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# A 4-week exercise and protein program improves muscle mass and physical functioning in older adults – A pilot study

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ABSTRACT

*Background:* Prehabilitation might attenuate common surgery-induced losses in muscle mass and physical performance. Beneficial effects of physical exercise with protein supplementation have been reported in older adults, but typically after an intervention of at least 12 weeks. The time-window for pre-surgery training is often limited to around 30 days, and it is not known if it is possible to achieve comparable results in such a short time window.
*Objectives:* The aim of this study was to pilot-test the effectiveness of a controlled four-week combined exercise and protein supplementation program on skeletal muscle-related outcomes in a Dutch older adult population.
*Design:* This study was a one-armed pilot trial.
*Participants:* Seventeen older men and women, aged 55-75y, not scheduled for surgery.
*Intervention:* A 4-week intervention program consisting of a twice-weekly supervised resistance and high-intensity aerobic exercise training of 75 min, combined with daily protein supplementation (2 doses of 15.5 g/day at breakfast and lunch).
*Measurement:* After two and four weeks, isometric quadriceps maximal voluntary contraction (MVC) was assessed via Biodex and quadriceps cross-sectional area (CSA) via magnetic resonance imaging. Other outcome measures were handgrip strength, chair rise time and maximal aerobic capacity ($VO_2$-max), as assessed from a submaximal exercise test.
*Results:* Compliance to the supervised training sessions (99.3%) and the protein supplementation (97%) was very high. The 4-week exercise and protein program led to an increase in quadriceps CSA of 2.3 ± 0.7 $cm^2$ ($P = 0.008$) in the dominant leg and 3.2 ± 0.7 $cm^2$ ($P < 0.001$) in the non-dominant leg. Isometric quadriceps MVC increased in the dominant leg (Δ14 ± 4 Nm, $P = 0.001$) and in the non-dominant leg (Δ17 ± 5 Nm, $P = 0.003$). Chair rise test time improved with −3.8 ± 0.5 s ($P < 0.0001$), and $VO_2$-max improved with 3.3 ± 1.1 ml/min/kg ($P = 0.014$). We observed no changes in body weight and handgrip strength.
*Conclusion:* A 4-week exercise and protein intervention led to improvements in muscle-related outcomes in older adults with low levels of physical activity.

## 1. Introduction

Surgery largely impacts the physical status of patients (Dronkers et al., 2016). A surgical procedure induces a stress response, which alters the functioning of different organs and physiological systems (Giannoudis et al., 2006). The surgery-induced stress response is

*Abbreviations:* BMI, Body Mass Index; bpm, beats per minute; CSA, cross-sectional area; HR, hear rate; HRR, heart rate reserve; METmin, metabolic equivalent minutes; MPC-80, milk protein concentrate, consisting of 80% protein; MRI, Magnetic Resonance Imaging; MVC, maximal voluntary contraction; Nm, Newton meter; PARQ, Physical Activity Readiness Questionnaire; rpm, rotations per minute; SQUASH, Short Questionnaire to Assess Health Enhancing Physical Activity; VO2-max, maximal aerobic capacity; W, Watt

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thought to serve as a protective mechanism, as it enables the body to provide the essential substrates needed for the healing process (Desborough, 2000). In order to provide these substrates, catabolic processes are activated, which lead to an increased breakdown of muscle proteins (Gillis and Carli, 2015; Weimann et al., 2017; Kehlet, 1997). The surgery-induced loss of muscle mass can have serious consequences on the daily functioning of older patients, who often already experience a certain degree of catabolism before surgery (Gillis and Carli, 2015; Gillis and Wischmeyer, 2019; Carli and Ferreira, 2018). Older adults with a compromised physical status before surgery are especially at risk of negative post-surgery outcomes such as prolonged hospitalisation, higher risk of infections, and death (Hewitt et al., 2018; Lieffers et al., 2012; Makary et al., 2010).

The number of older adults undergoing surgery is steadily increasing due to changing demographics (Lin et al., 2016). In the case of elective or semi-elective surgery, the surgery is planned and can be prepared for. The preparation window offers possibilities for physical training prior to surgery, a concept described in the literature as prehabilitation or “better in, better out” (Hoogeboom et al., 2014). The goal of this physical preparation is to improve the functional capacity of an individual to withstand the stressor of the surgical procedure (Ditmyer et al., 2002). A prehabilitation program typically consists of a multimodal approach in which aerobic exercise, resistance training, and increased protein intake are combined to employ their synergetic effects on muscle mass and physical performance (Carli and Ferreira, 2018). Recently, systematic reviews by Moran et al. (Moran et al., 2016) and Hughes et al. (Hughes et al., 2019) concluded that prehabilitation improves postoperative outcomes, but that there is a need to explore the potential for interventions that achieve maximum improvements in physical status within 30 days.

The short time window of around 30 days in which patients can be trained before surgery forms the main threat to the success of prehabilitation. Multiple studies have shown improvements in lean body mass and strength in older adults after a training period of around four months (Peterson et al., 2011; Peterson et al., 2010). It is not yet known if one month of training can already induce relevant benefits for this population. Therefore, before the further implementation of prehabilitation in older adults is warranted, we need to know whether improvements in muscle mass and physical performance can be achieved in such a short time window. The present study aims to assess the effects of an intense four-week combined exercise and protein supplementation program on muscle strength, muscle mass, and aerobic capacity in older adults.

