PCa patients on ADT has been reported in three studies [102, 106, 112] (Table 9). No effect was reported in any of the studies.

Table 9. Effect of strength training on aBMD in PCa patients on ADT.

					Within group change in aBMD (g/cm^2)		
First author	**Year**	**n**	**Group**	**Duration (weeks)**	**Femur neck**	**Spine**	**Hip**
Galvão et al.[102]	2006	10	STG	20	0.12		
Cormie et al.[106]	2015	32	STG	12		-0.16	-0.01
		31	CG	12		0.00	-0.01
Winters-Stone et al.[112]	2014	24	STG	52	-0.02	-0.01	-0.01
		12	CG	52	-0.01	-0.02	-0.01
Change within the STGs					**0.05**	**-0.06**	**-0.01**

At the time the PEPC trial was planned, only one study had reported on the effect of strength training on aBMD in PCa patients. Thus, the intervention duration was extended by 4 weeks, and several exercises that put load on the lower back and femur were included, to increase the likelihood of detecting increases in aBMD.

2.4.1.2 Effect of strength training on muscle cellular outcomes in PCa patients on ADT

No study to date has evaluated the effect of strength training at the muscle cellular level in PCa patients on ADT. Therefore, studies in healthy elderly men and/ or animal studies are described to provide a rationale for including the variables in the PEPC trial.

2.4.1.2.1 Muscle fiber cross-sectional area

Several studies have shown larger increases in muscle protein synthesis than breakdown after a bout of strength training (e.g. [119-122]). The increase in muscle protein synthesis occurs within hours after a bout of strength training [123], and may last for as long as 24 to 48 hours post training [119]. Thus, repeated bouts of strength training, e.g. 2-3 sessions per week, over a period of time, normally result in accumulation of contractile proteins. In turn this leads to an increased muscle fiber CSA [124-128]. Typically, greater increases are observed in type II fibers compared to type I fibers. On the other hand, testosterone supplementation has been shown to influence the two fiber types differently, with greater increases in type I fiber CSA [129], possibly due to the increased expression of AR in type I fibers compared to type II fibers [130]. This may also alter the response to strength training when testosterone is removed as in PCa patients on ADT.

2.4.1.2.2 Number of myonuclei per fiber

Increased muscle fiber CSA is typically paralleled by an increased number of myonuclei. Thus, the myonuclear domain (cytoplasmic volume to nucleus ratio) is kept relatively constant [131]. Whether or not the myonuclear addition is necessary for skeletal muscle hypertrophy has been an on-going debate in the literature (see [132] and connected publications), and an upper limit of 2000-2247 μm^2 cytosol per myonucleus has been suggested [72, 133-135]. Thus, an increase in muscle fiber CSA by >26% would typically create a demand for myonuclear addition [72, 134]. However, in previously active, or especially in previously strength-trained individuals, the number of nuclei per fiber may already be high. Thus, there may not be a need for an additional number of nuclei to support the growing fiber during “retraining.” Consequently, an increased number of myonuclei per fiber is not always seen after a strength training intervention in the elderly [126, 136].

Testosterone supplementation has also been shown to increase the number of myonuclei per fiber, but this was only observed at higher dosages of testosterone, which also induced the greatest increases in muscle fiber CSA [8, 70]. It has also been shown that testosterone sensitivity differs between the muscle fiber types, as more myonuclei express AR in type I fibers than in type II fibers [130].

The effect of strength training on myonuclei numbers during ADT has been investigated in young healthy men. In a placebo-controlled study, an increased number of myonuclei per fiber was seen in the placebo group, but not in the ADT group [137]. Thus, the testosterone-suppressed status of PCa patients on ADT may influence the myonuclear response to strength training, but this has not been investigated during long-term ADT as in PCa patients.

2.4.1.2.3 Number of satellite cells per fiber

Satellite cells become activated by a number of stimuli, such as strength training and muscle damage [138]. It has been shown that increased muscle fiber CSA during strength training is often preceded by an increased number of satellite cells per muscle fiber in both young [139, 140] and elderly subjects [72, 140-143]. Furthermore, Verney et al. (2008) [136], and Verdijk et al. (2009) [126] showed that the satellite cell response differs between fiber types, with the greatest increases found in type II fibers. According to Hanssen et al. (2012) training volume might also play a role in the satellite cell response to strength training [144].

ARs are expressed in satellite cells [145], and castration has been shown to decrease the number of satellite cells in young pigs [146]. On the contrary, testosterone supplementation increases the number of satellite cells in a dose-response dependent manner [8, 70]. Therefore testosterone seems to be important for satellite cell function, at least in men. Nevertheless, no differences in the satellite cell response to strength training was observed in a placebo-controlled study in young healthy men, where the groups were treated with ADT or placebo during the intervention [137]. Studies evaluating the effect of strength training, during long term ADT is lacking in the literature.

2.4.1.2.4 Content of androgen receptors in muscle

The effect of strength training during ADT on the levels of AR in muscle has not been investigated in any study to date. However, strength training showed no effect on the mRNA expression of AR in young healthy men on ADT [147], and similar results have

been reported on protein levels of AR in men not on ADT [148]. Therefore, it is not likely that strength training during ADT will change the content of AR in muscle.

2.4.1.2.5 Content of myostatin in muscle

The effect of strength training during ADT on the levels of myostatin in muscle has not been investigated in any study to date. However, one study show increased myostatin levels following strength training in elderly men [149]. But the effect of strength training during ADT on myostatin may differ from the healthy elderly.

2.4.1.2.6 Content of mitochondrial proteins in muscle

Although it is generally accepted that strength training does not increase mitochondrial function in young men [150, 151], increased activity of mitochondrial enzymes has been reported in healthy elderly men [152, 153] and in men suffering from mitochondrial myopathies [154]. The difference in training response between young and elderly subjects could rely on impaired basal levels in the elderly [155, 156].

On the other hand, testosterone seems to have direct effects on mitochondrial proteins, as castration has been shown to decrease the activity of COXIV [157]. Therefore, the result one might expect from strength training in PCa patients on ADT may differ from results in healthy elderly men, but this has not been elucidated in any study to date.

2.4.1.2.7 Content of heat shock proteins in muscle

It has been shown that elderly subjects may have higher baseline levels of HSPs than younger subjects [91]. Unpublished results from our lab indicate that strength training may reduce HSP levels in elderly subjects [158]. Reduced HSP levels with strength training in the elderly may indicate a normalization of the cellular environment. However, the effects of strength training during ADT on HSP levels have not been investigated in any study to date.

2.4.1.2.8 Content of free ubiquitin and ubiquitinated proteins in muscle

There are few studies in the literature that report on the effect of strength training on ubiquitin ligases or UPS activity. Trends towards reduced levels of ubiquitinated proteins were, however, observed in heart failure patients in another study [159].

Interestingly, the heart failure patients showed higher levels of ubiquitinated proteins than healthy controls at baseline. Also, strength training has been shown to normalize levels of the ubiquitin ligase MuRF-1 in chronic heart failure patients, who had higher baseline levels than healthy controls [160].

Importantly, testosterone is involved in regulation of UPS activity, as castration-induced atrophy in male rats has been shown to increase gene expression of atrogin-1 and MuRF-1, which returned to baseline after testosterone supplementation [93]. Thus the effects of strength training on ubiquitinated proteins in PCa patients on ADT may differ from those observed in other subjects, but this is not known.

3.0 Research aims

At the time when the PEPC trial was initiated, only two studies had evaluated the effect of strength training during ADT for PCa [102, 111], and no studies had investigated the effect of exercise on muscle cellular outcomes. Even though some studies have been published since the initiation of the PEPC trial, there is still a need for randomized studies to expand on the knowledge needed to prescribe exercise recommendations for PCa patients.

Therefore, the aim of the PEPC trial was to investigate the effects of strength training on changes in body composition, muscle strength and muscle cellular outcomes in PCa patients on ADT.

- The rationale and design are described in paper I.
- The primary aim of paper II was to investigate the effects of strength training on total LBM. Secondary aims were to investigate the effects of strength training on regional LBM, fat mass, aBMD, muscle strength functional tests and health-related quality of life (HRQoL)[1] in PCa patients during ADT.
- The primary aim of paper III was to investigate the effects of strength training on changes in muscle fiber CSA in PCa patients during ADT. Secondary aims were to investigate the effects of strength training on the number of myonuclei and satellite cells per fiber, content of AR and myostatin in muscle, and knee extensor muscle strength.
- The aim of paper IV was to investigate the effect of strength training on mitochondrial proteins and indicators of muscle cellular stress in PCa patients during ADT.

We hypothesized that strength training in PCa patients on ADT would:

1. Increase total LBM and regional LBM, with greater increases in upper and lower extremity LBM than in trunk LBM. We hypothesized increased aBMD, but no

[1] Although fatigue and HRQoL were included as secondary endpoints in paper I, it will not be included as an endpoint in the present thesis.

change in fat mass. Also, we hypothesized increased performance in all 1RM tests, all functional tests and the shuttle walk test.

2. Increases muscle fiber area in both fiber types, and increased numbers of myonuclei per fiber, thus the myonuclear domain would remain unchanged. We also hypothesized an increase in the number of satellite cells per fiber, but no changes in the content of AR or myostatin.
3. Increased content of mitochondrial proteins, and reduced muscle cellular stress indicated by a reduction in the content of heat shock proteins as well as reduced content of free ubiquitin and total ubiquitinated proteins.

4.0 Materials and Methods

The study was conducted in agreement with the Helsinki declaration, was approved by the Regional Committee for Medical and Health Research Ethics, South-East Region (protocol nr. 08/212b.2008/4062) and registered in ClinicalTrial.gov (NCT00658229).

4.1 Subjects

Patients were recruited from two different urologic units at Oslo University Hospital from January 2009 to September 2011.

4.1.1 inclusion and exclusion criteria

Patients with newly diagnosed PCa, with intermediate or high-risk profile referred to high-dose radiotherapy and (neo)-adjuvant ADT, under the age of 75 and able to understand and read Norwegian and living no further than one hour by car from the Norwegian School of Sport Sciences (NSSS) were eligible (Table 10).

Table 10. Inclusion- and exclusion criteria in the PEPC trial.

Inclusion criteria	Exclusion criteria
Newly diagnosed locally advanced prostate cancer (clinical stage T2 and T3)	Routine resistance training
<75 years of age	Medication for osteoporosis (i.e. Bisphosphonates)
Capable of reading and writing Norwegian	Conditions that contraindicate exercise without adjusted actions:
Treating oncologist has approved the subjects´ participation	Unregulated hypertension (>160/ 95 mmHg)
Lives within approximately 1 hour from Oslo by car or public transport	Uncontrolled cong. Heart failure (NYHA class >II)
Written informed consent received	Unstable angina pectoris
	Recent myocardial infarction (last 6 months)
	Cardiac arrhythmia
	Chronic obstructive pulmonary disease
	Severe asthma
	Recent stroke
	Epilepsy
	Insulin dependent diabetes mellitus
	Unstable bone lesions with high risk of fractures
	Mentally incompetent conditions:
	Severe anxiety or depression
	Dementia
	Known alcoholism or other abuse (liver test)
	Mentally retarded
	Conditions complicating ability to participate in a supervised training program:
	Uncontrolled pain
	Severe arthritis
	Scheduled hip or knee replacement
	Pathologic fractures last 6 months
	Amputation
	Walker or wheelchair user

Patients already performing resistance training on a regular basis were not eligible. Also, patients suffering from conditions that could compromise training adherence without adjustments being made to the training program were not eligible. Exclusion criteria are listed in Table 10.

4.2 Design and randomization

After singing an informed consent form, and completing baseline assessments, patients were computer randomized, in a 1:1 ratio, to a strength training group (STG) or a control group (CG) (Figure 1). The staff at the office of clinical research at OUS performed the randomization.

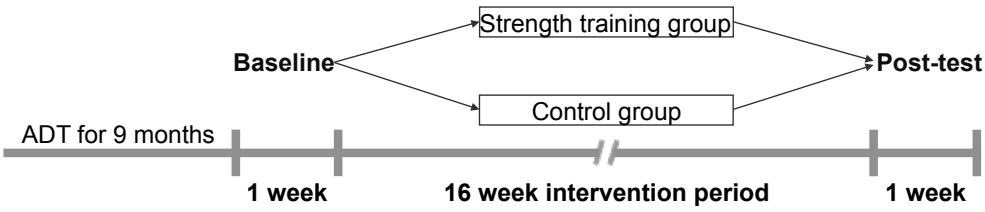


Figure 1. Design overview of the PEPC trial. After nine months of ADT PCa patients included in the study were randomly assigned to either a strength training group or a control group for 16 weeks.

4.2.1 Strength training group

Patients randomized to the STG underwent a strength training program designed to promote gains in LBM and strength. The program included five lower-body exercises: Smith machine half squat; leg press; Smith machine standing calf raises; knee flexion; and knee extension (Figure 2). Four upper-body exercises were also included: chest press; seated row; seated shoulder press; and biceps curl (Technogym, Gambettola, Italy). In addition, patients were encouraged to include two core exercises such as abdominal crunches and back extension at the end of the session.

Figure 2. Exercises included in the PEPC trial. From upper left: Smith machine half squat, leg press, Smith machine standing calf raises, knee flexion and knee extension, chest press, seated row and seated shoulder press. Seated biceps curl was also included.

The program was a modified version of that used by Segal and colleagues (2003) [111]. Compared to the original training program the duration was increased from 12 to 16 weeks, daily undulating periodization was applied, and the intensity was increased to optimize the training response (Table 11). The two first weeks were considered as familiarization, where the focus was on learning the correct technique in the exercises. Thereafter the training intensity was increased: the first weekly session was performed under the supervision of a training instructor at intensities equivalent to 10 repetitions maximum (10 RM) (i.e. the maximal load where the correct technique can be performed in 10 repetitions). The second session was performed alone or in the company of

another patient at a submaximal intensity, approximately 90% of 10RM in 10 repetitions. The last session was performed with an instructor with an intensity of 6 RM. Training volume was increased in a linear manner throughout the intervention to ensure progression (Table 11).

Table 11. Overview of the strength training program in the PEPC study.

Week	1. Session: heavy Monday	2. Session: moderate Wednesday	3. Session: heavy Friday
1 and 2	2 x 10 submaximal resistance Focus on correct technique	2 x 10 submaximal resistance Focus on correct technique	2 x 10 submaximal resistance Focus on correct technique
3 to 6	2 x 10 RM leg exercises 1 x 10 RM upper body	2 x 10 reps leg exercises 2 x 10 reps upper body (Resistance: 90% of 10 RM)	3 x 6 RM leg exercises 2 x 6 RM upper body
7 to 12	3 x 10 RM leg exercises 2 x 10 RM upper body	2 x 10 reps leg exercises 2 x 10 reps upper body (Resistance: 90% of 10 RM)	3 x 6 RM leg exercises 2 x 6 RM upper body
13 to 16	3 x 10 RM leg exercises 3 x 10 RM upper body	3 x 10 reps leg exercises 3 x 10 reps upper body (Resistance: 90% of 10 RM)	3 x 6 RM leg exercises 3 x 6 RM upper body

4.2.2 Control group

Patients randomized to the CG were encouraged to maintain their current habitual activity level, but were specifically asked not to undertake strength training. After the post tests patients in the control group were given access to the strength training facilities at NSSS and offered supervised strength training twice a week for 16 weeks.

4.3 Assessments

All assessments were carried out before and after the intervention. On Monday or Tuesday the patients underwent the biopsy procedure (optional) followed by familiarization with the physical tests. Then on the following Thursday or Friday the patients filled out a questionnaire and underwent the physical tests and a DXA scan

4.3.1 Physical tests

All physical tests were performed at the NSSS.

4.3.1.1 1 Repetition maximum tests

1 RM was determined in four strength exercises (in chronological order): leg press, knee extension, seated chest press and seated shoulder press (Technogym, Gambettola, Italy).

In order to familiarize patients with the tests, a familiarization session was performed three days prior to the actual test, in which range of motion (ROM) and equipment settings were standardized for each test exercise.

At both the familiarization session and the actual test, all patients underwent 10 minutes of warm-up on a stationary bike, or on a treadmill. Thereafter, the patients completed four warm-up sets for each exercise of increasing, but still submaximal, loads in 10, six, three and one repetition prior to the first 1 RM attempt. The patients were given at least 2 minutes of rest between all warm-up sets and 1RM attempts. At the 1 RM attempts patients were given oral encouragement to perform maximally. An effort was made to reach 1 RM within five attempts, and criteria for a successful 1 RM attempt for each exercise are described below.

A successful 1RM attempt in the leg press exercise occurred when the subjects started with full knee extension and lowered the load to a 90° knee flexion, and then were able to return the load back to full knee extension again. Pieces of wood, of different lengths, were used to restrict the ROM to fit the individual patient. The subjects were instructed to hold onto the handles, to prevent movement of the hip, and to take a deep breath to increase abdominal pressure.

ROM in knee extension was set to start with the knee flexed to a 90° angle, and was completed by reaching full extension. Subjects were instructed to hold on to the handles, and to apply force as rapidly and as hard as possible to complete the full ROM with the highest load possible.

Chair height in the seated chest press was standardized so the horizontal handles were aligned with the lower portion of *m. pectoralis major*. The exercise was performed as a concentric test, starting with the handles by the chest and full ROM was reached when the elbow was fully extended or, in tall subjects when the handles met. The subject was instructed to take a deep breath, and to abduct the shoulder joint so the elbow was in alignment with the handles.

Chair height in the shoulder press was adjusted so the handles were in line with the upper part of *m. deltorideus*. ROM was completed when the elbow reached full extension, or when the handles met.

4.3.1.2 Functional tests

The sit-to-stand test was performed using a 46 cm hardback chair without armrests. The subject started in a seated position leaning against the backrest, his feet positioned no wider than the chair and his hands crossed in front of him. One correct repetition was completed when the patient got up to fully extended knee and hip, and sat down again touching the backrest with his back. Arms had to be crossed at all times. The test leader counted the number of correctly performed repetitions in 30 seconds, timed with a stopwatch. The patients made two attempts separated by a two-minute rest, and the best result was registered.

The stair-climbing test was performed on a 20-step stair with a step height of 16 cm. The subjects started at the bottom of the stair, and ran up the stairs touching each step on the way without the use of handrails. Two sets of photocells (Brown timing system, USA) were used to time the subject up the stairs. After two attempts, two minutes apart, the subject was loaded with a 10 kg weight vest, and two 5 kg weight manuals, giving an additional load of 20 kg. The patients made two attempts at the loaded stair climb, separated by a two-minute rest.

To test cardio vascular fitness a shuttle walk test was performed. A distance of 10 meters was marked using two cones, and pace was determined by sound signals. The subject had to complete the distance between the beeps. Starting pace was set very slow: 30 meters in 60 seconds, and was gradually increased by 10 meters per 60 seconds every minute. As the pace increased the patient could run at his own initiative. The test lasted for a maximum of 12 minutes. Patients were informed of the option to quit if they felt too exhausted to continue, but were encouraged by the test leader to push themselves during the test.

4.3.2 Body composition

Body composition included total and regional LBM, fat mass, body weight, height, and body mass index (BMI). LBM and fat mass were measured using DXA (DELPHI, QDR

4500 series, or QDR 1000, Hologic, Waltham, MA). The total body scan provided total LBM (primary outcome in the PEPC trial), as well as LBM of the upper and lower extremities or appendicular skeletal muscle mass (ASM), and LBM of the trunk. Changes in upper and lower extremities and trunk were investigated separately to investigate possible differences in training responses between upper and lower muscles. Body weight was measured using a digital platform scale with a mounted stadiometer (Seca, Vogel & Halke, Hamburg, Germany), and body mass index (BMI) (weight/(height)^2) was calculated. Although not traditionally included in "body composition", aBMD was also measured by DXA.

4.3.3 Muscle biopsies

The subject lay in a supine position, and after injection of local anesthesia (Xylocain® adrenaline, 10 mg·ml^{-1} + 5 µg·ml^{-1}, AstraZeneca, London, UK), an incision in the skin and muscle fascia was made with a scalpel. A 6 mm Pelomi needle (Albertslund, Denmark) with manual suction was used to obtain tissue samples (~150 mg) from the mid-section of the right m. vastus lateralis. The procedure was performed twice in each incision, using one cut in the distal and one cut in the proximal direction. The post biopsy was placed approximately 3 cm proximal to the baseline biopsy, and care was taken not to obtain tissue within the same area twice. The total amount of tissue was divided for subsequent analysis.

For immunohistochemistry (IHC) a piece of tissue was dissected free from visual fat, the edge was trimmed for easier cryostat handling and it was embedded in Tissue-Tec (O.C.T. Compound, Sakura Finetek, Torrance, CA, USA), before being frozen at -160°C in isopentane on liquid nitrogen, within 5 minutes after the tissue was collected. The specimen was then stored at -80°C for further analysis.

Pieces for immunoassays were rinsed in ice-cold isotonic physiological saline (0,9% NaCl, Braun, Melsungen, Germany), and carefully dissected free of visual fat, connective tissue and blood. Pieces, each of 50 mg, were frozen in isopentane on dry ice and stored at -80°C for later homogenization.

4.3.3.1 Immunohistochemistry

Serial cross transverse sections (8 µm thick) were cut in a cryostat microtome (Leica CM3050, Nussloch GmbH, Germany) at -22°C, mounted on Superfrost Plus microscope glass slides (Menzel-Gläser, Braunschweig, Germany) and stored at -80°C until further analysis.

The glass slides were acclimated to room temperature (RT) for 30 minutes, before being incubated with phosphate buffered saline containing 0.05% Tween 20 (PBS-t) for 5 minutes at RT. Thereafter, the slides were blocked with 1% bovine serum albumin, in PBS-t, for 30 minutes at RT, before primary antibody was applied and incubated overnight at +4˚C. The sections were then washed 3 x 10 minutes in PBS-t and incubated for 30 minutes at RT with appropriate secondary antibodies (Alexa fluor 488/594, A11001/A11005/A11008/A11012, Invitrogen, Carlsbad, CA, USA). Thereafter, the washing procedure was repeated and cover slides mounted using Prolong Gold Antifade reagent with DAPI (Invitrogen, Carlsbad, CA, USA). Images of the stained cross-sections were captured by light microscope (Olympus BX61 TRF, Tokyo, Japan) connected to a fluorescence light source (EXFO X-cite 120, Mississauga, Canada) with a camera (Olympus DP72, Tokyo, Japan) attached.

All biopsy analyses were blinded, meaning the researcher had no knowledge of which group the samples belonged to.

4.3.3.1.1 Muscle fiber cross-sectional area

Primary antibodies towards dystrophin (polyclonal, Abcam, ab15277, Cambridge, UK), and type II fibers (monoclonal, SC71, gift from Dr. Schifiano) were used according to the protocol described above (section 4.3.3.1). The Tema Image-Analysis System (Scan Beam, Hadsund, Denmark) was used to analyze the muscle fiber CSA by tracking the inner rim of the dystrophin staining (Figure 3a) of each muscle fiber type. The total muscle fiber CSA was displayed as the two fiber types combined.

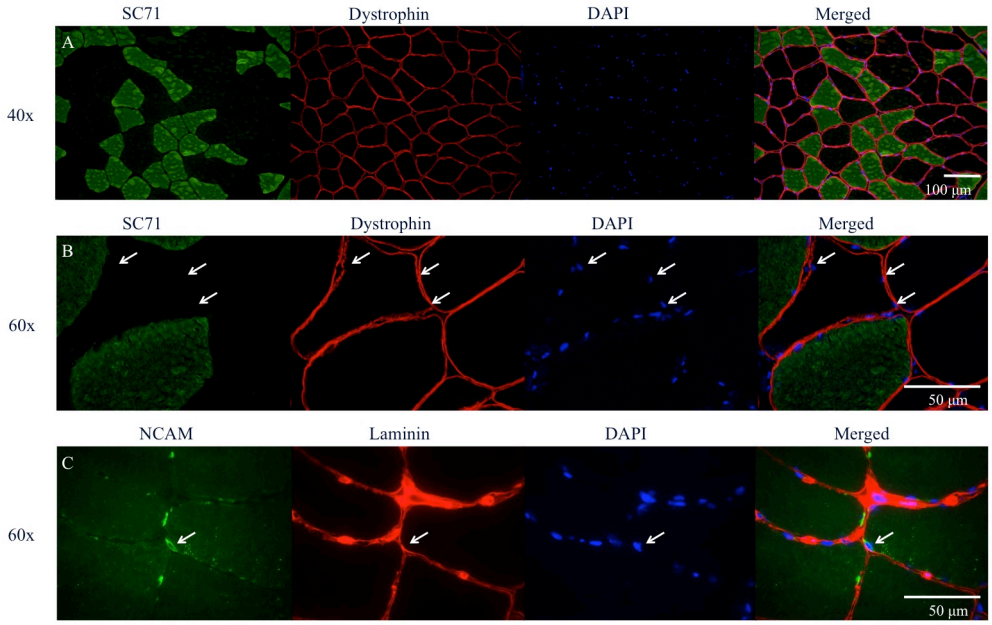


Figure 3. Immunohistochemical analysis of muscle biopsy cross-sections. A: Fiber type specific muscle fiber area was calculated as area inside the dystrophin staining. SC71 stains all type II fiber in human muscle. B: The number of myonuclei was counted as at least 2/3 of the DAPI staining (nucleus) inside the dystrophin staining, and related to muscle fiber type. C: The number of satellite cells was counted as at least 2/3 of a ring-like NCAM staining inside the laminin staining, and related to muscle fiber type on the adjacent cross-section.

4.3.3.1.2 Myonuclei and myonuclear domain

The number of myonuclei per muscle fiber was counted as DAPI staining inside the plasma membrane, visualized by dystrophin, and related to either fiber type I or II (Figure 3b). Care was taken not to include nuclei outside of the membrane staining by applying a stringent counting criterion: 2/3 of the DAPI staining had to be located inside the dystrophin staining. As an indicator of myonuclear domain muscle fiber CSA was divided on the number of myonuclei per fiber.

4.3.3.1.3 Satellite cells

Antibodies towards Ncam (Abcam, ab9272, Cambridge, UK) and laminin (Daco Denmark AS, 20097, Glostrup, Denmark) were used as described above, using the cross-section adjacent to the one used for CSA and myonuclei analysis. The number of satellite cells

was counted as ring-like Ncam staining encircling at least 2/3 of a nucleus (DAPI staining) located inside the laminin staining (Figure 3c).

