DISSERTATION FROM THE
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Knut Sindre Mølmen

The impact of vitamin D_3 supplementation, chronic obstructive pulmonary disease and exercise load on resistance training-associated adaptations in older adults

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Summary

Background. Lifestyle therapy with resistance training is a potent measure to counteract age-related loss in muscle strength and mass. Unfortunately, many individuals fail to respond in the expected manner to such treatment. This phenomenon is particularly common among older adults and those with chronic diseases such as chronic obstructive pulmonary disease (COPD) and may involve endocrine characteristics such as low vitamin D status and low-grade inflammation, as well as suboptimal training protocols.

Aims. The Granheim COPD Study consisted of two studies; a *preparatory study* and a *RCT study*. COPD is associated with impaired cardiorespiratory capacity, but it remains uncertain if this affects muscular performance. Therefore, in the *preparatory study*, the aim was to compare muscular performance in three resistance exercises of the legs involving different amounts of active muscle mass in COPD and healthy control (Healthy) persons (**Paper I**). In the *RCT study*, the aim was to investigate the effects of 12 weeks of vitamin D_3 supplementation-only, followed by 13 weeks of combined vitamin D_3 supplementation and resistance training, on muscle functional and biological training-associated adaptations in a mixed group of older adults, and also to compare the muscle functional and biological effects of resistance training for COPD and Healthy, as well as high-load vs low-load resistance training (**Paper II-IV**).

Participants and methods. In the *preparatory study*, 11 COPD (GOLD grade II/III; forced expiratory volume in first second (FEV_1), 53±14% of predicted value; age 66±8 years) and 12 Healthy (FEV_1, 117±12% of predicted value; age 62±7 years) participants performed tests of muscular performance in three resistance exercises with different complexity and physiological demand; (i) one-legged knee extension, (ii) one- and (iii) two-legged leg press. In the *RCT study*, 95 older individuals (56-77 years) were randomly assigned to receive either vitamin D_3 or placebo supplementation, stratified by health status (COPD, n=24; Healthy, n=71) and sex. The intervention was initiated by 12 weeks of supplementation-only (two weeks with 10 000 international units (IU) vitamin $D_3 \cdot day^{-1}$, thereafter 10 weeks with 2 000 $IU \cdot day^{-1}$), followed by 13 weeks of combined supplementation (2 000 $IU \cdot day^{-1}$) and supervised whole-body resistance training (twice weekly). In the training sessions, leg exercises were performed unilaterally, with one leg randomized to high-load training (10 repetitions maximum; RM) and the contralateral leg randomized to low-load training (30RM). This unilateral training protocol served two purposes: i) to circumvent issues relating to conduction of training with two-legged exercises and ii) to investigate the relative efficacy of two different training modalities. Outcome measures included multiple assessments of muscle strength ($n_{variables}$=7), endurance performance ($n_{variables}$=6), muscle mass ($n_{variables}$=2), muscle quality, muscle biology (*m. vastus lateralis*; muscle fiber characteristics, RNA content including transcriptome) and health-related variables (body composition, lung function, blood, health-related quality of life). For a subset of participants (COPD, n=11; Healthy, n=12), outcome measures also included mitochondrial quantity (citrate synthase activity) and respiratory capacity. For core outcome domains (muscle strength/mass/quality and lower-limb/whole-body endurance performance), weighted combined factors were calculated from the range of singular assessments.

Main results. In the *preparatory study*, muscular performance was impaired for COPD in two-legged leg press compared to Healthy, but not in one-legged leg press, suggesting that the cardiorespiratory

limitations inherent to the disease seems to negatively influence the performance in resistance exercises involving larger amounts of active muscle mass (>one-legged leg press) (**Paper I**). In the *RCT study*, 13 weeks of resistance training increased muscle strength (13%), muscle mass (9%) and endurance performance (one-legged, 23%; whole-body, 8%), assessed as weighted combined factors, and were associated with beneficial changes in health variables (e.g. visceral fat, -6%; low-density lipoprotein levels, -4%) and muscle tissue characteristics such as muscle fiber type proportions (e.g. IIX, -3%-points), myonuclei·fiber^{-1} (30%), total RNA/rRNA abundances (15%/6-19%), and transcriptome profiles (e.g. 312 differentially expressed genes). Vitamin D_3 supplementation did not affect training-associated changes for any of the main outcome domains, despite robust increases in serum 25(OH)D levels (Δ49% vs placebo) (**Paper II**). In secondary analyses, resistance training with vitamin D_3 supplementation resulted in higher expression of gene sets involved in vascular functions in muscle tissue and larger strength gains in participants with high fat mass, compared to resistance training-only (**Paper II**). In the *RCT study*, COPD participants displayed well-known disease-related pathophysiologies compared to Healthy at baseline, including impaired lung function, higher levels of systemic low-grade inflammation (serum c-reactive protein levels), lower muscle mass and functionality, and muscle biological aberrancies such as lower mitochondrial oxidative capacity, higher proportions of muscle fiber type IIA and IIX and genome-wide differences in transcriptome profiles (differential mRNA expression of 227 genes) (**Paper III-IV**). However, despite these adversities, COPD participants showed similar or larger improvements to resistance training for health and muscle functional and biological variables compared to Healthy (**Paper III-IV**). 10RM and 30RM training were associated with similar ratings of perceived exertion. When combining the data from the two study clusters (i.e. COPD and Healthy), 30RM training led to more pronounced increases in lower-body muscle mass compared to 10RM, while 10RM training led to a larger fiber type conversion from IIX to IIA and larger improvements in cycling economy compared to 30RM, but this was not associated with differential changes in muscle strength and muscle performance between the two exercise modalities. Furthermore, 10RM resistance training was associated with improved ability to maintain bone mineral density compared to 30RM resistance training.

Conclusions. Vitamin D_3 supplementation did not affect muscular responses to resistance training. This rejects the notion that vitamin D_3 supplementation is necessary to obtain adequate muscular responses to resistance training in the general older population, at least for the enrolled clusters of COPD and Healthy participants with mostly sufficient vitamin D levels at pre-RCT. Although COPD participants showed clear functional and biological deviations compared to Healthy at baseline, which previously has been speculated to be associated with impaired training responsiveness, they did not show such impaired responses to resistance training in this training setting. Generally, low-load resistance training was associated with larger lower-body muscle mass gains and similar muscle strength and performance improvements compared to high-load resistance training, and can therefore be advocated as an effective resistance training modality alternative for older adults. Importantly, the beneficial effects of high-load resistance training on bone health, emphasizes that resistance training programs for this population should include elements of such training. In general, the training intervention was associated with pronounced health effects, emphasizing the potency of resistance training for preventing/relieving sarcopenia in the general older population and for improving COPD-specific pathophysiologies.

Sammendrag

Bakgrunn. Styrketrening er et effektivt livsstilstiltak for å motvirke aldersrelatert tap av muskelstyrke og -masse. Effektene av slik type behandling ser imidlertid ut til å være av individuell karakter hvor flere ikke oppnår betydningsfulle fysiske forbedringer. Dette fenomenet er spesielt vanlig blant eldre personer og de med kroniske sykdommer som kronisk obstruktiv lungesykdom (KOLS). Det er tidligere foreslått at dette kan settes i sammenheng med bl.a. hormonelle variabler som lav vitamin D-status, systemisk inflammasjon og suboptimale treningsprotokoller.

Formål. The Granheim COPD Study bestod av to studier; en *forberedende studie* og en *RCT-studie*. KOLS er assosiert med redusert kardiorespiratorisk kapasitet, men det er foreløpig usikkert om dette kan påvirke den muskulære prestasjonen. I den forberedende studien var formålet derfor å sammenligne muskulær prestasjon blant KOLS-rammede (KOLS) og friske kontrollpersoner (Friske) i tre styrketreningsøvelser for beina som involverer ulik mengde aktiv muskelmasse (**Artikkel I)**. I RCT-studien var formålet å undersøke effektene av 12 uker med vitamin D_3-tilskudd, etterfulgt av 13 uker med kombinert vitamin D_3-tilskudd og styrketrening, på muskelfunksjons- og muskelbiologiske treningstilpasninger i en gruppe med eldre personer med og uten KOLS (**Artikkel II)**, samt å sammenlikne treningseffektene hos KOLS og Friske (**Artikkel III-IV),** og undersøke betydningen av høymotstands- sammenlignet med lavmotstandstrening for de samme variablene.

Deltakere og metode. I den forberedende studien gjennomførte 11 personer med KOLS (GOLD grad II/III; ekspirasjonsvolum på ett sekund (FEV_1), 53±14% av forventet verdi; alder 66±8 år) og 12 Friske (FEV_1, 117±12% av forventet verdi; alder 62±7 år) deltakere tester av muskulær prestasjon i tre styrketreningsøvelser med ulik kompleksitet og fysiologiske krav; (i) ettbeins kneekstensjon, (ii) en- og (iii) tobeins beinpress. I RCT-studien ble 95 eldre individer (56-77 år) tilfeldig fordelt til å motta enten vitamin D_3 eller placebo-tilskudd. Dette ble stratifisert etter helsestatus (KOLS, n=24; Friske, n=71) og kjønn. Intervensjonen ble startet med 12 uker med kun tilskudd (to uker med 10 000 internasjonale enheter (IE) vitamin $D_3 \cdot dag^{-1}$, deretter 10 uker med 2 000 $IE \cdot dag^{-1}$), etterfulgt av 13 ukers kombinert tilskudd (2 000 $IE \cdot dag^{-1}$) og veiledet helkropps styrketrening to ganger i uken. Under treningsøktene ble beinøvelsene utført unilateralt, hvor ett tilfeldig bein trente med høy motstand (10 repetisjoner maksimum; RM), mens det kontralaterale beinet trente med lav motstand (30RM). Denne unilaterale treningsprotokollen hadde to formål: i) å omgå potensielle kardiorespiratoriske begrensninger ved gjennomføring av trening med tobeins styrkeøvelser hos KOLS og ii) for å kunne sammenligne effektene av to ulike styrketreningsmetoder. Utfallsvariablene inkluderte flere mål på muskelstyrke ($n_{variabler}$=7), utholdenhetsprestasjon ($n_{variabler}$=6), muskelmasse ($n_{variabler}$=2), muskelkvalitet, muskelbiologi (*m. vastus lateralis*; muskelfiberegenskaper, RNA-mengde med transkriptom) samt helserelaterte variabler (kroppssammensetning, lungefunksjon, blodvariabler, helserelatert livskvalitet). For et utvalg av studiedeltakere (KOLS, n=11; Friske, n=12) ble også mitokondriemengde (sitrat syntase-aktivitet) og mitokondriell respirasjonskapasitet målt. For RCT-studiens kjerneutfallsdomener (muskelstyrke/-masse/-kvalitet og ettbeins og helkropps utholdenhetsprestasjon), ble vektede kombinerte faktorer kalkulert utfra enkeltvariablene.