## 2. Methods

### 2.1. Study design and participants

This 4-week single-arm repeated measurements pilot-study included 18 untrained healthy, not physically active men and women who were not scheduled for surgery. Potential participants were recruited from a volunteer database from Wageningen University and Research, and eligibility was assessed via five questionnaires: (Dronkers et al., 2016) a screening questionnaire regarding the inclusion and exclusion criteria, (Giannoudis et al., 2006) a screening questionnaire regarding the demographics of the participants, (Desborough, 2000) the Short Questionnaire to Assess Health Enhancing Physical Activity (SQUASH) (Wendel-Vos et al., 2003) to assess current activity level, (Gillis and Carli, 2015) the Physical Activity Readiness Questionnaire (PARQ) and (Weimann et al., 2017) a Magnetic Resonance Imaging (MRI) screening questionnaire.

Eligible participants were those between 55 and 75 years, who performed less than 30 min of physical activity of at least a moderate intensity on at least five days per week, and who did not participate in a structured exercise program the last three months before recruitment. Exclusion criteria were; having an allergy for (or being sensitive to) milk proteins, being lactose intolerant, being diagnosed with renal insufficiency (estimated glomerular filtration rate < 60 ml/min/ 1.73 m2), being diagnosed with cancer for which currently treated, having a diet which affects protein intake, having a contraindication for MRI scanning or exercise training, participating in another intervention trial, not being able to understand Dutch, and not having a general physician. Study outcome assessment (at week 0, 2 and 4) and supervised training sessions were performed at hospital Gelderse Vallei in Ede, The Netherlands, between April 2016 and July 2016. The study was approved by the medical research ethics committee and is registered at The Netherlands National Trial Register (NTR5701). For each participant, informed consent was obtained.

### 2.2. Intervention

The intervention consisted of an exercise program and protein supplementation. The exercise program contained both supervised exercise training sessions and unsupervised home-based exercise guidelines. The supervised exercise training of approximately 75 min took place at the department of physical therapy of the Gelderse Vallei Hospital in Ede and was given twice a week. An experienced physiotherapist supervised the training sessions. During these training sessions, the participants performed 30 min of resistance training, followed by 30 min of high-intensity interval aerobic training. Resistance training included the following exercises: Leg Press (Technogym, Rotterdam, The Netherlands), Leg Extension, Latissimus Dorsi (Lat) Pulldown, and Chest Press (Lifefitness, Barendrecht, The Netherlands). All exercises were performed in sets of three, with 12 repetitions each set and with one-minute rests between the sets. Resistance exercises were performed at 12-Repetition Maximum (12-RM) with a perceived exertion rate between 13 and 15 on the Borg-scale (Scherr et al., 2013). If needed, resistance was increased to assure that the 13–15 Borg-scale perceived exertion rate was reached during all training sessions.

High-intensity interval aerobic training was performed on a cycle-ergometer (Ergoline, Bitz, Germany) and consisted of four high-intensity bouts aimed to reach 90% of the calculated heart rate reserve (HRR) and a perceived exertion rate of 15–17 on the Borg scale. Each bout lasted one to three minutes and was alternated by three minutes of cycling at a lower intensity of 50–60% of the calculated HRR and perceived exertion rate between 11 and 13 on the Borg-scale. All training sessions included a 10-min warm-up and a five-minute cool down on a cycle-ergometer with an intensity of 20–30 W at 65–80 rotations per minute (rpm). Between the supervised training sessions, there was a rest period of at least 48 h. Between supervised training sessions and outcome measurements, a rest period of at least 24 h was scheduled. Participants were divided into two groups. Participants in group one attended the training sessions every week on Monday from 2.30 p.m. to 3.45 p.m. and on Thursday from 11.15 a.m. to 12.30 p.m. Participants in group two trained every week on Monday from 3.45 p.m. to 5 p.m. and on Thursday from 1 p.m. to 2.15 p.m.

With regard to the home-based exercise guidelines, participants were instructed to be moderately active for a minimum of 30 min (dividable into parts of at least 10 min) on all days that no supervised training was performed. Participants were given a Borg scale, along with the explanation that moderate exercises are those scoring 12 to 15 on this scale. Examples of typical moderate-intensity activities such as walking and cycling were given. Participants were instructed to register their daily physical activity in a diary with the corresponding perceived exertion on a Borg scale.