4.3.3.2 Protein quantification

Total protein was extracted from muscle samples using a commercially available kit (T-PER® Tissue Protein Extraction Reagent, cat.no.78510, Thermo Scientific, Waltham, MA), and according to the manufacturer's instructions. Furthermore, 2% protease and phosphatase inhibitor cocktail (Halt™ Protease and Phosphatase Inhibitor Cocktail, cat.no.78440, Thermo Scientific, Waltham, MA) and 2% Ethylenediaminetetraacetic acid (EDTA) was added to the lysate dilution according to the manufacturer's instructions.

A second muscle sample was fractionated into cytosol-, membrane-, cytoskeletal- and nuclear fractions using a commercially available fractionation kit (ProteoExtract Subcellular Proteo Extraction Kit, Cat.no.539790, Calbiochem, EMD Biosciences, Darmstadt, Germany) according to the manufacturer's instructions. Protein concentrations were determined using a commercial kit (BioRad DC protein micro plate assay, Cat.no.0113, Cat.no.0114, Cat.no.0115, Bio-Rad, Hercules, CA, USA), a filter photometer (Expert 96, ASYS Hitech, Butterfield, Luton, UK) and the provided software (Kim, ver. 5.45.0.1, Daniel Kittrich). The protein standard was γ-globulin, ranging from 0.125 to 1.5 mg per ml. The protein standard curve and samples were analyzed in triplicate with the standard curve having an r^2>0.9.

4.3.3.2.1 Western blot

Equal amounts of protein (10- 40 µg per well, depending on the fraction loaded) were loaded and separated on precast NuPAGE Novex 4-12% Bis-tris Midi gels (Cat. No NP0321, Invitrogen, Carlsbad, CA, USA) for 35-45 minutes at 200 volts in cold MES running buffer (NuPAGE MES SDS running buffer, cat. No. NP0002, Life technologies, Invitrogen, Carlsbad, CA, USA). All subcellular fractions were routinely loaded on the same gel and run for the same time period, to enable comparison. Separated proteins were transferred on to immune-blot PVDF membrane (Immuno-blot, Cat.no.162-0177, Bio-Rad, Hercules, CA, USA), at 30 volts for 90 min in cold transfer buffer (NuPAGE transfer buffer, Cat.no.NP0006-1, Life technologies, Invitrogen, Carlsbad, CA, USA). Membranes used for HSP70 and alphaB-crystallin (Table 11) were blocked over night at

4 ^{0}C in a 5% fat-free skimmed milk and 0.05% TBS-t solution (TBS, Cat.no.170-6435, Bio-Rad, Hercules, CA, USA; Tween 20, Cat.no.437082Q, VWR International, Radnor, PA, USA; Skim milk, Cat.no.1.15363, Merck, Darmstadt, Germany). Membranes used for HSP60, COX IV, CS, and ubiquitination (Table 12) were blocked at room temperature (RT) for 2 hours. Blocked membranes were then incubated with monoclonal primary antibodies for 2 hours at RT, or overnight at 4^{0}C. Thereafter, membranes were incubated with secondary antibody (goat anti-mouse, Cat.no.31430, Thermo Scientific/Pierce Biotechnology, Rockford, IL, USA) diluted 1:30 000 or 1:2000 (anti-rabbit IgG, Cat.no. 7074, Cell Signalling, Boston, MA, USA) at RT for 1 hour. All antibodies were diluted in a 1% fat-free skimmed milk and 0.05% TBS-t solution. Between stages the membranes were washed with 0.05% TBS-t. Protein bands were visualized, using an HRP-detection system (Super Signal West Dura Extended Duration Substrate, Cat.no.34076, Thermo Scientific/Pierce Biotechnology, Rockford, IL, USA). Chemiluminescence was measured using a CCD image sensor (Kodak image station 2000R, Eastman Kodak company, Rochester, NY, USA) and band intensity was calculated using the Carestream molecular imaging software (v. 5.0.7.2.2, Carestream Health, New Haven, CT, USA). All samples were analyzed in duplicate, and mean values were used for statistical analysis. Representative blots are shown in Figures 4 and 5.

Table 12. Overview of antibodies used for Western blot.

	Cat.nr.	LOT	Host	Dillution	Manufacturer
HSP70	ADI-SPA-810	12071118	Mouse	1:4000	Enzo Life Sciences (USA)
AlphaB-crystalline	ADI-SPA-222	9011032	Mouse	1:4000	Enzo Life Sciences (USA)
HSP27	ADI-SPA-800	9061109	Mouse	1:10000	Enzo Life Sciences (USA)
HSP27	ADI-SPA-803	3020917	Rabbit	1:10000	Enzo Life Sciences (USA)
HSP60	SPA-807-E	12409	Mouse	1:4000	Stressgen Biotechnologies (Canada)
COXIV	Ab14744	GR67749-1	Mouse	1:1000	Abcam (UK)
Citrate synthase	ab96600	GR134613-9	Rabbit	1:12000	Abcam (UK)
Ubiquitin	SPA-203	B405440	Mouse	1:2000	Nordic Biosite AB (Sweden)

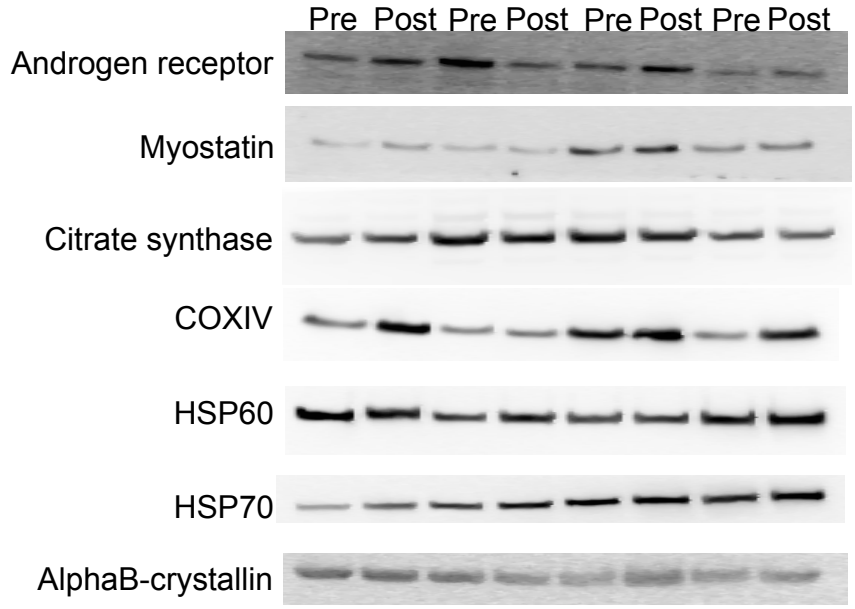


Figure 4. Representative blots for androgen receptor, myostatin, citrate synthase, COXIV, HSP60, HSP70 and alphaB-crystallin.

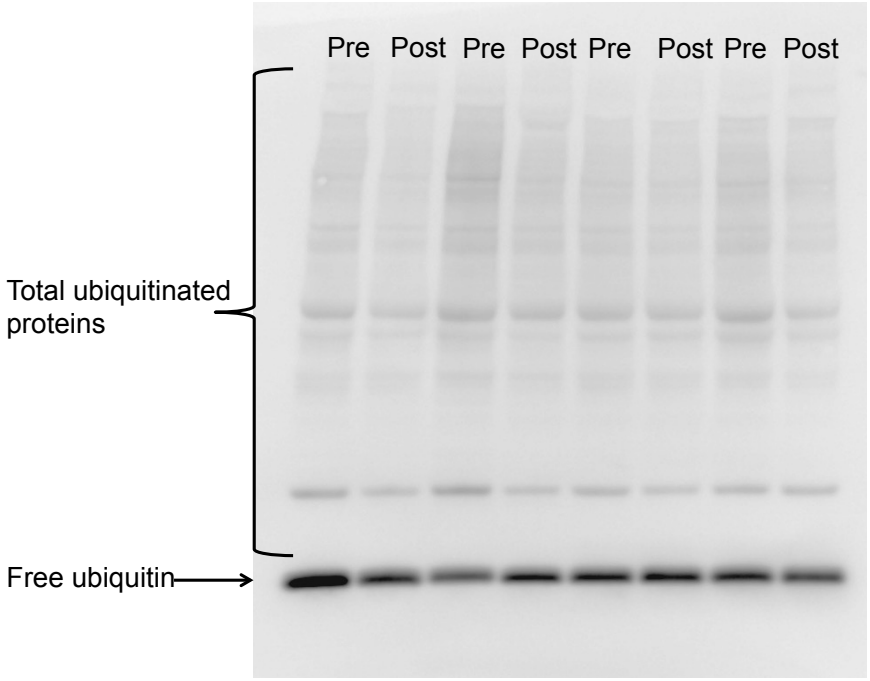


Figure 5. Representative western blots for free ubiquitin and total ubiquitinated proteins.

4.3.3.2.2 Enzyme linked immunosorbent assay (ELISA)

Protein quantification of HSP27 was performed using a double-antibody sandwich ELISA developed in our lab. Each sub-cellular fraction was analyzed separately and later combined to give the total cellular amount. A monoclonal capture antibody against HSP27 (25 ng per well; mouse anti-HSP27,Enzo Life Sciences, Plymouth Meeting, PA, USA) and a polyclonal detection antibody against HSP27 (rabbit anti-HSP27, Enzo Life Sciences, Plymouth Meeting, PA, USA) were diluted 1:10 000 and horseradish peroxidase conjugate was used as the secondary antibody, diluted 1:10 000 (Amersham Biosciences; GE Healthcare Life Sciences, Buckinghamshire, UK). The HSP27 assay was performed in high-binding polystyrene microplates (Costar, Inc., Corning, NY, USA) using tetramethylbenzidine (TMB Solution, Calbiochem, EMD Biosciences, Darmstadt, Germany) as the substrate, and 2N sulfuric acid as the stop solution. Recombinant HSP27 (Enzo Life Sciences, Plymouth Meeting, PA, USA) was used as the standard (0.0975–25 ng/mL). All samples were diluted 1:300 (cytosolic fraction), 1:100 (membrane fraction) or 1:50 (cytoskeletal fraction) and analyzed in triplicate (CV < 10%). The amount of HSP27 was determined using a filter photometer measuring optical density at 450 nm.

4.4 Statistics

4.4.1 Sample size calculations

At the time the PEPC-trial was planned, there was a limited number of strength training studies available that had investigated PCa patients on ADT. Sample size was therefore estimated based on findings by Kvorning et al. 2006 [161] who reported an increase in lean mass following strength training in young men on ADT, and studies that reported loss of lean mass in PCa patients on ADT [5]. Thus, we expected a 3 kg difference in mean change between the two groups. With a two-sided significance level of 5% and a power of 90%, 22 patients were required in each group, assuming a standard deviation (SD) of 3 kg. To allow for dropouts we planned to include about 30 patients in each group.

Using total CSA for our power calculation, a total of 37 patients would have a power of > 90% to detect an effect size of 0.8 (difference/SD). Due to drop-outs and insufficient tissue quality for immunohistochemistry the power was reduced to approximately 80%.

4.4.2 Paper II

Between-group differences were assessed by analysis of covariance (ANCOVA) with the change from baseline included as the dependent variable, group assignment (STG versus CG) as a fixed factor, and baseline score as a covariate. In addition mean change from baseline within treatment groups with corresponding 95% CIs were also estimated. Missing data were imputed by an intention-to-treat approach using the last observation carried forward. We also performed sensitivity analyses, including patients with complete data sets only (per-protocol). A p-value <0.05 was considered statistically significant. Data were analyzed using SPSS version 18.0.

4.4.3 Paper III

Only patients with both baseline and post-intervention biopsies were included in the analysis. For the IHC-data, between-group differences were assessed by analysis of covariance (ANCOVA) with the change from baseline to post-test included as the dependent variable, group assignment (STG versus CG) as a fixed factor, and baseline score as a covariate. By calculating the individual changes from baseline to post-test, we estimated the mean changes within each group and the corresponding 95 % CIs, using paired sample t-tests. The association between changes in muscle strength and muscle fiber CSA was analyzed by linear regression. Data were analyzed using SPSS version 18.0.

Normality of the WB-data (AR and myostatin) was assessed by visual inspection of normality plots, as well as the D'Agostino-Pearson omnibus normality test. Between-group differences in change were analyzed with a two-sample t-test. Within-group changes were analyzed using a pair-sample t-test and visualized in graphs by means and 95% CIs. Western blot data were analyzed using GraphPad Prism 5. A p-value <0.05 was considered statistically significant.

4.4.4 Paper IV

Only patients with both baseline and post-intervention biopsies were included in the analysis. Normality was assessed by visual inspection of plots as well as D'Agostino-Pearson omnibus normality tests. Data that passed the normality test were analyzed by comparing changes between groups using a two-sample t-test, and data that failed the normality test (COXIV and total ubiquitinated proteins) were analyzed by a nonparametric Wilcoxon matched-pairs signed-rank test. Within-group changes are

visualized in graphs by individual relative changes, and within-group mean changes and 95% CIs. Extreme outliers were identified using Bland-Altman plots, and removed from the dataset. Removal of outliers did not alter any between- or within-group changes. Data were analyzed using GraphPad Prism 5 (version 5, San Diego, CA, USA, http://www.graphpad.com).

5.0 Results and discussion

5.1 Methodological discussion

Before engaging in a discussion of the findings from the PEPC-trial, methodological considerations will be discussed to place our results into the proper context.

5.1.1 Internal validity

The internal validity of a study depends on factors other than the treatment given that could have influenced the observed effects. Threats to internal validity are often related to study design, assessment procedures and other efforts to minimize bias [162].

5.1.1.1 Design considerations

The PEPC trial was a RCT, which is considered the "gold standard" when investigating the effect of a treatment. Thus, the design included in the PEPC trial seems to be appropriate for detecting the effect of strength training in PCa patients.

5.1.1.2 Assessments

To evaluate the effect of our intervention on body composition, DXA was found to be the appropriate method. DXA has been validated with computer tomography (CT) [163], which has shown excellent correlation (r=0.99) with actual tissue weight in cadaver validation studies [164]. Also, age does not alter the validity of DXA for estimating skeletal muscle mass [165]. According to the latest CONSORT-statement [166] RCTs should be performed in a blinded manner. Therefore, personnel performing the DXA scans were blinded to the group allocation of the patients.

The basic principle behind DXA relies on the attenuation of high- and low-energy x-ray beams as they pass through the different tissues [167]. The majority of the trunk volume is occupied by lungs and internal organs, and to a comparable smaller degree by skeletal muscle. Therefore, the apparatus would have to be highly sensitive to detect small changes in skeletal muscle mass here. Thus, the ability of DXA to quantify LBM in the trunk is somewhat uncertain [165, 168]. MRi might therefore be preferable to investigate effects on trunk LBM in future studies. This may have influenced our results

for changes in trunk LBM. Consequently, these results need to be validated by more sensitive measurements before firm conclusions can be reached.

To assess the effect of strength training to improve muscle function in PCa patients on ADT, we choose to evaluate muscle function as maximal strength, or the maximal load by which a full range of motion of a given exercise can be completed once (one repetition maximum, or 1RM). The rationale for this decision was that 1RM testing is considered as the gold standard for assessing muscle strength [169]. In addition it is relatively simple to perform, and good test-retest reliability has been shown both for multi-joint exercises and for single joint exercises [170-172]. Also, to increase the clinical impact of our results, commonly-used functional tests were included.

It has been shown that unblinded studies report larger 1RM increases than blinded designs [173]. Therefore, ideally the test leader overseeing the physical tests should have been blinded to group allocation. However, due to the planned two-year duration of the intervention, it was not possible to recruit one independent test leader who would be able to perform all the tests in the PEPC trial. To ensure high test-retest reliability all tests were performed under supervision of the same test leader (the author of the present thesis). To reduce the potential threat of our non-blinded tests, the test leader was unaware of the baseline test result prior to the post-test. Consequently, all post-tests were conducted as independently as possible. However, it cannot be ruled out, objectively, that knowing the group allocation of the patient at the post-test has biased the test leader.

To increase the reliability of the physical tests all subjects underwent standardized warm-up procedures, and performed all test exercises in a standardized order. Importantly, prior to all tests (both baseline and post-test) and for both groups, a familiarization session was performed three days prior to the actual test. The familiarization session was used to find an appropriate starting weight for the actual 1RM-test, and to familiarize the patients with the technique required in each test. An additional familiarization session might have increased the test-retest reliability of our 1RM tests even further [174]. However, it has been shown that one familiarization

session prior to the strength test is sufficient to ensure reliability in middle-aged men [169]. Therefore, it is not likely that this would have influenced our results.

In the present study we included muscle biopsies, to evaluate the effects of strength training in PCa patients on ADT on the muscle cellular level. Although the muscle biopsy procedure is an invasive technique and thus is associated with infection risk, the Bergstrøm muscle biopsy technique reduces the risk of infection to a minimum compared to alternative procedures (e.g. open biopsy technique) [175]. Furthermore, in our lab we have adopted the Bergstrøm needle biopsy technique with manual suction, which gives the largest amount of tissue per "clip" [175, 176]. We also used the 6mm Bergstrøm needle, which has been shown to increase the amount of tissue per clip more than the more commonly used 5mm needle [177]. In turn, this reduces the risk of complications as it reduces the number of clips needed to obtain a sufficient amount of tissue. Furthermore, to ensure patient recruitment in the PEPC trial, muscle biopsies were made optional. Therefore, the biopsy procedure included in the present study seems to be appropriate, and ethically defensible.

In paper II we used immunohistochemistry to evaluate the effect of strength training on several muscle cellular outcomes during ongoing ADT. Immunohistochemistry is first and foremost limited by the subjective nature of the method: the observer has to decide whether or not to include a given structure in the analysis (intra-observer validity), which could differ from other observer's opinion (inter-observer validity). Consequently, there are concerns related to the use of immunohistochemistry as a diagnostic tool [23, 178]. However, it is important to distinguish between evaluating tissue cross-sections for diagnostic purposes and our usage of immunohistochemistry: whereas the entire cross-section, with varying degrees of pathology, is evaluated (graded) as a whole for diagnostic purposes, we identified and quantified specific structures within the cross-section. The latter method has been shown to be reliable, with good intra- and inter-observer validity [179, 180]. Importantly, the observer was blinded to the group allocation of the patients, until all analysis was completed. This was done to keep the observer unbiased, since knowing the group allocation could have influenced his decision to count a given staining or structure.

To ensure good intra-observer validity in the present study, the observer was given appropriate training before he started analyzing muscle tissue obtained from the PCa patients. Also, inter-observer validity was secured by having a trained observer re-count some of the cross-sections evaluated by the observer in the present study. Unfortunately, we did not perform any statistics to evaluate inter-observer reliability, but a qualitative evaluation of the counts was performed. The next step to secure reliable results in immunohistochemistry is to use proper antibodies to the proteins of interest. There are several antibodies available to quantify satellite cells in muscle cross-sections [181]. In order to compare our results to previous studies, we applied antibodies commonly used in similar studies in the elderly (e.g. [126, 136]).

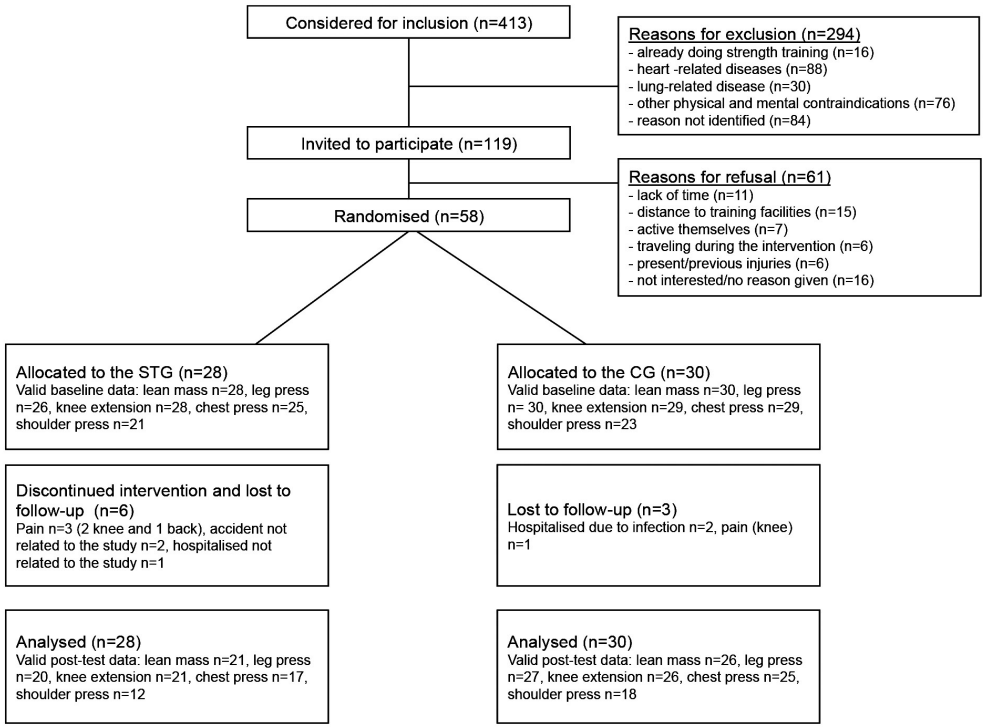
In paper III we used the western blot technique to evaluate the effect of strength training on the content of AR and myostatin in muscle, and in paper IV effects were evaluated on mitochondrial proteins and markers of cellular stress. Traditionally western blot experiments generate two pieces of data: changes in the expression of the protein of interest and changes in the expression of a loading control, often a housekeeping protein (ß-actin, ß-tubulin or glyceraldehyde 3-phospate dehydrogenase (GAPDH)). The loading control is applied to ensure equal loading between samples, thus making the different samples comparable. However, when using housekeeping proteins as loading controls, it is assumed that they are not altered during the experiment. This is, however, not the case during a 16-week strength training intervention, as exercise has been shown to induce changes in housekeeping proteins such as ß-tubulin [182] and GAPDH [183]. Furthermore, differences in muscle fiber type composition between patients might also influence the content of GAPDH [184], but not ß-actin [185], which could have led to additional noise in our data. Therefore, in the absence of more sensitive loading controls, normalization of the protein of interest to the total protein content of the sample, rather than a single housekeeping protein, has been suggested [186, 187]. This method was also applied in the present study, where equal amount of total protein was loaded.

5.2 Patient characteristics

5.2.1 Patient flow

Between 2009 and 2011, 1818 PCa patients received curative high-dose radiotherapy and (neo)-adjuvant ADT at OUS. The main reasons for not being invited to participate in the PEPC trial were: residence too far from the training facilities (1000 patients); different stage of PCa or received other treatment options (297); too old to participate (99 patients); or were not invited for other reasons (9). Therefore, 413 were considered for inclusion, and 119 patients were invited to join the PEPC trial, of whom 58 patients agreed to participate. The overview of reasons for exclusions and refusals, and patient flow are listed in Figure 6.

Figure 6. Consort diagram.



5.2.2 Generalizability

Strict inclusion and exclusion criteria were important to eliminate potential confounders and to ensure the feasibility of the intervention. However, it may be difficult to generalize the study results to other populations, thus strict inclusion and exclusion

criteria represent threats to external validity. Since there were few RCTs on strength training during ADT available when the PEPC trial was planned [102, 111], we included several inclusion and exclusion criteria to make sure that the intervention would be safe. The main reasons for excluding PCa patients from the present study, except for travelling distance to the training facilities, were age, diabetes, heart or lung disease, or other physical or mental contraindications (Figure 6). In fact, our exclusion criteria excluded 72% of the eligible population. Thus, patients in the PEPC trial represent a highly selected group of PCa patients, and generalization of our intervention and results to the general population of PCa patients is therefore limited.

First and foremost, health-related issues excluded 194 patients (Figure 6). Therefore, the results reported from the PEPC trial may only be representative of the healthiest of PCa patients. Secondly, the incidence of PCa increases strongly with increasing age [188]. According to the Cancer Registry of Norway only 4% of all new PCa cases in Norway (2008) were younger than 54 years old, and the mean age of diagnosis was 72 years old [189]. In the PEPC trial PCa patients were excluded if they were over the age of 75 years. This may have influenced the feasibility of the PEPC trial. Thus, results reported from the PEPC trial may only be representative of the youngest PCa patients.

5.2.3 Patient characteristics

Of the 58 patients in the PEPC trial, six patients dropped out of the STG and three dropped out of the CG. There was no difference in dropout rates between the groups ($p=0.23$) during the intervention. Baseline characteristics are listed in Table 13.

With regard to papers III and IV, 31 of the 58 patients agreed to undergo muscle biopsies. Only patients with both baseline and post-intervention biopsies were included in the analysis. Unfortunately, some muscle biopsies were of poor quality due to freezing damage, or provided too few muscle fibers, and had to be excluded from the analysis. This resulted in 11 patients in the STG and 12 patients in the CG being available for the IHC analysis in paper III

Table 13. Baseline characteristics.

	STG (*n* = 28)	CG (*n*=30)
Age (years)		
Mean (range)	66 (54-76)	66 (54-76)
SD	6.6	5
Risk profile groups		
Intermediate (proportion of patients)	50	50
High-risk (proportion of patients)	50	50
Total time on ADT (months)		
Mean (range)	17.0 (8-34)	18.0 (8-28)
SD	8.7	8.2
Time on ADT at baseline (months)		
Mean (range)	9.0 (7-12)	9.0 (5-12)
SD	1.6	1.8
Time from rad. to baseline (months)		
Mean (range)	3.0 (1-7)	3.0 (1-6)
SD	1.3	1.3
Testosterone at baseline (mmol/l)		
Mean (range)	< 0.6 (0.4-1.5)	< 0.6 (0.4-1.3)
SD	0.3	0.2

STG, strength training group; CG, control group;
SD, standard deviation; BMI, body mass index;
ADT, androgen deprivation therapy; Rad, radiotherapy.

The level of higher education has been shown to influence the time spent doing leisure time physical activity level [190-192], and also physical fitness levels [193]. In the present study 54% of patients in the STG, and 45% of patients in the CG, reported more than four years of higher education. This is more than twice as high as the education level in the general population in the Oslo area, where 20% of men between 60 and 66 years of age, and 18.3% of men older than 67 years of age reported four or more years of higher education, according to reports by SSB [194]. Thus, the patients in the PEPC trial may represent the proportion of PCa patients that are more highly educated, and thus (in theory) are more likely to be active.

5.3 Intervention

At the time when the PEPC trial was planned limited knowledge existed on the trainability of PCa patients, and no studies had been able to induce gains in LBM in PCa patients [102, 111]. Therefore, the training program included in the present study was based on Segal et al. (2003) [111], but training intensity and volume were increased.