Hovedresultater. I den forberedende studien ble den muskulære prestasjonen redusert for KOLS ved tobeins beinpress, men ikke ved ettbeins beinpress. Dette tyder på at de kardiorespiratoriske begrensningene ved KOLS ser ut til å påvirke den muskulære prestasjonen negativt ved styrkeøvelser

som engasjerer en større mengde aktiv muskelmasse (>ettbeins beinpress) (**Artikkel I**). I RCT-studien førte 13 ukers styrketrening til økt muskelstyrke (13%), økt muskelmasse (9%) og forbedrede utholdenhetsprestasjoner (ettbeins utholdenhetsprestasjon, 23%; helkropps utholdenhetsprestasjon, 8%). Treningsintervensjonen var også assosiert med gunstige endringer i helsevariabler (f.eks. visceralt fett, -6%; konsentrasjon av LDL-kolesterol, -4%) og muskelkarakteristikker som endret muskelfibersammensetning (f.eks. andelen IIX, -3%-poeng), antall muskelcellekjerner·fiber^{-1} (30%), total RNA/rRNA-mengde (15%/6-19%) og endret transkriptom (f.eks. 312 differensielt uttrykte gener). Tilskudd av vitamin D_3 påvirket ikke treningsresponsen for noen av kjerneutfallsdomenene, til tross for at vitamin D_3-supplementeringen førte til en solid økning i 25(OH)D-serumnivå (Δ49% sammenlignet med placebo) (**Artikkel II**). I sekundære analyser ble det observert at styrketrening med vitamin D_3-tilskudd førte til et høyere uttrykk av gensett involvert i vaskulære funksjoner i muskelvev, samt større forbedring av muskelstyrke for deltakere med høy fettmasse, sammenlignet med styrketrening alene (**Artikkel II**). Deltakerne med KOLS i RCT-studien hadde kjente KOLS-relaterte patofysiologier ved studiestart. Dette inkluderte nedsatt lungefunksjon, høyere nivåer av systemisk, lavgradig betennelse (serumnivåer av c-reaktivt protein), mindre muskelmasse og dårligere muskelfunksjon, samt at de hadde muskelbiologiske forstyrrelser som lavere mitokondriell, oksidativ kapasitet, større andel muskelfibertype IIA og IIX og ulikt transkriptom (227 gener hadde forskjellig mRNA-uttrykk mellom KOLS og Friske) (**Artikkel III-IV**). Til tross for disse biologiske uregelmessighetene, viste imidlertid studiedeltakerne med KOLS enten like eller større styrketreningseffekter for alle helse-, samt muskelfunksjonelle- og biologiske variabler sammenlignet med Friske (**Artikkel III-IV**). 10RM og 30RM-styrketrening førte til lik grad av opplevd anstrengelse. Ved å se på resultatene etter sammenslåing av dataene fra de to studiegruppene (KOLS og Friske), så man at 30RM-styrketrening førte til større økning av underkroppsmuskelmasse enn 10RM, mens 10RM-styrketrening førte til større fibertypeovergang fra type IIX til IIA, samt større forbedringer i arbeidsøkonomi ved sykling. Disse ulike responsene mellom 10RM- og 30RM-styrketrening førte imidlertid ikke til ulike forbedringer i muskelstyrke og muskulær prestasjon mellom de to treningsmetodene, men 10RM-styrketrening var assosiert med økt evne til å opprettholde beinmineraltettheten sammenlignet med 30RM-styrketrening.

Konklusjoner. Tilskudd av vitamin D_3 påvirket ikke de muskulære effektene av styrketrening. Dette motbeviser at vitamin D_3-tilskudd er nødvendig for å oppnå optimale muskulære effekter av styrketrening for den generelle, eldre befolkningen, iallfall for disse studiedeltakerne som stort sett hadde suffisiente vitamin D-nivåer ved studiestart. Selv om deltakerne med KOLS viste tydelige funksjonelle og biologiske forskjeller sammenlignet med Friske på variabler som tidligere har blitt assosiert med å redusere treningseffekten, viste de ingen tegn til slik redusert effekt av styrketrening i denne treningssettingen sammenliknet med effektene hos Friske. Generelt var lavmotstandstrening (dvs.30RM) assosiert med større økning av muskelmasse i underkroppen, samt lignende effekter som høymotstandstrening (dvs. 10RM) for å forbedre muskelstyrke og muskulær prestasjon. Lavmotstandstrening kan derfor ses på som et effektivt alternativ til høymotstandstrening for den eldre befolkning. Høymotstandstrening på sin side var assosiert med gunstige effekter på beinhelsen. Dette understreker at styrketreningsprogrammer for denne gruppen mennesker bør inneholde innslag av slik type trening. Generelt var treningsintervensjonen assosiert med gunstige helseeffekter. Dette understreker potensialet til styrketrening for å forebygge og lindre utviklingen av sarkopeni i den generelle eldre befolkningen, samt for å forbedre KOLS-spesifikke patofysiologier.

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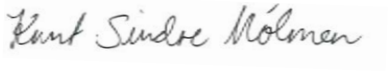

List of papers

The dissertation is based on the following research papers, which are referred to in the text by their Roman numerals:

I. **Mølmen KS***, Evensen Thy J*, Thallaug Dalane S, Ellefsen S, Falch GS. Muscular performance decreases with increasing complexity of resistance exercises in subjects with chronic obstructive pulmonary disease. *Transl Sport Med*. 2020;3(1):26-33.

II. **Mølmen KS**, Hammarström D, Pedersen K, Lie ACL, Steile RB, Nygaard H, Khan Y, Hamarsland H, Koll L, Hanestadhaugen M, Eriksen AL, Grindaker E, Whist JE, Buck D, Ahmad R, Strand TA, Rønnestad BR, Ellefsen S. Vitamin D_3 supplementation does not enhance the effects of resistance training in older adults. *J Cachexia Sarcopenia Muscle*. 2021; published online ahead of issue publication (doi: 10.1002/jcsm.12688).

III. **Mølmen KS**, Hammarström D, Falch GS, Grundtvig M, Koll L, Hanestadhaugen M, Khan Y, Ahmad R, Malerbakken B, Rødølen TJ, Lien R, Rønnestad BR, Raastad T, Ellefsen S. Chronic obstructive pulmonary disease does not impair responses to resistance training. *In review*.

IV. Oberholzer L*, **Mølmen KS***, Hammarström D, Falch GS, Lundby A-KM, Rønnestad BR, Ellefsen S**, Lundby C**. Resistance exercise training increases skeletal muscle mitochondrial respiration in COPD. *Submitted*.

* Authors share first authorship

** Authors share senior authorship

Abbreviations

COPD	chronic obstructive pulmonary disease
CSA	cross-sectional area
DXA	dual-energy x-ray absorptiometry
FEV_1	forced expiratory volume in one second
FVC	forced ventilatory capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
IHC	immunohistochemistry
IU	international units
mRNA	messenger ribonucleic acid
O_2	oxygen
RCT	randomized controlled trial
RT	resistance training
$\dot{V}O_2max$	maximal oxygen consumption per unit time
RM	repetition(s) maximum
RNA	ribonucleic acid
ROS	reactive oxygen species
rRNA	ribosomal ribonucleic acid
qPCR	quantitative polymerase chain reaction
VDR	vitamin D receptor
vitamin D_3	cholecalciferol
$1,25(OH)_2D$	1,25-dihydroxycholecalciferol
25(OH)D	25-hydroxycholecalciferol
Δ	difference

1 Introduction

Aging is associated with progressive loss of muscle strength and mass, accompanied by declines in physical performance. In 2016, ~11 million Europeans (>65 years of age) were estimated to have sarcopenia,[1] a formally recognized disease characterized by severe loss of muscle quantity and quality.[1] Sarcopenia increases the likelihood of adverse health events such as falls, fractures, physical disability, morbidity and mortality,[2,3] which further fuels muscle deterioration, resulting in a spiraling decrease in overall health and health-related quality of life.[4–6] In Europe, the prevalence of sarcopenia is expected to increase to at least ~19 million by 2045,[1] coinciding with increasing proportions of older adults, potentiated by suboptimal nutrition and increasing incidences of causal morbidities such as systemic inflammatory diseases.[7,8] For elderly to stay healthy, active and independent, efficient lifestyle measures to prevent, treat and reverse sarcopenia are warranted.[7,8] To this end, lifestyle therapy with resistance training is an attractive, low-cost and potent intervention.[9,10] Unfortunately, the benefits of such interventions are not always consistent, especially in the older population, with selected individuals and populations showing impaired abilities to increase muscle strength and mass.[11,12] At present, this training-response-spectrum is of unknown causality, though it interdepends on factors such as genetics,[13,14] epigenetics,[14] and composites of the inner physiological milieu, including nutrition,[15,16] endocrine variables (e.g. vitamin D),[17,18] and hallmarks of health such as low-grade chronic inflammation,[19] oxygen saturation levels,[20] and potentially also the type of training program (e.g. training with different exercise loads).[21] This makes chronically diseased populations such as persons with chronic obstructive pulmonary disease (COPD) particularly vulnerable, as they show deviant levels for several of the potential determinants of training responses, and indeed typically display accelerated decay in muscle strength and mass.[22,23] To circumvent such issues, combinatorial lifestyle protocols targeting and correcting such suboptimal factors may be necessary for adequate resistance training-induced muscle adaptations to occur, thus ensuring efficient treatment for both preventing and rehabilitating sarcopenia.

Vitamin D and its impact on muscle function and biology. Over the last two decades, vitamin D has emerged as a potential determinant of muscle functionality and biology.[24] There seems to be a robust relationship between heterogeneity in vitamin D status and traits such as physical performance[25–27] and susceptibility to falling,[28] suggesting a causal association between vitamin D and muscle functions, and potentially also the risk of developing sarcopenia.[29] Vitamin D insufficiency is particularly prevalent in older adults, measured as 25-hydroxycholecalciferol (25(OH)D) serum levels <50 $nmol \cdot L^{-1}$, and especially in older adults living in the Northern Hemisphere,[30,31] where cutaneous vitamin D synthesis is minuscule or absent during winter months.[32] Accordingly, exogenous vitamin D supplementation is gaining momentum as a potential ergogenic aid for preventing and treating sarcopenia.[29] Unfortunately, the presumed benefits of vitamin D supplementation deduced from observational studies are not necessarily supported by data from interventional studies. While some studies and meta-analyses report favorable effects of vitamin D supplementation *per se* on muscle strength[33–35] and falling incidences,[36,37] with benefits being more pronounced in persons with low baseline values (<30 $nmol \cdot L^{-1}$)[38] and in older persons,[38] others do not.[39–42] These discrepancies may not be surprising, as exercise training is arguably necessary to provoke improvements in muscle functions.[43] However, a similar ambiguity is present in the few studies that have assessed the effects of vitamin D supplementation on outcomes of

resistance training.[44–47] Indeed, none of the existing studies report clear benefits of vitamin D supplementation for alterations in muscle strength,[44–47] muscle mass,[45–47] or incidences of falling.[44,46] Still, a recent meta-analysis concluded that vitamin D supplementation provides benefits for training-associated changes in lower body muscle strength.[43]

Consequently, we have limited and conflicting knowledge about the combined effects of vitamin D supplementation and resistance training for muscle functions and biology in humans. The present confusion may partly be attributed to methodological uncertainties in available studies, potentially lowering their ecological validity and explaining their lack of coherence with the resulting meta-analysis data.[43] This includes heterogeneous study populations (varying from young adults[45,47] to older adults[47] to elderly[44,46]) with large differences in baseline 25(OH)D levels (average 31 $nmol \cdot L^{-1}$ [46] – 71 $nmol \cdot L^{-1}$ [47]), large variation in vitamin D dosage (from 400 $IU \cdot day^{-1}$ [46] – 4 000 $IU \cdot day^{-1}$ [45]), lack of familiarization to maximal muscle strength tests,[44,46] suboptimal training protocols[44,46] (failing to comply to current guidelines, advocating resistance training with controlled maximal effort[48,49]), low compliance to training,[44,46] and a lack of dietary assessment during the intervention.[44,46,47] Also, neither of the studies has included a period of vitamin D supplementation prior to resistance training, which may be necessary to prime muscle cells for adaptations, potentially acting to alter epigenetic signatures, which has been observed in other cell types, such as T-cells[50] and oral cancer cells.[51] Furthermore, the effects of vitamin D supplementation on muscle fiber characteristics and biology remain poorly understood and unclear.[52]

In theory, vitamin D may potentiate muscle fiber responsiveness in two ways. Either directly by acting through vitamin D receptors in muscle fibers or progenitor cells, perhaps inducing intramuscular signaling pathways such as the p38 mitogen-activated protein kinase pathway,[53,54] or indirectly by interacting with systemic signaling cascades, for example by inducing testosterone signalling[55] and thereby facilitating muscle plasticity. Our lack of insight is underlined by the longstanding uncertainty of the presence of vitamin D receptors in muscle tissue,[56] though several indications advocate its expression. *First*, there seem to be associations between mutations in the vitamin D receptor and muscle weakness in both humans and mice.[57,58] *Second*, muscle-specific knock-out of the vitamin D receptor in mice deteriorates muscle strength and mass in a manner that resembles sarcopenia.[59,60] The prevailing uncertainty is fueled by a seeming lack of effects of vitamin D supplementation *per se* on the muscle transcriptome in vitamin D-insufficient frail elderly, though also in that study, the vitamin D dosage was relatively low (400 $IU \cdot day^{-1}$).[61]

To date, a mere single study has assessed the effects of vitamin D supplementations on resistance-training induced muscle biological adaptations in humans, and as such assessing only a limited selection of traits and failing to disclose conclusive findings.[47] Furthermore, to date, no studies has elucidated on and distinguished between the effects of vitamin D_3 supplementation *per se* on muscle functionality and biology and its concerted effects together with resistance training. Such an investigation is arguably possible using a study design where an initial vitamin D supplementation period is followed by a resistance-training period. If the vitamin D supplementation-only protocol prior to resistance training successfully increases vitamin D status compared to placebo supplementation, this would enable assessment of the effects of resistance training in participants with pre-training differences in vitamin D status. Indeed, this would also be a plausible necessity for muscle cells to adapt to resistance training. Therefore, there is clearly a need for more research investigating the broad range of potential muscle biological implications of

combined vitamin D supplementation and resistance training, especially for the older population where combinational interventions aiming to counteract the age-related decline in muscle mass and function are particularly warranted.