During the intervention period, all participants consumed 250 ml of a protein supplement (FrieslandCampina Consumer Products Europe, Wageningen, The Netherlands) twice a day in addition to their habitual diet. Per portion, the protein supplements contained 397.8 kJ, 15.5 g of protein (coming from milk protein concentrate (MPC-80)), 7.3 g of lactose and 0.5 g of fat (Table 1). With this protein dose, the aim was to achieve (1) a protein intake of 1.2–1.5 g/kg/d, (2) a breakfast and

**Table 1**
Nutritional profile of the protein supplement.

| Nutrient | Per portion (250 ml) |
|---|---|
| Energy (kcal) | 95 |
| Fat (g) | 0.5 |
| Lactose (g) | 7.3 |
| Calcium (g) | 0.4 |
| Protein (g) | 15.5 |
| Alanine (g) | 0.5 |
| Arginine (g) | 0.7 |
| Aspartic acid (g) | 1.2 |
| Cysteine (g) | 0.1 |
| Glutamic acid (g) | 3.3 |
| Glycine (g) | 0.3 |
| Histidine (g) | 0.4 |
| Isoleucine (g) | 0.8 |
| Leucine (g) | 1.5 |
| Lysine (g) | 1.3 |
| Methionine (g) | 0.4 |
| Phenylalanine (g) | 0.7 |
| Proline (g) | 1.6 |
| Serine (g) | 0.9 |
| Threonine (g) | 0.7 |
| Tryptophan (g) | 0.2 |
| Tyrosine (g) | 0.8 |
| Valine (g) | 1.1 |

lunch protein intake of ≥25 g, while (3) remaining below an acceptable level of 25% of daily energy from protein. The participants ingested the protein supplements directly after breakfast and lunch. On supervised training days, the participants consumed the protein supplements within 30 min after training instead of directly after lunch. Participants were instructed to return empty bottles and to record supplement intake on a calendar for compliance monitoring.

### 2.3. Outcome measurements

Quadriceps strength, expressed as the maximal voluntary contraction (MVC), was assessed via isometric dynamometry (Biodex System 3, Biodex Medical Systems, New York). Participants were seated with their knees flexed at 60 degrees, stabilised by straps at the hip, shoulders, and upper leg to prevent unwanted movements. Participants performed one submaximal contraction to familiarise with the technique, followed by three 5 s MVC's with 30 s rest between contractions for each leg. The highest torques (Nm) of the dominant and non-dominant leg were used for analysis.

Quadriceps mass, expressed as quadriceps cross-sectional area (CSA), was measured using MRI scanning with a 3-T Siemens Magnetom Verio Syngo MR B19 MRI scanner (Siemens AG, Erlangen, Germany). Participants lay supine in the MRI scanner, with a vitamin E capsule taped on the right leg at the midpoint between the iliac crest and the upper edge of the patella. The vitamin E capsule was used as a mark during the analysis of the MRI images. A body coil was placed over the legs, with the midpoint of the coil above the vitamin E marker. During each measurement, both upper legs were imaged. The MRI scanner used 2D spin-echo pulse sequences to obtain T1-weighted images (TR: 700 ms, TE: 10 ms, flip angle: 140 degrees, turbo factor: three, acquisition time: three minutes). Eventually, 50 slices of five mm were obtained for each leg, and for each leg, the slice in which the vitamin E capsule was best visible was selected for analysis. Analysis of the MRI images (TIFF format, 16-bit grayscale) was performed in the software program ImageJ (Bonekamp et al., 2007) by one analyst. Quadriceps muscle tissue was manually distinguished from other muscles and other tissues. When necessary, a threshold was manually set to obtain a clear border between quadriceps muscles and other tissues. After all area measurements were performed, the analyses of the first six slices were discarded and repeated by the same analyst to reduce possible learning-induced intra-observer variation that likely occurred. A second observer re-analysed a random subgroup of eleven images to assess inter-observer variation as well, which was less than 5% between both observers. In this way, from all measurement time points, mid-quadriceps CSA in $cm^2$ of both legs was assessed and used for analysis.

Muscle quality was expressed as torque per $cm^2$ of quadriceps CSA and was calculated for both legs by dividing peak torque by quadriceps CSA. Quadriceps power was assessed via the 10-times chair rise test on a 45-cm high chair (Csuka and McCarty, 1985). Handgrip strength (kg) of both hands was measured with the use of a hand dynamometer (Jamar®, Jackson, Michigan) (Roberts et al., 2011), in a seated position with the elbow flexed at 90 degrees. Participants completed three measurements per hand, starting with the non-dominant hand and alternating between hands. The maximum reading per hand was used for analysis. During the screening procedure, height (m) of each participant was obtained with the use of a stadiometer. In addition, weight (kg) was obtained during each visit with a calibrated analogue scale. Participants were instructed not to wear heavy clothing when weighting. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Physical activity levels were obtained with the use of an accelerometer (PAM AM300, PAM BV). All participants wore the accelerometer during two periods of seven consecutive days: before the start of the intervention period and during the final week of the intervention period. From the results of the accelerometer, the total metabolic equivalent minutes (METmin) per day that contributed to all daily activities were calculated for each participant.