However, it has been shown that androgen supplementation (DHEA) reduces recovery time after mixed exercise [195]. Thus, the testosterone-suppressed status of our PCa patients might have affected recovery between sessions. Therefore, it can be speculated whether the trainability differs from that of healthy men.

5.3.1 Intervention characteristics

To expand on our speculations about reduced training adaptation and recovery in PCa patients on ADT, information from the training logs recorded by the trainers was analyzed. Progression in training load (Figure 7) and training volume (Figure 8) were analyzed separately for the upper and lower body.

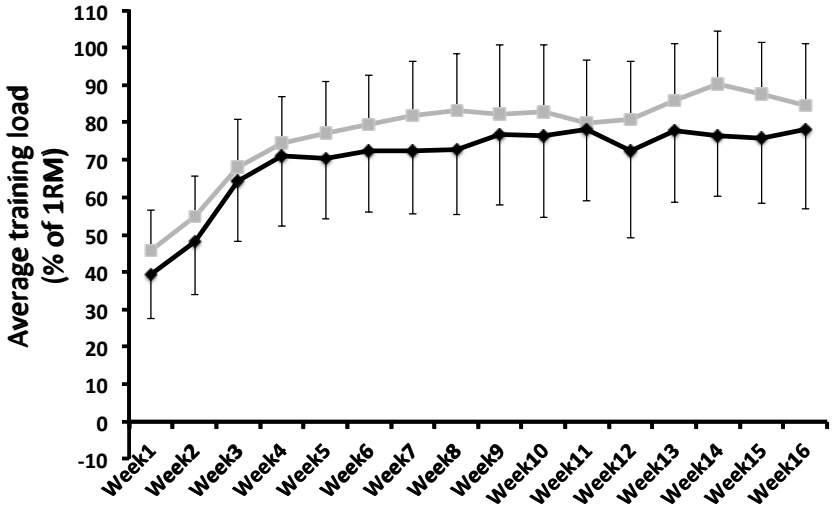


Figure 7. Progression of training load in leg exercises (grey line) and upper body exercises (black line), relative to the baseline 1RM.

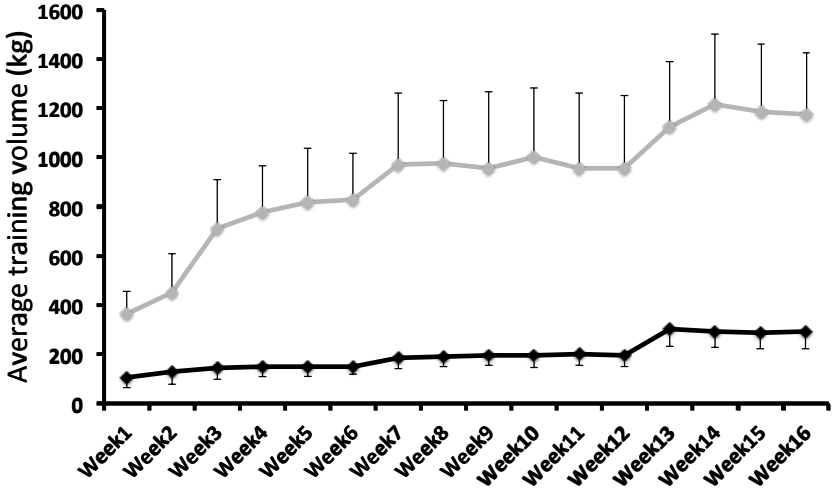


Figure 8. Progression in training volume in leg exercises (grey line) and upper body exercises (black line). The average weekly training volume was calculated as the training load in leg- and upper body exercises, multiplied with the total number of reps per sessions, divided on the number of sessions per week (three).

Patients in the PEPC trial seemed to show normal progression both in training load and training volume in both the leg exercises and the upper body exercises during the intervention. The average weekly training volume was increased three-fold during the intervention, from 400 kg to 1200 kg in leg exercises and from 100 kg to 300 kg in upper body exercises. Thus, the progression in both training intensity and training volume do not seem to be blunted in the PEPC trial. However, comparing recovery after a standardized training session between PCa patients and healthy elderly men might help expand the knowledge on strength training adaptations during ADT.

5.3.2 Training adherence

Patient adherence to the training program can strongly affect the outcomes in a training study. Furthermore, adherence may also imply whether the training program is feasible or not.

The training instructor adjusted the training load or instructed the patient to leave out certain exercises if training-related joint pain occurred during or after the training sessions. Therefore, training adherence was calculated for upper- and lower body exercises separately. Adherence to the training program was similar between upper- and lower body exercises, with patients completing 87.9% (range 68-100%) and 87.6%

(range 65-98%) of the strength training sessions for upper and lower body exercises respectively. Adherence was calculated as the achieved number of sessions relative to the intended number of sessions. The adherence rate in the PEPC trial indicates that our training program was well tolerated. However, it is important to keep in mind that the patients included in the PEPC trial performed all heavy training sessions under supervision, were relatively healthy and somewhat younger than the average PCa patient.

5.2.3 Adverse events

In addition to the exclusion criteria included in the PEPC trial, the feasibility of our training program could also be evaluated based on the number of drop-outs from the STG. Accidents and events not related to the intervention accounted for all three dropouts in the CG and three out of six dropouts in the STG. One of the remaining three participants dropping out of the STG had a history of injuries that were not picked up during the inclusion. Although comprehensive adjustments were made in the training program, he still experienced back pain that did not recover when the training load was removed over a period of time. Unfortunately this led to exclusion from the trial. Another patient experienced knee pain that did not cease when the training load was removed. Results from an MRi scan revealed a small stress fracture in the distal part of the femur. Although previous stress on the knee joint may have primed the femur for a stress fracture, we cannot rule out that this occurred as a consequence of the training load. The last patient who dropped out of the STG also experienced knee pain. However, at the time of inclusion the patient had severe mobility challenges due to joint stiffness. Adjustment in the training protocol was made, but he was unfortunately not able to continue the intervention. We cannot rule out that this was related to the intervention. The three dropouts represent approximately 10% of all the patients included in the STG, which implies that the training program was well tolerated by most patients.

5.4 Discussion of main findings

5.4.1 Changes in body composition

5.4.1.1 Total lean body mass

Contrary to our hypothesis, we did not observe any significant effect of 16 weeks of strength training on total LBM. (+0.5 kg, p=0.16). The effects of strength training on

changes in LBM and muscle volume in PCa patients on ADT have been investigated in six previous studies [101-103, 106, 109, 113]. In line with our results, no effects were reported in two studies [101, 102], and contrary to our results significant effects were reported in the four other studies [103, 106, 109, 113]. Our finding of no significant intervention effect on total LBM was somewhat surprising, especially since the training program was performed with a relatively high training volume, an instructor supervised every hard session, and the intervention period was of a sufficient duration to induce LBM gains under normal circumstances.

Two of the studies reporting significant intervention effects on LBM also report significant increases within the STGs [103, 109]. It might be an important observation that the PCa patients included in these studies had been on ADT for some time at baseline (60 and 186 weeks). By comparison, in the two other studies, initiated shortly after the onset of ADT (1 and 15 weeks), the intervention effect seems to be prevention of the LBM loss observed within the CGs [106, 113]. The PEPC trial was initiated after 36 weeks on ADT, and no significant LBM change was observed in the CG during the intervention. Prevention of LBM loss is, of course, of equal clinical importance, but might shed some light on the ability to adapt to strength training during ADT.

Two studies compared the effect of strength training in PCa patients on or off ADT: one reported no difference in LBM change [113], and the other reported no difference in the effect on muscle volume [101]. However, the change in LBM within the STGs in most studies [106, 109, 113], and in the present study, seems to be smaller than what is normally observed in healthy individuals (e.g. [114, 196]). Therefore, we extracted data from healthy elderly men (HEM) participating in a comparable randomized trial at the NSSS [196], and compared the effect of the HEM trial to the effects in the PEPC trial. There were some differences between the two trials: compared to the PEPC trial, the intervention in the HEM trial was four weeks shorter in duration, and the subjects included were on average 10 years older. Nevertheless, we merged the data files from the HEM trial and the PEPC trial, and analyzed the differences in training effect on total LBM by ANCOVA, with baseline score and age as covariates and group assignments (STG versus CG, and PEPC versus HEM) as fixed factors (per protocol).

Results from this analysis showed that men in the HEM trial achieved 1.13 kg (p=0.05) larger gain in total LBM than the PCa patients in the PEPC trial (Figure 7). Furthermore, when looking at the individual training effects (Figure 7), it is clear that the variability in the training response is greater in PCa patients on ADT than in healthy men. In fact, three patients (10%) in the PEPC STG lost LBM despite 16 weeks of strength training.

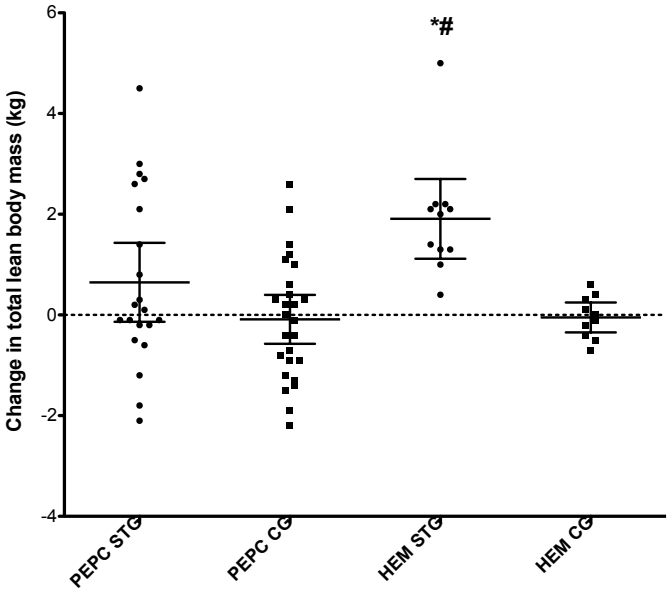


Figure 9. Comparison of change in total lean body mass between the groups in PEPC- and HEM trial. Bars represent mean value and 95% confidence intervals. Data points represent individual changes. *=Significant within-group changes from baseline to post. #=Significant difference in change between the intervention groups (PEPC STG *vs.* HEM STG) from baseline to post.

In two of the studies reporting significant intervention effects on total LBM in PCa patients on ADT [106, 113], the decrease within the CGs seems to be more pronounced than what we observed in the PEPC trial. In fact, Figure 7 reveals quite large variations in the change in LBM within the CG in the PEPC trial (range 2.6 to -2.2 kg). Two patients in the CG seem to have increased their LBM by more than 2 kg. The reason for this is unclear, and we initially suspected contamination of the intervention by patients in the CG actually performing strength training. Inspection of the activity level reported by the patients in the CG (Godin Leisure-time exercise questionnaire) found that no change in moderate activity was reported by one patient, but an increase from two to four hours of moderate activity per week was reported by the other (data not shown). Also, the DXA

prints were manually checked, but no obvious deviations were observed. Therefore, one patient might have been more active during the intervention, but the reasons for the LBM increase in the other patient in the CG remains unclear.

Our sample size calculation was based on an expected 3 kg difference in total LBM change from baseline to post-test between the groups, with an SD of 3 kg. Based on studies initiated after the PEPC trial, we acknowledge that a 1 kg difference in change from baseline to post-test between groups, with a SD of 1.25, would be more realistic [109]. As the ratio of SD and hypothesized difference is very similar whatever assumptions are made, sample size estimation would have been affected to a limited extent. More importantly, inspection of the estimated confidence intervals for efficacy demonstrates that it is not likely that large effects have gone undetected.

5.4.1.2 Regional lean body mass

In agreement with our hypothesis and previous studies [106, 109], a significant effect of strength training was observed in the upper extremities (0.2 kg, p=0.05), the lower extremities (0.5 kg, p=0.00), and thus ASM (0.6 kg, p=0.00). However, no difference in mean change in trunk LBM was observed between the STG and CG during the intervention (-0.2 kg, p=0.40). This was partly in line with our hypothesis, as we expected smaller increases here than in the extremities.

Effects of strength training on trunk LBM in PCa patients on ADT have not been reported previously. However, changes in trunk LBM can be estimated by subtracting change in ASM from the change in total LBM, as reported by Galvão et al. (2010) [109]. From our calculations, there seemed to be a 0.2 kg increase in trunk LBM both in the training group and in the control group [109].

It is, however, important to interpret these findings with caution, since the reliability of DXA in quantifying muscle mass is best in the extremities [165]. Nevertheless, it is an interesting finding that fits well with results from other studies: Snyder et al. (1999) reported increased LBM especially in the trunk region after supplementing elderly subjects with testosterone [115]. Also, by comparing the immunohistochemical analyses of biopsies obtained from *m. trapezius* and *m. vastus lateralis*, Kadi et al. (2000) reported

a higher number of myonuclei expressing ARs in the trunk muscle compared to the lower body muscle [116]. Overall, these findings may indicate higher sensitivity for androgens in trunk muscles. Thus, removal of testosterone may affect adaptations to strength training in the trunk muscle to a greater degree than in the extremities. Although, this remains speculative to date, it may warrant further research to explore our findings.

5.4.1.3 Fat mass

In agreement with our hypothesis, and inline with studies published after the initiation of the PEPC trial [102, 103, 109, 113], no group difference in mean change was observed between the two groups in fat mass or fat percentage. So far only one study, involving a combined intervention of both aerobic and strength training at an early stage of ADT, has been successful in preventing the fat mass gains observed in the control group [106]. Thus from a clinical standpoint, the focus may be on preventing increases in fat mass from occurring. Also, including endurance training, ideally in combination with nutritional counseling, may be a more efficient strategy for influencing fat mass than strength training alone.

5.4.1.4 Bone mass

In disagreement with our hypothesis, strength training did not influence aBMD. This is, however, in line with previous studies [102, 106]. The explanation for the lack of positive effects of exercise on aBMD during ADT could be related to the training modality, as well as the duration of the intervention. However, a 1-year-long intervention combining both impact stimuli and strength training showed no significant effect on aBMD in PCa patients on ADT[112], whereas significant effects of the same intervention were observed in postmenopausal breast cancer patients (lumbar spine)[197]. Thus, a negative effect of ADT on training-induced changes in aBMD may be postulated. Thus more studies examining the effects of strength training on bone health during ADT are needed..

5.4.2 Changes in muscle strength and functional tests

In agreement with our hypothesis, significant improvements were observed in all 1 RM tests that were included in the present study (Table 14).

Table 14. Effects of strength training on muscle strength (1RM) and functional tests.

		Baseline		Post test		Within groups mean change from baseline		Group difference in mean change from baseline*		
	n	Mean	SD	Mean	SD	Mean	[95% CI]	Mean	[95% CI]	*P*
Muscle strength (kg)										
Leg press										
STG	26	184	44	228	61	44	[30 - 57]	42	[29 - 55]	<0.001
CG	30	168	42	168	42	0	[-3 - 4]			
Knee extension										
STG	28	53	12	61	13	8	[5 - 11]	8	[5 - 11]	<0.001
CG	28	52	10	52	12	0	[-2 - 2]			
Chest press										
STG	25	50	12	55	12	6	[3 - 8]	6	[3 - 8]	<0.001
CG	29	48	11	48	11	0	[-1 - 1]			
Shoulder press										
STG	20	23	7	29	10	6	[3 - 8]	5	[3 - 8]	<0.001
CG	23	23	9	24	8	0	[-1 - 2]			
Functional tests										
Sit to stand (Number of reps. in 30 sec.)										
STG	26	16	3	18	3	2	[1 - 3]	2	[1 - 3]	< 0.001
CG	30	16	3	16	3	0	[-0 - 1]			
Shuttle walk (meters)										
STG	25	779	188	804	204	24	[-3 - 52]	39	[-2 - 80]	0.064
CG	29	756	176	741	189	-15	[-45 - 16]			
Stair climbing (sec.)										
Unloaded										
STG	25	5.8	0.9	5.5	1.0	-0.3	[-0.41 - -0.10]	-0.23	[-0.45 - -0.00]	0.047
CG	30	5.9	1.0	5.9	1.0	0.0	[-0.20 - 0.12]			
Loaded (20 kg)										
STG	25	6.5	1.2	6.2	1.1	-0.3	[-0.51 - -0.17]	-0.27	[-0.50 - 0.04]	0.024
CG	30	6.6	1.1	6.5	1.1	-0.1	[-0.24 - 0.09]			

Analysis on patients with valid post test showed same trends
STG= strength training group; CG= control group; SD = standard deviation; CI = confidence interval;
Reps = repetitions; Sec = seconds
* analysis adjusted for baseline values

The gains in muscle strength observed within the STG in the PEPC trial (Table 15) is comparable to other studies reporting on changes in 1RM following strength training in PCa patients on ADT [102, 103, 106, 109].

The intervention also had significant beneficial effects on performance in the sit-to-stand test, in the unloaded and loaded stair-climbing test, and a borderline significant effect on the shuttle walk test (Table 15). These results are in line with other studies reporting the effects of strength training on comparable tests [102, 103, 106, 109].

5.4.3 Changes in muscle cellular outcomes

5.4.3.1 Muscle fiber cross-sectional area

Partly in agreement with our hypothesis, we observed an 11% increase in muscle fiber area for both fiber types combined in the STG, which resulted in a strong trend towards a statistically significant difference in mean change between the groups (p=0.06).

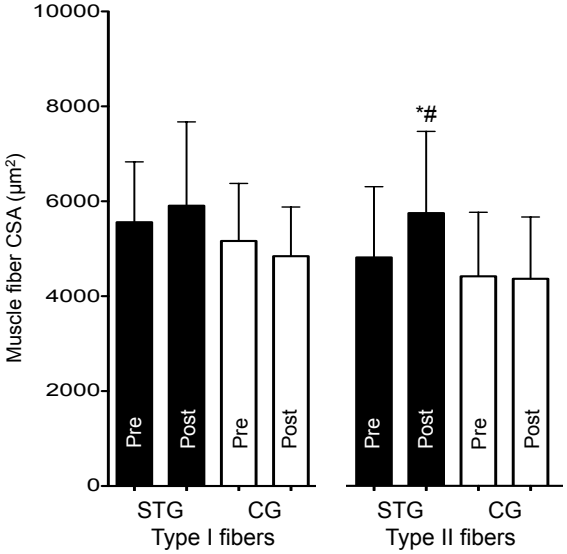


Figure 10. Cross-sectional area of muscle fibers in the PEPC trial. *= Significant within-group difference from baseline to post. #= Significant difference in mean change between STG and CG. Data are presented as mean group values and standard deviations.

As shown in healthy elderly men in previous studies [126, 128], only minor, non-significant differences were observed in the type I fibers. The largest increase was observed in the type II fibers, where we observed a 19% increase in CSA, which resulted in a statistically significant difference in mean change between the groups (p=0.03) (Figure 8). This was in line with our hypothesis, and previous studies in healthy elderly men [124-128]. However, the increases in type II fiber CSA are at the lower end of what have been reported in healthy elderly males, as increases of approximately 30% are commonly reported [124-127]. In the present study we observed a moderate, but significant (B=0.004, p=0.02), relation between changes in knee extension 1RM and changes in muscle fiber CSA (Figure 9). This indicates that the increase in fiber CSA was translated into improved function.

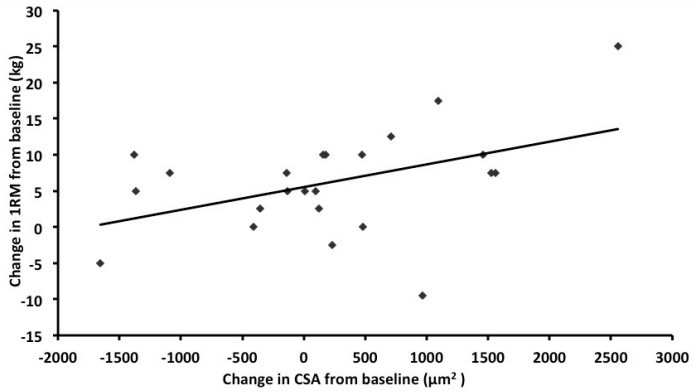


Figure 11. Relation between change in knee extension 1RM and change in muscle fiber CSA of both fiber types combined (B=0.004, p=0.02).

There are indications in the literature that the response to androgens is different between the different fiber types. By using immunohistochemistry, Hulmi et al. (2008) observed more intense AR staining within or near type I fibers than in type II fibers [130]. Furthermore, Üstünel et al. (2003) reported larger increases in type I fiber CSA after testosterone administration in rats [129]. As described above we did not observe any increase in type I fiber CSA in the present study, which is in line with these studies. This has, however, also been described in healthy populations [126, 128]. More importantly, we observed a trend towards decreased type I fiber CSA of 6% in the control group (p=0.10). It could be speculated that this is due to higher androgen sensitivity in type I fibers, and removal of testosterone could therefore be more detrimental to these fibers.

5.2.3.2 Number of myonuclei per fiber and myonuclear domain

In line with our hypothesis, we observed a significant group difference in mean change from baseline to post-test in the number of myonuclei per fiber when both fiber types were combined (p=0.04).

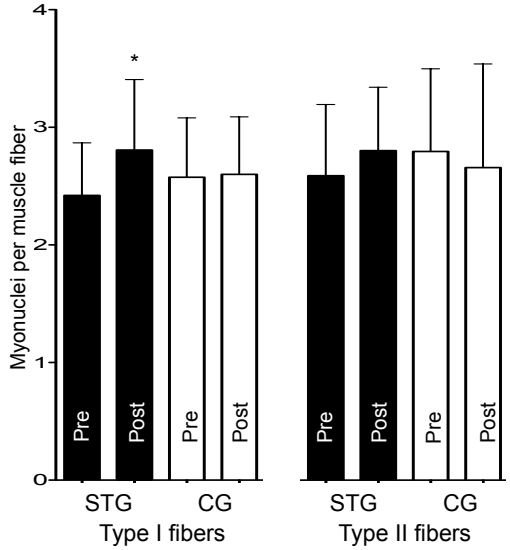


Figure 12. Number of myonuclei per muscle fiber in the PEPC trial. *= Significant within-group difference from baseline to post. Data are presented as mean group values and standard deviations.

Possibly due to lack of statistical power, we only detected weak trends towards group differences in mean change when we analyzed data from type I fibers (p=0.10) and type II fibers (p=0.10) separately (Figure 10).

By using paired sample t-tests, we explored within-group changes in the number of myonuclei per fiber. While no significant increase was observed in type II fibers (p=0.15), a significant increase was observed in type I fibers (p=0.01). This finding was somewhat surprising, as it has been suggested that an increase in CSA of approximately 26% would be needed to create a demand for additional nuclei in healthy subjects [72, 134]. This was contrary to our hypothesis, as the largest increase in muscle fiber CSA was observed in type II fibers.

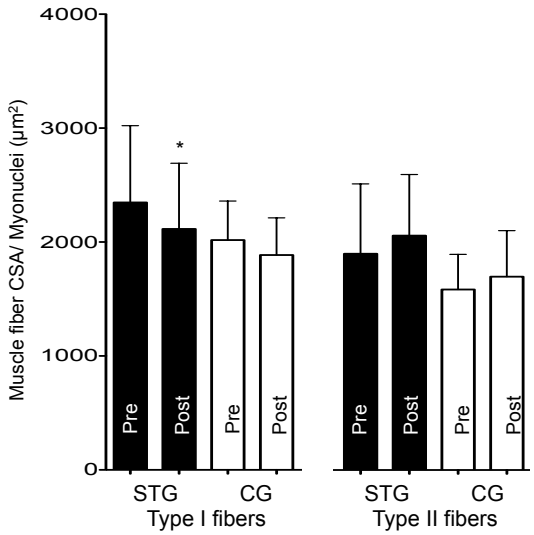


Figure 13. Myonuclear domain sizes in the PEPC trial. CSA= cross-sectional area. *= Significant within-group difference from baseline to post. Data are presented as mean group values and standard deviations.

Partly in agreement with our hypothesis, the myonuclear domain in type II fibers was unchanged (p=0.25), but the myonuclear domain actually decreased in type I fibers (p=0.05) (Figure 11). Unchanged myonuclear domains following strength training are normally reported in the literature [134, 136, 198]. Therefore, the decreased myonuclear domain in type I fibers was somewhat surprising. Together with the trend towards a reduced fiber CSA of the type I fibers observed in the CG (p=0.10), this could indicate that ADT affects the type I fibers to a greater extent than the type II fibers, and we speculate on decreased nuclear efficacy of type I fibers in the absence of testosterone. To the best of our knowledge this has not previously been elucidated in any study.

On the other hand, several studies in healthy men show that increases in the number of myonuclei do not always take place during strength training [126, 136]. However, the number of myonuclei per fiber at baseline in these studies was somewhat higher (3.6 for type I fibers, 2.8 for type II fibers and 3.0 when combined) than we observed in our PCa patients on ADT. Thus, there is a need to evaluate the effect of ADT on muscle fiber CSA and myonuclei to establish proper baseline vales. This should be of interest in future studies.

5.4.3.3 Number of satellite cells per fiber

Contrary to our hypothesis, and to previous studies in healthy elderly men [126, 136], the number of satellite cells did not increase in the present study. There was no group difference in mean change (p=0.56 for both fiber types combined) between the STG and CG, or within groups from baseline to post intervention in the STG (p=0.77, for both fiber types combined) (Figure 12).

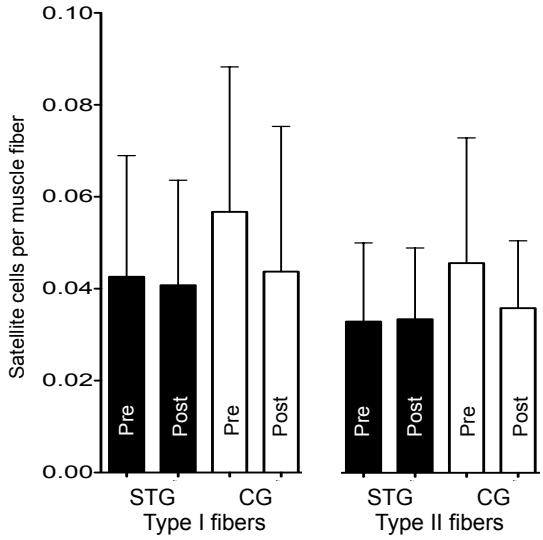


Figure 14. Number of satellite cells per muscle fiber in the PEPC trial. Data are presented as mean group values and standard deviations.

Compared to other studies that have included similar counting criteria and antibodies [126, 136], we report a relatively low number of satellite cells per fiber. The reason for this remains unclear and speculative since we did not include a biopsy prior to the ADT, but it could imply an important role for testosterone in normal satellite cell proliferation and maintenance of the satellite cell pool in men.