Training protocols and COPD rehabilitation. For persons with COPD, limb muscle dysfunction is highly prevalent and has important clinical implications such as reductions in functional capacity, health-related quality of life and also life expectancy.[62–64] For its prevention and reversal, physical training is recognized as a prerequisite and the most potent intervention available.[63,64] However, the magnitude of response to exercise training in COPD is highly variable, with some persons showing only small or no benefits.[65] Some evidence indicates that such suboptimal responses to exercise training may be linked to the cardiorespiratory limitations inherent to the disease,[63] leaving COPD persons with inability to tolerate sufficient intensity and/or duration of exercise to provoke muscle cell adaptations. Despite this, whole-body endurance exercise (e.g. cycling and walking), which requires a substantial cardiorespiratory demand during execution, is the most commonly applied exercise modality in pulmonary rehabilitation.[64] To resolve this issue, resistance exercise is a readily available exercise modality, activating smaller amounts of muscle mass over a shorter time span than whole-body endurance exercises,[66] and thus requires less from the cardiorespiratory system. This strategy should ensure optimal muscle activation regardless of blood oxygenation levels, enabling activation of key cellular signaling pathways and thus induce favorable muscle adaptations. Even positive effects on skeletal muscle mitochondria has recently been displayed after resistance training in COPD, in which increased citrate synthase activity and hydroxyacyl coenzyme A dehydrogenase protein levels has been shown after eight weeks of low-load resistance training.[67] Thus, resistance training may provide a stimulus to augment muscular oxidative capacity in COPD. However, whether that is also reflected in increased skeletal muscle mitochondrial respiration and whether the response is only specific to low-load resistance training remains to be elucidated.

Generally, the magnitude of resistance training-associated adaptations of muscle strength and muscle function remains largely ambiguous in COPD, with available studies displaying a large span of variation in training adaptations.[65,68–72] This heterogeneity in training responses may result from differences in study design, including differences in resistance training protocols. Indeed, the cardiorespiratory limitations of COPD patients may call for specific modifications of resistance training exercises in order to further reduce the physiological demand.[73] At present, we know little about this perspective, with only a couple of studies investigating the acute responses to different resistance exercise modalities.[74,75] These studies show that, using elastic bands, unilaterally performed leg resistance exercises result in superior exercise workloads compared to conventional bilateral exercises in severe to very severe COPD (GOLD[76] grade III/IV, predicted forced expiratory volume in one second (FEV_1) <50%), but not in healthy persons, which indeed indicates a cardiorespiratory exercise limitation in COPD persons. However, no analysis was performed of the interaction between difference in exercise workload$^{\text{-leg}}$ from single- to two-limb exercises and study clusters (COPD vs. healthy participants). Thus, it is still uncertain if COPD persons show progressively lowered muscular performance in resistance exercises with increasing complexity and active muscle mass compared to healthy persons. It also remains unknown if this applies to COPD of less severity (e.g. GOLD grade II/III, $FEV_{1predicted}$=30-80%), and if it is applicable to isolated resistance exercises performed in apparatus.

Training responsiveness in COPD vs healthy persons. Persons with COPD are particularly prone to accelerated decay of muscle strength and mass with advancing age. This deterioration is accompanied by systemic co-morbidities such as reduced levels of testosterone,[77] vitamin D[28,78] and oxygen saturation levels,[20] and elevated levels of low-grade inflammation.[79] These pathophysiologies can arguably leave COPD persons in a state of anabolic resistance,[80] resulting in impaired abilities to adapt to exercise training.[43,81,82] This is to some extent also supported by the available literature, showing that muscular responses to exercise training may be attenuated in COPD compared to healthy control persons.[83–85] After whole-body endurance training, fewer genes were significantly altered in COPD compared to healthy persons,[84] and the muscle angiogenic training response seems to be blunted.[85] Importantly, this may be ascribed the inability to achieve sufficient exercise intensities to enable muscle cell adaptations during whole-body exercises in COPD. However, also during resistance training with a low amount of active muscle mass (i.e. 30 maximal repetitions of one-legged knee extensions executed with a knee angular speed of 180°·sec^{-1}), which should enable similar muscle-specific exercise intensities in COPD and healthy participants, COPD was still associated with blunted increases in proteins related to catabolic, anabolic and transcription processes, although changes in mRNA expressions for a selection of genes were broadly similar.[83] Notably, for proteins regulating myogenesis (i.e. MyoD, myogenin and myostatin), similar protein level responses to resistance training were shown in COPD and healthy participants.[83] Thus, the muscle biological responsiveness to resistance training in COPD remains equivocal. Moreover, this is also the case for the observations of muscle functional responses to resistance training in COPD, with available studies ranging from negligible or trivial training responses[68,69] to substantial and clinically relevant responses.[70,71] Indeed, the COPD population is reported to have a high prevalence of non-responders to pulmonary rehabilitation programs including exercise training,[62,86,87] which once more indicates that training responsiveness may be limited in COPD.

However, only a mere single study has previously compared functional and biological adaptations to resistance training between COPD and healthy controls (ISRCTN ID: *22764439*),[83,88,89] and as such was conducted with a relatively short training intervention (8 weeks), a rather untraditional training protocol with little clinical and practical relevance (isokinetic knee extensions conducted in a dynamometer), and a limited selection of outcome variables. Whereas the study failed to disclose COPD-related impairments in muscle strength and muscle growth responses, it seems premature to dismiss the notion that COPD-related pathophysiologies may impair resistance training responsiveness,[22,63] especially because of the blunted protein responses observed in that particular study.[83] Consequently, the assumed impaired resistance training responsiveness in COPD obviously warrants further investigation.

Exercise load and its impact on resistance training-associated adaptations. The external exercise load is one of the most common adjustable variables during resistance exercise, and is clearly of importance for the amount and type of muscle functional and biological adaptations resulting from such training.[48] Current training guidelines recommend relatively high exercise loads, i.e. 60-100% of one-repetition maximum (1RM) performed with quite few repetitions per exercise set (4-12 repetitions), as the most potent strategy to achieve muscle strength and hypertrophy in anyone from novices to resistance-trained individuals.[48] This has been claimed based on the postulate that heavy loading is required to fully recruit higher threshold motor units,[90] and consequently it has been reasonable to assume that optimal improvements in muscle strength and hypertrophy can only

be achieved through the use of high loads. However, in recent years, this view has been challenged by the scientific community, at least for young healthy individuals.[91–93] For that population, resistance exercise with low-loads conducted to muscular failure seems to translate into similar long-term training-induced increases in muscle mass as high-load resistance training.[91–93] This seems as such to be decoupled from the degree of voluntary muscle activation during exercise, for which low-load resistance exercises carried out to muscular failure consistently show lower mean and peak electrical amplitude (using surface electromyography) compared to exercises conducted with high-loads.[94–97] Possibly, this may be explained by greater alterations of other important factors for muscle hypertrophy during low-load resistance training, e.g. exercise volume[98] and degree of metabolic perturbations, [95,99] as well as longer time under tension for the muscle fiber type I low-threshold motor units which may possess a greater stimulus for muscle fiber type I hypertrophy.[100,101] Importantly, comparison of muscle functional and biological adaptations to low-load and high-load resistance training remains largely unstudied in other populations such as in older adults and those with chronic diseases. The training effects in these populations may not necessarily reflect those seen in young healthy adults, as e.g. aging may influence the degree of voluntary muscle activation.[102–104] Older adults also show some dissimilar muscle transcriptional and translational responses to resistance training compared to those seen in young adults,[105–107] which seems to result in a reduced anabolic response compared to young counterparts.[108,109] These potential divergences between older and young individuals makes it therefore difficult to employ the prevailing resistance training guidelines to ensure optimal training responses for this population. For different patient groups such as the COPD population, current training prescriptions are even more difficult to employ as exercise responses may be affected by disease-specific pathophysiologies as well, such as increased low-grade inflammation and lower oxygen availability in COPD.

2 Research aims and hypotheses

The overall aim of The Granheim COPD Study was to investigate the impact of vitamin D_3 supplementation, chronic obstructive pulmonary disease and exercise load on resistance training-associated adaptations in older adults. Two separate studies were conducted; a *preparatory study* and a randomized controlled trial (*RCT study*).

The specific aims of the papers were:

I. To compare muscular performance in three resistance exercises of the legs involving different amounts of active muscle mass in COPD and healthy control persons (**Paper I**, *preparatory study*)

II. To investigate the effects of 12 weeks of vitamin D_3 supplementation-only, followed by 13 weeks of combined vitamin D_3 supplementation and resistance training, on muscle functional and biological training-associated adaptations in a mixed group of older adults with stable COPD or normal lung function (**Paper II**, *RCT study*)

III. a) To investigate the inherent differences in muscle functionality and biology between the COPD and healthy control (Healthy) study clusters
b) To compare the effects of 13 weeks of resistance training for the COPD and the Healthy study participants on muscle functional and biological outcomes
c) To investigate the interaction between high-load and low-load resistance training (10 vs 30 repetitions maximum, RM) and training responsiveness for the two study clusters separately (**Paper III**, *RCT study*)

IV. To determine the effects of 13 weeks of resistance training on mitochondrial respiratory capacity in *m. vastus lateralis* for COPD and healthy control persons, and to investigate the potential influence of resistance training load (10RM vs 30RM) (**Paper IV**, *RCT study*)

Additional aim (only elucidated in this thesis):

To compare the muscle functional and biological effects of high-load and low-load resistance training (10RM vs 30RM) for a mixed group of older adults with stable COPD or normal lung function (*RCT study*)

Main hypotheses:

A. Muscular performance in COPD persons would be increasingly impaired with increasing amount of active muscle mass compared to Healthy persons (**Paper I**, *preparatory study*)
B. Vitamin D_3 supplementation would enhance the muscle functional and muscle biological resistance training-associated effects compared to resistance training-only (**Paper II**, *RCT Study*)
C. COPD persons would display impaired muscle functional and muscle biological resistance training-associated responses compared to Healthy persons (**Paper III**, *RCT Study*)
D. Resistance training would increase mitochondrial respiration in both COPD and Healthy persons (**Paper IV**, *RCT Study*)
E. High-load (10RM) and low-load (30RM) resistance training would result in similar muscular adaptations (*RCT Study*)

3 Methods

Detailed description of study designs and methods for *the preparatory study* (Paper I) and *the RCT study* (Paper II-IV) in The Granheim COPD Study are provided in Papers I and II, respectively. In addition, for the RCT study, the data also resulted in a qualitative paper written in Norwegian (**Appendix I**), which is not included in the assessment of the thesis.