Aerobic capacity, expressed as maximal oxygen consumption ($VO_2$-max), was estimated with the Åstrand-Rhyming submaximal exercise test on a stationary bicycle (Ergoline, Bitz, Germany) (Åstrand and Ryhming, 1954). During a warm-up phase, the initial workload (50 W) was increased every minute by 50 W for males and 25 W for females until a steady heart rate around 120 bpm was achieved. When this steady heart rate was achieved, the 6-min exercise test started. Heart rate (HR) was recorded every minute with a Polar HR sensor. VO2-max was estimated with the use of the mean HR of the fifth and the sixth minute, the Åstrand-nomogram, and a validated correction factor for age (Noonan and Dean, 2000; Astrand, 1960). If the HR differed more than five beats between minute five and six, the test was prolonged with a maximum of three minutes to achieve a more steady HR. Over the repeated measurements, room and time of day were kept identical for each participant.

Dietary intake was assessed by self-administered web-based 24-h recalls (Compl-eat, Wageningen University, Wageningen, NL), on two random days in the week before the start of the intervention, and on two random days in the final week of the intervention. Trained dietitians checked the recalls, and calculated total energy and nutrient intake by using the 2013 Dutch food composition database.

### 2.4. Statistical analysis

Sample size ($n = 18$) was calculated by using power calculation software (G*Power, Heinrich-Heine-University, Düsseldorf), by using a two-tailed alpha of 0.05, a beta of 80%, a drop-out rate of 20% and an estimated difference of 20 ± 25 Nm in peak isometric knee extension torque. This difference was estimated by using an expected clinically relevant increase of 10% from normal average maximum isometric knee extension torque of 200 Nm as measured by Biodex in adults aged ≥50 years (Harbo et al., 2012).

Changes over time in primary and secondary outcomes were assessed by linear mixed models, with a random intercept and a variance components covariance structure. Time was added as a fixed factor, participant as a random factor and models were adjusted for gender. Model assumptions were checked via visual inspection of QQ-plots and scatterplots. In case of a significant time-effect, Bonferroni-adjusted posthoc analyses were performed. Differences in dietary intake, exercise performance and physical activity levels between baseline and

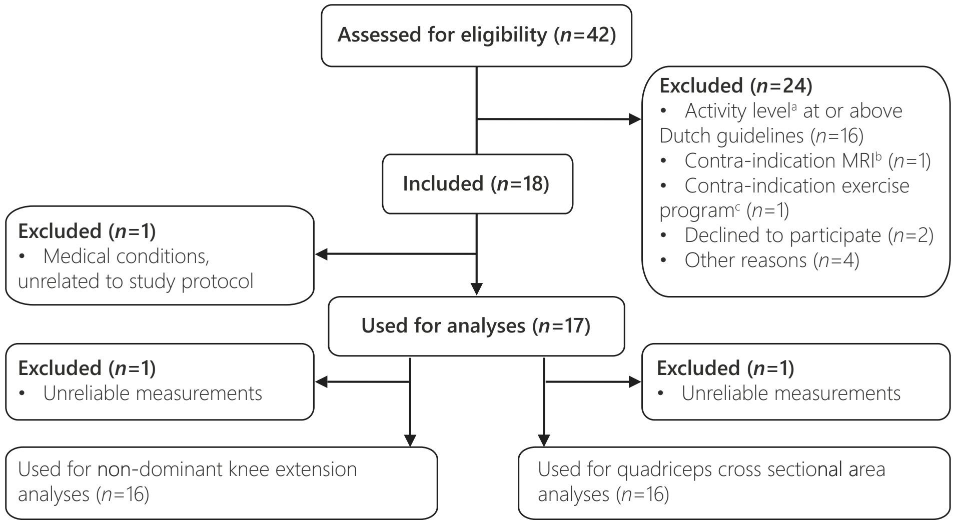


**Fig. 1.** Flowchart of participants. [a] Assessed via the Short Questionnaire to Assess Health Enhancing Physical Activity (SQUASH) questionnaire. [b] Assessed via the magnetic resonance imaging screening questionnaire. [c] Assessed via the Physical Activity Readiness Questionnaire (PARQ) questionnaire.

end of follow-up were assessed by paired sample *t*-tests. All analyses were carried out in SAS 9.4 (SAS Institute Inc.) in per-protocol fashion. Descriptives are presented as mean ± SD or median (p25 – p75), and differences are presented as mean ± SEM.

## 3. Results

A total of 18 participants were enrolled in this study (Fig. 1). One participant dropped out before the week 2 measurement due to medical conditions unrelated to the study protocol. The remaining 17 participants completed all measurements, but one participant was excluded for the non-dominant knee extension analysis, due to the inability to perform a maximum effort caused by pain complaints. Another participant was excluded from quadriceps cross-sectional analyses due to failed slice identification. Baseline characteristics of the 17 completers are presented in Table 2. The study population consisted of an equal proportion of males and females, with a median age of 71 years and a median BMI of 26.0 kg/m$^2$. Participants were active for, on average, 430 ± 134 METmin per day.