Contrary to our findings, Kvorning et al. (2014) reported an increased number of satellite cells, but not an increase in myonuclei per fiber, after an 8-week strength training intervention in healthy young men treated with ADT [137]. This could indicate a timing effect in our data, since we report an increased number of myonuclei but not satellite cells per fiber from our intervention, which was twice the duration of the intervention used by Kvorning et al. Also, it is important to keep in mind that the

increased number of myonuclei in the present study shows that the ability to recruit satellite cells for nuclear donation was intact. If the satellite cell function indeed is impaired during ADT, this could be of clinical importance if the PCa patient is hospitalized, or immobilized for other reasons, and has to go through re-training.

5.4.3.4 Content of androgen receptor and myostatin in muscle

In line with our hypothesis, no difference in mean change of AR concentration was observed between the groups (p=0.96) in the present study. Also, we did not observe any within-group changes (p=0.55 for STG, and p=0.78 for CG) in AR content (Figure 13a).

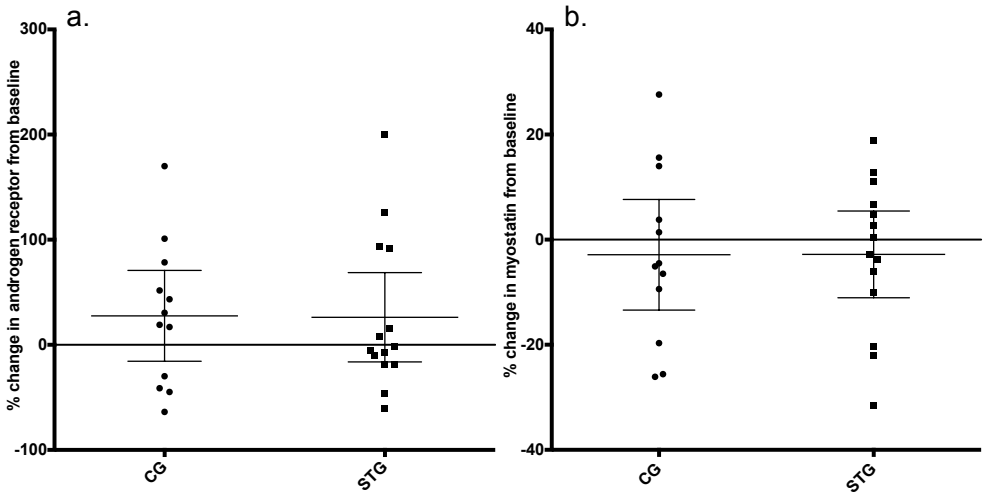


Figure 15. Change in the content of androgen receptor (a.) and myostatin (b.) in the PEPC trial. Bars represent mean value and 95% confidence intervals. Data points represent individual changes.

The concentration of AR in muscle has been shown to increase after functional overload in rat models [199], especially when overload is accompanied with administration of anabolic steroids [200]. However, long-term strength training does not seem to alter the content of AR in skeletal muscle [148, 201]. This is in agreement with our results.

The greatest limitation in interpreting our western blot (WB) data is the lack of a healthy control group, or a muscle biopsy prior to ADT in the PCa patients. Therefore, we cannot rule out that ADT may have induced changes in some of our WB variables

prior to the baseline biopsy. Nevertheless, strength training during ADT did not alter the concentration of AR.

In agreement with our hypothesis, we observed no significant difference between mean group changes (p=0.21) (Figure 13b) in myostatin in the present study. Nor did we observe within-group changes from baseline to post-intervention in either of the two groups. Age may play a role in the myostatin response as unchanged myostatin mRNA expression has been observed in young men, whereas increased expression is seen in elderly [149]. Interestingly, levels of myostatin mRNA and protein have been shown to be 2-fold higher in elderly compared to young men [202]. Similar age differences in myostatin mRNA expression have also been shown in women [203]. in summary, these studies point to a possible age difference in myostatin levels. Testosterone supplementation, on the other hand, has been shown to decrease myostatin levels [204]. However, the effects of castration on baseline values of myostatin are presently unknown. This complicates interpretation of our results to some extent, as our results seem to differ from other reports involving elderly subjects. Nine months of ADT prior to our baseline biopsy could have altered myostatin levels in our PCa patients and thus made comparison with studies in healthy men difficult. Nevertheless, strength training during ADT did not alter the content of AR or myostatin in skeletal muscle.

5.4.3.5 Content of mitochondrial proteins in muscle

Contrary to our hypothesis, no difference in mean change in the levels of citrate synthase (CS) (p=0.37 and 0.40), cytochrome c oxidase IV (COXIV) (p=0.37) or heat shock protein 60 (HSP60) (p=0.98) was observed between the STG and CG during the intervention (Figure 14a-c). There was, however, a tendency towards decreased levels of citrate synthase within the CG (p=0.09). Thus it seems that the testosterone-suppressed state of the PCa patients on ADT blunted the effects of strength training, previously seen in healthy men [152, 205].

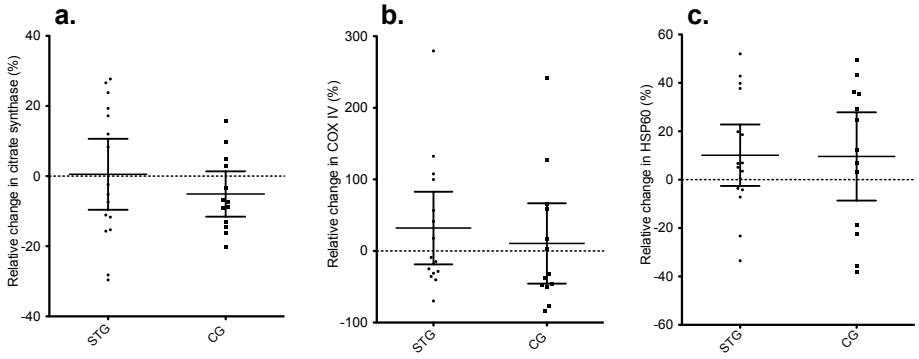


Figure 16. Change in the content of citrate synthase (a.), COXIV (b.) and HSP60 (c.) in the PEPC trial. Bars represent mean value and 95% confidence intervals. Data points represent individual changes.

Most studies evaluating the effect of training on mitochondrial enzymes report on enzyme activity, rather than the amount of mitochondrial enzymes. In the present study we were, however, unable to measure enzyme activity and report on changes in the amount of mitochondrial enzymes. Importantly, it has been shown that enzyme activity is dependent up on the total amount of enzymes [206, 207]. Although it is generally accepted that strength training does not increase mitochondrial function in young men [150, 151], increased activity of mitochondrial enzymes has been reported in healthy elderly men [152, 153]. One explanation to the difference in training response between young and elderly subjects could be that the basal levels are impaired in the elderly [155, 156]. Also, the elderly in general are thought to be more sedentary compared to younger participants, thus participation in a strength training intervention will introduce a greater increase in activity level in elderly subjects.

On the other hand, testosterone seems to have direct effects on mitochondrial enzymes, as castration has been shown to decrease the activity of COXIV and testosterone supplementation increases enzyme activity [157]. Therefore, less effects of exercise in general, and specifically from strength training, might be expected during ADT for PCa.

5.4.3.6 Content of heat shock proteins in muscle

Contrary to our hypothesis, no difference in mean change between the two groups in any of the HSPs was observed in the present study (Figure 15a-c). There were, however,

some within-group changes: in line with our hypothesis HSP70 decreased by 6% (p=0.01) in the STG, and contrary to our hypothesis, a non-significant decrease of 8% (p=0.10) in alphaB-crystallin levels was observed in the CG.

It has been shown that aged muscle contains higher baseline values of some HSPs than younger muscle [91, 208]. This is also inline with unpublished results from our lab, showing decreased levels in the elderly after twelve weeks of strength training [158]. Thus it was argued that strength training could lead to normalization of the cellular environment. It is difficult to draw firm conclusions from our results, but strength training seems to have minor effects on the content of HSPs in PCa patients on ADT.

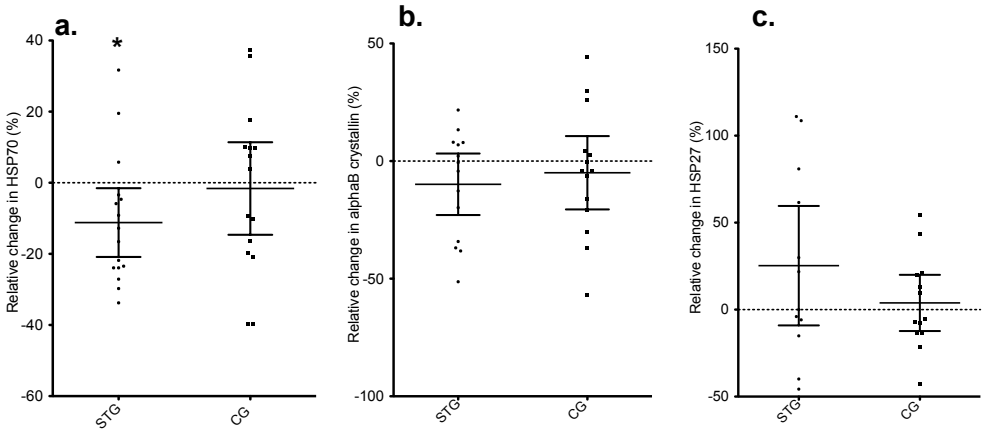


Figure 17. Change in the content of HSP70 (a.), alphaB-crystallin (b.) and HSP27 (c.) in the PEPC trial. Bars represent mean value and 95% confidence intervals. *= Significant within-group difference from baseline to post. Data points represent individual changes.

Contrary to our hypothesis the levels of alphaB-crystallin did not change significantly over the intervention period. However, one study, published after the completion of the WB analysis in the present study, shows that alphaB-crystallin in exercised muscles is mainly affected by endurance training and not by strength training [209]. Although strength training has been shown to increase alphaB-crystallin levels in untrained young men [210], this could indicate that the stimulus given in the PEPC trial might not be appropriate for altering alphaB-crystallin levels.

5.4.3.7 Content of free ubiquitin and total ubiquitinated proteins in muscle

Contrary to our hypothesis, and to trends reported in hart failure patients [159] we observed no change in either the level of ubiquitinated proteins or in the level of free ubiquitin (Figure 16).

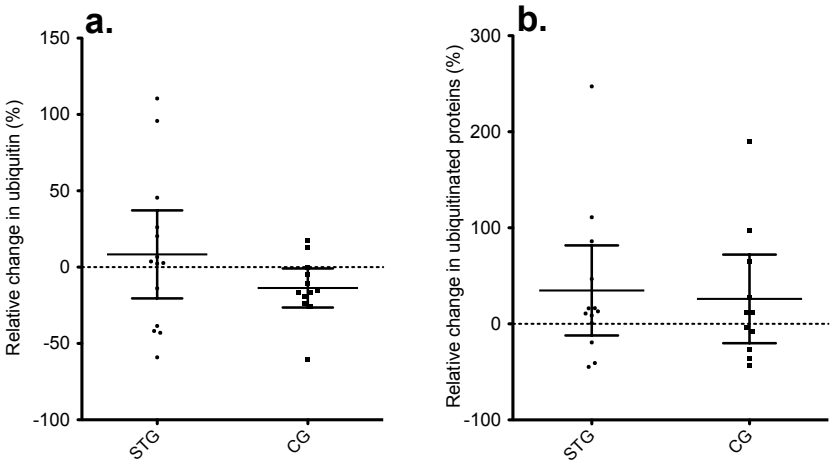


Figure 18. Change in the content of ubiquitin (a.) and ubiquitinated proteins (b.) in the PEPC trial. Bars represent mean value and 95% confidence intervals. Data points represent individual changes.

In a similar western blot set up as in our lab, elevated levels of ubiquitinated skeletal muscle protein have been observed after heart failure was induced in rats, and aerobic exercise led to a decrease in these levels [159]. However, heart failure patients only showed tendencies towards elevated levels of ubiquitinated muscle proteins compared to healthy controls, and aerobic exercise led to non-significant reductions [159]. Importantly, the activity of the 26s proteasome was significantly elevated in heart failure patients and aerobic exercise normalized these levels [159]. This was not measured in the PEPC trial.

Expression levels of ubiquitin ligases are more commonly reported in the literature, and acute increases after a strength training session are often noted [211-213]. On the other hand, protein levels of ubiquitin ligases are more rarely studied, but have been shown to follow the same pattern as mRNA expression [212]. So far, the effect of a strength intervention on ubiquitin ligases has only been evaluated at the mRNA level, where no changes have been reported in young subjects [214, 215], and increased levels of MuRF-

1, but not Atrogin-1, have been reported in older adults [216]. However, mRNA data from intervention studies should be interpreted with caution, as mRNA levels may not always reflect changes in protein level. Due to technical difficulties in measuring protein levels of ubiquitin ligases in our lab, and most likely also in other labs as reflected by the lack of reports in the literature, we were not able to investigate such changes in our study.

Testosterone suppression has been shown to increase the expression of MuRF-1 and Atrogin-1 in skeletal muscle [93], which, at least in theory, would lead to increased ubiquitination of muscle proteins. This could explain the results in the present study, where we found no effect of strength training on the level of ubiquitinated muscle proteins. In an attempt to describe participants with different responses to ubiquitination, we investigated the relationship between the changes in ubiquitinated protein levels and ubiquitin levels and changes in muscle fiber CSA. However, no relationship was found using Pearson's correlation coefficient (data not shown).

6.0 Conclusions

1. No statistically significant intervention effect was observed in total or trunk LBM, but statistically significant differences were found between groups for LBM in the upper and lower extremities, and in all muscle strength- and functional tests. No intervention effect was seen on fat mass or on aBMD.
2. Significant intervention effects were observed in muscle fiber CSA, with greater increases in type II fibers than in type I fibers, and in the number of myonuclei per fiber, with the greatest increases seen in type I fibers. Within the STG, the myonuclear domain of type I fibers was reduced, whereas the myonuclear domain of type II fibers remained unchanged. No effect of the intervention was observed on the number of satellite cells in any muscle fiber type or in the content of AR or myostatin.
3. No effect of the intervention was seen in the content of mitochondrial proteins, content of HSPs, content of free ubiquitin or in the total ubiquitinated proteins. However, citrate synthase tended to decrease within the CG and the content of HSP70 was reduced within the STG.

7.0 Suggestions for further research

Compared to breast cancer, the effect of exercise during treatment for PCa is a somewhat understudied topic. Therefore there is a general need for additional training intervention studies to confirm and expand on current knowledge. Furthermore, experiences from the PEPC study show that the training response seems heterogeneous among PCa patients, as indicated by the large standard deviations in our results. Thus, it could be of importance to include larger cohorts to identify and describe the characteristics of patients who respond to a greater degree to strength training and patients who experience less beneficial effects from such intervention.

Even though the training program in the PEPC trial was developed to increase the likelihood of LBM gains we were still not able to detect any additional benefit from our intervention compared to what was seen in other studies using a lower training volume [106, 109, 113]. Therefore, more studies comparing the effects of different training programs on PCa patients could be of relevance to determine "optimal" or "sufficient" training doses while on ADT. It would be of particular interest to compare different training programs (e.g. different training loads or training frequency) between PCa patients.

There are two studies in the literature comparing the effects of strength training in PCa patients on or off ADT [101, 113], and no differences in training effect on LBM and muscle volume were reported. However, when comparing the effects on the patients in the PEPC trial with those on healthy elderly men participating in a study in our institution, we speculate that PCa patients on ADT experience less effect from strength training than healthy elderly men. Therefore, a direct comparison of the effects and feasibility of the same training program between PCa patients on ADT and age-matched controls might be of interest. Also, comparing the recovery period after a training session between PCa patients and healthy subjects could give some information on trainability and perhaps also some recommendations on prescriptions for training frequency during ADT.

8.0 References

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Paper I

Thorsen L., Nilsen T.S., Raastad T., Courneya K.S., Skovlund E,. Fosså S.D.: **A randomized controlled trial on the effectiveness of strength training on clinical and muscle cellular outcomes in patients with prostate cancer during androgen deprivation therapy: rationale and design**. *BMC Cancer* 2012, **12**(1):123

Thorsen et al. BMC Cancer 2012, 12:123
http://www.biomedcentral.com/1471-2407/12/123

BMC Cancer

STUDY PROTOCOL Open Access

A randomized controlled trial on the effectiveness of strength training on clinical and muscle cellular outcomes in patients with prostate cancer during androgen deprivation therapy: rationale and design

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Abstract

Background: Studies indicate that strength training has beneficial effects on clinical health outcomes in prostate cancer patients during androgen deprivation therapy. However, randomized controlled trials are needed to scientifically determine the effectiveness of strength training on the muscle cell level. Furthermore, close examination of the feasibility of a high-load strength training program is warranted. The Physical Exercise and Prostate Cancer (PEPC) trial is designed to determine the effectiveness of strength training on clinical and muscle cellular outcomes in non-metastatic prostate cancer patients after high-dose radiotherapy and during ongoing androgen deprivation therapy.

Methods/design: Patients receiving androgen deprivation therapy for 9-36 months combined with external high-dose radiotherapy for locally advanced prostate cancer are randomized to an exercise intervention group that receives a 16 week high-load strength training program or a control group that is encouraged to maintain their habitual activity level. In both arms, androgen deprivation therapy is continued until the end of the intervention period.

Clinical outcomes are body composition (lean body mass, bone mineral density and fat mass) measured by Dual-energy X-ray Absorptiometry, serological outcomes, physical functioning (muscle strength and cardio-respiratory fitness) assessed with physical tests and psycho-social functioning (mental health, fatigue and health-related quality of life) assessed by questionnaires. Muscle cellular outcomes are a) muscle fiber size b) regulators of muscle fiber size (number of myonuclei per muscle fiber, number of satellite cells per muscle fiber, number of satellite cells and myonuclei positive for androgen receptors and proteins involved in muscle protein degradation and muscle hypertrophy) and c) regulators of muscle fiber function such as proteins involved in cellular stress and mitochondrial function. Muscle cellular outcomes are measured on muscle cross sections and muscle homogenate from muscle biopsies obtained from muscle vastus lateralis.

Discussion: The findings from the PEPC trial will provide new knowledge on the effects of high-load strength training on clinical and muscle cellular outcomes in prostate cancer patients during androgen deprivation therapy.

Trial registration: ClinicalTrials.gov: NCT00658229

Keywords: Strength training, Prostate cancer, Androgen deprivation therapy, Clinical and muscle cellular outcomes

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Background

Prostate Cancer (PC) is the most frequent diagnosed malignancy in men in Europe and North America. The risk of PC increases with age, and the median age of onset is approximately 70 years. Treatment depends on stage, histology and the serum PSA level, beside the patients' general health and age. Radiotherapy combined with androgen deprivation therapy (ADT) is used in patients with locally advanced tumors and/or those with high Gleason scores, characterized as intermediate or high-risk profiles [1]. ADT has potentially negative effects on several clinical outcomes, such as muscle atrophy [2].

Physical exercise during and after cancer treatment has been shown to be effective to reduce several negative clinical consequences followed by cancer and cancer treatment [3,4]. Compared to breast cancer patients relatively few studies in PC patients have been published [4,5]. More evidence on the efficacy of strength training on clinical outcomes in PC patients is therefore desirable. At the same time more attention should be paid to the effect of ADT on the muscle cell level because the reduced testosterone levels influence the underlying regulators of muscle mass and muscle function. Furthermore, the effects of strength training on the same regulators are of particular interest during ADT, and as far as we know they have not previously been explored in PC patients.

Ageing is associated with a reduction in muscle mass, typically by 1-2% per decade from the age of 30 to the age of 50. Thereafter the rate of muscle loss increases progressively to 10% per decade [6]. It is well known that decreased levels of serum testosterone are followed by reduced muscle mass [7,8]. In PC patients treated with ADT for 6-12 months, lean body mass (LBM) has been reported to decrease by 3% [9]. This reduction may affect muscle strength markedly because muscle mass is the dominant tissue in LBM.

Loss of bone mineral density (BMD) has been observed in PC patients on ADT [10,11]. It has also been shown that ADT increases body weight and fat mass in these patients [9]. Decreased levels of testosterone and body changes during ADT, may also influence mental health, fatigue and health-related quality of life (HRQOL) in PC patients [12,13].

Total skeletal muscle mass reflects the size of all individual muscle groups in the body. Furthermore, the size of each muscle group is primarily determined by the size of the individual muscle fibers[1], and to a lesser extent by the number of fibers. Regulation of muscle fiber size is first and foremost driven by an increase in the number and size of myofibrils (contractile proteins) within the muscle fiber (Figure 1). This process is normally supported by an increased number of satellite cells and (often) increased number of myonuclei (Figure 1). Importantly, satellite cells seem to play a significant role in regulation of muscle fiber size. Satellite cells are mononuclear progenitor cells that are found between the sarcolemma and the basement membrane in the muscle fibers (Figure 1 and Additional file 1). Satellite cells are normally in a quiescent state, but are activated by adequate stimuli (e.g. strength training). Upon activation the satellite cells donate their nuclei to the existing muscle fibers and thus facilitate skeletal muscle hypertrophy by increasing the protein synthesis [14]. During regeneration after muscle injury, satellite cells may also fuse and thereby form new mature muscle fibers [14]. In addition, satellite cells seem to support muscle hypertrophy by production of local growth factors (e.g. IGF-1) [15].

In the muscle tissue, testosterone stimulates both the muscle protein synthesis in muscle fibers and activation of satellite cells through the interaction with the androgen receptor [16]. Consequently, graded dosage of testosterone has been shown to both increase the muscle fiber size, reflected by increased muscle fiber cross sectional area, and number of satellite cells in a dose dependent pattern [17,18]. Suppression of testosterone reduces the positive stimuli on muscle protein synthesis, and patients treated with ADT experience reduced LBM [19,20]. Low levels of testosterone might also influence the regulation of muscle mass through its inhibition of the ubiquitin-proteasome system [21]. Consequently, ADT might facilitate muscle atrophy both by inhibiting important pathways involved in muscle hypertrophy, as well as stimulating pathways involved in protein degradation.

Muscle strength is mainly determined by the size of muscles, but other qualities of muscles, such as endurance and stress tolerance, are more related to the content and function of stress proteins and proteins involved in the mitochondrial function [22,23]. Interestingly, castration has been shown not only to reduce muscle fiber size, but also to affect the mitochondrial structure negatively in rat muscles [24]. Furthermore, castration seems to reduce the stress induced up-regulation of stress proteins in heart muscle [25]. Whether castration also affects the stress protein response and mitochondrial function in human skeletal muscles is currently not known.

Effects of strength training

Strength training has the potential to increase muscle mass and BMD in both young and elderly males and females [26-29]. Although the response to strength training differs among individuals, most studies report an increase in muscle fibre cross sectional area by 10-

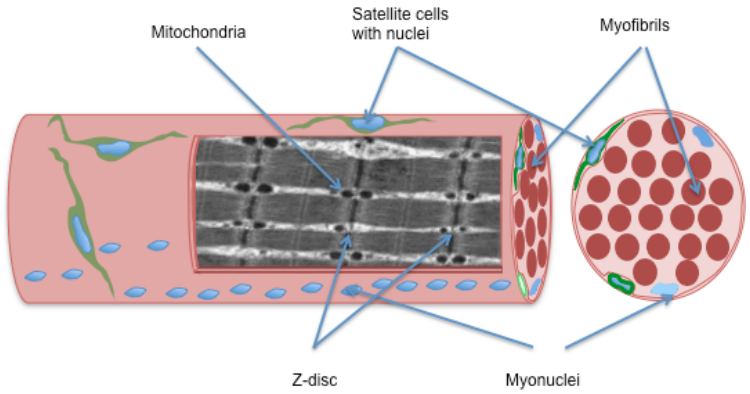

Figure 1 A schematic drawing of a muscle fiber (muscle cell) in longitudinal- and cross sectional plane. The muscle fiber is surrounded by two membranes, the ***plasma membrane*** (inner) and the ***basal lamina*** (outer). ***The satellite cells*** are located between these two membranes, and just beneath the ***plasma membrane*** lays the ***myonuclei***. The contractile proteins in the muscle cell are arranged in ***myofibrils***. In the longitudinal plane you see that the ***myofibrils*** are organized into sarcomeres separated by the z-disc and ***the mitochondria*** are seen as circular spots between the myofibrils.

60% over 9-30 weeks of training [30]. Furthermore, an increase in BMD from 1 to 4% has been observed over 16-52 weeks of strength training [27,31-33].

Effectiveness of exercise on clinical outcomes has been reported in numerous studies in cancer patients. Research has so far shown promising effects on among others muscle strength, aerobic fitness, fatigue, anxiety and quality of life [3,4]. However, the effect of exercise during ADT in PC patients has been less studied [4,5]. These studies have shown positive results in muscular and aerobic endurance, fatigue and QoL [5,34,35]. Less promising is the effect of physical exercise on body-composition endpoints. It could therefore be hypothesized that ADT compromises some important cellular signals regulating the exercise induced increase in lean body mass with strength training.

The increase in muscle fiber size in response to strength training seems to be dependent, at least to some extent, on satellite cell activation in order to incorporate new myonuclei in the growing muscle fiber [14]. In healthy subjects, strength training increases the number of myonuclei and the number of satellite cells in both young and elderly individuals [36,37] (Figure 2). The mechanisms behind the activation of satellite cells during strength training in humans are not fully understood, but changes in local growth factors and testosterone interactions seem to be important [38]. Whereas reduced levels of testosterone during ADT negatively affect the regulation of muscle mass, strength training might counteract these detrimental effects on muscle fibres (Figure 2). On the other hand, it might be that the very low testosterone levels, comparable with castration, blunt the effects of strength training in these patients. Interestingly, healthy young men treated with a GnRH analog over 12 weeks showed the same increase in mRNA of important local growth factors in response to strength training as placebo treated controls [39]. Nevertheless, the accumulation of muscle mass during the strength training intervention was reduced in the testosterone-suppressed group compared to the placebo treated controls [40].

The effects of strength training on mitochondrial function and protection against cellular stress in muscle fibers are less studied than the effect on muscle size. Nevertheless, in previously untrained healthy men strength training has been observed to positively affect both mitochondrial proteins and stress proteins [41,42]. Consequently, strength training has the potential to counteract several negative effects of ADT on muscle size and function.