3.1 Study ethics

Both studies (i.e. the preparatory study and the RCT study) were approved by the Regional Committee for Medical and Health Research Ethics – South-East Norway (reference no. *2013/1094*) as parts of The Granheim COPD Study, preregistered at clinicaltrials.gov (ClinicalTrials.gov identifier: *NCT02598830*), and conducted according to the *Declaration of Helsinki*. All participants were informed about the potential risks and discomforts associated with the study and gave their written informed consent prior to study enrolment.

3.2 Participants

Persons with either a medical diagnosis of stable, moderate COPD (GOLD grade II or III, predicted FEV_1 between 30-80% and FEV_1/FVC (forced ventilatory capacity) <70% after reversibility testing) or normal lung function (Healthy) were separately recruited for the preparatory study and the RCT study (**Table 1**). Each participant conducted only one study protocol, i.e. the preparatory study or the RCT study. For CONSORT flowchart of the RCT study, see **Appendix II**. For a more detailed overview of baseline characteristics for supplementation arms and study clusters in the RCT study, see Paper II (Table 1) and Paper III (Table 1), respectively.

Table 1 *Participant characteristics*

Study	Cluster/arm	N	Age (years)	Body mass (kg)	BMI ($kg \cdot m^{-2}$)	FVC (L)	FEV_1 (% pred.)	FEV_1/FVC (%)
Prep. study	COPD cluster	11 (6 ♀)	66 ± 8	70 ± 14	26 ± 5	2.7 ± 1.1	53 ± 14	49 ± 13
	Healthy cluster	12 (7 ♀)	62 ± 7	76 ± 12	25 ± 3	4.1 ± 0.8	117 ± 12	72 ± 6
RCT study	Vitamin D_3 arm	34 (20 ♀; 9 COPD)	68 ± 5	74 ± 17	26 ± 4	3.4 ± 0.8	86 ± 24	66 ± 14
	Placebo arm	44 (25 ♀; 11 COPD)	67 ± 4	76 ± 16	26 ± 5	3.7 ± 1.0	96 ± 26	70 ± 13
	COPD cluster	20 (8 ♀)	69 ± 5	73 ± 18	25 ± 5	3.2 ± 0.9	57 ± 11	47 ± 8
	Healthy cluster	58 (37 ♀)	67 ± 4	76 ± 16	26 ± 5	3.6 ± 0.9	104 ± 16	75 ± 6

Characteristics of the participants completing the study protocols. For the RCT study, participant characteristics are presented as both per supplementation arm and per study cluster. BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; ♀, females; ♂, males.

3.3 Study designs

Preparatory study. All participants in the preparatory study attended 7 days of performance testing, distributed over 4 weeks (**Figure 1**). Test days were separated by at least 48 hours. On test days 1-3, 1RM tests were conducted in one-legged knee extension, one-legged leg press and two-legged leg press. On these test days, participants alternated between starting the test session with one-legged (knee extension and leg press) and two-legged exercises (leg press), giving each participant one attempt with fully rested lower limbs for each test modality. These data were subsequently utilized

to calculate relative workload for tests of muscular performance, which were defined as the number of repetitions achieved over the course of three sets, with 2 minutes of rest in-between, at a load corresponding to 60% of 1RM. Tests of muscular performance were performed on test days 4-7 (**Figure 1**; two separate test days for one-legged exercises and two separate test days for the two-legged exercise). The choice of one-legged knee extension, as well as one- and two-legged leg press as test exercises were motivated by the different amounts of active muscle mass and thus dissimilar cardiorespiratory demands associated with these exercises. For each of the three muscular performance tests, the best result was used in further analyzes.

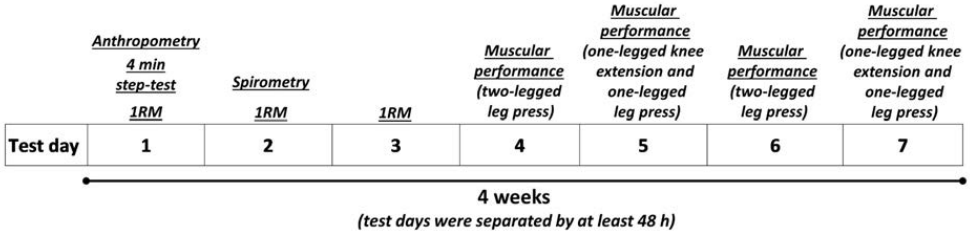

Figure 1. Protocol for *the preparatory study*. 1RM, tests of one repetition maximum in one-legged knee extension, and one- and two-legged leg press.

RCT study. Participants were randomly assigned into one of the two study arms (vitamin D_3 vs placebo arm), using concealed allocation, stratified by sex and health status (COPD vs Healthy). The RCT was initiated by 12 weeks of supplementation-only (two weeks with 10 000 IU vitamin $D_3 \cdot day^{-1}$ followed by 10 weeks with 2 000 $IU \cdot day^{-1}$, or placebo supplementation), followed by 13 weeks of combined supplementation (2 000 IU vitamin $D_3 \cdot day^{-1}$ or placebo) and resistance training. Like the vitamin D_3 capsules, the placebo capsules contained cold-pressed olive oil and were as such identical in appearance to the vitamin D_3 capsules. Pharma Nord ApS (Vejle, Denmark) procured the two supplements. All participants consumed 500 mg $calcium \cdot day^{-1}$ (Nycoplus, Takada AS, Asker, Norway). Throughout the entirety of the study, participants completed a weekly health survey every Sunday evening, which included information about experienced health and potential discomforts with the nutritional supplementation. For a timeline of the study protocol, see **Figure 2**.

The training intervention consisted of two weekly full-body training sessions for all participants. Leg exercises (knee extension, leg press, knee flexion) were performed unilaterally, with one of the legs of each participant being randomly assigned to three sets of 10RM and the contralateral leg to perform three sets of 30RM. Upper-body exercises (chest press, lat pulldown) were performed bilaterally, consisting of two sets of 10RM. All sessions were supervised by qualified personnel. The effectiveness of the training intervention was assessed as a wide range of outcome measures (**Figure 2**), including multiple assessments of endurance performance, muscle strength and mass, measures of lung function, one-legged/two-legged maximal oxygen consumption ($\dot{V}O_2max$), oxygen cost/gross efficiency, health-related quality of life, and collection of blood and *m. vastus lateralis* biopsies (both legs).

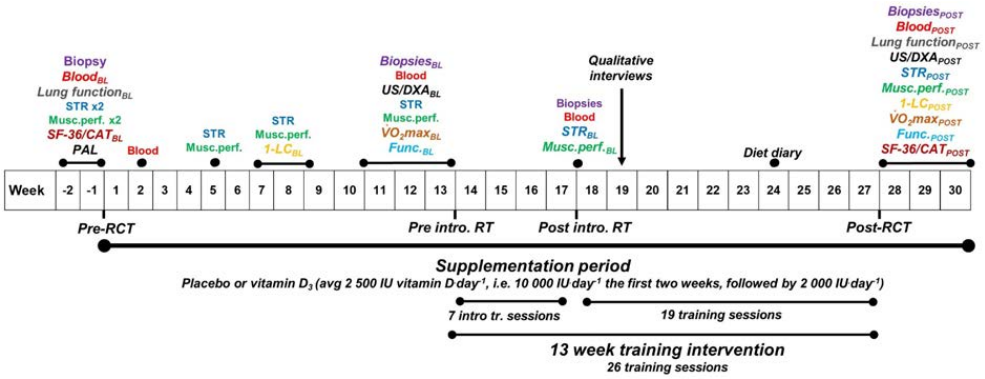

Figure 2. Timeline of the study protocol (BL and POST indicates the defined baseline and post-test measurement for the specific outcome measure, respectively). Methodological notes on retrieval of outcome measures: **i)** *Blood and muscle measurements*. Prior to collection of blood and muscle biopsies, participants were instructed to attend an overnight fast and to avoid heavy physical activity for the last 48 h. Blood samples were analyzed for serum concentrations of 25-hydroxycholecalciferol (25(OH)D), 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$), hormones, lipids, and markers of iron metabolism and tissue damage, as previously described.[110] Muscle biopsies were analyzed for muscle fiber type proportions, myonuclei content, muscle fiber cross-sectional area (CSA), and rRNA and mRNA content (total RNA, rRNA subspecies, myosin heavy chain isoforms I, IIA and IIX, and whole-genome transcriptome), as previously described.[110–112] Transcriptome analysis was restricted to a subset of participants (COPD, n=19; Healthy, n=34). **ii)** *Lung function*. Spirometry testing was performed following the guidelines from the American Thoracic Society and the European Respiratory Society.[113] Participants with COPD were tested before and after inhalation of two bronchodilators (salbutamol/ipratropiumbromid). **iii)** *Muscle strength and performance* (STR and Musc. perf). Muscle strength was assessed as one-repetition maximum (1RM) in unilateral knee extension and leg press, bilateral chest press, and handgrip. Muscle performance was defined as the number of repetitions achieved at 50% of pre-study 1RM and was assessed using unilateral knee extension and bilateral chest press. Isokinetic unilateral knee-extension torque was tested at three angular speeds (60°, 120° and 240°·sec^{-1}; Humac Norm, CSMi, Stoughton, MA, USA). **iv)** *Health-related quality of life* (SF-36 and CAT). All participants completed the Short Form (36-item) Health Survey (SF-36^{TM}). COPD participants also completed the COPD Assessment Test (CAT®) questionnaire. **v)** *Physical activity level* (PAL). All participants completed a questionnaire (self-produced) regarding regular weekly activity habits. The results (time spent for different activities) were translated into energy expenditure (kcals·week^{-1}) during activities using number of metabolic equivalents (METs) provided in Jetté, Sydney, and Blümchen.[114] **vi)** *One-legged cycling and bicycling performance* (1-LC and $\dot{V}O_2$max). Participants conducted one-legged cycling tests (Excalibur Sport, Lode BV, Groningen, the Netherlands) to assess O_2-costs and mechanical efficiency[115] during submaximal cycling, and maximal one-legged oxygen consumption ($\dot{V}O_2$max) and maximal workload. Maximal two-legged cycling $\dot{V}O_2$max and workload were tested on a separate day. Oxygen consumption was measured using the JAEGER Oxycon Pro^{TM} system (Carefusion GmbH, Höchberg, Germany). **vii)** *Muscle thickness and body composition* (US/DXA). Muscle thickness of *m. vastus lateralis* and *m. rectus femoris* were measured using B-mode ultrasonography (SmartUs EXT-1M, Telemed, Vilnius, Lithuania). Body composition was measured using dual-energy X-ray absorptiometry (DXA; Lunar Prodigy, GE Healthcare, Madison, WI, USA). **viii)** *Functional performance* (Func.). Functional tests were conducted as the maximal number of sit-to-stands during one minute (seat height: 45 cm) and as the number of steps onto a 20 cm step box during 6 minutes. *Qualitative interviews* were performed for a subset of participants in the COPD cluster (n=8; Week 19). The interviews were recorded with a dictation machine and subsequently transcribed and analyzed using systematic text condensation.[116] During week 24, all participants conducted a *dietary registration*, in which they logged their dietary intake for three days, including one weekend day.

3.4 Statistical analyses

Data in text and tables are presented as means with standard deviations, unless otherwise stated. In figures, error bars denote 95% confidence limits of the mean. Statistical significance was set to

p<0.05. Statistical analyses were performed using SPSS Statistics package version 24 (IBM, Chicago, IL, USA) and R software.[117] Figures were made using Prism Software (GraphPad 8, San Diego, CA, USA) and Microsoft Office PowerPoint 2016 (Microsoft Corp., Redmond, WA, USA).