Changes in primary and secondary outcomes are presented in Fig. 2 and Table 3. Quadriceps CSA increased between week 0 and week 4 by 2.3 ± 0.7 cm$^2$ ($P$ = 0.008) in the dominant leg and by 3.2 ± 0.7 cm$^2$ ($P$ < 0.001) in the non-dominant leg. In the non-dominant leg, a significant increase in quadriceps CSA was observed after two weeks (Δ2.1 ± 0.7 cm$^2$, $P$ = 0.005). Isometric knee extension torque increased significantly in the dominant leg (Δ14 ± 4 Nm, $P$ = 0.001) and the non-dominant leg (Δ17 ± 5 Nm, $P$ = 0.003) between week 0 and week 4. In the total sample, muscle quality only tended to increase in the dominant leg (Δ0.15 ± 0.06 Nm/cm$^2$ = 0.054; P for time-effect 0.044). The time needed to complete the 10-times repeated chair rise test decreased by 4 ± 1 s ($P$ < 0.0001) between week 0 and week 4. Estimated $VO_2$Max increased with 3.3 ± 1.1 ml/min/kg ($P$ = 0.014). Handgrip strength and body weight did not change over the 4 weeks.

Individual responses to the training program are shown in Fig. 2. Quadriceps CSA decreased in both legs in two participants (Fig. 2A and C, ID = 2 and ID = 10). Muscle quality increased by 1.0 Nm/cm$^2$ in the non-dominant leg of one participant (Fig. 2A-D, ID = 11), as a result of a 45% increase in non-dominant knee extension torque and a 3% increase in quadriceps CSA.

Compliance to the supervised exercise sessions was 99.3%. The workload of the leg-press exercise increased on average by 41 ± 3 kg ($P$ < 0.0001), and the workload of the high-intensity interval training increased by 7 ± 3 W ($P$ = 0.027). During the high-intensity intervals, the 90% HRR was reached during at least one out of four intervals by $n$ = 5 in week 1, $n$ = 10 in week 2, $n$ = 7 in week 3 and $n$ = 8 in week 4. Participants increased their physical activity level on average by 56 ± 24 METmin/day between week 0 and week 4 ($P$ = 0.031). One supervised training sessions accounted for approximately 363 METmin, meaning that any increase lower than 100 METmin/day on average for the final week indicates a decrease in non-supervised activity, and thus low compliance to home-based exercises.

On average, participants consumed 54 of the 56 protein supplements (97% compliance, range: 91–100%). Protein intake increased from 0.9 ± 0.3 g/kg/d in week 0 to 1.2 ± 0.2 g/kg/d in week 4 (Δ0.4 ± 0.1 g/kg/d, P < 0.0001), while total energy intake did not increase (week 0, 6942 ± 2296 KJ/d; week 4, 6816 ± 2400 KJ/d; Δ-125 ± 538 KJ/d, $P$ = 0.819) The proportion of daily energy received from protein increased from 16 ± 3% to 22 ± 4% over the course of the intervention (Δ6 ± 1%, P < 0.0001). Habitual energy and protein intake, without the energy and protein from the supplements, did not change between week 0 and week 4 (Energy intake week 0, 6942 ± 2296 KJ/d; week 4, 6626 ± 2400 KJ/d; Δ-315 ± 538 KJ/d, $P$ = 0.566; Protein intake week 0, 0.9 ± 0.3 g/kg/d; week 4, 0.8 ± 0.2 g/kg/d; Δ-0.1 ± 0.1 g/kg/d, $P$ = 0.442). Between week 0 and 4, protein intake during breakfast increased from 10 ± 8 g to 25 ± 7 g (Δ15 ± 1 g, P < 0.0001), and protein intake during lunch

**Table 2**
Baseline characteristics.

| | Mean ± SD (unless stated otherwise) |
|---|---|
| Male, % (n) | 53 (9) |
| Age (y), median (IQR) | 71 (69–74) |
| Body weight (kg) | 76.8 ± 9.2 |
| Height (cm) | 169 ± 8 |
| BMI (kg/m$^2$), median (IQR) | 26.0 (25.6–28.6) |
| Physical activity (METmin/day) | 430 ± 134 |
| Living situation, % (n) | |
| Independent, alone | 35 (6) |
| Independent, with roommate or partner | 65 (11) |
| Smoking, % (n) | |
| Current smoker | 6 (1) |
| Former smoker | 53 (9) |
| Non-smoker | 41 (7) |

BMI, body mass index; IQR, interquartile range; METmin, metabolic equivalent minutes; SD, standard deviation.