To our knowledge no previous studies have investigated the effect of strength training on the regulation of muscle size and of muscular function on a cellular level in PC patients during ADT. Furthermore, previous clinical data need to be confirmed. Here, we present the design and methods of an ongoing trial called the Physical Exercise and Prostate Cancer (PEPC) trial, which aims to explore the effects of strength training on clinical outcomes as well as explore mechanisms of the effect on muscle cellular outcomes in patients with PC during ongoing (neo)-adjuvant ADT.

Aims

The overall aims of the PEPC trial are to evaluate the effectiveness of a high-load 16 week strength training

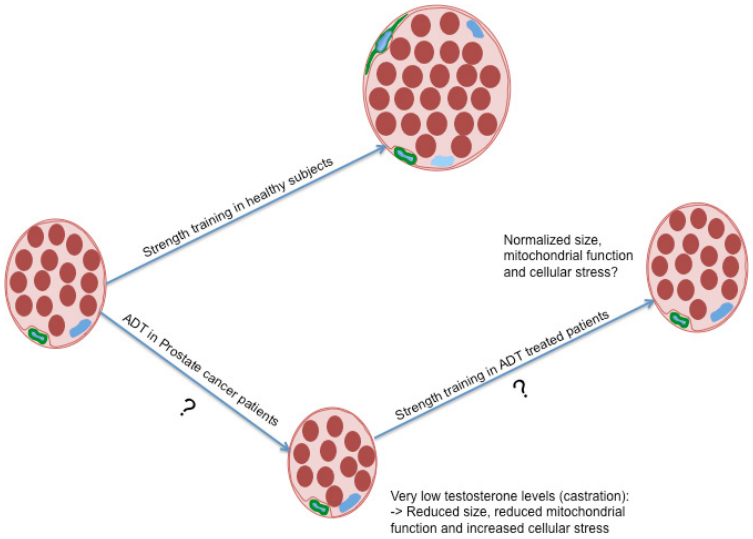

Figure 2 Schematic muscular adaptations to strength training in healthy men and PC patients on ADT. Schematic muscular adaptations to strength training in healthy men (**A**), possible consequences of ADT on muscle fibers in PC patients (**B**), and possible muscular adaptations to strength training in PC patients on ADT (**C**). In A), the muscle fiber cross sectional area is increased as a result of an increase in the number and size of myofibrils within the muscle fiber, and this increase in size is supported by an increased number of satellite cells and (often) increased number of myonuclei. In B), ADT results in decreased muscle fiber cross sectional area and reduced muscle function. In C), muscle fiber cross sectional area and muscle function is normalized in ADT treated PC patients on strength training.

program on a) clinical outcomes including serological parameters such as lipid profile (low and high density lipoprotein cholesterol) and low grade inflammation (C-reactive protein (CRP)) b) muscle cellular outcomes as muscle fiber size, regulators of muscle fiber size and regulators of muscle fiber function and c) feasibility of a high-load strength training program in PC patients who at intervention start have discontinued their high-dose radiotherapy and are on ongoing (neo)-adjuvant ADT throughout the intervention period.

Methods/design

This study is a randomized clinical trial with two arms comparing an exercise intervention group (EG) that receives a 16 week high-load strength training program with a control group (CG) that is encouraged to maintain their habitual activity level and not start strength training. The study has been approved by the Regional Committee for Medical and Health Research Ethics, South-East Region (protocol nr. 08/212b.2008/4062).

Participants

Patients are included from two oncological units at Oslo University hospital, the Norwegian Radium Hospital (NRH) and Ullevaal University Hospital (UUH). All patients must fulfill the following eligibility criteria 1) PC cancer of intermediate or high-risk profiles, 2) high-dose radiotherapy with or without two high-dose-rate brachytherapy fractions [43], 3) (neo)-adjuvant ADT by commercially available LHRH analogue for 9-36 months. The most important inclusion and exclusion criteria are 1) less than one hour car drive between the patient's residence and the place of training or 2) current regular strength training. All inclusion and exclusion criteria are listed in Additional file 2.

In addition to written information, eligible patients are verbally informed about the study by their responsible radiotherapist and the study coordinator usually during or immediately after radiotherapy. After giving their written consent agreeing patients are scheduled for pre-intervention assessments usually 1-2 months after radiotherapy, which are repeated one week after the intervention period (Figure 3).

Randomization

At completion of the pre-intervention assessments patients are randomized in a 1:1 ratio to the EG or CG, stratified for hospital. Randomization is computerized and performed by the staff at the clinical research office at NRH.

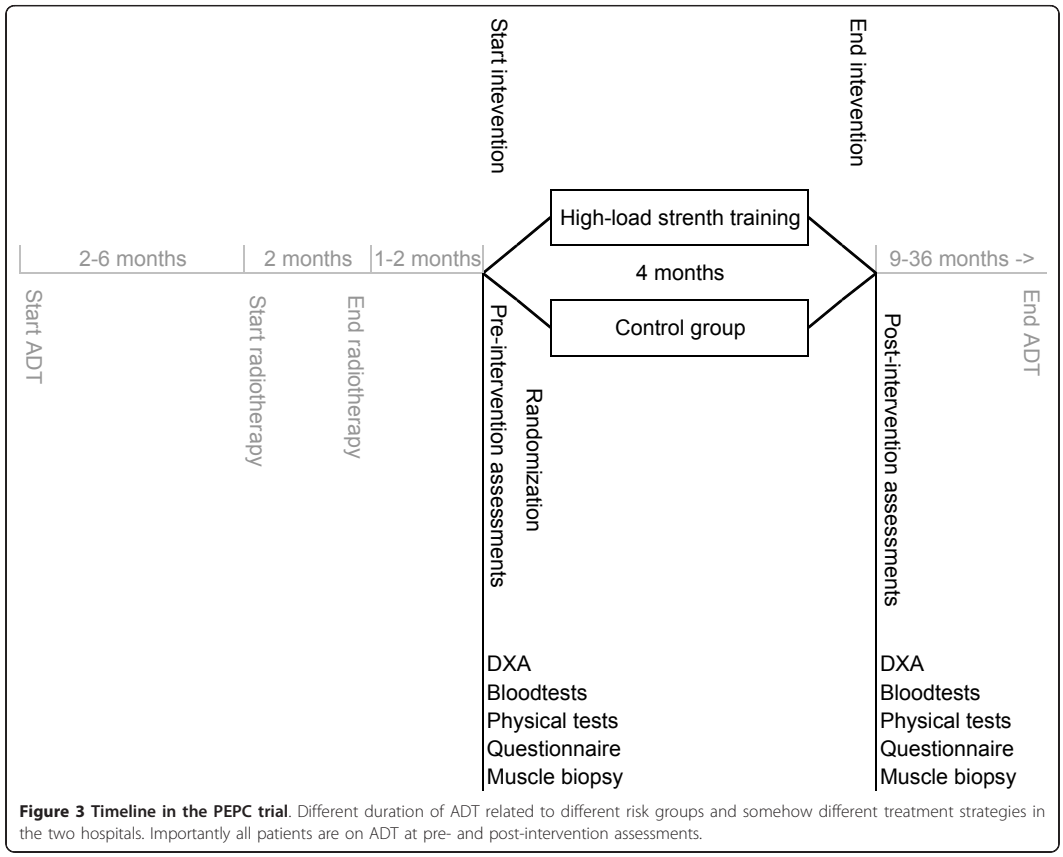

Figure 3 Timeline in the PEPC trial. Different duration of ADT related to different risk groups and somehow different treatment strategies in the two hospitals. Importantly all patients are on ADT at pre- and post-intervention assessments.

Intervention

The exercise intervention starts 5-10 months on ADT, one week after the pre-intervention assessment (Figure 3). The intervention is performed at the Norwegian School of Sport Sciences (NSSS) in Oslo.

The EG follows a modified resistance exercise program originally tested by Segal et al. [44]. Compared to the original program the intensity (training load) and duration are increased in order to potentially increase the effect of the strength training on LBM/body composition (Table 1). The patients perform three strength training sessions per week. Two of these sessions are performed under supervision by qualified instructors, to ensure safety, technique and progression in training load, with a maximum of three patients per instructor. The mid-week session, without the instructor, is performed at moderate intensity alone or together with other trial participants at NSSS. In each training set, the patient documents the training load and rate of exhaustion in an exercise log.

Each session consists of one to three sets of nine strength training exercises, performed at an intensity of 6 or 10 repetitions maximum (6-10 RM: the load that induces technique failure in 6 or 10 repetitions). The two first weeks of the program are considered to represent familiarization to the exercise protocol for the patients, and are performed at a light load (40-50% of one repetition maximum (1 RM)) in sets of 10 repetitions. The exercises performed are: smith machine squat, leg press, standing calf raises, knee flexion, knee extension, chest press, seated rows, shoulder press and preacher biceps curl (training equipment provided by Technogym, Italia).

Prior to the strength training session the patients perform 10 minutes of warm up on an exercise ergometer. In addition, the patients complete a sub maximal set of 10 repetitions as a specific warm-up in the squat exercise prior to every training session. After the two first weeks the patients are instructed to gradually increase the training load, in order to perform the exercise with

Table 1 The strength training program

Week	1. Session: heavy intensity Monday	2. Session: moderate intensity Wednesday	3. Session: heavy intensity Friday
1 and 2	2 × 10 sub maximal resistance Focus on correct technique	2 × 10 sub maximal resistance Focus on correct technique	2 × 10 sub maximal resistance Focus on correct technique
3 to 6	2 × 10 RM leg exercises 1 × 10 RM upper body	2 × 10 reps. leg exercises 2 × 10 reps. upper body (resistance: 90% of 10 RM)	3 × 6 RM leg exercises 2 × 6 RM upper body
7 to 12	3 × 10 RM leg exercises 2 × 10 RM upper body	2 × 10 reps. leg exercises 2 × 10 reps. upper body (resistance: 90% of 10 RM)	3 × 6 RM leg exercises 2 × 6 RM upper body
13 to 16	3 × 10 RM leg exercises 3 × 10 RM upper body	3 × 10 reps. leg exercises 3 × 10 reps. upper body (resistance: 90% of 10 RM)	3 × 6 RM leg exercises 3 × 6 RM upper body

RM - repetitions maximum, reps - repetitions

the highest load possible during the prescribed number of repetitions per set on the two sessions with heavy intensity. From experience, this means that they increase the resistance by 2-5% per week through the 16 weeks period.

Patients in the CG are encouraged to maintain their habitual activity level and not start strength training. In order to increase the participation rate and reduce the dropout rate, the patients in the CG are offered the exercise intervention after the post-intervention assessment.

Outcomes and assessments

Both clinical and muscle cellular outcomes are collected before the intervention (pre-intervention assessments) and after the intervention (post-intervention assessments) (Figure 3). All outcomes, specific variables and assessments in the PEPC trial are listed in Additional file 3.

Clinical outcomes

Body composition LBM, BMD and fat mass are measured by dual-energy X-ray absorptiometry (DXA) using a Hologic multiple detector, fan-beam bone densitometer (Discovery QDR series). LBM is measured in arms, legs, trunk and total body. Changes in upper and lower body LBM are investigated separately because of differences in androgen sensitivity in leg muscles compared to neck, chest and shoulder muscles [45]. Body weight is measured by a digital platform scale and height by a stadiometer and body mass index (weight/(height)^2) is calculated.

Serological outcomes Fasting blood tests are taken between 8:00 am and 9:00 am. The tests analyzed are listed in Additional file 3. A biobank for frozen serum and full blood (EDTA) is established.

Physical functioning Muscle strength is measured by 1 RM test, sit-to-stand test and stair-climbing test [46] and cardio-respiratory fitness is measured by Shuttle Walk test.

To secure validity of the physical tests, all patients undergo a session of familiarization to the actual tests 3-4 days prior to the pre- and post intervention assessments. Both sessions are performed based on the same guidelines, but after the familiarization session the load of each exercise is adjusted to match the expected 1 RM. Additional description of the physical functioning assessments is provided in Additional file 4.

Psycho-social functioning Mental health are self-rated by the Hospital Anxiety and Depression scale (HADS) [47], fatigue is assessed by the Norwegian version of Fatigue Questionnaire (FQ) [48] and HRQOL by The European Organization and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ C-30) [49]. Additional description of the psycho-social functioning assessments is provided in Additional file 4.

Muscle cellular outcomes

Muscle biopsies are obtained from approximately half of the patients included in the study. Patients not willing to undergo biopsy are still eligible for trial participation.

With the patients in a supine position, a 6 mm Pelomi-needle (Albertslund, Denmark) with manual suction is used to obtain muscle samples (≈ 200 mg), under local anaesthesia (Xylocain® adrenaline, 10 mg·ml^{-1} + 5 μg·ml^{-1}, AstraZeneca, Södertälje, Sweden). Before the intervention the biopsy is obtained from the mid-section of the right vastus lateralis, and after the intervention the biopsy is obtained 3 cm proximal to the pre-intervention biopsy.

Muscle fibre size and regulators of muscle fibre size *Muscle fiber size*, measured as muscle fiber cross sectional area, represents the primary muscle cellular outcome. Secondary muscle cellular outcomes reflecting *regulators of muscle fibre size* are a) number of myonuclei per muscle fiber b) number of satellite cells per

muscle fiber, c) number of satellite cells and myonuclei positive for androgen receptors and d) proteins involved in muscle protein degradation (muscle breakdown); Forkhead Box Protein O (FOXO), Ubiquitin ligase E2 and Myostatin and muscle hypertrophy; androgen receptors and growth factors such as Insulin like growth factor 1 (IGF1) and Mechano growth factor (MGF).

Muscle fibre cross sectional area and regulators of muscle fibre size are analysed by immunohistochemistry on cross sections of muscle biopsies and by western blots and enzyme-linked immunosorbent assay (ELISA) in muscle homogenate.

Muscle fibre cross sectional area will be measured by cutting transverse serial sections of the muscle biopsy (8 μm thick) with a cryostat microtome (Microm, Germany) at -22°C and mounted on glass slides. Serial sections are immunohistochemically stained for fibre types (type I and type II) (used to measure muscle fibre cross sectional area), number of satellite cells, number of myonuclei and number of satellite cells and myonuclei positive for androgen receptors. Muscle fibre cross sectional area is measured for the different fibre types separately. An image of a satellite cell and basal lamina staining on a cross section from a muscle biopsy is shown in Additional file 1.

Regulators of muscle fibre function Regulators of muscle fibre function studied in this project are proteins involved in the protection against cellular stress; heat shock proteins (HSP) 27 and HSP 70), as well as enzymes involved in mitochondrial function; Cytochrome C Oxidase 4 (Cox 4), Hsp 60 and Citrate synthase. These regulators are measured by western blots and ELISA.

Feasibility

The feasibility of the PEPC trial is investigated by examining the eligibility, compliance, attrition and safety among those considered for inclusion and those participating. This involves registration of a) the number of eligible patients among all PC patients receiving radiotherapy combined with ADT, b) the number of patients willing to participate among the eligible patients and c) compliance and adherence to the intervention programs and reasons for missing exercise sessions and discontinuation

Background variables

The patients provide information about partnership, number and age of children living at home, education, work and sick leave by filling out a questionnaire. Information about medical situation as time points for treatment, stage of disease and comorbidity are collected from the medical record. Past illnesses and other medical problems are also reported in the questionnaire.

Lifestyle outcomes The level of physical exercise is assessed by a modified Norwegian version of the Leisure Score Index (LSI) from the Godin Leisure-Time Exercise Questionnaire (GLTEQ) [50]. Dietary habits are assessed by a modified version of the SmartDiet - a short food questionnaire [51]. Smoke- and snuff habits are measured by two single questions; Do you smoke? "Yes daily", "Yes, now and then", "I smoked earlier but I have stopped" and "No, I have never smoked" and Do you take snuff? "Yes, daily", "Yes, now and then", "I snuffed earlier but I have stopped" and "No, I have never snuffed".

Sample size and statistical considerations

LBM is the primary endpoint of the study. Sample size calculation is based on comparing the clinically relevant difference in change before and after the 16 weeks period in LBM between the EG and CG. To detect a 3 kilograms difference in change between the groups, and assuming a standard deviation (SD) of 3 kilograms, 22 patients are needed in each group with a significance level (two sided) of 5% and a power of 90%. Due to dropouts we plan to include 30 patients in each group. According to previous studies, this number should also be sufficient to detect differences in the cellular muscle outcomes (Sinha-Hikim et al., 2006).

Changes in outcome variables from start of intervention will be compared between the EG and CG groups by analysis of covariance using the baseline measurement as a covariate. P-values below 5% will be regarded as statistically significant. If obvious deviations from normal distributions are detected, changes in outcome will be compared between groups by Wilcoxon-Mann-Whitney test. The primary analysis population will be the intention to treat population using last observation carried forward to impute any missing values. In addition a per-protocol analysis including only patients with no missing observations of the variable of interest will be performed. Should imbalances in important variables be detected sensitivity analyses will also be added including these as covariates in the model.

Discussion

Previous research has examined the effect of physical exercise on clinical outcomes such as body composition, physical function, mental health, fatigue and quality of life in PC patients [3-5]. Still research on the effect of physical exercise in this patients group is less extensive than in other cancer groups, such as for example patients with breast cancer. Two of the most pronounced clinical side effects of ADT are the negative effect on muscle mass and muscle strength. However, studies investigating the effects of ADT and exercise at the muscle cell level are still lacking. Expanded knowledge in this field among PC patients is therefore required.

The clinically indicated ADT in PC patients enables the study of testosterone's role in regulation of muscle mass during strength training. The main question is whether normal adaptations to strength training are disturbed when the level of testosterone is below 1 nmol/L (castration level); e.g. is the activation of satellite cells and hypertrophic effect of strength training blunted in these patients? Furthermore, by investigating important factors for the regulation of muscle mass and muscle function, the cellular effects of ADT on muscle tissue will be elucidated. Currently, three other ongoing trials are investigating the effect of exercise on muscular outcomes assessed by muscle biopsies in patients with breast, lung and testicular cancer receiving chemotherapy [52-54]. However, the effects of high-load strength training on muscular adaptations in PC patients during ADT have not previously been investigated.

Compared to previous studies in PC patients the intensity and/or duration in our strength training protocol is somewhat increased [44,55,56]. The rationale for choosing a strenuous strength training protocol is the fact that the effect on total muscle mass has been moderate or absent in previous studies on PC patients. One possible explanation for negligible effects on muscle mass in previous studies may be that the stimuli for muscle hypertrophy have been too low. In healthy men, it is suggested that a training intensity of 75-80% of 1 RM (6-12 RM sets) combined with multiple sets in each exercise (starting from 1-2 and progressing to 3-6 sets per exercise), give optimal stimuli for muscle hypertrophy when performed 2-3 times per week [57]. Consequently, in order to maximize the stimuli for muscle hypertrophy we chose to implement this strength training protocol in our patients. Importantly the training protocol should still be tolerable and safe.

Positive findings from the PEPC trial will help in the construction of more effective training protocols to counteract the negative effects of ADT on muscle tissue, BMD and physical function. More importantly, the results on the cellular effects of ADT in muscle tissue will provide novel understandings of muscle mass regulation by testosterone. Such knowledge will improve our understanding on how aging in general and reduced testosterone production during ADT specifically, induces loss of muscle mass (sarcopenia). In turn this knowledge can be used to establish more effective strategies against sarcopenia in both healthy elderly and in patient populations at risk. New strategies could include both new medical treatments and training strategies focused to overcome the cellular changes inducing sarcopenia.

Summary

Knowledge of the effect of exercise on clinical outcomes such as physical functioning and quality of life outcomes has increased over the last decades. Studies testing aerobic exercise in breast cancer patients are most frequent. Studies in other cancer groups testing the effects of strength training program are still lacking. The PEPC trial focuses on the effect on a high-load strength training program on both clinical and muscle cellular outcomes in PC patients during ADT. As far as we know this is the first study having a muscular cellular outcome in these patients. By testing a high-load strength training program the study will provide new knowledge on optimal training programs in PC patients.

Endnote

[1]The term muscle fiber (muscle cell) originates from the embryonic formation of mature muscle cells where mononuclear myoblasts fuse to the giant multinuclear muscle cells.

Additional material

Additional file 1: An image of a satellite cell and basal lamina staining on a cross section from a muscle biopsy.

Additional file 2: Inclusion and exclusion criteria.

Additional file 3: Outcomes, specific variables and assessments.

Additional file 4: Descriptions of assessments.

Acknowledgements

The study is funded by the Norwegian Foundation of Health and Rehabilitation and the Norwegian Cancer Society, the south Norway Regional Health Authority and the Norwegian School of Sports Sciences, K.S. Courneya is supported by the Canada Research Chairs Program

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Authors' contributions

All authors read and approved the final manuscript. LT: provided substantial contribution to the conception and design of the study and drafted the manuscript, TSN: provided substantial contribution to the conception and design of the study and drafted the manuscript, TR: provided substantial contribution to the conception and design of the study and helped to draft the manuscript, KSC: provided substantial contribution to the conception and design of the study and helped to draft the manuscript, ES: provided substantial contribution to the design of the study and helped to draft the manuscript and SDF: provided substantial contribution to the conception and design of the study and helped to draft the manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 29 February 2012 Accepted: 29 March 2012
Published: 29 March 2012

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Pre-publication history

The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2407/12/123/prepub

doi:10.1186/1471-2407-12-123
Cite this article as: Thorsen *et al.*: A randomized controlled trial on the effectiveness of strength training on clinical and muscle cellular outcomes in patients with prostate cancer during androgen deprivation therapy: rationale and design. *BMC Cancer* 2012 **12**:123.

Paper II

Nilsen T.S., Raastad T., Skovlund E., Courneya K.S., Langberg C.W., Lilleby W., Fosså S.D., Thorsen L.: **Effects of strength training on body composition, physical functioning and quality of life in prostate cancer patients during androgen deprivation therapy.** Accepted by ACTA Oncologica.

Acta Oncologica, 2015; Early Online: 1–9

informa
healthcare

ORIGINAL ARTICLE

Effects of strength training on body composition, physical functioning, and quality of life in prostate cancer patients during androgen deprivation therapy

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ABSTRACT

Background. Androgen deprivation therapy (ADT) increases survival rates in prostate cancer (PCa) patients with locally advanced disease, but is associated with side effects that may impair daily function. Strength training may counteract several side effects of ADT, such as changes in body composition and physical functioning, which in turn may affect health-related quality of life (HRQOL). However, additional randomised controlled trials are needed to expand this knowledge.

Material and methods. Fifty-eight PCa patients on ADT were randomised to either 16 weeks of high-load strength training ($n = 28$) or usual care ($n = 30$). The primary outcome was change in total lean body mass (LBM) assessed by dual x-ray absorptiometry (DXA). Secondary outcomes were changes in regional LBM, fat mass, and areal bone mineral density (aBMD) measured by DXA; physical functioning assessed by 1-repetition maximum (1RM) tests, sit-to-stand test, stair climbing test and Shuttle walk test; and HRQOL as measured by the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30.

Results and Conclusion. No statistically significant effect of high-load strength training was demonstrated on total LBM ($p = 0.16$), but significant effects were found on LBM in the lower and upper extremities (0.49 kg, $p < 0.01$ and 0.15 kg, $p < 0.05$, respectively). Compared to usual care, high-load strength training showed no effect on fat mass, aBMD or HRQOL, but beneficial effects were observed in all 1RM tests, sit-to-stand test and stair climbing tests. Adherence to the training program was 88% for lower body exercises and 84% for upper body exercises. In summary, high-load strength training improved LBM in extremities and physical functioning, but had no effect on fat mass, aBMD, or HRQOL in PCa patients on ADT.

The combination of radiotherapy and adjuvant androgen deprivation therapy (ADT) increases survival rates among prostate cancer (PCa) patients with locally advanced disease [1]. However, castrate levels of testosterone are associated with negative effects on body composition [2,3]. Reduced lean mass, increased fat mass, loss of bone mass, as well as reduced physical functioning and health-related quality of life (HRQOL) are commonly reported following ADT [4,5].

Physical exercise, especially strength training, has been suggested as a beneficial strategy to counteract such side effects. The latest review on this topic identified 10 studies, and the authors concluded that exercise was safe and beneficial in regard to muscular strength and lean body mass (LBM), whereas fat mass, bone mass, and HRQOL were highlighted as outcomes for future studies [6]. Only three of the 10 studies were randomised controlled trials (RCT) on strength training in PCa patients on

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(Received 12 December 2014; accepted 24 March 2015)

ISSN 0284-186X print/ISSN 1651-226X online © 2015 Informa Healthcare
DOI: 10.3109/0284186X.2015.1037008

ADT the results of which showed inconsistent effects on body composition [7–9]. RCTs are therefore highly needed to replicate previous studies in order to give specific physical exercise recommendations regarding training load, frequency, duration and timing to PCa patients on ADT [10].

By increasing the training volume, and duration of the intervention compared to previous randomised strength training trials in PCa patients on ADT, we expected larger effects than previously reported, particularly in LBM. Furthermore, androgen sensitivity has been shown to differ in trunk- and extremity muscles [11]. This may have consequences with regard to strength training adaptation in different body regions, but no previous studies have explored these consequences in PCa patients during ADT.

The Physical Exercise and Prostate Cancer trial (The PEPC trial) was a RCT examining the effect of a 16-week high-load strength training programme on clinical and muscular cellular outcomes in PCa patients on ADT [12]. Here, we report the results on the primary outcome (total LBM) and selected secondary outcomes [regional LBM, areal bone mineral density (aBMD), fat mass, physical functioning and HRQOL] in the PEPC trial. We hypothesised that PCa patients on ADT would experience beneficial effects of strength training on all outcomes compared to PCa patients receiving usual care, and that regional differences in androgen sensitivity would interfere with training adaptations in trunk lean mass. The effects of the intervention on serological and muscle cellular outcomes will be published elsewhere.

Material and methods

Participants

Patients were recruited from two units at Oslo University Hospital from December 2008 to December 2011. Eligible patients were PCa patients with an intermediate- or high-risk profile, which was determined based on serum levels of prostate specific antigen and the histology and extent of the primary tumour [13]. All patients had been referred to high-dose radiotherapy, which started 2–6 months after the initiation of neo-adjuvant ADT, followed by adjuvant ADT, which continued for 9–36 months. ADT was administered by subcutaneous injections by a GnRH analogue (Zoladex®Astra Zeneca) every three months. Other eligibility criteria were age ≤ 75 years, ability to understand Norwegian, and residence less than 1 hour by car from the training facility. Exclusion criteria were regular strength training (≥ 1 session per week), use of osteoporosis medication, and/or medical conditions that could complicate participation. There was only limited literature on strength training in PCa patients on ADT when the PEPC trial was planned. The high age in this patient population added to the risk of injury, thus the exclusion criteria were relatively strict [12].