Preparatory study. Differences between COPD and Healthy in muscular performance were examined using mixed-design ANOVAs with study clusters (i.e. COPD and Healthy) acting as the between-clusters factor and type of exercise (i.e. one-legged knee extension, one-legged leg press and two-legged leg press) acting as within-cluster factors. When a significant *F* value occurred, a Sidak *post hoc* test was used to determine differences between and within clusters.

RCT study. Investigation of the effects of vitamin D_3 supplementation on a diverse set of research questions and outcome measures was the primary objective of this study, as defined in the pre-registration of the study protocol (ClinicalTrials.gov Identifier: *NCT02598830*). As also described in the pre-registration, this was performed using different defined baseline time points and time frames (outlined in **Figure 2**; see the *Methodological considerations* paragraph for rationales underlying the different baseline time points). Alongside the results presented in this thesis, a more thorough analysis of the vitamin D_3 RCT perspective are covered in **Paper II**, while the objectives of the RCT study related to the investigation of the muscle functional and biological adaptations to resistance training in COPD and Healthy participants are reported in this thesis, as well as in **Paper III** and **IV**. For the last objective of the RCT study, i.e. to compare the muscle functional and biological adaptations to 10RM and 30RM resistance training for all participants in the RCT study combined, this is only highlighted in this thesis' *Results and discussion* chapter.

In **Paper II**, for continuous variables, linear mixed-effects models were used to examine the effects of vitamin D_3 supplementation (compared to placebo), with relative change scores from baseline being defined as the dependent variable and the supplementation arms being defined as the fixed effect of interest. The two different exercise loads (10RM and 30RM) were added to the models as repeated measures/observations (for unilateral outcome measures), and baseline values were used as co-variates. For all participants, random intercepts were specified. For all unilateral leg variables, interaction effects were explored between the fixed effect and study clusters (COPD/Healthy) and exercise loads (10RM/30RM). For other variables, interactions were investigated between the fixed effect (vitamin D_3 vs placebo) and study clusters. For non-continuous variables, such as muscle fiber type proportions (IHC and qPCR), rRNA and mRNA content (qPCR and transcriptome), and variables from the weekly health survey, generalized linear mixed-effects models were used to examine differences in responses for the fixed effect (vitamin D_3/placebo supplementation).

In **Paper III**, linear mixed-effects models were used to examine differences between study clusters (i.e. COPD and Healthy) both at baseline and as responses to resistance training. For continues data, additional analyses were performed in order to examine if adaptations to resistance training were decoupled from the inherent disease-related study cluster differences; statistical models with both relative and absolute change scores from baseline were conducted. The effect of sex (female/male) was implemented into all models, and analyses included evaluation of interaction effects with sex and exercise loads (10RM/30RM) when applicable.

In **Paper IV**, linear mixed-effects models were used to examine the effects of resistance training on mitochondrial function in COPD and Healthy participants, and also to separately

investigate the potential influence of exercise load (10RM/30RM) on changes in mitochondrial function in the COPD and Healthy study clusters.

For analyses only presented in this thesis, i.e. the comparison of 10RM and 30RM resistance training on muscle functional and biological adaptations for all participants in the RCT study combined, linear mixed-effects models were used to examine differences in relative changes from baseline for each outcome variable, with exercise load (10RM/30RM) defined as the fixed effect. The two different exercise loads (10RM and 30RM) were added to the models as repeated measures/observations. Interaction effects between the fixed effect and sex (female/male) and study clusters (COPD/Healthy) was also explored.

Generally, for most outcome measures, the main effect of time (i.e. to check if there was a significant change from baseline for an outcome measure irrespective of supplementation arm, study cluster and exercise load) was examined using mixed modelling, using absolute values of the dependent variable and time points as repeated measures/observations.

For selected outcome measures, specific considerations had to be integrated. For transcriptome analyses, genes were regarded as differentially expressed when the absolute $\log_2$ fold change/difference were greater than 0.5 and the adjusted *p*-value (false discovery rate adjusted *per* model coefficient) was below 5%.[111] Moreover, enrichment analyses were performed on hallmark, KEGG and gene ontology gene sets, using two approaches. *First*, a non-parametric rank test was performed based on gene-specific minimum significant differences (MSD). *Second*, gene set enrichment analysis (GSEA) was performed to quantify directional regulation of the gene set. Consensus results between the two tests were interpreted as having larger biological meaning. All gene sets were retrieved using the molecular signature database (version 7.1.).[118] For all immunohistochemical variables (muscle fiber CSA, fiber type proportion, and myonuclear content), statistical models were weighted for numbers of counted fibers *per* biopsy. This was done to account for the reduced reliability accompanying fewer observations/fibers.[110] Notably, for myonuclear content analyses, we also experienced suboptimal immunostaining for a large proportion of the biopsies. Consequently, ~50% of the biopsies could not be processed be the automated CellProfiler software[119] used for myonuclei counting (see **Appendix III**, supplementary material for Paper II), leaving the myonuclei analyses with reduced statistical power. Some caution is thus warranted for interpretation of these data.

3.5 Methodological considerations

For the RCT study, a number of methodological considerations formed the basis for how the study protocol eventually was performed, as well as for how the collected data was analyzed.

Study design-measures to increase the validity of muscle functional outcome variables. To ensure valid analyses of training-associated effects on muscle-related features in the RCT study, some precautionary measures were deemed necessary. For muscle strength and muscle performance measures, baseline levels were defined to be equivalent to values collected after 3 ½ weeks of introduction to resistance training (**Figure 2**), rather than values collected before its onset, as noted in the preregistration of the study (*NCT02598830*). At this time point, the initial adaptations to training were likely to have occurred, preferably non-hypertrophic effects relating to technical, psychological and neural learning effects,[120] phenomena that are particularly prominent in older persons.[121] Using this time point as baseline arguably strengthens the association between changes

in muscle strength and muscle mass. To further improve validity and minimize the confounding effects of non-hypertrophic increases in strength and performance, all participants conducted a series of repeated tests prior to baseline tests, including five repeated 1RM and muscular performance tests in knee extension and chest press, three familiarization tests for 1RM leg press and two familiarization tests for isokinetic strength.

Dietary supplementation. The vitamin D_3 RCT study consisted of two periods; a supplementation-only period (12 weeks), succeeded by a combined supplementation and resistance-training period (13 weeks). Two motives were emphasized for the initial supplementation-only period. *First*, the period was implemented to investigate the effects of vitamin D_3 supplementation *per se* on markers of vitamin D biology (e.g. 25(OH)D and 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$), i.e. the storage form and the bioactive form of vitamin D, respectively), as well as its effects on muscle function and biology. In this manner, we wanted to elucidate on and distinguish between the effects of vitamin D_3 supplementation *per se* and the effects of vitamin D_3 supplementation in concert with resistance training. *Second*, if the vitamin D_3 supplementation-only protocol successfully would increase the vitamin D status compared to placebo supplementation, this would enable investigation of the effects of resistance training in participants with differences in vitamin D status prior to the resistance training intervention. It seems plausible that the pre-training supplementation period may be necessary to prime muscle cells for adaptations, potentially acting by changing epigenetic traits, which has been observed in other cell types, such as T-cells[50] and oral cancer cells.[51]

In order to increase the integrity of the vitamin D_3 supplementation RCT results, a number of precautionary measures were incorporated into the study protocol. 1) During study conduct, all participants were instructed to restrict vitamin D intake from food sources to <400 $IU \cdot day^{-1}$ and to abstain from solarium and travels to southern and/or sunny areas. 2) The intervention was conducted in Lillehammer, Norway (latitude 61°N) from September to May, ensuring low or no natural vitamin D synthesis by the skin from sunlight UVB radiation.[32] 3) Placebo capsules were identical in appearance to vitamin D_3 capsules, which ensured that it was impossible for the participants to differentiate between the two supplements. 4) Daily supplementation of calcium was incorporated to the study protocol for all participants to ensure adequate calcium levels, a chemical element important for some vitamin D effects to take place.[122]

To aid recovery and ensure adequate protein intake after training, participants ingested half a protein bar immediately after each training session (~15g protein; Big 100, Proteinfabrikken, Sandefjord, Norway).

Contralateral exercise design. A contralateral lower-limb exercise design was chosen to compare the effects of two different resistance training modalities, 10RM and 30RM. Such a study design has previously been highlighted to provide greater statistical power and reducing the time and cost of a study,[123] as such an approach reduces the between-person variability and enables within-person comparison, but has also been criticized for certain aspects. The main criticism is related to the hypothesized crossover training effects that occurs between the exercised limb and the contralateral limb, i.e. that unilateral resistance training induces an increase in systemic, anabolic hormones (e.g. growth hormone, insulin-like growth factor, testosterone) and releases different myokines from the exercising muscle, which theoretically can influence the contralateral limb.[123] However, these potential effects are in all likelihood negligible, as neither mRNA

abundance,[123] mitochondrial content,[124–126] capillarization,[127] muscle protein synthesis[128] nor muscle hypertrophy[129,130] responses in the exercised/trained limb seems to translate into responses in the non-exercised/trained limb. For this reason, we considered it to be unlikely that the contralateral design in the current study confounded the muscle biological measures. However, neural learning effects, i.e. factors related to motor unit recruitment, are seemingly more prone to crossover-limb training effects.[123] As previously noted, to prevent such effects from affecting analyses, an extensive training and testing familiarization protocol was performed.

Another rationale for choosing a one-legged exercise protocol was the lower amount of active muscle mass compared to conventional two-legged exercises, and thus the reduced cardiorespiratory demands of such exercises. This was regarded as favorable for the COPD persons performing the training protocol. Indeed, COPD persons seems to show larger training volumes[-leg] and performance in one-legged exercises compared to two-legged exercises. This may translate into superior training adaptations if the inherent low cardiorespiratory fitness makes it difficult to achieve the necessary exercise intensities during two-legged exercises to provoke muscle cell adaptations.[22,63] Indeed, the preparatory study was conducted with the aim to compare the muscular performance between COPD and Healthy in three resistance exercises with different cardiopulmonary demands and complexity.

Analytical considerations. The participants in the RCT study constituted a quite heterogeneous study group, which included both female and male participants, and persons with and without a COPD diagnosis. This was in line with our intention to study responses to vitamin D_3 supplementation and resistance training in the general population of resistance training-naïve older adults, potentially increasing the ecological validity and impact of the RCT results. However, to the contrary, some people will claim that introducing a patient group will lead to a possible risk of *selection bias*, i.e. that proper randomization is not achieved, which implies that the study group is not representative of the population intended to be studied.[131] E.g. it may be conceivable to argue that COPD persons, which suffer from pathophysiologies such as reduced oxygen saturation levels and elevated levels of low-grade inflammation may have an interaction effect with vitamin D_3, and the diagnosis may thus interfere the vitamin D_3-analyses. To circumvent this possible issue, two precautionary measures were deemed necessary: *First*, randomization to vitamin D_3 and placebo arms were stratified by COPD diagnosis (yes/no) and sex (female/male), ensuring that both supplementation arms had the same proportion of female and male participants with and without a COPD diagnosis. *Second*, during statistical analyses, a mixed modelling-approach was employed, as it enables to examine multiple *between-person* and *within-person* (also referred to as *repeated-measures*) factors. That feature enabled to, based on the fixed factor of interest (i.e. vitamin D_3/placebo, COPD/Healthy, 10RM/30RM), also check for possible interaction effects with other relevant factors. For transparency, all statistical analyses of main effects and interaction effects for the vitamin D_3 vs placebo supplementation RCT-perspective are provided in **Appendix III** (supplementary section of Paper II). This rigorous overview is not provided for the ancillary studies of resistance training-associated changes in COPD vs Healthy participants and 10RM vs 30RM resistance training, but are instead commented on in the main text whenever relevant. Moreover, for analyses of vitamin D_3 vs placebo supplementation and COPD vs Healthy responses to resistance training, we used the mixed modelling-approach to specify two different observations of the dependent variable (i.e. *the response to resistance training*; pre to post measures) *per* participant,

i.e. both the response to 10RM and 30RM resistance training. This arguably increased the statistical power of these analyses.[132] Importantly, a check for interactions with exercise load were performed.