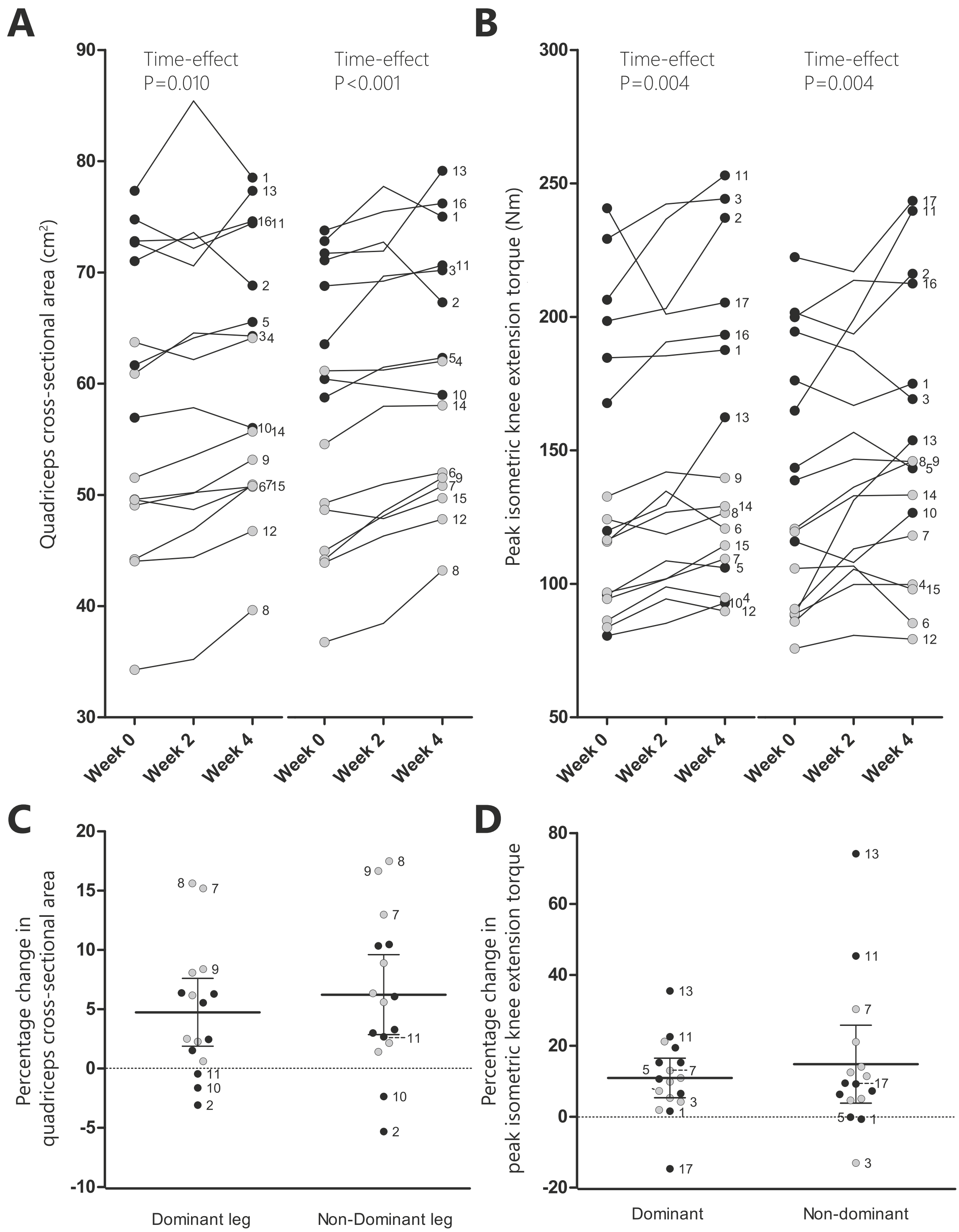


**Fig. 2.** Individual changes from week 0 to week 4 in quadriceps cross-sectional area (Panel A, overall time effects for dominant leg $P = 0.010$ and for non-dominant leg $P < 0.001$) and isometric knee extension torque (Panel B, overall time effect for both legs $P = 0.004$). Panel C and D represent relative changes, with a thick line representing the mean and error bars representing 95% confidence intervals. Grey symbols represent females, and black symbols represent males. Identification numbers are added to all participants in the top figures, and to extreme responders in bottom figures (negative responders and responders with > 15% change in cross-sectional area or > 30% change in torque).

increased from 19 ± 14 g to 31 ± 11 g (Δ12 ± 4 g, $P = 0.008$). Protein intake during dinner and snacks was 36 ± 22 g in week 0 and 37 ± 14 g in week 4 (Δ0 ± 5 g, $P = 0.949$).

All participants reported mild muscle ache at least one time during the training period. In most of the cases (61%), the muscle ache disappeared after one day, and in the other cases, the muscle ache disappeared after two (25%) or three (14%) days. Muscle ache represented 49 of all 64 adverse events reported in this study. The other adverse events consisted of falls ($n = 4$), flu-like complaints ($n = 3$), flatulence ($n = 2$), constipation (n = 2), joint pain (n = 2), back pain ($n = 1$) and light-headedness during the cycling training (n = 1).