Randomisation

After radiotherapy, included PCa patients were randomised to either a strength training group (STG) or a control group (CG) in a 1:1 ratio, stratified for hospital units. Randomisation was computerised and performed by the staff at the clinical research office at Oslo University Hospital. The study was conducted in accordance with the Helsinki Declaration and approved by the Regional Committee for Medical and Health Research Ethics, South-East Region (protocol nr. 08/212b.2008/4062), and registered in ClinicalTrial.gov (NCT00658229). All patients willing to participate in the PEPC-trial signed an informed consent form, prior to enrolment.

High-load strength training program

To avoid acute troublesome bowel side effects from high-dose radiotherapy, the high-load strength training programme was initiated at least one month after radiotherapy, corresponding to five months or more after initiation of ADT depending on the duration of the neo-adjuvant part of ADT.

The training programme was a modified version of the programme tested by Segal et al. 2003 [9], with increased training volume and duration of the programme. The patients performed three sessions per week for 16 weeks. Each session included nine exercises (Smith machine half squat, leg press, Smith machine standing calf raises, knee flexion, knee extension, chest press, seated row, seated shoulder press, and biceps curl). After two weeks of familiarisation, including low resistance corresponding to 40–50% of one repetition maximum (1 RM, i.e. the maximal load that can be lifted once with full range of motion in the exercise) in two sets of 10 repetitions, the training programme followed a daily undulating periodisation model, with a linear progression in training volume through the intervention period: from one to three sets of 10RM on Mondays, and from two to three sets of 6RM on Fridays. A submaximal session was carried out on Wednesdays, with 10 repetitions with 80–90% of 10RM in 2–3 sets. An instructor supervised all "heavy" sessions, to ensure that the prescribed training load was used. The instructor also recorded any kind of pain during the intervention period and adjusted the training load if necessary. Further details of the training programme have been previously described [12]. Patients randomised to CG were encouraged to maintain

their habitual activity level and not to initiate strength training.

Assessments

Outcomes were assessed the week before and after the intervention. Importantly all post-intervention assessments were performed while the patients were still on ADT. The primary outcome in the PEPC trial was difference in mean change from baseline to post-test between the STG and the CG for total LBM. Secondary outcomes were group differences in mean change in other body composition variables [regional LBM (trunk, lower extremities, upper extremities and appendicular skeletal muscle), aBMD (total, total lumbar spine, total hip, trochanter and femoral neck), fat mass (total and trunk fat mass), fat percentage, body mass and body mass index (BMI)], physical functioning (muscle strength and cardio-respiratory fitness), HRQOL and fatigue.

LBM, aBMD and fat mass were assessed by dual x-ray absorptiometry (DXA) using a Hologic multiple detector, fan-beam bone densitometer (Discovery QDR series). Muscle strength was measured by 1RM in leg press, chest press and shoulder press. Function in activities of daily living was measured by the sit-to-stand test and stair climbing test [14], and cardio-respiratory fitness by Shuttle walk test (see [12] for details). HRQOL was measured by the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) [15].

All patients underwent a familiarisation session three days prior to the actual test, where all test exercises were introduced and performed to a "somewhat hard" load. Personnel performing DXA scans were blinded to group allocation. To ensure inter-tester reliability all 1RM-tests was supervised by the same test leader.

Adherence to the intervention was calculated based on training logs, where the instructor recorded the training load for each exercise. Adherence rates were calculated separately for the upper- and lower body, as the proportions of completed exercise sessions, relative to the scheduled number (48).

Sample size calculation and statistics

At the time the PEPC-trial was planned, there were a limited number of strength training studies available in PCa patients on ADT. Sample size was therefore estimated based on findings from Kvorning et al. 2006 [16] who reported an increase in lean mass following strength training in young men on ADT, and studies that reported loss of lean mass in PCa patients on ADT [2]. Thus, we expected a 3 kg difference in mean change between the two groups. With a two-sided significance level of 5% and a power of 90%, 22 patients were required in each group, assuming a standard deviation (SD) of 3 kg. The study would have 80% power to detect a difference of 2.55 kg between groups. To account for dropouts we planned to include about 30 patients in each group.

Between group differences were assessed by analysis of covariance (ANCOVA) with the change from baseline included as the dependent variable, group assignment (STG vs. CG) as a fixed factor, and baseline score as a covariate. In addition mean change from baseline within treatment groups with corresponding 95% CIs were also estimated. Missing data were imputed by an intention-to-treat approach using the last observation carried forward. We also performed sensitivity analyses, including patients with complete data sets only. A p-value < 0.05 was considered statistically significant. Data were analysed by SPSS version 18.0.

Results

Among 119 invited PCa patients, 58 were randomised. Reasons for exclusion, refusal, and dropouts are listed in the consort diagram (Figure 1). At trial inclusion (baseline) the mean age was 66 years and the average ADT duration was nine months (Table I).

Changes in body composition

Statistically significant differences in mean change from baseline between groups were not found for total LBM (STG 0.50 kg vs. CG −0.07 kg, difference 0.56 kg, $p = 0.16$) or LBM in the trunk (STG −0.02 kg vs. CG 0.14 kg, difference −0.17 kg, $p = 0.40$), but significant intervention effects were observed for LBM in the lower extremities (STG 0.28 kg vs. CG −0.21 kg, difference 0.49 kg, $p = 0.002$) and in the upper extremities (STG 0.20 kg vs. CG 0.04 kg, difference 0.15 kg, $p = 0.05$) and consequently also for appendicular skeletal muscle (STG 0.47 kg vs. CG −0.17 kg, difference 0.64 kg, $p = 0.001$) (Table II). No group differences in mean change in total or trunk fat mass, fat percentage, body mass or BMI or total or regional aBMD, emerged (Table II). Analyses in patients who completed both pre- and post-intervention assessments showed results that were similar to the intention-to-treat analyses.

Changes in physical functioning and HRQOL

Statistically significant effects of the intervention were observed for 1RM in leg press, chest press, and

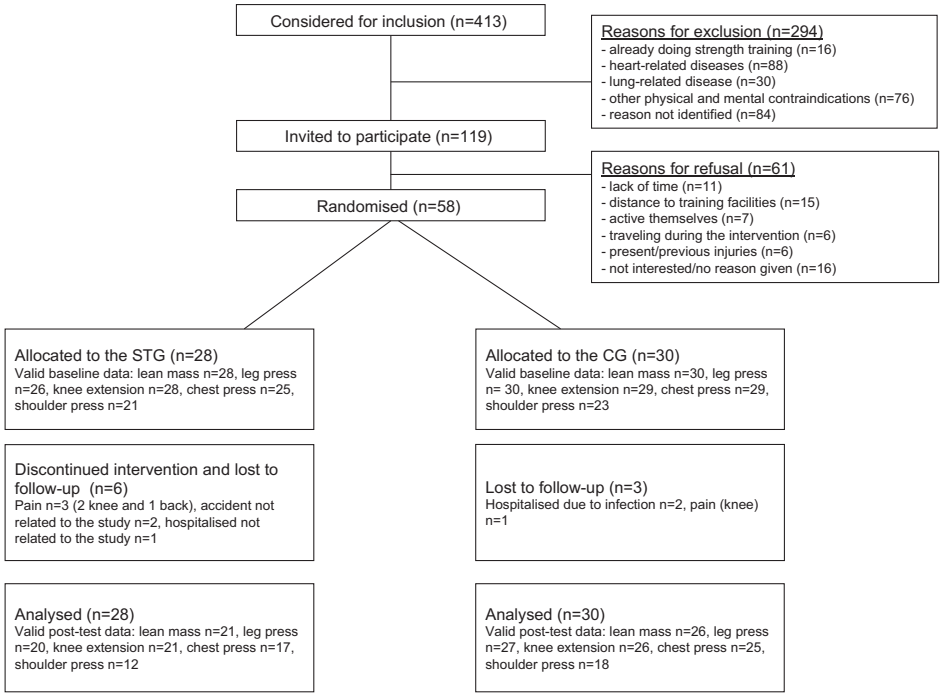

Figure 1. Consort diagram. CG, control group; STG, strength training group.

Table I. Baseline characteristics.

	STG ($n = 28$)	CG ($n = 30$)
Age (years)		
Mean (range)	66 (54–76)	66 (54–76)
SD	6.6	5
Risk profile groups		
Intermediate (proportion of patients)	50	50
High-risk (proportion of patients)	50	50
Total time on ADT (months)		
Mean (range)	17.0 (8–34)	18.0 (8–28)
SD	8.7	8.2
Time on ADT at baseline (months)		
Mean (range)	9.0 (7–12)	9.0 (5–12)
SD	1.6	1.8
Time from rad. to baseline (months)		
Mean (range)	3.0 (1–7)	3.0 (1–6)
SD	1.3	1.3
Testosterone at baseline (mmol/l)		
Mean (range)	<0.6 (0.4–1.5)	<0.6 (0.4–1.3)
SD	0.3	0.2

STG = strength training group; CG = control group; SD = standard deviation; BMI = body mass index; ADT = androgen deprivation therapy; Rad = radiotherapy.

shoulder press, and in the sit-to-stand test and stair climbing test, whereas a borderline effect was observed in the Shuttle walk test (Table III). No intervention effect was observed for HRQOL (Table IV). Analyses in patients who completed both pre- and post-intervention assessments showed results that were similar to the intention-to-treat analyses

Exercise adherence and adverse events

Patients in the STG completed a mean of 88% of the strength training sessions for the lower-body exercises (range: 64–98%) and 84% for the upper-body exercises (range: 69–98%, excluding one outlier of 31%). Three patients in the STG discontinued the intervention due to pain, two due to pain in the knee and one patient due to back pain.

Discussion

The PEPC-trial is one of only a few RCTs examining the effects of strength training in PCa patients on ADT. Surprisingly, we did not observe any statistically significant between group effect from 16 weeks of high-load strength training on total LBM, but the

Table II. Effects of strength training on lean body mass, fat mass and areal bone mineral density.

		Baseline		Post test		Within groups mean change from baseline		Group difference in mean change from baseline*		
	n	Mean	SD	Mean	SD	Mean	[95% CI]	Mean	[95% CI]	*p*
Lean body mass (kg)										
Total body										
STG	28	59.8	6.9	60.3	7.6	0.5	[−0.1–1.1]	0.5	[−0.2–1.2]	0.157
CG	30	57.9	6.6	57.9	6.7	−0.1	[−0.5–0.4]			
Trunk										
STG	28	29.9	4.0	29.9	4.5	0.0	[−0.4–0.4]	−0.2	[−0.7–0.3]	0.403
CG	30	28.5	3.4	28.7	3.4	0.1	[−0.2–0.5]			
Lower extremities										
STG	28	18.7	2.3	19.0	2.4	0.3	[0.1–0.5]	0.5	[0.2–0.8]	0.002
CG	30	18.5	2.3	18.3	2.4	−0.2	[−0.4–−0.0]			
Upper extremities										
STG	28	6.5	0.8	6.7	0.8	0.2	[0.1–0.3]	0.2	[0.0–0.3]	0.048
CG	30	6.4	1.0	6.4	1.1	0.0	[−0.1–0.1]			
Appendicular skeletal muscle mass										
STG	28	25.2	2.9	25.7	3.1	0.5	[0.2–0.8]	0.6	[0.3–1.0]	0.001
CG	30	24.8	3.2	24.7	3.4	−0.2	[−0.4–0.1]			
Fat mass (kg)										
Total										
STG	28	26.5	6.5	26.4	6.3	0.0	[−0.5–0.4]	−0.3	[−0.9–0.4]	0.402
CG	30	26.4	6.6	26.7	6.5	0.2	[−0.3–0.7]			
Trunk										
STG	28	14.7	4.2	14.6	4.1	−0.1	[−0.3–0.2]	−0.2	[−0.6–0.2]	0.355
CG	30	14.6	4.2	14.7	4.1	0.2	[−0.2–0.5]			
Percent (%)										
STG	28	29.5	4.6	29.3	4.3	−0.2	[−0.6–0.3]	−0.4	[−1.0–0.2]	0.164
CG	30	30.0	4.0	30.2	4.0	0.2	[−0.2–0.6]			
Body mass										
STG	28	88.9	12.1	89.3	12.7	0.4	[−0.1–1.0]	0.3	[−0.5–1.1]	0.509
CG	30	87.1	12.5	87.2	12.5	0.1	[−0.5–0.7]			
BMI										
STG	28	29.1	3.9	29.2	3.9	0.1	[−0.2–0.3]	0.1	[−0.2–0.4]	0.594
CG	30	28.4	3.4	28.4	3.4	0.0	[−0.2–0.2]			
Areal bone mineral density (mg/cm^2)										
Total body										
STG	28	1.17	0.13	1.16	0.12	−0.01	[−0.02–−0.00]	0.00	[−0.02–0.01]	0.520
CG	30	1.16	0.12	1.16	0.13	−0.01	[−0.01–0.00]			
Total lumbar spine										
STG	28	1.05	0.19	1.04	0.19	0.00	[−0.01–0.01]	0.00	[−0.02–0.01]	0.847
CG	30	1.02	0.16	1.01	0.17	0.00	[−0.01–0.01]			
Total hip										
STG	28	1.02	0.15	1.01	0.15	0.00	[−0.01–0.00]	0.00	[−0.01–0.01]	0.690
CG	30	0.99	0.11	0.99	0.11	0.00	[−0.01–0.01]			
Trochanter										
STG	28	0.77	0.13	0.77	0.14	−0.01	[−0.01–−0.00]	0.00	[−0.01–0.00]	0.221
CG	30	0.74	0.11	0.74	0.11	0.00	[−0.01–0.00]			
Femoral neck										
STG	28	0.82	0.13	0.81	0.13	−0.01	[−0.02–0.00]	0.00	[−0.02–0.01]	0.467
CG	30	0.79	0.10	0.79	0.10	0.00	[−0.01–0.00]			

Analysis on patients with valid post test showed same trends.
STG = strength training group; CG = control group; SD = standard deviation; CI = confidence interval; BMI = Body mass index.
*analysis adjusted for baseline values.

intervention led to significant beneficial effects on LBM in the extremities, muscle strength, and physical function. No intervention effects were observed for aBMD, fat mass, or HRQOL.

To increase the likelihood of intervention effects, particularly for the primary outcome, we increased the weekly training volume (three sessions per week) and the duration (16 weeks) compared to most prior strength training studies in PCa patients on ADT. However, contrary to our hypothesis and to prior studies [8,17,18], no statistically significant effect of high-load strength training on total LBM emerged.

Table III. Effects of strength training on muscle strength (1RM) and functional tests.

		Baseline		Post test		Within groups mean change from baseline		Group difference in mean change from baseline*		
	n	Mean	SD	Mean	SD	Mean	[95% CI]	Mean	[95% CI]	*p*
Muscle strength (kg)										
Leg press										
STG	26	184	44	228	61	44	[30–57]	42	[29–55]	<0.001
CG	30	168	42	168	42	0	[−3–4]			
Chest press										
STG	25	50	12	55	12	6	[3 – 8]	6	[3–8]	<0.001
CG	29	48	11	48	11	0	[−1–1]			
Shoulder press										
STG	20	23	7	29	10	6	[3–8]	5	[3–8]	<0.001
CG	23	23	9	24	8	0	[−1–2]			
Functional tests										
Sit to stand (Number of reps. in 30 sec.)										
STG	26	16	3	18	3	2	[1–3]	2	[1–3]	<0.001
CG	30	16	3	16	3	0	[−0–1]			
Shuttle walk (meters)										
STG	25	779	188	804	204	24	[−3–52]	39	[−2–80]	0.064
CG	29	756	176	741	189	−15	[−45–16]			
Stair climbing (sec.) Unloaded										
STG	25	5.8	0.9	5.5	1.0	−0.3	[−0.41–−0.10]	−0.23	[−0.45–−0.00]	0.047
CG	30	5.9	1.0	5.9	1.0	0.0	[−0.20–0.12]			
Loaded (20 kg)										
STG	25	6.5	1.2	6.2	1.1	−0.3	[−0.51–−0.17]	−0.27	[−0.50–0.04]	0.024
CG	30	6.6	1.1	6.5	1.1	−0.1	[−0.24–0.09]			

Analysis on patients with valid post test showed same trends.
STG = strength training group; CG = control group; SD = standard deviation; CI = confidence interval; Reps = repetitions; Sec = seconds; *analysis adjusted for baseline values.

Interestingly, two strength training sessions per week in the study by Galvão et al. (2010) led to a significant 0.76 kg difference in total LBM change between the intervention and control group in 12 weeks [8], compared to 0.56 kg in our study. The largest training effect on LBM in PCa patients on ADT is, however, reported in the study by Hanson et al. (2012), where three weekly strength training sessions increased LBM by 1.7 kg, using drop-sets in the training protocol [18]. Thus, the optimal training strategy for strength training and its effect during ADT is therefore still to be determined.

The LBM in the CG in our study remained unchanged in the present study, and one could speculate on contamination of the intervention by participants in the CG were actually engaging in strength training. However, this is unlikely since the CG reported no change in high intensity exercise during the intervention period (measured by Godin leisure time physical activity questionnaire, data not shown).

Our sample size calculation was based on an expected 3-kg difference in total LBM change from baseline to post-test between the groups, with a SD of 3 kg. Based on studies initiated after the PEPC trial, we acknowledge that a 1 kg difference in change from baseline to post-test between groups, with a SD of 1.25, would be more realistic [8]. As the ratio of SD and hypothesised difference is very similar whatever assumptions made, sample size estimation would have been affected to a limited extent. More importantly, inspection of the estimated confidence intervals for efficacy demonstrates that it is not likely that large effects have gone undetected.

The fact that our strength training programme, which involved large muscle groups in the upper body, had no impact on trunk LBM is worth attention. We speculate that ADT may interfere differently with LBM gains in the trunk compared to the extremities due to differences in androgen sensitivity [11]. No previous studies have directly reported on the effects of strength training on trunk LBM in PCa patients on ADT. However, no change in trunk LBM was revealed in the study by Galvão et al. (2010) by subtracting appendicular LBM increase from the increase in total LBM [8]. However, we cannot rule out that the DXA scan lacks the sensitivity to detect small changes in trunk LBM, and further studies with more accurate measures of muscle mass (e.g. magnetic resonance imaging) are therefore warranted.

Due to increased risk for diabetes and cardiovascular diseases, it is important to counteract gains in

Table IV. Effects of strength training on health-related quality of life.

		Baseline		Post test		Within groups mean change from baseline		Group difference in mean change from baseline*		
	n	Mean	SD	Mean	SD	Mean	[95% CI]	Mean	[95% CI]	*p*
EORTC QLQ-C30 function scales										
Physical functioning										
STG	28	87.1	11.7	88.3	11.8	1.2	[−1.72–4.10]	0.7	[−3.63–5.01]	0.750
CG	30	81.6	17.2	83.6	14.8	2.0	[−1.76–5.76]			
Role functioning										
STG	28	85.7	19.6	89.9	17.8	4.2	[−2.56–10.89]	1.3	[−6.83–9.50]	0.744
CG	30	76.1	28.9	84.4	19	8.3	[−0.43–17.10]			
Emotial functioning										
STG	27	91.4	14.9	92.9	11.5	1.5	[−3.21–6.29]	3.1	[−1.71–7.88]	0.203
CG	30	85.0	18.2	85.0	18.2	0.0	[−2.58–2.58]			
Cognitive functioning										
STG	27	87.7	9.9	92.0	10.7	4.3	[−0.00–8.64]	2.7	[−3.32–8.75]	0.371
CG	30	83.9	19.3	86.7	18.8	2.8	[−2.15–7. 70]			
Social functioning										
STG	27	74.7	23.7	77.2	22.2	2.5	[−5.44–10.38]	0.6	[−8.05–9.21]	0.893
CG	30	72.8	24.2	75.6	18.9	2.8	[−4.36–9.92]			
Global health status and quality of life										
STG	27	76.5	17.3	79.6	17.0	3.1	[−1.12–7.29]	−6.9	[−13.90–0.12]	0.054
CG	30	66.7	19.6	78.9	20.7	12.2	[6.52–17.93]			
EORTC QLQ-C30 symptom scales										
Fatigue										
STG	28	34.5	15.2	33.7	16.1	−0.8	[−6.41–4.82]	2.3	[−5.84–10.54]	0.568
CG	30	36.5	14.9	33	22.3	−3.5	[−9.74–2.70]			
Nausea and vomiting										
STG	28	4.2	13.3	2.4	12.6	−1.8	[−3.82–0.25]	−0.7	[−3.32–1.90]	0.588
CG	30	1.7	5.1	1.1	4.2	−0.6	[−2.55–1.43]			
Pain										
STG	28	18.5	17.8	20.2	25.8	1.8	[−6.15–9.72]	2.5	[−8.17–13.07]	0.645
CG	30	19.4	25.9	18.3	21.1	−1.1	[−10.05–7.83]			
Dyspnea										
STG	28	25	25.1	17.9	19.2	−7.1	[−16.68–2.40]	−4.0	[−15.68–7.78]	0.502
CG	30	37.8	22.7	27.8	27.8	−10.0	[−19.33–−0.67]			
insomnia										
STG	25	25.3	30.9	24	29.7	−1.3	[−10.63–7.96]	2.1	[−10.46–14.72]	0.735
CG	28	33.3	30.1	27.4	31.5	−6.0	[−15.94–4.03]			
Appetite loss										
STG	28	4.8	14.9	3.6	13.9	−1.2	[−5.47–3.09]	0.0	[−4.15–4.18]	0.993
CG	30	4.4	11.5	3.3	10.2	−1.1	[−3.38–1.16]			
Constipation										
STG	27	12.3	21	9.9	18.1	−2.5	[−7.54–2.61]	1.9	[−5.02–8.88]	0.579
CG	30	14.4	24.3	8.9	15	−5.6	[−13.62–2.51]			
Diarrhea										
STG	27	35.8	31.9	35.8	35.7	0.0	[−7.31–7.31]	11.2	[−1.46–23.80]	0.080
CG	30	32.2	29.7	22.2	26.7	−10.0	[−21.39–1.39]			

Analysis on patients with valid post test showed same trends.
STG = strength training group; CG = control group; SD = standard deviation; CI = confidence interval.
*analysis adjusted for baseline values.
0 indicates the lowest function and 100 the best in the function scales; 0 indicates fewest symptoms and 100 the most in the symptom scales; 10 points or more is considered to be of clinical relevance.

fat mass observed in PCa patients on ADT [19]. Combined endurance and strength training for 3 months, immediately after starting ADT, has shown beneficial effects on fat mass in PCa patients [17]. We observed no effect on fat mass with our high-load strength training intervention. A mixed intervention, using both strength and endurance training, would probably have greater chances to affect fat mass. Exercise in combination with nutritional counselling should be expressly considered, as the greatest potential to influence energy balance lies in energy intake.

In the present study, as well as in previous studies initiated immediately after starting ADT [17], or after one year on ADT [7], aBMD was not influenced by strength training, which is otherwise seen in healthy men [20]. The explanation for the lack of positive

effects of exercise on aBMD could be related to the training modality, as well as to short intervention periods. However, a one-year long intervention combining both impact stimuli and strength training showed no significant effect on aBMD in PC patients on ADT [21], whereas significant effects of the same intervention were observed in postmenopausal breast cancer patients (lumbar spine) [22]. Thus more studies examining the effects of strength training on bone health during ADT are needed.

An important finding in our study was that the increased muscle strength translated into improved physical functioning. However, these improvements did not seem to influence the patients' HRQOL or the level of fatigue. This is contrary to other RCTs that have investigated the effect of strength training, where improvements in various aspects of HRQOL are often reported [8,9,17,23]. However, our baseline values showed that patients in the STG were already at a higher level of HRQOL than PCa patients on ADT from other studies [24], thus one could not expect large increases here [25].

Even though the exercise instructors adjusted the programme when the patients reported training-related pain, the high-load strength training programme used in the PEPC trial still might have been too intensive for three patients who dropped out of the STG due to pain. Further studies comparing strength training programmes with different intensities are therefore warranted.

A limitation in the PEPC trial is the modest sample size, which might have affected the power and generalisability of results for the secondary outcomes. This needs to be taken into consideration when interpreting our results. Due to strict exclusion criteria, the patients in the present study may represent the healthiest PCa patients and thus our results may be representative of only the healthiest PC patients on ADT. This might also have contributed to the lack of effect on total LBM, as the potential for LBM gains may be higher among more fragile patients.

In summary, our results suggest that PCa patients on ADT can benefit from high-load strength training, in terms of increased LBM in the extremities and increased muscle strength and physical functioning. More studies are needed to confirm our findings regarding the apparent lack of effects of strength training on total LBM and HRQOL. Although reduced fat mass and increased aBMD were not observed in the present study, in clinical practice, PCa patients should still be encouraged to perform strength training during ADT as maintenance of muscle strength is important for activities of daily living.

Acknowledgements

This work was supported by the Norwegian Foundation of Health and Rehabilitation and the Norwegian Cancer Society (HF-57004/001) and co-funded by the Regional Health Authority in Southern Norway. Additional funding was also given by Eckbo's legacy, The Radium Hospital's legacy and Trivselsanlegget's legacy. Thanks to all patients for participating and to Melanie-Birte Schulz, Stine Solli, Siri Kjellaas, Jon Reidar Iversen, Nils K. Raabe, Kjell Magne Russnes and Gunnar Tafjord for their help identifying and including patients. Thanks also to Karol Axcrona for useful comments regarding the design and to Arne Høiseth for evaluating the DXA scans. The authors also thank the Department for Clinical Research Support, Oslo University Hospital, for their assistance with tasks related to the data management of this study.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Paper III

Nilsen T.S., Thorsen L., Fosså S.D., Wiig M., Kirkegaard C., Skovlund E., Benestad H.B., Raastad T.: **Effect of strength training on muscle cellular variables during androgen deprivation for prostate cancer: a randomised trial.** *Accepted by Scandinavian Journal of Medicine and Science in sports.*

Scand J Med Sci Sports 2015: ••: ••–••
doi: 10.1111/sms.12543

SCANDINAVIAN JOURNAL OF
MEDICINE & SCIENCE
IN SPORTS

Effects of strength training on muscle cellular outcomes in prostate cancer patients on androgen deprivation therapy

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Accepted for publication 20 July 2015

Androgen deprivation therapy (ADT) improves life expectancy in prostate cancer (PCa) patients, but is associated with adverse effects on muscle mass. Here, we investigated the effects of strength training during ADT on muscle fiber cross-sectional area (CSA) and regulators of muscle mass. PCa patients on ADT were randomized to 16 weeks of strength training (STG) ($n = 12$) or a control group (CG; $n = 11$). Muscle biopsies were obtained from m. vastus lateralis and analyzed by immunohistochemistry and western blot. Muscle fiber CSA increased with strength training (898 μm^2, $P = 0.04$), with the only significant increase observed in type II fibers (1076 μm^2, $P = 0.03$). There was a trend toward a difference in mean change between groups myonuclei number (0.33 nuclei/fiber, $P = 0.06$), with the only significant increase observed in type I fibers, which decreased the myonuclear domain size of type I fibers ($P = 0.05$). Satellite cell numbers and the content of androgen receptor and myostatin remained unchanged. Sixteen weeks of strength training during ADT increased type II fiber CSA and reduced myonuclear domain in type I fibers in PCa patients. The increased number of satellite cells normally seen following strength training was not observed.