The meta-analysis perspective. The inclusion of a heterogeneous study group is also underlined by the rationale behind the general biobank *The Trainome*,[133] which is situated at Inland Norway University of Applied Sciences (INN), campus Lillehammer, within which samples from the present study are integrated. The biobank represents the long-term strategy (2014-2039) of the research environment at INN-Lillehammer and aims to decipher the causality behind individual variations in responses to lifestyle therapy, with the overall objective to develop computational frameworks for personalized lifestyle therapy prescription. At present, four comparable training intervention studies with different participant characteristics have been completed or are currently being conducted (n=185 participants). One of these other training intervention studies, i.e. *The Alpha & Omega Study* (ClinicalTrials.gov identifier: *NCT04279951*), which is in its data collection phase (April 2021), are using the same training protocol as in the RCT study of The Granheim COPD Study, though conducted using different study clusters (resistance training-naïve persons in the age of 30-60 years, with or without obesity). Together, these two data sets will enhance our knowledge about how different factors and person characteristics (e.g. age, sex, obesity, COPD) affect muscle functional and biological adaptations to 10RM and 30RM resistance-training loads. Notably, for analyses of data from The Granheim COPD Study, the general effects of 10RM vs 30RM resistance training are not presented in debt in any of the papers accompanying this thesis, but are rather highlighted in the Results and discussion chapter.

The choice of primary outcomes in vitamin D_3-based analyses and the rationale behind weighted combined-factors analyses. In retrospect, the pre-identified primary objective for the vitamin D_3 RCT-perspective of the study was not ideal (i.e. the effects of resistance training with vitamin D_3 supplementation on muscle fiber cross-sectional area (CSA) and proportions; *NCT02598830*). The underlying rationale behind the choice was to investigate the effects of vitamin D_3 supplementation on a set of unbiased biological variables, adhering to the existing notion that vitamin D may affect muscle fiber size and fiber type proportions.[134] We thus clearly underestimated the reliability issues riddled with histological measures, which indeed were evident in the data set (**Figure 3**). Accordingly, in order to achieve reliable assessment of muscle hypertrophy, and thus to avoid relying on muscle fiber CSA data alone, we developed a lower-body muscle mass factor, in which change scores from a collection of muscle mass-related outcomes were combined in a weighted manner (**Table 2**). Similar approaches have previously been used to reduce the variability associated with singular outcome measures when investigating biological phenotypes related to high- and low-responders to resistance training.[135–137] Careful investigation of the computed muscle mass factor suggested that it increased the biological value of muscle mass-related analyses (for more information, see **Appendix III** and **IV**, supplementary material to Paper II and III, respectively). Following this logic, combined factors were also computed for other outcome domains, including lower-body maximal muscle strength, lower-body muscle quality, and one-legged and whole-body endurance performance (**Table 2**; see the table text for a brief description of how the factors were computed) and are presented as core outcome domains in Paper II, III and IV. Importantly, neither of these factors have been independently validated, but factor analyses revealed correlations between the underlying outcome variables for all factors, indicating biological coherence (**Appendix III** and **IV**). In Paper II, muscle fiber CSA was included in the muscle mass factor. This was not continued in Paper III, IV and this thesis,

due to its poor association with other muscle mass measures in cluster-specific analyses. Importantly, the removal of muscle fiber CSA from the muscle mass factor in Paper II did not alter any results or interpretations.

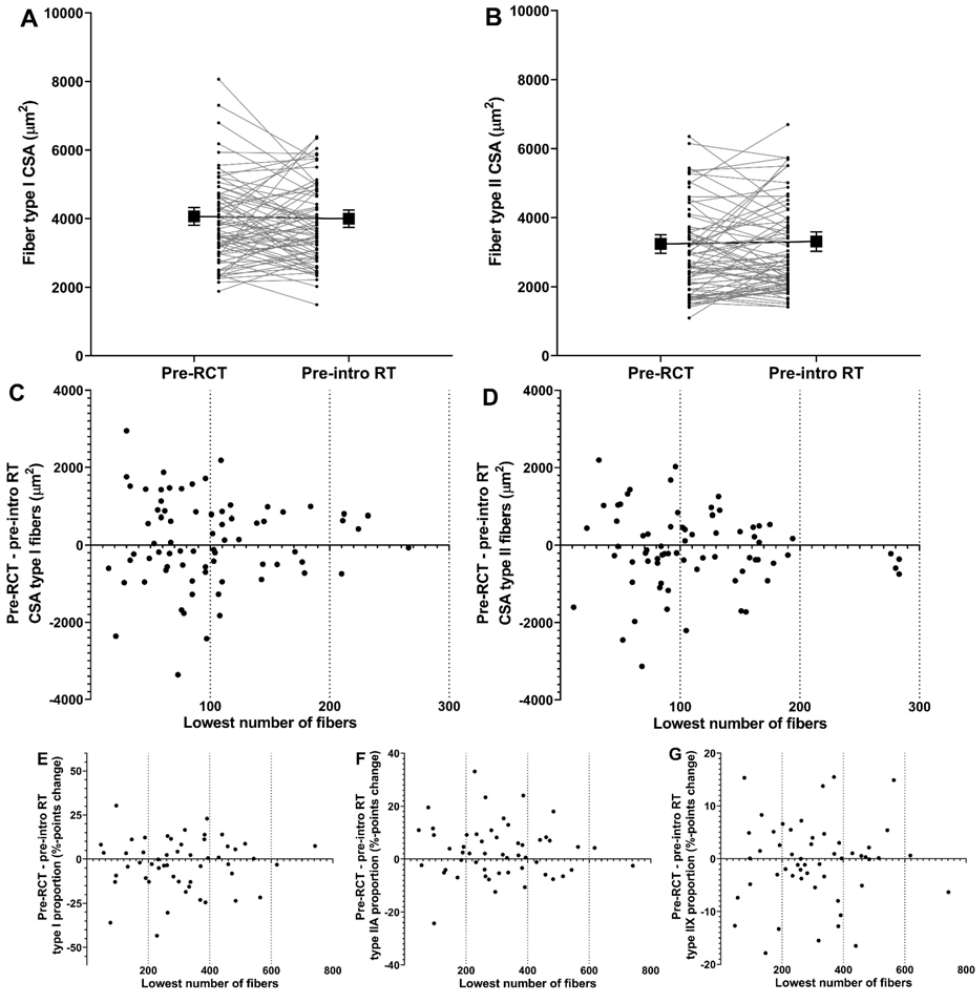

Figure 3. Sample-resample reliability measures of immunohistochemical assessments of muscle fiber cross-sectional area (**A-D**) and muscle fiber proportions (**E-G**) in *m. vastus lateralis* sampled at pre-RCT and pre-introduction to resistance training (pre-intro RT; i.e. no resistance training conducted between the two sampling events). In A-B, data are presented as means with 95% confidence limits. In C-G, data are presented as individual values in *p-plots*, emphasizing the relationship between differences in muscle fiber characteristics measured at the two time points and the lowest number of fibers counted at any time point. In general, these data display increasing differences in sample-resample muscle characteristics with decreasing number of analyzed fibers. RT, resistance training. Rough analyses suggested that we would have needed >250 fibers of each fiber type to achieve a reliable assessment of CSA and >600 fibers to achieve reliable assessment of fiber type proportions, of which our material contained an average of 118±64/137±69 fibers (type I/type II, range 0-428/11-424) and 462±265 fibers (range 26-1982), respectively.

Factors	Lower-body muscle strength 1RM knee extension and leg press *and* peak torque knee extension at 60, 180 and 240°/sec	Lower-body muscle mass Leg lean mass *and* *V. lateralis* and *r. femoris* thickness	Lower-body muscle quality Muscle strength factor *divided by* muscle mass factor	One-legged endurance performance Maximal workload $cycling_{1\text{-leg}}$ *and* # of repetitions at 50%of 1RM knee $extension_{pre\text{-}RCT}$	Whole-body endurance performance Maximal workload bicycling *and* # of steps achieved in a 6-min test *and* # of sit-to-stands in a 1-min test
Vitamin D arm	♂0.6±0.1 ♀0.4±0.1	♂0.7±0.1 ♀0.6±0.1	♂0.8±0.1 ♀0.6±0.1	♂0.3±0.1 ♀0.3±0.1	♂0.5±0.1 ♀0.5±0.1
Placebo arm	♂0.6±0.1 ♀0.4±0.1	♂0.7±0.1 ♀0.6±0.1	♂0.8±0.1 ♀0.6±0.1	♂0.4±0.1 ♀0.3±0.1	♂0.6±0.2 ♀0.5±0.1
COPD cluster	♂0.5±0.1 ♀0.3±0.1	♂0.6±0.1 ♀0.5±0.1	♂0.7±0.1 ♀0.6±0.1	♂0.2±0.0 ♀0.2±0.0	♂0.4±0.1 ♀0.3±0.1
Healthy cluster	♂0.6±0.1 ♀0.4±0.1	♂0.7±0.1 ♀0.6±0.1	♂0.9±0.1 ♀0.7±0.1	♂0.4±0.1 ♀0.3±0.1	♂0.7±0.1 ♀0.6±0.1

Table 2. Combined factors for the core outcome domains *lower-body muscle strength*, *lower-body muscle mass*, *one-legged endurance performance* and *whole-body endurance performance* were computed from various outcome measures, as defined in the upper panel. In the table, baseline characteristics of supplementation arms and study clusters in the RCT study. *Brief description of how the factors were computed:* First, for each singular outcome measure, each study participants' values (baseline and post) were normalized to the highest value recorded during the study of any participant, resulting in individual scores ≤1. Thereafter, outcome domain factors were calculated as the mean of the normalized values for each variable for each participant.

Ethical considerations. Despite the fact that both the preparatory study and the RCT study were approved by The Regional Committee for Medical and Health Research Ethics – South-East Norway and were carried out according to the *Declaration of Helsinki*, participation in such interventions may include some potential risks and discomforts. All participants were informed about these issues both written and orally and gave their informed consent prior to study enrolment.

In the RCT study, muscle biopsies from *m. vastus lateralis* and venous blood samples were sampled in order to measure muscle and blood characteristics and changes thereof with vitamin D_3 supplementation and resistance training. Taken into account the invasive nature of such assessments, a potential risk of infection was present. However, these risks were minimized by using the microbiopsy procedure for muscle sampling.[138] This method does not require incision through the skin, as the skin is rather punctuated using the needle; thereby markedly reducing the invasiveness of this method compared to the more commonly used Bergström method. The use of disposable needles, sterile conditions and experienced operators further secured the muscle and blood sampling procedures. No infections were reported after the 539 biopsies and the 392 blood samples collected in the current study. Nevertheless, some participants displayed hematomas with subsequent thigh pain for days after the biopsy sampling, and a handful of the muscle sampling events were associated with thigh pain lasting from a few days to 3 weeks. In those occasions, participants received daily follow-up from the study manager and, if required, participants were examined by the responsible medical doctor in the study.

Vitamin D_3 supplementation can, in extreme cases, lead to vitamin D toxicity (i.e. hypervitaminosis D), normally defined as serum 25(OH)D levels >375 $nmol \cdot L^{-1}$.[139] Such levels are associated with consumption of vitamin D_3 in the range of >40 000 – 50 000 $IU \cdot day^{-1}$ for ≥6 weeks,[140,141] i.e. a far larger dosage than in the present RCT. Adding to this, no significant increases in serum calcium levels were observed, commonly known as the first manifestation of vitamin D_3 toxicity,[139] and the vitamin D_3 arm did not report any pronounced digestion or sleep problems,

dermal irritations or issues with the urinary system or the vestibular system in their weekly health survey compared to those reported in the placebo arm.