**Table 3**
Means of primary and secondary outcomes per visit.

| | Week 0 | Week 2 | Week 4 | Time-effect |
|---|---|---|---|---|
| Quadriceps cross-sectional area ($cm^2$) | | | | |
| Dominant leg ($n$ = 16) | 58.4 ± $1.9^{a}$ | 59.5 ± $1.9^{ab}$ | 60.7 ± $1.9^{b}$ | P = 0.010 |
| Non-dominant leg (n = 16) | 57.8 ± $1.6^{a}$ | 59.8 ± $1.6^{b}$ | 60.9 ± $1.6^{b}$ | P < 0.001 |
| Isometric knee extension (Nm) | | | | |
| Dominant leg (n = 17) | 138 ± $11^{a}$ | 144 ± $11^{ab.}$ | 152 ± $11^{b}$ | P = 0.004 |
| Non-dominant leg (n = 16) | 135 ± $9^{a}$ | 145 ± $9^{ab}$ | 152 ± $9^{b}$ | P = 0.004 |
| Muscle quality ($Nm/cm^2$) | | | | |
| Dominant leg (n = 16) | 2.3 ± $0.2^{a}$ | 2.5 ± $0.2^{a}$ | 2.5 ± $0.2^{a}$ | P = 0.044 |
| Non-dominant leg ($n$ = 15) | 2.3 ± 0.2 | 2.4 ± 0.2 | 2.4 ± 0.2 | P = 0.283 |
| Grip strength (kg) | | | | |
| Dominant hand ($n$ = 17) | 27 ± 1 | 28 ± 1 | 29 ± 1 | P = 0.204 |
| Non-dominant hand (n = 17) | 28 ± 2 | 28 ± 2 | 29 ± 2 | P = 0.181 |
| 10-times repeated chair rise test (s) (n = 17) | 20 ± $1^{a}$ | 18 ± $1^{b}$ | 16 ± $1^{c}$ | P < 0.0001 |
| Estimated maximum oxygen consumption (ml/min/kg) (n = 17) | 29 ± $1^{a}$ | 31 ± $1^{ab}$ | 32 ± $1^{b}$ | P = 0.016 |
| Body weight (kg) (n = 17) | 75.8 ± 1.9 | 76.3 ± 1.9 | 76.4 ± 1.9 | P = 0.076 |

Data presented as adjusted means ± standard error. Superscript characters represent results of posthoc analyses in case of a significant time-effect. Different letters represent significant differences after Bonferroni adjustment.

## 4. Discussion

Prehabilitation is effective to improve postoperative outcomes, but the time-window in which patients can be trained is limited to around 30 days. Therefore, there is a need to explore potential interventions that achieve maximum improvements within this short time window (Moran et al., 2016). The present study revealed that a 4-week intervention consisting of supervised resistance exercise and high-intensity interval training, protein supplementation and increased daily physical activity, leads to substantial improvements in quadriceps cross-sectional area, quadriceps strength and power, and aerobic capacity in older adults.

Our 4-week intervention resulted in improvements in quadriceps strength of 10% in the dominant leg and 12% in the non-dominant leg. Earlier studies in older adults have shown that resistance exercise, with or without protein supplementation, leads to improvements in muscle strength after 12 weeks (Tieland et al., 2012; Francis et al., 2017; Holm et al., 2008; Lixandrao et al., 2016; Kongsgaard et al., 2004; Fiatarone et al., 1994). Two of these studies measured isometric knee extension strength and showed improvements of 12.7% (Francis et al., 2017) and 14.7% (Kongsgaard et al., 2004) after 12 weeks of training. The reason that we achieved similar improvements in only four weeks might be due to differences in the training program, as these studies either did only use body-weight or TheraBand resistance exercises (Francis et al., 2017) or did not provide concurrent protein supplementation (Kongsgaard et al., 2004). While our program was rather intensive, combining resistance exercise and high-intensity interval training, twice daily protein supplementation and increased daily physical activity.

We observed improvements in quadriceps CSA of 3.9% in the dominant leg and 5.4% in the non-dominant leg. To the best of our knowledge, we are one of the first to report significant increases in muscle mass in older adults after already four weeks of exercise training and protein supplementation. In many studies, changes in muscle mass are assessed for the first time after 10 to 12 weeks of intervention. One study that did report vastus lateralis CSA changes in older adults ($n$ = 6) after four weeks of resistance exercise found a similar increase of around 4%, but this increase was not significant, likely due to their limited sample size (Lixandrao et al., 2016). Another study investigated the effects of home-based exercises in a one-armed pilot study among 12 older adults, and did find a small increase of 2.6% in quadriceps CSA, but not in whole-body skeletal muscle mass (Cegielski et al., 2017). Our findings suggest that a more intense program or a concurrent improvement in nutritional status via increased protein intake is needed to achieve substantial improvements in muscle mass in the short term.

The role of protein supplementation to augment the effects of exercise programs in older adults is subject to ongoing debate (Thomas et al., 2016; Finger et al., 2015). In young volunteers, protein supplementation can improve responses to resistance exercise training (Morton et al., 2018) and early evidence suggests that it can also improve responses to aerobic exercise training (Knuiman et al., 2019). However, in older adult populations, the response to protein supplementation seems to be dependent on nutritional status at baseline (Yoshimura et al., 2017) and population (Thomas et al., 2016; Finger et al., 2015; Yoshimura et al., 2017). Although we did not assess nutritional status in our study, the nutritional intervention did improve the protein intake of our participants. Overall daily protein intake increased from 0.9 ± 0.3 g/kg/d body weight at baseline to 1.2 ± 0.2 g/kg/d during the trial. An intake of 1.2 g/kg/d is the recommended level for older individuals to support muscle mass and function with ageing (Bauer et al., 2013; Deutz et al., 2014). Moreover, the proportion of participants that reached the recommended 25 g of protein during main meals (Bauer et al., 2013; Paddon-Jones and Rasmussen, 2009) increased from 6% to 41% during breakfast, and from 17% to 65% during lunch. The increased protein intake achieved in our study might explain the differences in findings compared to the study by Dronkers et al. (Dronkers et al., 2010). In their study, no improvements in chair rise test performance or $VO_2$Max were observed, while we observed a 9% improvement in chair rise test performance and an 8% improvement in $VO_2$Max. Their participants followed an exercise training regimen that was similar to ours but without protein supplementation. In older adults, maximal muscle protein synthesis seems to be achieved by supplementing 30 to 40 g of post-exercise protein (Yang et al., 2012; Holwerda et al., 2019), considerably higher than our used protein dose of 15 g. Future prehabilitation studies might consider increasing the post-exercise protein dose to stimulate muscle mass accumulation even more.