Androgen deprivation therapy (ADT) increases life expectancy for prostate cancer (PCa) patients (Bolla et al., 2010), but is also associated with a number of side effects such as loss of muscle mass (Storer et al., 2012). This could in turn lead to impaired muscle function and daily function. Strength training has been shown to increase muscle function during ADT and could therefore be an effective countermeasure (Gardner et al., 2013), but so far, little is known about the muscle cellular responses to strength training during ADT.

The effects of testosterone on muscle mass have been known for decades (Kochakian & Murlin, 1935). On the muscle cellular level, testosterone stimulates synthesis of proteins involved in cell growth and survival through the androgen receptor (AR; Haren et al., 2011; Vicencio et al., 2011), and inhibits muscle protein degradation (Yin et al., 2009). Consequently, removal of testosterone by ADT in PCa patients results in reduced muscle mass (Storer et al., 2012). On the other hand, gain in muscle mass following strength training in humans is achieved by increased cross-sectional area (CSA) of individual muscle fibers, and to a lesser extent by an increased number of fibers (Folland & Williams, 2007). Typically, type II fibers respond with larger increases in CSA than type I fibers during strength training (Hikida et al., 2000; Kosek et al., 2006; Verdijk et al., 2009). However, since the AR content seems to be higher in type I fibers (Hulmi et al., 2008), the fiber types may respond differently to strength training during ADT.

An increased CSA of a muscle fiber is often paralleled by an increased number of myonuclei, and thereby keeping the cytoplasm-to-nuclei-ratio, termed myonuclear domain, relatively constant (Bruusgaard et al., 2010). Because of the post-mitotic status of the myonuclei, an increased number of myonuclei depends on nuclear donation from satellite cells, which are muscle progenitor cells found in a quiescent state between the basal lamina and the sarcolemma (Kadi et al., 2004). It has been shown that increased muscle fiber CSA induced by testosterone supplementation (Sinha-Hikim et al., 2006) and by strength training (Mackey et al., 2007; Snijders et al., 2009) is paralleled with an increased number of satellite cells per muscle fiber. Short-term ADT in healthy young men has been shown to attenuate muscle mass gains (Kvorning et al., 2006) and the increase in myonuclear numbers (Kvorning et al., 2014) following strength training. The effects of strength training during long-term ADT as in PCa patients on testosterone responsive muscle cellular outcomes are, however, not known.

Whereas testosterone has a profound positive effect on muscle mass through its action on AR in muscle cells, myostatin provides an inhibitory effect on muscle growth by repressing protein synthesis signaling (Schiaffino et al., 2013). Therefore, AR and myostatin represent key regulators of muscle mass, with opposite effects. The content of AR in muscle have been shown to decrease acutely after a strength training session (Vingren et al., 2009), but baseline levels seem to recover thereafter and be unchanged after a period of strength training (Ahtiainen et al., 2009). Acutely after a bout of strength training, the mRNA expression of myostatin seems to decrease (Mascher et al., 2008; Hulmi et al., 2009; Dalbo et al., 2011). There are conflicting reports on the effect of strength training over a period of time on the expression of myostatin, where decreased levels and unchanged levels have been reported (Roth et al., 2003; Hulmi et al., 2009). The response might differ with age, as the expression of myostatin has been reported to increase in elderly, but not in young subjects, following strength training (Mero et al., 2013). On the other hand, suppression of testosterone might decrease AR levels (Antonio et al., 1999) and increase myostatin levels (Mendler et al., 2007). Thus, strength training might change the contents of AR and myostatin in muscle differently in PCa patients on long-term ADT and healthy individuals.

Therefore, the primary aim of the present study was to investigate the effect of 16 weeks of strength training on muscle fiber CSA in PCa patients on ADT. Secondary outcomes were number of myonuclei and satellite cells per fiber, as well as the content of AR and myostatin in muscle, as well as knee extensor muscle strength. We hypothesized beneficial effect of strength training on muscle fiber CSA, and on the number of myonuclei and satellite cells per muscle fiber and muscle strength in PCa patients during ADT, but not in content of AR or myostatin.

Methods

Setting and participants

This study was conducted as a part of the Physical Exercise and Prostate Cancer trial (The PEPC trial; Thorsen et al., 2012). Eligible patients were PCa patients with intermediate or high-risk profile (D'Amico et al., 2003) referred to high-dose radiotherapy and (neo)adjuvant ADT (9–36 months). Other eligibility criteria were ≤ 75 years of age, ability to understand Norwegian, and residence less than 1 h by car from the training facilities. Exclusion criteria were regular strength training (≥ one weekly session), use of osteoporosis medication, and/or other conditions that could complicate participation without major adjustment in the training program. See (Thorsen et al., 2012) for additional information on exclusion criteria.

Design and randomization

The PEPC trial was a two-armed randomized controlled trial. At least 1 month after radiotherapy and still on ADT, the patients were assigned (ratio 1:1) to a strength training group (STG) or a control group (CG). The study was conducted in accordance with the Helsinki declaration, and was approved by the Regional Committee for Medical and Health Research Ethics, South-East Region [protocol nr. 08/212b.2008/4062 (ClinicalTrials.gov: NCT00658229)].

Strength training program

To avoid troublesome bowel side effects from high-dose radiotherapy during strength training, the program was initiated at least 1 month after radiotherapy. Depending on the duration of the neoadjuvant part of ADT, the strength training program started 5 months or more after initiation of ADT.

The program was performed three times a week for 16 weeks to increase the intervention duration compared with existing literature when the PEPC was planned, and included nine exercises (Smith machine half squat, leg press, Smith machine standing calf raises, knee flexion, knee extension, chest press, seated row, seated shoulder press, and biceps curl). After 2 weeks of familiarization, using low resistance (40–50% of one repetition maximum (1 RM) in two sets of 10 repetitions), the training program followed a daily undulating periodization model, where the training volume increased during the intervention period; from one to three sets of 10RM on Mondays, and from two to three sets of 6RM on Fridays. On Wednesdays, a submaximal session was carried out, with 10 repetitions with 80–90% of 10RM in two to three sets. An exercise physiologist supervised all heavy sessions (for details, see Thorsen et al., 2012). Patients in the control group (CG) were encouraged to maintain their habitual activity level but not initiate strength training.

Outcomes and assessments

All outcomes were assessed the week before and after the intervention. Importantly all post-intervention assessments were performed while the patients were still on ADT.

Muscle biopsy procedures and analysis

The muscle biopsies were collected from the mid-part of m. vastus lateralis, separated by at least 3 cm under local anesthesia (xylocain-epinephrine 10 mg/mL + 5 μg/mL, AstraZeneca, Södertälje, Sweden). All muscle biopsies were collected at the same time a day (morning), in a non-fasted state. The post-biopsy was obtained 72–96 h after the last training session. An incision was made in the skin and the fascia before biopsies were obtained using a 6-mm Pelomi-needle with manual suction (Albertslund, Denmark, the Bergström technique). The muscle biopsy was divided in several pieces for different analysis. All biopsy analyses were blinded, and the researcher had no knowledge of group allocation.

Histology

The piece selected for immunohistochemistry was dissected free from visual fat, and was within 3–5 min aligned in a biopsy cryomold, embedded in O.C.T. compound (Tissue-Tek; Sakura Finetek Europe, Zoeterwoude, the Netherlands) and frozen by immersion in isopentane, precooled (−160 °C) by liquid nitrogen and stored in −80 °C for later analysis. Later, the piece was cryosectioned at 8 μm (Leica CM 3050, Nußloch, Germany), mounted on glass slides, and blocked by 1% bovine serum albumin, in PBS-t, for 30 min at RT, before primary antibody (table) was applied and incubated overnight at +4 °C. The sections were then washed 3 × 10 min in PBS-t and incubated for 30 min at

RT with appropriate secondary antibodies. Thereafter, the washing procedure was repeated and cover slides mounted using Prolong Gold Antifade reagent with DAPI (Invitrogen, Eugene, OR, USA). Images of the stained cross sections were captured by light microscope (Olympus BX61 TRF, Tokyo, Japan) connected to a fluorescence light source (EXFO X-cite 120, Mississauga, Canada) with a camera (Olympus DP72) attached.

Muscle fiber CSA

Primary antibodies toward dystrophin (polyclonal, Abcam, ab15277, Cambridge, UK), and type II fibers (monoclonal, SC71, gift from Dr. Schifiano) were used as described above. Tema Image-Analysis System (Scan Beam, Hadsund, Denmark) was used to analyze the muscle fiber CSA by tracking the inner rim of the dystrophin staining (Fig. 1a) of each muscle fiber type. The total muscle fiber CSA is displayed as the two fiber types combined. Cross sections with fewer than 50 fibers of each fiber type were excluded from analysis. An average of 157 ± 78 (mean ± SD) and 184 ± 115 type I fibers, and 223 ± 98 and 289 ± 148 type II fibers were included from each patient in the fiber area analysis at the pre- and post-measurements, respectively. There were no significant differences in the number of included fibers between the two groups.

Myonuclei and myonuclear domain

A myonucleus was counted when 2/3 of the DAPI staining was located inside the dystrophin staining, and related to either fiber type I or II (Fig. 1b). As an indicator myonuclear domain, muscle fiber CSA was divided on the number of myonuclei per fiber. An average of 59 ± 11 and 63 ± 14 type I fibers, and 87 ± 30 and 96 ± 39 type II fibers were included from each patient in the myonuclear counting at the pre- and post-measurements, respectively. Apart from the post-biopsy, where more type II fibers were included for the CG ($P = 0.01$), there were no significant differences in the number of included fibers between the two groups.

Satellite cells

Antibodies toward Ncam (Abcam, ab9272, UK) and laminin (Daco Denmark AS, 20097, Glostrup, Denmark) used as described above, on the adjacent cross section to the one used for CSA and myonuclei analysis. The number of satellite cells was counted as ring-like Ncam staining encircling at least 2/3 of a nucleus (DAPI staining) located inside the laminin staining (Fig. 1c) and related to either fiber type I or II. An average of 254 ± 120 and 293 ± 190 type I fibers and 357 ± 183 and 468 ± 279 type II fibers were included from each patient in the satellite cell counting at the pre- and post-measurements, respectively. There were no significant differences in the number of included fibers between the two groups.

Homogenization

Muscle biopsy pieces for immunoassays were rinsed in ice-cold isotonic physiological saline (0.9% NaCl, Braun, Melsungen, Germany), and carefully dissected free of visual fat, connective tissue, and blood. Pieces of 50 mg were frozen in isopentane on dry ice and stored at −80 °C for later homogenization.

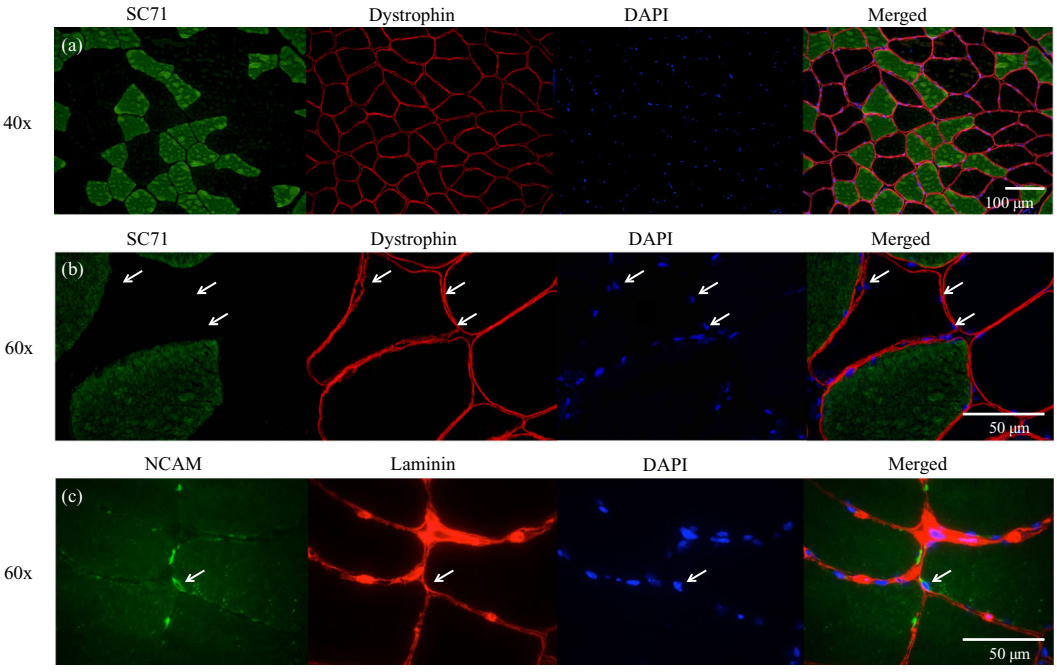

Fig. 1. Immunohistochemical analysis of muscle biopsy cross sections. (a) Fiber type-specific muscle fiber area calculated as area inside the dystrophin staining. SC71 stains all muscle fiber type II in human muscle. (b) When at least 2/3 of the DAPI staining (nucleus) was located inside of the dystrophin staining, it was counted a myonucleus. The number of myonuclei per fiber was related to muscle fiber type. (c) The number of satellite cells was counted as at least 2/3 of a ring-like NCAM staining inside the laminin staining, and related to muscle fiber type on the adjacent cross section.

Total protein was extracted from muscle samples using a commercial available kit (T-PER® Tissue Protein Extraction Reagent, cat. no. 78510, Thermo Scientific, Rockford, IL, USA), according to the manufacturer's procedures. Furthermore, 2% protease and phosphatase inhibitor cocktail (Halt™ Protease and Phosphatase Inhibitor Cocktail, cat. no. 78440, Thermo Scientific) and 2% EDTA was added to the lysate dilution according to the manufacturer's procedures.

Protein concentrations were determined, using a commercial kit (BioRad DC protein micro plate assay, cat. no. 0113, cat. no. 0114, cat. no. 0115, Bio-Rad, Hercules, CA, USA), a filter photometer (Expert 96, ASYS Hitech, Cambridge, UK) and the provided software (Kim, ver. 5.45.0.1, Daniel Kittrich). γ-globulin was used as standard protein, ranging from 0.125 to 1.5 mg per mL. Protein standard curve and samples were analyzed in triplicates; standard curve: $r^2 > 0.9$.

Western blot

Twenty-five milligrams of protein were loaded and separated by precast NuPAGE Novex 4–12% Bis-tris Midi gels (Lot. no. 11042274, Invitrogen, USA) for 35–45 min at 200 volts in cold MES running buffer (NuPAGE MES SDS running buffer: Life Technologies, Invitrogen, California, USA). Both time points for each patient were routinely loaded on the same gel, to enable comparison. Separated proteins were transferred on to immunoblot PVDF membrane (Immuno-blot, cat. no. 162–0177, Bio-Rad), at 30 volts for 90 min in cold transfer buffer (NuPAGE transfer buffer, cat. no. NP0006-1, Life Technologies). Thereafter, membranes were blocked overnight at 4 °C in a 5% fat-free skimmed milk and 0.05% TBS-t solution (TBS, cat. no.170–6435, Bio-Rad; Tween 20, cat. no. 437082Q, VWR International, Radnor, PA, USA; skim milk, cat. no. 1.15363, Merck, Germany), and incubated with monoclonal primary antibodies toward AR (Abcam, Ab9474, UK; diluted 1:1000) or myostatin (Abcam, ab98337, UK; diluted 1:100) for 2 h at RT. After washing, membranes were incubated with secondary antibody (goat anti-mouse, cat. no. 31430, Thermo Scientific/Pierce Biotechnology, Rockford, IL, USA) diluted 1:30 000 at RT for 1 h. All antibodies were diluted in a 1% fat-free skimmed milk and 0.05% TBS-t solution. Between stages, the membranes were washed with 0.05% TBS-t. Protein bands were visualized, with HRP-detection system (Super Signal West Dura Extended Duration Substrate, cat. no. 34076, Thermo Scientific/Pierce Biotechnology). Chemiluminescence was measured using a CCD image sensor (Kodak image station 2000R, Eastman Kodak Company, Rochester, New York, USA) and band intensity was calculated using the Carestream molecular imaging software (v. 5.0.7.2.2 Carestream Health, New Haven, Connecticut, USA). All samples were analyzed in duplicates, and mean values were used for statistical analysis.

Muscle strength

Muscle strength was measured as one repetition maximum (1RM: the maximum load that can be lifted once) in knee extension (Technogym, Gambettola, Italy). See Thorsen et al. (2012) for details.

Statistics

Using total CSA for our power calculation, a total of 37 patients would have a power of > 90% to detect an effect size of 0.8 (difference/SD). Because of dropouts and insufficient tissue quality for immunohistochemistry, the power was reduced to approximately 80%. Only patients with both baseline and post-intervention biopsies were included in the analysis.

Between-group differences were assessed by analysis of covariance (ANCOVA) with the change from baseline to the posttest included as the dependent variable, group assignment (STG vs CG) as a fixed factor, and baseline score as a covariate. By calculating the individual changes from baseline to posttest, we estimated the mean changes within each groups and corresponding 95% CIs, with paired sample *t*-test. The association between changes in muscle strength and muscle fiber CSA was analyzed by linear regression. Data were analyzed by SPSS version 18.0 (SPSS, Inc., Chicago, Illinois, USA).

Normality of the western blot data (AR and myostatin) was assessed by visual inspection of normality plots as well as D'Agostino-Pearson omnibus normality test. Between-group differences in change were analyzed with a two-sample *t*-test. Within-group changes were analyzed by a pair-sample *t*-test and are visualized in graphs by mean and 95% CIs. Western blot data were analyzed using GraphPad Prism 5. A *P*-value less than 0.05 were considered statistically significant.

Results

Baseline characteristics

Between 2009 and 2011, 104 eligible patients were invited to the PEPC trial and 58 agreed to participate. Of these, 37 patients were willing to undergo muscle biopsies, of which two patients dropped out because of accidents not related to the study and four refused to undergo post-biopsies because of discomfort (Fig. 2). Furthermore, eight patients were excluded because of reduced quality of the muscle tissue needed to perform immunohistochemistry (low number of fibers or freeze damage). Mean duration of ADT at baseline was approximately 9 months; other baseline values for the 12 patients in the STG and 11 in the CG are listed in Table 1.

Muscle fiber CSA

There was a statistically significant difference in mean change in total muscle fiber CSA between STG and CG (898 μm^2, $P = 0.04$; Table 2). Separate analyses in type I and II fibers indicated a larger effect in type II fibers (mean increase: 1076 μm^2, $P = 0.03$) than in type I fibers (mean increase: 723 μm^2, $P = 0.11$; Fig. 3a).

Myonuclei

There was a trend toward a difference in mean change between groups in number of myonuclei when type I and type II fibers were taken together (0.33 nuclei/fiber, $P = 0.06$; Table 2). Within the STG, the number of myonuclei per type I fibers increased on average by 0.39 nuclei per fiber (17%) from baseline to posttest ($P = 0.01$; Fig. 3b). No statistically significant change was observed in the number of myonuclei for type II fibers.

Myonuclear domain

No difference in mean change in myonuclear domain was observed between groups when type I and type II

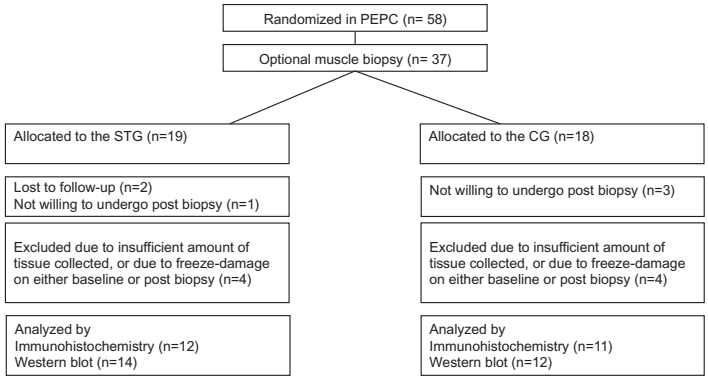

Fig. 2. Consort diagram.

Table 1. Baseline characteristics

	STG (n = 12)	CG (n = 11)
Age (years)		
Mean (range)	67 (54–76)	64 (54–76)
SD	7	6
Height (m)		
Mean	1.78 (1.70–1.87)	1.75 (1.52–1.88)
SD	0.06	0.1
Weight (kg)		
Mean	88.9 (75.1–106.9)	89.3 (70.9–118.3)
SD	9.4	15.14
Body mass index (kg/m^2)		
Mean	28.0 (24.1–31.2)	29.8 (25.0–35.8)
SD	2.3	3.5
Risk profile groups		
Intermediate (proportion of patients)	43	33
High-risk (proportion of patients)	57	67
Time on ADT at baseline (months)		
Mean (range)	9.1 (7.0–12)	9.4 (6.0–12.0)
SD	1.8	1.9
Total time on ADT (months)		
Mean (range)	18.0 (8.0–28.0)	20.3 (8.0–28.5)
SD	8.4	8.3
Time from rad. to baseline (months)		
Mean (range)	2.9 (1.0–7.0)	2.8 (1.0–6.0)
SD	1.4	1.4
Testosterone at baseline (mmol/L)		
Mean (range)	0.58 (< 0.40–1.50)	0.63 (< 0.40–1.30)
SD	0.29	0.27

ADT, androgen deprivation therapy; BMI, body mass index; rad, radiotherapy; SD, standard deviation.

fibers were combined (Table 2). There was a significant reduction in myonuclear domain in type I fibers within the STG (−233 μm^2/nucleus, $P = 0.05$), with no apparent change within the CG (Table 2). The myonuclear domain in type II fibers was unchanged from baseline to posttest in both groups (Fig. 3c).

Satellite cells

There were no significant changes in the number of satellite cells in any group, neither when types I and II fibers were combined (Table 2), nor when they were analyzed separately (Fig. 3c).

AR and myostatin

Western blot analysis of whole muscle homogenate showed no difference in mean change between the groups in content of AR ($P = 0.96$) or myostatin ($P = 0.99$) during the intervention (Fig. 4).

Muscle strength

There was a significantly larger increase in knee extensor strength (measured as 1RM) in STG compared with CG from baseline to posttest ($P < 0.01$), with a 21(± 11)% increase in the STG ($P < 0.01$), and no change in the CG [2(± 8)%, $P = 0.90$]. The change in 1RM from baseline was associated with the change in muscle fiber CSA (B = 0.004, $P = 0.02$; Fig. 5).

Discussion

This is the first randomized controlled study to evaluate the effect of strength training at the muscle cellular level in PCa patients during ADT. Strength training for 16 weeks increased CSA of the muscle fibers, especially in type II fibers, and there was a tendency toward an increased number of myonuclei per fiber. Contrary to our hypothesis, strength training did not change the number of satellite cells per fiber. No effect of strength training was observed on the content of AR and myostatin. The change in muscle fiber CSA was associated with the muscle strength changes.

Although no previous studies have examined the effects of strength training on muscle fiber CSA in PCa patients on ADT, increased CSA of both types I and type

Table 2. Effects of strength training on fiber area, and number of myonuclei and satellite cells per fiber

	n	Baseline		Posttest		Within groups mean change from baseline			Group difference in mean change from baseline*		
		Mean	SD	Mean	SD	Mean	[95% CI]	*P*	Mean	[95% CI]	*P*
Cross-sectional area (μm^2)											
STG	12	5186	1313	5828	1661	641	[–108–1391]	0.09	898	[51–1744]	0.04
CG	11	4791	1108	4604	966	–187	[–613–238]	0.35			
Number of myonuclei											
STG	12	2.50	0.50	2.80	0.54	0.30	[0.04–0.56]	0.03	0.33	[–0.01–0.67]	0.06
CG	11	2.68	0.53	2.63	0.62	–0.06	[–0.30–0.19]	0.62			
Myonuclear domain											
STG	12	2123	628	2086	531	–37	[–249–175]	0.71	68	[–202–340]	0.60
CG	11	1801	280	1792	338	–9	[–219–200]	0.93			
Number of satellite cells											
STG	12	0.04	0.02	0.04	0.01	0.00	[–0.01–0.01]	0.90	0.00	[–0.01–0.01]	0.81
CG	11	0.05	0.03	0.04	0.01	–0.01	[–0.03–0.00]	0.15			

*Analysis adjusted for baseline values.CI, confidence interval; CG, control group; SD, standard deviation; STG, strength training group.

II muscle fibers have been observed in healthy men following strength training (Hikida et al., 2000). However, not all interventions showed an increased CSA of type I fibers (Kosek et al., 2006; Verdijk et al., 2009). This is in line with the results of the present study, where the only significant effect was observed for the type II fibers CSA. The effects in CSA of type II fibers that we observed from our strength training intervention seems to be somewhat lower than effects reported in healthy men (Hikida et al., 2000; Kosek et al., 2006; Verdijk et al., 2009), which could be due to the castrate levels of testosterone in the PCa patients included in the present study. Nevertheless, the increase of type II fiber CSA achieved with strength training can probably be valuable to PCa patients because the fast type II fibers are the most important contributors to muscle power, a critically determinant of physical function, especially in older adults (Reid & Fielding, 2012).

In the present study, there was a weak tendency toward a decreased CSA of the type I fibers in the CG ($P = 0.10$), while the type II fiber CSA remained unchanged. The largest atrophy during normal aging is found in the type II fibers (Thompson, 1994), but type I fibers might be more sensitive to changes in testosterone levels (Sinha-Hikim et al., 2002). Therefore, our findings might suggest that ADT influences muscle fibers differently from normal ageing. However, we are unable to conclude on this matter, since the first biopsy in the PEPC trial was obtained 9 months (average) after ADT was initiated. Prospective studies exploring the effects of ADT in muscle, which includes muscle biopsies, are waranted.