Heavy resistance training involves a potential risk of injury. All training sessions were therefore supervised by qualified personnel to ensure correct technical execution. If pain was still experienced during exercise, modifications were made to the training program such as brief periods of reduced training volume or even not perform training exercises on the problematic leg were provided (n=3).

4 Results and discussion

4.1 Characteristics of COPD vs Healthy participants

In the RCT, COPD participants displayed clear and well-known disease-related aberrancies compared to Healthy participants, including impaired lung function, higher levels of systemic low-grade inflammation (c-reactive protein$_{serum}$ levels), lower levels of bone mineral density, muscle mass and muscle functionality, as well as muscle biological aberrancies such as lower mitochondrial oxidative capacity, higher proportion of muscle fiber type IIX and differential mRNA expression of 227 genes (**Figure 4A**). The COPD phenotype thus resembled observations previously made in the COPD population, [22,83,142–144] with exception of the larger muscle fiber type I CSA in COPD compared to Healthy (**Figure 4A**). This contrasts previous studies, who have reported smaller or similar CSA of type I fibers in COPD.[143,145,146] If this is representative for the COPD population, this may point to a compensatory mechanism for a more accelerated loss of motor units in these COPD participants than during normal aging in healthy persons,[147] whereby reduced quantities of muscle fibers are compensated for by increased sizes of remaining fibers, as previously reported in rodents.[148]

In the preparatory study, the COPD phenotype clearly had an impact on muscular performance. Generally, COPD was associated with impaired muscular performance compared to Healthy (**Figures 4B, 4D**), and this was exacerbated during the two-legged leg press exercise, which was the exercise performed associated with the largest physiological demand. More specifically, for Healthy, muscular performance increased progressively with increasing complexity of the exercise, and thus with the amount of active muscle mass: one-legged knee extension < one-legged leg press < two-legged leg press (**Figures 4B, 4D**). For COPD, a similar increase was seen going from one-legged knee extension to one-legged leg press, but not from one-legged leg press to two-legged leg press, where no significant increase occurred (**Figures 4B, 4D**). This progressive increase was highlighted in a subset of analyses where we calculated one- and two-legged leg press performance as relative performance compared to one-legged knee extension (**Figures 4C, 4E**). In these analyses, there were significant interaction effects between study cluster and two-legged leg press performance (**Figure 4C**, **Figure 4E**), highlighting that muscular performance was impaired during two-legged leg press in COPD compared to Healthy participants.

The results suggest that for persons with moderate COPD (GOLD grade II/III), the cardiorespiratory limitations inherent to the disease has negative consequences for performance in resistance exercises involving larger amounts of active muscle mass (>one-legged leg press). Previously, similar observations have been made for COPD persons with more severe diagnoses,[74,75] but not in the present population of moderate COPD, and not in connection with isolated resistance exercises performed in apparatus. Additionally, the findings provide support for the use of unilateral training protocols in the RCT study, although it is largely unstudied if a larger acute muscle-specific exercise volume will translate into superior long-term resistance training adaptations for this population. But indeed, one-legged resistance training in COPD has recently been associated with greater resistance training-associated effects on functional capacity (6-minute walking distance) compared to a conventional two-legged resistance exercise training-approach.[67] The results may also be interpreted as supportive for combining the Healthy and COPD clusters in the vitamin D_3 RCT analyses, as the COPD persons apparently were not limited by their cardiorespiratory fitness in such exercises as performed during the training intervention in the RCT study.

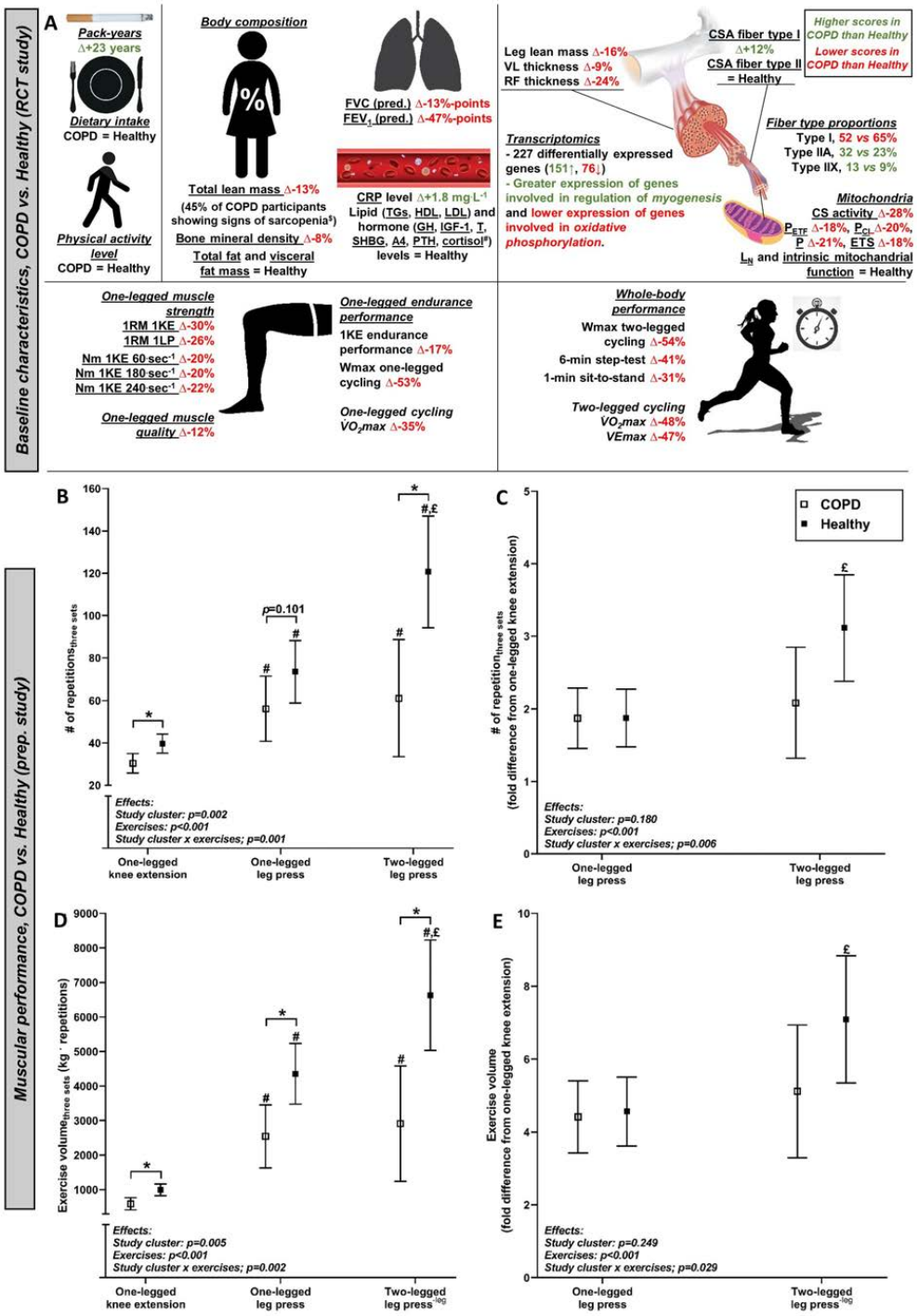

Figure 4. A) Comparison of baseline characteristics for COPD and Healthy participants in the RCT study, including body composition, lung function, blood variables, muscle characteristics, muscle strength and endurance performances. $, as defined by Baumgartner *et al.*[149]; #, cortisol levels were significantly lower in

COPD compared to Healthy at pre-intro RT; Δ, average difference between COPD and Healthy (COPD – Healthy). Green and red text denotes higher scores in COPD compared to Healthy and vice versa, respectively. Alpha level at $p<0.05$. **B-E)** Muscular performance in resistance exercises for COPD and Healthy participants performed as three sets to exhaustion at 60% of 1RM. Muscular performance was measured as **B**, total number of repetitions to exhaustion, **C**, number of repetitions to exhaustion in one-legged and two-legged leg press relative to one-legged knee extension, **D**, total exercise volume (kg · repetitions) *per* leg and **E**, total exercise volume for one-legged leg press and two-legged leg press^{-leg} relative to one-legged knee extension. Data are means with 95% confidence levels. Two-legged leg press^{-leg}, two-legged leg press divided by two; CS, citrate synthase; P_{ETF}, fatty acid oxidation capacity; P_{CI}, complex-1 linked respiration; P, total oxidative phosphorylation capacity; ETS, maximal uncoupled respiration; L_N, leak respiration; *, significant difference between study clusters; #, significant different from one-legged knee extension; £, significant different from one-legged leg press. Alpha level at $p<0.05$.

4.2 General observations on the conduct and quality of the RCT, and the effects of vitamin D_3 supplementation *per se* on muscle function and biology

Before assessing the results of the RCT, it is vital to reaffirm that the protocols used held sufficient quality, with particular emphasize on whether the training protocol and the vitamin D_3 supplementation were conducted successfully. It is also of importance to rule out the effects of vitamin D_3 supplementation *per se* (weeks 1-12 of the RCT, **Figure 2**), before embarking on the analyses of the main objective of the RCT, i.e. the effects of combined vitamin D_3 supplementation and resistance training on muscle functional and biological training-associated adaptations. In this way, we can arguably differentiate between the effects of vitamin D_3 supplementation *per se* and vitamin D_3 supplementation + resistance training.

General quality and efficacy of the resistance training protocol. The training protocol was associated with a low drop-out rate (n=4, 5%; COPD, n=2) and high adherence (98%, range 81-100%), likely ensured by close follow-up from qualified personnel, including supervision of all training sessions (for details, see **Appendix I**: *A qualitative analysis of motivational factors for resistance training in chronic obstructive pulmonary disease: experiences from The Granheim COPD Study*). Overall, for all study participants taken together, this was reflected by considerable increases in exercise volume throughout the training intervention. Exercise volume (kg · repetitions) increased by 20% (knee extension) and 30% (leg press) from the 1st to the 4th week of training, by 48% and 54% from the 1st to the 8th week, and by 65% and 68% from the 1st to the last week of training, which resembles or exceeds training progression in similar studies on previously untrained participants.[91,150] These increases (knee extension and leg press combined) were similar between vitamin D_3 and placebo supplementation arms (p=0.199-0.478) and COPD and Healthy study clusters (p=0.091-0.142), with exception of the change from the 1st to the 4th training week, which was different between study clusters (COPD, 18%; Healthy, 28%; p=0.023). For both knee extension and leg press exercises, exercise volume was generally higher for 30RM compared to 10RM (Δ18%, p=0.001; Δ76%, p<0.001), which is typically seen in young healthy adults as well.[93] For 1RM muscle strength improvement *per* session, the intervention showed relative efficiencies of 0.9% (knee extension) and 1.4% (leg press) improvement, which resemble or exceeds previous findings in untrained older adults (i.e. 0.5-1.0% per session).[151–153] The intervention also led to pronounced improvements in whole-body functional performance, including maximal workload achieved during two-legged cycling (12 watts/8%↑, p<0.001), 6-min step test performance (14 steps/7%↑, p<0.001), and 1-min sit-to-stand

performance (2 sit-to-stands/8%↑, p<0.001). This was accompanied by reductions in cycling oxygen cost (4%↓; p<0.001) and improved gross efficiency (0.6%-points↑, p=0.001).

The arguably successful completion of the resistance training intervention was accompanied by marked muscle biological adaptations when combining the results from all study participants. This included significant increases in muscle mass (lean body mass, 0.7 kg/1.4%↑, p<0.001; leg lean mass, 0.14 kg/1.9%↑, p<0.001; *m. vastus lateralis* thickness, 1.4 mm/7%↑, p<0.001; *m. rectus femoris* thickness, 1.9 mm/14%↑, p<0.001), muscle fiber-CSA (type I, 360 μm^2/14%↑, p<0.001; type II, 599 μm^2/22%↑, p<0.001), increases in myonuclei number *per* fiber (type I, 36%↑, p=0.018; type II, 20%↑, p=0.011), alterations in muscle fiber proportions (e.g. type IIX muscle fiber proportion changed from 10% to 7%, p<0.001), and robust alterations in muscle transcriptome profiles (499 and 312 differentially expressed genes compared to baseline at 3 ½ weeks and post-RCT, respectively). Further, the study intervention was associated with beneficial health effects such as reduced serum levels of triglycerides and low-density lipoprotein/LDL, reduced fat mass (total and visceral fat), and improved self-reported health and health-related quality of life.