The clinical relevance of the increase in quadriceps CSA and leg strength is dependent on expected losses during surgery and hospitalisation that in practice will follow after prehabilitation. Based on a study that quantified muscle atrophy in the first week of hospitalisation after elective hip surgery, a 3.4% decrease in quadriceps CSA was observed (Kouw et al., 2019). In the case of a pre-surgery increase in CSA of 3.9%, as observed in our study, the assumed 3.4% decrease in CSA in the first week after hospitalisation would not have resulted in a net quadriceps CSA loss. In a different study, isometric knee torque showed to decrease by 11.2% after ten days of bed rest (Kortebein et al., 2008). The 10% increase in dominant knee torque achieved by prehabilitation in our study would result in a slight net loss of leg strength in case of an assumed 11.2% loss during hospitalisation. These speculative extrapolations suggest that our prehabilitation program might be successful in preventing large losses in quadriceps CSA and knee strength in the period of −4 to +1 week around surgery. To further prevent muscle mass loss in the following weeks after hospitalisation, postoperative strategies should be added to the prehabilitation program.

Our participants showed excellent compliance to the supervised training sessions and the protein supplementation. On the other hand, the compliance to the home-based exercise was low, as indicated by an average increase of only 56 METmin/day, measured in week four of the program. Attending the supervised training sessions of that week alone would have led to an increase of around 100 METmin/day, indicating that participants decreased their unsupervised physical activity. This suggests that increasing activity beyond supervised exercise training and protein supplementation is not necessary to achieve improvements similar to our findings and that short-term training programs should focus on reaching adequate intensity rather than increasing the overall activity volume.

A clear limitation of this study is the lack of a control group. For some outcome measures, we are unable to rule out the possibility of a learning effect. The outcome measures that are most prone to a learning effect are isometric knee extension strength, chair rise test and handgrip strength. We observed no changes in handgrip strength, so this was probably not affected by a learning effect. Moreover, it is unlikely that the effects found in quadriceps strength and power are solely addressable to a learning effect, as these results are very much in line with the results of quadriceps cross-sectional area, an objective outcome that cannot be influenced by learning effects. The VO2-max test is typically prone to a learning effect. However, we used a heart rate based Åstrand test, in which physiological phenomena influence the estimated VO2-max more than mental determinants.

This study was performed in healthy older untrained adults with a low level of physical activity, who were not scheduled for surgery. The extrapolation of our findings to patient populations should be done with caution. Physiological mechanisms that are related to the reason for surgery likely alter the generalisability of our findings from one population to another. This notion is illustrated by the work of Boereboom et al., who showed that a 31-day HIIT training improved VO2max in healthy older adults (Boereboom et al., 2016), but not in colorectal cancer patients (Boereboom et al., 2019). Moreover, compliance to the intense training protocol could be compromised in an actual patient population. Explorative inspection of our data suggests that the increase in quadriceps cross-sectional area is mainly driven by the female participants, who showed average crude increases of 7% in the dominant leg and 8% in the non-dominant leg. For males, the crude increase was only 0.2% in the dominant leg and 2% in the non-dominant leg. The differential change could be explained by the lower baseline quadriceps cross-sectional area in female participants. Previous work found similar trends, although to a smaller extent (Churchward-Venne et al., 2015). Interestingly, we observed no sex differences in the relative increase in torque. More data regarding sex differences in response to short-term training protocols are needed to understand if sex-specific strategies are necessary.

To conclude, this study shows that it is possible to improve muscle mass, muscle strength and aerobic capacity already within four weeks of supervised resistance exercise training in combination with protein supplementation. Future studies are needed to confirm the findings of this pilot-study versus a control group, in preoperative patients.

## CRediT authorship contribution statement

**Pol Grootswagers:** Formal analysis, Writing - Original Draft, Writing - Review & Editing **Margot de Regt:** Conceptualization, Investigation **Jacintha Domić:** Formal analysis, Writing - Original Draft **Jaap Dronkers:** Conceptualization **Marlieke Visser:** Conceptualization, Investigation **Ben Witteman:** Conceptualization, Supervision **Maria Hopman:** Conceptualization, Supervision **Marco Mensink:** Conceptualization, Supervision.

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## Ethical approval

The study was approved by the medical research ethics committee and is registered at The Netherlands National Trial Register (NTR5701). For each participant, informed consent was obtained.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

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