In the present study, the muscle fiber CSA increased within the STG, and was accompanied by an increased number of myonuclei per fiber. It has been shown that even low dosages of testosterone supplementation alone can increase the number of myonuclei in elderly men (Sinha-Hikim et al., 2006). Our results show a borderline significant increase in the number of myonuclei as an effect of strength training. Consequently, although testosterone has been shown to promote cell fusion of human muscle precursor cells *in vitro* (Sculthorpe et al., 2012), it does not seem to be essential for nuclear addition in response to strength training. Nevertheless, ADT might reduce the myonuclear accretion in response to strength training, and this possible negative effect of ADT on muscular adaptations should be further investigated.

The baseline myonuclear domain size observed in the present study seems to be comparable with sizes reported in previous studies in healthy, elderly men (Petrella et al., 2006; Verney et al., 2008; Walker et al., 2012). The myonuclear domain within the STG remained unchanged in the type II fibers, but was actually reduced in the type I fibers. This was due to a significantly increased number of myonuclei per type I fiber, without a concomitant increase in fiber CSA. Some studies in rodents report that the increase in myonuclei precedes the increase in muscle fiber CSA (Bruusgaard et al., 2010), and our findings in type I fibers could be in line with this. However, the baseline myonuclear domain was smaller in the type II fibers compared with type I fibers. This may explain why an increase in myonuclear number was not seen here, as a larger increase in CSA would be needed to create a demand for more myonuclei (Bruusgaard et al., 2012). This has previously also been reported in humans where increased myonuclear numbers were seen in young subjects but not in elderly subjects, which had smaller myonuclear domain size at baseline (Petrella et al., 2006). On the other hand, a greater number of myonuclei in type I fibers expresses AR than in type II fibers (Hulmi et al., 2008). Therefore, removal of testosterone, as during ADT, might affect type I fibers to a greater extent than type II fibers. One could speculate that the decrease in myonuclear domain of muscle fiber type I within the STG, and the tendency toward a decreased type I fiber CSA within the CG ($P = 0.10$) indicate a more deleterious effect of ADT on type I than in type II fibers. However, prospective studies following PCa patients

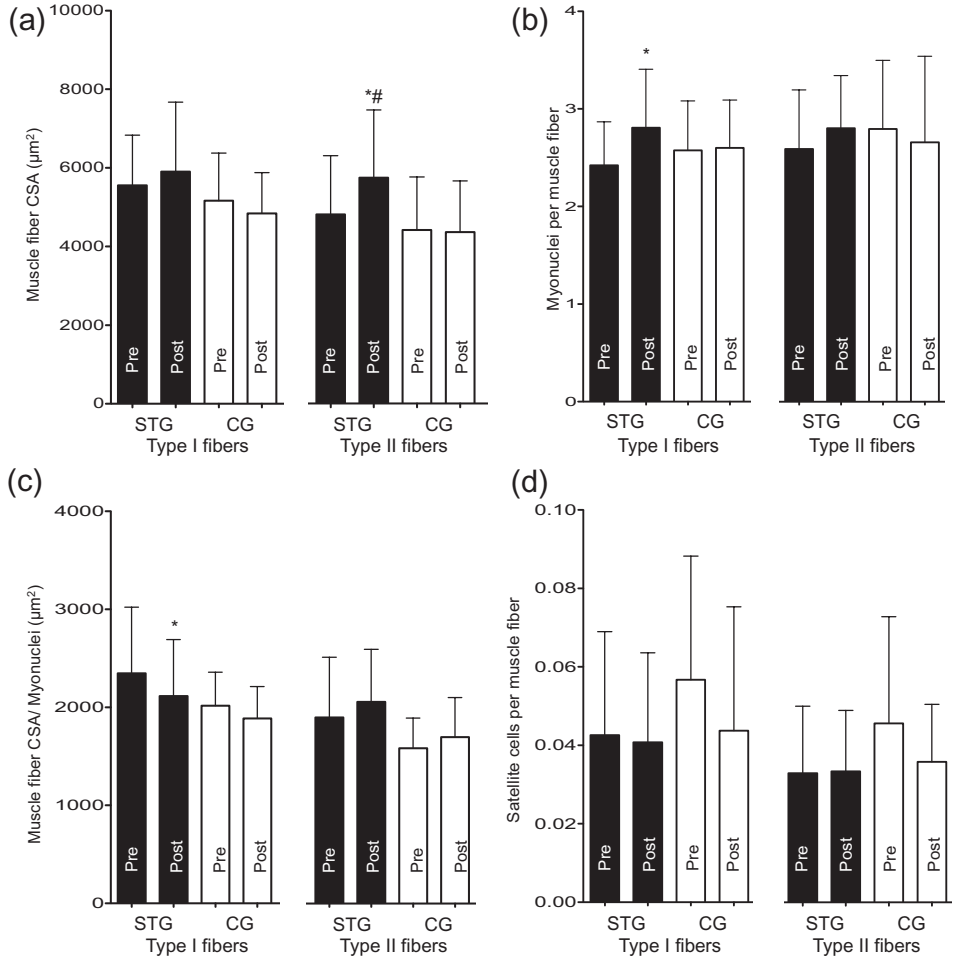

Fig. 3. Pre-post values of myofiber cross-sectional area (CSA) (a), number of myonuclei per fiber (b), myonuclear domain (c), and number of satellite cells per fiber (d), for fiber type I and II separately. #Difference in mean change from baseline to post-intervention between groups ($P \leq 0.05$). *Within-group change from baseline to post-intervention ($P \leq 0.05$).

during ADT over a longer duration than 16 weeks and with a larger sample size are needed to evaluate any fiber type-specific effects of ADT.

The increased number of satellite cells normally observed around type II fibers following strength training in healthy elderly men (Verney et al., 2008; Verdijk et al., 2009), was not seen in the present study. Satellite cell numbers did not differ when analyzed as satellite cells per muscle fiber CSA or as a percentage to the number of myonuclei (data not shown). Furthermore, it has been shown that testosterone supplementation increases the number of satellite cells in elderly men, even without strength training (Sinha-Hikim et al., 2006). Consequently, the absence of effect of strength training during ADT on the number of satellite cells, in any fiber type, points to a possible impairment of satellite cell function during ADT. Importantly, the satellite cell population seems to be capable of furnishing muscle cells with new nuclei. Therefore, the results in the present study could indicate an important role of testosterone in satellite cell proliferation in men. Another possible explanation for the lack of increase in satellite cell number in the present study could be due to a timing effect. It has been showed that the number of satellite cells per fiber increased, but the number of myonuclei remained unchanged in young healthy men performing strength training for 8 weeks while on ADT (Kvorning et al., 2014). This is the opposite of what we are reporting from our 16-week intervention. Although this remains as speculation, we might have missed the peak in satellite cell numbers, and caught the increased in number of myonuclei instead. Nevertheless, our results are in contrast to previously published

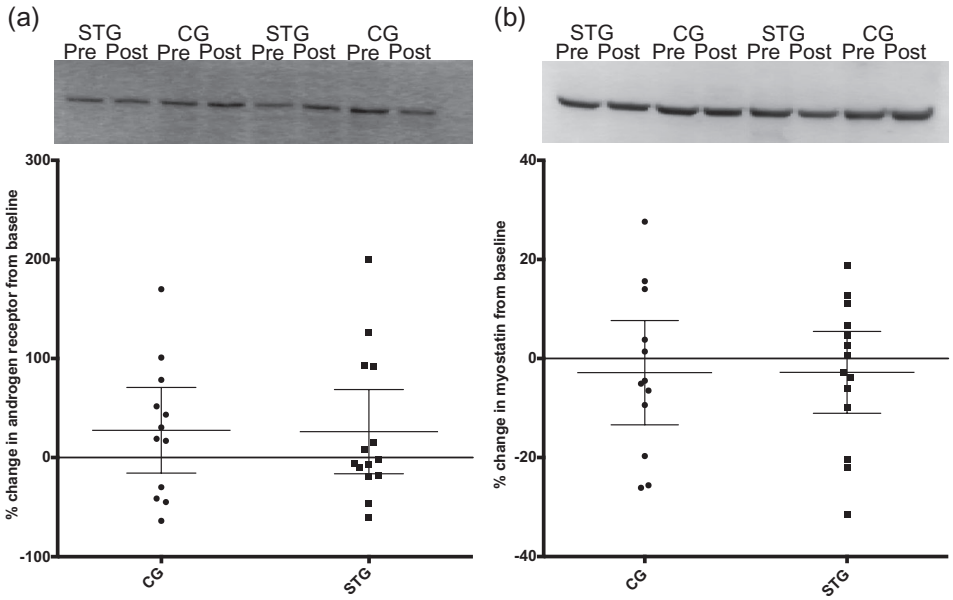

Fig. 4. Change in the content of androgen receptor (a) and myostatin (b). Figures include representative blots. Data points represent individual change from baseline; bars represent mean and 95% confidence interval.

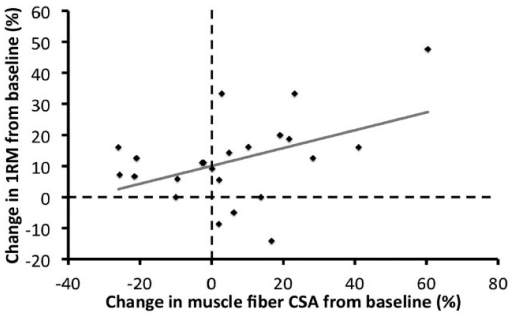

Fig. 5. Relation between the relative change in muscle strength and relative change in muscle fiber cross-sectional area (CSA) for both fiber types combined (B = 0.004, $P = 0.02$).

results from strength training in elderly (Verney et al., 2008; Verdijk et al., 2009).

In the present study, strength training did not alter the content of AR in PCa patients on ADT. The effect of strength training during ADT on protein levels of AR and myostatin has not been investigated previously. However, the AR mRNA expression remained unchanged both in the ADT group and the placebo group after strength training in young men (Kvorning et al., 2007). Furthermore, strength training does not seem to alter AR protein content in healthy men (Ahtiainen et al., 2009). On the other hand, Ahtiainen et al. (2009) reported a significant relation between the change in AR content and the change in muscle fiber CSA. However, a Pearson correlation analysis revealed that this correlation was not present in our PCa patients on ADT ($r = 0.32$; data not shown), which could indicate that the AR plays a smaller role in strength training adaptation during ADT. This, however, remains a speculation.

The protein levels of myostatin remained unchanged in the present study. There are few strength training studies reporting on protein levels of myostatin, but our results are in agreement with studies that report on changes in mRNA levels of myostatin following strength training in young healthy men (Hulmi et al., 2009), and in young healthy men on ADT (Kvorning et al., 2007). However, in elderly men, increased levels of myostatin mRNA was observed after strength training (Mero et al., 2013). The reason for this apparent discrepancy is unclear, but the effect on protein level, in the present study, may differ from the effect of mRNA level, in the cited study. Also, the castrate levels of testosterone might have influenced the results in the present study (Mendler et al., 2007). Nevertheless, the strength training program included in our study was insufficient to induce changes in the myostatin levels in PCa patients on ADT.

Although no previous studies have dealt with the effect of strength training on the muscle fiber CSA and change in knee extensor 1RM in PCa patients on ADT, there are several studies in healthy elderly men (Hikida et al., 2000; Kosek et al., 2006; Verdijk et al., 2009). All interventions were successful in increasing the maximal strength in the knee extension exercise. An important finding in our study was that the strength training

successfully increased fiber CSA in type II fibers, improving both the strength and power-generating capacity in the muscles. In addition, there was a moderate, but significant, relation between changes in muscle strength and changes in muscle fiber CSA in the present study.

In conclusion, this is the first randomized controlled trial to investigate the effect of strength training on the muscle cellular level in PCa patients on ADT. Sixteen weeks of strength training led to an increased muscle fiber CSA, and when analyzed separately the only significant CSA increase was observed in the type II fibers. Despite apparently unchanged type I fiber CSA, these fibers still had additional myonuclei, and thus the myonuclear domain was reduced. The increased number of satellite cells per fiber normally reported during strength training in healthy men was absent in the present study.

Perspectives

Loss of muscle mass, reflected by loss of lean body mass, is commonly reported in PCa patients on ADT (Storer et al., 2012), and might be counteracted by strength training (Gardner et al., 2013). Increased muscle fiber CSA after strength training, as showed in the present study, reflects this on the muscle cellular level. However, the effects seem to be somewhat smaller than expected in healthy elderly men (Kosek et al., 2006; Verdijk et al., 2009). The observed increase in type II fiber CSA may be of special importance for functional performance. Contrary to reports on healthy elderly (Verney et al., 2008; Verdijk et al., 2009), we did not observe any increased number of satellite cells per fiber in the present study. Any clinical implication of reduced satellite cell response as observed in the persent study is unclear. Nevertheless, strength training had beneficial effects on muscle cellular outcomes in PCa patients on ADT and is therefore recommended.

Key words: Androgen receptor, myonuclei, myostatin, satellite cells, testosterone, exercise, resistance training.

Acknowledgements

Thanks to all the patients for participating and to the doctors and nurses at OUS for their help with identifying and including patients. Thanks also to Ingrid Ugelstad for the invaluable help in muscle biopsy handling. The authors also thank the Department for Clinical Research Support, Oslo University Hospital for their assistance with tasks related to the data management of this study. The PEPC trial was supported by the Norwegian Foundation of Health and Rehabilitation and the Norwegian Cancer Society (HF-57004/001) and by the Regional Health Authority in Southern Norway. Additional funding was also given by Eckbo's legacy, The Radium Hospital's legacy and Trivselsanlegget's legacy.

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Paper IV

Nilsen T.S., Thorsen L., Kirkegaard C., Ugelstad I., Fosså S.D., Raastad T.: **The effect of strength training on indicators of muscle cellular stress during testosterone suppression in prostate cancer patients.** Manuscript in preparation for Journal of Endocrinology.

Appendix

Approval from the Regional Committee for Medical and Health Research Ethics

Patient information and consent form

UNIVERSITETET I OSLO

DET MEDISINSKE FAKULTET

Stipendiat Lene Thorsen
Radiumhispotalet
Montebello
0310 Oslo

Regional komité for medisinsk og helsefaglig forskningsetikk Sør-Øst B (REK Sør-Øst B)
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NO-0318 Oslo

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Dato: 17.04.2008
Deres ref.:
Vår ref.: 08/212b.2008/4062

08/212b.2008/4062 Fysisk trening og prostatakreft - (The PEPC study) Fysisk trening for pasienter med prostatakreft under hormonbehandling - en randomisert intervensjonsstudie

Komiteen behandlet søknaden i sitt møte den 10. april 2008. Prosjektet er vurdert etter lov om behandling av etikk og redelighet i forskning av 30. juni 2006, jfr. Kunnskapsdepartementets forskrift av 8. juni 2007 og retningslinjer av 27. juni 2007 for de regionale komiteer for medisinsk og helsefaglig forskningsetikk.

Saksfremstilling
Omtrent halvparten av menn som får prostatakreft hvert år i Norge får hormonbehandling. Behandlingen kan påvirke fysisk og psykisk helse og dermed helserelatert livskvalitet. Studiens hypotese er at trening kan ha en positiv effekt på flere av disse bivirkningene.

Forskningsetisk vurdering
Komiteen kan ikke se at studien reiser etiske betenkeligheter.

Forskningsbiobank
Komiteen har ingen merknader til søknad om opprettelse av forskningsbiobank og tilrår at denne opprettes.

Informasjonsskriv/Samtykkeerklæring
Komiteen har ingen merknader til informasjonsskrivet.

Vedtak
Prosjektet godkjennes. Komiteen vil videresende skjema for opprettelse av forskningsbiobank, informasjonsskriv samt komiteens vedtak til Helsedirektoratet for endelig behandling av spørsmålet om opprettelse av forskningsbiobank.

Komiteens avgjørelse var enstemmig.

Komiteens vedtak kan påklages (jfr. Forvaltningslovens § 28) til Den nasjonale forskningsetiske komité for medisin og helsefag. Klagen skal sendes til REK Sør-Øst B (jfr. Forvaltingslovens § 32). Klagefristen er tre uker fra den dagen du mottar dette brevet (jfr. Forvaltningslovens § 29). Det bes presisert hvilke vedtak/vilkår som påklages og den eller de endringer som ønskes. Se informasjon om klageadgang og partsinnsynsrett på http://www.etikkom.no/REK/klage

Med vennlig hilsen

Tor Norseth
Leder

Julianne Krohn-Hansen
Komitésekretær

Kopi: Sosial- og helsedirektoratet

UNIVERSITETET I OSLO

DET MEDISINSKE FAKULTET

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Dato: 08.07.2008
Deres ref.:
Vår ref.: 08/212b.2008/4062

08/212b.2008/4062 Fysisk trening og prostatakreft - (The PEPC study) Fysisk trening for pasienter med prostatakreft under hormonbehandling - en randomisert intervensjonsstudie

Vi viser til e-post mottatt 24.06.08 med forespørsel om godkjenning av diverse endringer i prosjektet og med følgende vedlegg: Protokoll, versjon 24. juni 2008 og reviderte informasjonsskriv.

Komiteen har ingen merknader til de foreslåtte endringene i studien.

Vi ønsker lykke til videre med prosjektet.

Med vennlig hilsen

Tor Norseth
Leder

Julianne Krohn-Hansen
Komitésekretær

Kopi: Sosial- og helsedirektoratet

RADIUMHOSPITALET
Nasjonalt kompetansesenter for studier av langtidseffekter
etter behandling for kreft

Skannes til **J5**

Forespørsel om deltakelse i forskningsprosjektet

"Fysisk trening og prostatakreft"

Bakgrunn og hensikt
Dette er et spørsmål til deg om å delta i en forskningsstudie for å undersøke hvilken effekt styrketrening har på fysisk og psykisk helse under hormonbehandling for prostatakreft. Tidligere undersøkelser har vist at hormonbehandling kan påvirke bl.a. fysisk funksjon (styrke og kondisjon), kroppssammensetning (muskelmasse, bentetthet og fettprosent), tretthet og psykisk helse. Dette kan påvirke livskvalitet. Basert på tidligere studier er vår hypotese at styrketrening kan redusere disse potensielle bivirkningene. Det er derfor vi nå tar kontakt med pasienter som får hormonbehandling for nydiagnostisert prostatakreft ved Oslo universitetssykehus, Radiumhospitalet og Ullevål.

Nasjonalt kompetansesenter for studier av langtidseffekter etter behandling for kreft, Oslo universitetssykehus, Radiumhospitalet er ansvarlig for undersøkelsen.

Hva innebærer studien?
Dersom du ønsker å delta i studien vil vi undersøke helsen din etter fjerde hormonsprøyte. Dette vil vi gjøre ved hjelp av spørreskjemaer, fysiske tester, dexa-scan, blodprøver og muskelbiopsi (mer informasjon se vedlegg A). Etter denne testen vil du trekkes til å være med i enten en treningsgruppe eller en kontrollgruppe. Dersom du blir trukket til å være med i treningsgruppen skal du i 16 uker gjennomføre 3 styrketreningsøkter per uke (á ca. 60 min). To av disse øktene vil gjennomføres med personlig trener og den tredje vil gjennomføres som egentrening. All trening vil foregå på Norges idrettshøgskole, som ligger ved Sognsvann i Oslo. Dersom du trekkes til kontrollgruppen skal du fortsette med samme trening og mosjon som du har gjort til nå, men du vil få tilbud om samme program som treningsgruppen senere. Umiddelbart etter avsluttet treningsperiode vil alle deltagerne testes på nytt. Eventuelle medisinske grunner for ikke delta vil bli undersøkt og avklart av behandlende onkolog.

Mulige fordeler og ulemper
Dersom du blir trukket til treningsgruppen vil du følges opp av erfarne trenere. Du vil få et tilpasset styrketreningsprogram, som vil justeres slik at du stadig blir sterkere. Du må imidlertid være klar over at det å trene 3 ganger i uken til tider *kan* oppleves som en belastning.

(fortsettelse baksiden av arket)

Det er 50 % sjanse for at du blir trukket til å være i kontrollgruppen. Da kan du fortsette med din normale trening og mosjon, men ikke starte med et strukturert styrketreningsprogram før etter tredje evaluering. Da vil du få tilbud om det samme treningsprogrammet som treningsgruppen. Du vil på samme måten som treningsgruppen gjennomføre alle testene. Dette vil gi en mulighet til å følge med på egen helsetilstand. Av testene som skal gjennomføres er det bare muskelbiopsien som kan være ubehagelig. Denne trenger du imidlertid ikke ta, selv om du ønsker å delta i studien.

Hva skjer med prøvene og informasjonen om deg?
Prøvene som blir tatt av deg og informasjonen som registreres om deg skal bare brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine personlige opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Det vil ikke være mulig å identifisere deg til resultatene av studien når disse publiseres. All informasjon som er registrert om deg vil slettes når studien er avsluttet og data er lagret en viss tid etter at prosjektet er avsluttet for mulig kontroll og etterprøvning. Dette vil skje i løpet av 2020.

Frivillig deltagelse
Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for din videre behandling.

Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte prosjektleder Lene Thorsen på telefonnummer 22 93 51 81.

Ytterligere informasjon om studien finnes i kapittel *A – utdypende forklaring av hva studien innebærer.*
Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring.*

Samtykkeerklæring følger etter kapittel B.

Kapittel A- utdypende forklaring av hva studien innebærer

Spørreskjema og fysiske tester
Du vil bli bedt om å svare på et spørreskjema som inneholder spørsmål knyttet til, demografiske forhold, tidligere aktivitetsnivå, kosthold, angst og depresjon, tretthet og helse-relatert livskvalitet. I tillegg til å svare på spørreskjemaet, skal du gjennomføre noen fysiske tester. Dette skal gjøres ved to ulike tidspunkter på Norges idrettshøgskole (ved Sognsvann i Oslo) og Sentrum Røntgeninstitutt (Oslo City i Oslo).

I tillegg til blodprøver vil følgende målinger/tester foretas:
- Kondisjon
    - Kondisjon vil måles ved hjelp av en såkalt *"Shuttle walk" test*. Du vil bli bedt om å gå med jevn fart, frem og tilbake, langs en strekning på 10 meter. Et signal vil indikere når du skal nå motsatt side. Tiden mellom signalene reduseres fortløpende og du må derfor gå raskere. Testen avsluttes når du ikke klarer å opprettholde nødvendig fart. Pulsen din vil måles og registreres umiddelbart før og etter testen.
    - Kondisjon vil også måles ved hjelp av en *"trappetest".* Du skal gå så fort du kan en etasje (20 trinn av 16 cm). Tempoet tilpasses slik at testen gjennomføres trygt uten bruk av rekkverk. Du vil også bli bedt om å gjennomføre samme testen med belastning.

- Muskelstyrke
    - Benstyrke vil måles ved en såkalt *"Sitte-til-stå test"*. Du sitter på en stol uten armlener. Du skal reise deg og sette deg igjen så mange ganger du klarer på 30 sekunder. Dersom det er behov for å bruke armene vil det være mulig.
    - Muskelstyrke vil også måles ved hjelp av styrketreningsapparater. To tester for overkropp og to tester for bena vil gjennomføres. Testene vil gjøres med gradvis økende belastning, du vil bli bedt om å gjennomføre øvelsene til du ikke greier å gjennomføre testen, p.g.a. for tung belastning.

- Kroppssammensetning
    - Kroppssammensetning vil måles ved hjelp av dexa-scan. Dette er et røntgenbasert instrument som måler bentetthet. I tillegg gir undersøkelsen svar på fordeling av muskler, fett og benvev i kroppen din. Undersøkelsen gjennomføres på Sentrum Røntgeninstitutt i Oslo, den er smertefri og medfører liten strålebelastning. I tillegg vil høyde, vekt og hofte-liv mål registreres.

- Muskelsammensetning
    - Muskelsammensetning vil måles ved hjelp av muskelbiopsi. En liten bit av lårmuskelen din vil tas ut ved hjelp av en sprøyte. Du vil få lokal bedøvelse og erfaringer fra Norges Idrettshøgskole viser minimale plager for den som testes. Undersøkelsen vil gjøres av lege eller annet helsepersonell med lang erfaring med denne type undersøkelse. Denne testen er imidlertid helt frivillig, selv om du ønsker å være med på undersøkelsen.

(fortsettelse baksiden av arket)

Treningsprogrammet
Dersom du vurderer å si ja til å være med må du være inneforstått med hva deltagelse i prosjektet innebærer. Det er 50 % sjanse for at du trekkes til treningsgruppen, uten at prosjektansvarlige har noen innflytelse på hvem dette blir. Det er viktig at du på forhånd er motivert for å gjennomføre hele treningsprogrammet og alle testene.

Deltagere i treningsgruppen:
- skal starte treningsprogrammet etter fjerde hormonsprøyte
- skal fullføre et 16 uker langt styrketreningsprogram
- skal trene totalt tre timer per uke, fordelt over tre dager på Norges idrettshøgskole
- vil få et tilrettelagt styrketreningsprogram, som skal justeres etter hvert som du blir sterkere
- skal gjennomføre to økter med trener og en alene
- skal testes totalt to ganger
- vil få tett oppfølging av trener
- må selv dekke utgifter knyttet til transport til og fra testene og treningene

Kontrollgruppen:
- skal fortsette med samme aktivitetsnivå som de har frem til de fikk høre om denne studien
- må ikke starte systematisk styrketrening før etter tredje test/evaluering
- skal testes totalt to ganger
- vil få tilbud om samme treningsprogram som treningsgruppa etter tredje evaluering.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern
Opplysninger som registreres om deg er knyttet til din fysiske og psykiske helse. Du vil på to ulike tidspunkter bli bedt om å svare på et spørreskjema, gjennomføre tester for å kartlegge kondisjon, styrke og kroppssammensetning, samt ta blodprøver for å undersøke hormonnivå og lipidprofil. Medisinske data vil hentes fra din sykehusjournal. Noen utvalgte personer i prosjektgruppa vil ha tilgang på datamaterialet. Oslo universitetssykehus ved administrerende direktør er databehandlingsansvarlig.

Biobank
Blodprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank ved Oslo universitetssykehus, Radiumhospitalet. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Prof. dr.med. Sophie D. Fosså er ansvarlig for biobanken. Biobanken planlegges å vare til 2020. Etter dette vil materiale og opplysninger bli ødelagt etter interne retningslinjer.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi
Det er ingen utfordringer knyttet til etiske eller praktiske sider ved økonomien i prosjektet. Prosjektleders lønn er finansiert gjennom forskningsmidler fra Helse og Rehabilitering, gjennom Kreftforeningen. Det finnes ingen interessekonflikter mellom sponsorer og studien.

Forsikring
Du er forsikret i henhold til reglene i Pasientskadeloven og Norsk pasientskadeerstatning (NPE-ordningen).

Informasjon om utfallet av studien
Du har rett til å få informasjon om utfallet/resultatet av studien.

(fortsettelse baksiden av arket)

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)