Sarcopenia. Overall, the resistance training intervention proved effective for treating age-related loss in muscle mass, leading to 1.4% increases in total lean body mass. This reduced the number of participants that could be defined as sarcopenic from 16% (11 persons) to 12% (8 persons), with sarcopenia being defined as appendicular lean mass (kg)/m^2 greater than two standard deviations below the sex-specific means of young adults.[149] Speculatively, the increase in total lean mass was supported by increased levels of serum creatinine in both supplementation arms (+6%). Although serum creatinine is generally used for evaluation of renal function,[154] creatinine production and levels also increase with increases in total muscle mass.[154,155]

During the training intervention neither of the participants experienced training-related injuries, and only five participants (6%) reported discomforts with training towards the end of the intervention. Of the four participants that withdrew from the study during the resistance training intervention, neither were associated with training injuries.

General efficacy of the vitamin D_3 supplementation protocol. The initial 14 days of high doses of vitamin D_3 (10 000 IU·day^{-1}) efficiently increased vitamin D status (measured as serum 25(OH)D levels) in the vitamin D_3 arm, which was subsequently maintained at a high level during the rest of the study intervention using maintenance doses of vitamin D_3 (2 000 IU·day^{-1}) (**Figure 5**). Conversely, in the placebo arm, 25(OH)D levels either declined or was unaltered compared to baseline levels (**Figure 5**). This led to robustly elevated levels in the vitamin D_3 arm compared to the placebo arm during the entirety of the study conduct (Δ45-49 nmol·L^{-1}) (**Figure 5**). In the vitamin D_3 arm, all participants were vitamin D-sufficient at the onset of resistance training (as well as at all other time points), as classified by the National Academy of Medicine ([25(OH)D] >50 nmol·L^{-1}),[31] while in the placebo arm, 5-13 participants were vitamin D-insufficient during the study intervention. After the initial 14 days with high daily doses of vitamin D_3, the marked increase in 25(OH)D in the vitamin D_3 arm were accompanied by robust increases in 1,25$(OH)_2$D levels (the bioactive form of vitamin D) compared to the placebo arm (**Figure 5**). However, the rapid elevation of 1,25$(OH)_2$D levels was subsequently reversed towards baseline levels during the rest of the study conduct when the participants in the vitamin D_3 arm consumed lower doses of vitamin D_3 (**Figure 5**).

According to the weekly health survey, vitamin D_3 supplementation was not associated with adverse health issues compared to placebo supplementation, including digestion problems, sleep problems, urinary system issues, dermal irritations or vestibular system issues.

Habitual dietary intake. During the training intervention, the habitual dietary intake was similar between the two supplementation arms, as well as between the two study clusters regarding protein (vitamin D_3, 1.3 $g \cdot kg \cdot day^{-1}$; placebo, 1.3 $g \cdot kg \cdot day^{-1}$; COPD, 1.2 $g \cdot kg \cdot day^{-1}$; Healthy, 1.3 $g \cdot kg \cdot day^{-1}$), fat (vitamin D_3, 1.0 $g \cdot kg \cdot day^{-1}$; placebo, 1.0 $g \cdot kg \cdot day^{-1}$; COPD, 1.0 $g \cdot kg \cdot day^{-1}$; Healthy, 1.0 $g \cdot kg \cdot day^{-1}$) and carbohydrate consumption (vitamin D_3, 2.5 $g \cdot kg \cdot day^{-1}$; placebo, 2.9 $g \cdot kg \cdot day^{-1}$; COPD, 2.6 $g \cdot kg \cdot day^{-1}$; Healthy, 2.7 $g \cdot kg \cdot day^{-1}$), emphasizing equal nutritional status.

Effects of 12 weeks of vitamin D_3 supplementation-only (weeks 1-12) on muscle strength, performance and characteristics. Vitamin D_3 supplementation itself had no effect on upper- and lower-body muscle strength and performance. Surprisingly, the only exception was 1RM knee extension, for which vitamin D_3 led to negative changes compared to placebo (Δ-8.4%; p=0.008), opposing the seemingly accepted dogma that vitamin D supplementation *per se* exerts positive effects on leg muscle strength.[38,156] Notably, for all muscle strength and muscular performance variables, the initial 12 week supplementation period without resistance training was associated with improved performance in all performance tests. In this time period, an extensive test-retest protocol with five test sessions with assessment of 1RM muscle strength and muscular performance were conducted. These improvements occurred without any apparent changes in muscle cell characteristics in thigh muscle, including muscle fiber CSA (type I, 4%, p=0.573; type II, 9%, p=0.312), muscle fiber type proportions (p=0.127-0.901), and total RNA/rRNA expression (p=0.604-1.000). They were hence likely caused by technical, psychological and neural learning effects,[120] effectuated by repeated exposure to testing prior to and during the supplementation period (see **Figure 2**). Such effects have previously been seen to be more pronounced in older persons,[121] in which these results further emphasizes the importance of familiarization to performance tests to ensure stable and less confounded baseline measurements.

Overall, the 12-weeks supplementation-only period did not lead to marked changes in mRNA transcriptome profiles when combining values from the two supplementation arms. Vitamin D_3 supplementation was, however, associated with differential changes in the expression of a selected genes compared to placebo; 27 genes↑ and 27 genes↓. This included increased expression of B-cell lymphoma 6 and prolyl 4-hydroxylase subunit alpha-1 (*BCL6* and *P4HA1*), both of which are known to oppose accumulation of reactive oxygen species (ROS),[157–159] and decreased expression of angiopoietin-like protein 4 (*ANGPTL4*), which is closely correlated with levels of mitochondrial respiration.[160] These findings were reaffirmed by gene enrichment analyses, which showed a general reduction in the expression of gene sets relating to both oxidative and glycolytic metabolism in the vitamin D_3 arm. This is in line with previous observations whereby vitamin D has been shown to counteract ROS and mitochondrial oxidative stress.[161] The seemingly negative effect of vitamin D_3 supplementation for expression of mitochondrial genes may thus be due to reduced mitochondrial turnover, albeit this is clearly a speculative interpretation. Of note, expression of the vitamin D receptor (VDR) was identified in the data set, but was not affected by supplementation.

4.3 The impact of vitamin D_3 supplementation on resistance training-associated adaptations

Effects of vitamin D_3 supplementation on resistance training-associated changes in myofiber cross-sectional area and proportions (primary outcomes). In contrast to the main hypothesis, vitamin D_3 supplementation did not enhance resistance training-associated increases in muscle fiber cross-sectional area or changes in muscle fiber proportions (**Figure 5**, *Primary outcomes*). Hence, the results does not support the prevailing notion that vitamin D affects such variables in a favorable manner (e.g. elucidated in the review from Ceglia[134]), at least not in the enrolled group of study participants (older adults with and without COPD) and within the time frame of the study.

Effect of vitamin D_3 supplementation on training-associated changes in maximal muscle strength and lower-limb muscle mass. Participants in both vitamin D_3 and placebo arms showed increases for all measure of muscle strength and mass (except handgrip strength), assessed from baseline (i.e. after 3 ½ weeks of resistance training) to after finalization of the resistance training intervention: 12-25% for upper- and lower body 1RM muscle strength, 6-11% for maximal leg muscle torque, 7-19% for muscle thickness, and 1-3% for leg lean mass (**Figure 5**, *Secondary outcomes*). As expected, after combining these measures into weighted muscle strength and muscle mass factors, and the derived muscle quality factor (Δmuscle strength factor/Δmuscle mass factor), similar increases were observed (**Figure 5**, *Core outcomes*). Overall, vitamin D_3 supplementation did not affect any of these outcome measures compared to placebo in the participants. This was primarily evaluated as changes in the calculated weighted muscle strength, muscle mass and muscle quality factors (**Figure 5**, *Core outcomes*), and secondarily as changes in each of the underlying outcome measures (**Figure 5**, *Secondary outcomes*). Vitamin D_3 supplementation thus had no main effect on training-associated changes in muscle functionality or gross muscle biology. While this conclusion coheres with the few comparable studies assessing the effect of combined vitamin D_3 intake and resistance training,[43,45–47] it contrasts the conclusion drawn in the only available meta-analysis on this subject, wherein vitamin D_3 supplementation was associated with augmented increases in muscle strength in older adults.[44] Notably, among the selection of ten specific outcome measures, two did not conform with the main finding. Vitamin D_3 was associated with beneficial effects for changes in 1RM knee extension (**Figure 5**, *Secondary outcomes*) and muscle thickness of *m. rectus femoris* (**Figure 5**, *Secondary outcomes*). For 1RM knee extension, the effect was interrelated with the negative development seen from pre-RCT to pre-introduction to training in the vitamin D_3 arm (see *Effects of 12 weeks of vitamin D_3 supplementation-only (weeks 1-12) on muscle strength, performance and characteristics*). Indeed, when assessing the effect of vitamin D_3 on 1RM knee extension from pre- to post-RCT (rather than from baseline at post-introduction to training), no beneficial effect was observed compared to placebo (Δ-2% (95% CI, -12, 7), p=0.628). As for muscle thickness in *m. rectus femoris*, we did not collect data pre-RCT and can thus not deduce if this variable followed the same pattern as 1RM knee extension. The observed benefits of vitamin D_3 supplementation for changes in *m. rectus femoris* thickness contrasts observations made for *m. vastus lateralis thickness* (**Figure 5**, *Secondary outcomes*), and even oppose those made for lean mass of the legs, which tended to increase less in the vitamin D_3 arm compared to the placebo arm (**Figure 5**, *Secondary outcomes*, p=0.090).

Effects of vitamin D_3 supplementation on training-associated changes in one-legged and whole-body endurance performance. Participants in both vitamin D_3 and placebo arms showed improvements in one-legged and whole-body endurance performance over the course of the resistance training

intervention: 37-52% increases in one-legged knee extension and bilateral chest press performance, 7-9% increases in maximal power output in one- and two-legged cycling , 3-5 % reductions in O_2 costs of submaximal one-legged cycling, and 6-10% increases in functional performance (sit-to-stand test and 6-min step test) (**Figure 5**, *Secondary outcomes*). In accordance with this, marked increases were observed in weighted one-legged and whole-body endurance performance factors (**Figure 5**, *Core outcomes*). These effects cohere well with previously observed benefits of resistance training for endurance variables in older adults.[162–164] However, vitamin D_3 supplementation did not affect any of these outcome measures compared to placebo, neither for weighted endurance performance factors (**Figure 5**, *Core outcomes*), nor for any of the specific outcome measures (**Figure 5**, *Secondary outcomes*).

Effects of vitamin D₃ supplementation on training-associated changes in muscle fiber characteristics and transcriptomics. Participants in both vitamin D_3 and placebo arms showed marked changes in muscle fiber characteristics over the course of the training intervention. These included decreased type IIX muscle fiber proportions from 10% to 7%, increased type IIA proportions from 26% to 29%, increased type IIA/IIX hybrid fibers abundances from 2.6% to 3.2%, and 25-48% increases in myonuclei number *per* muscle fiber (**Figure 5**). Changes in IIX and IIA proportions were verified using qPCR, showing decreased levels of type IIX mRNA abundance and increased levels of type IIA mRNA (**Figure 5**, *Secondary outcomes*), calculated using the gene family-profiling approach.[165] These analyses also revealed increased proportions of type I mRNA after the training intervention, potentially caused by increased type I protein turnover. The observed changes in muscle fiber-type characteristics in response to resistance training corroborate well with previous studies in older adults,[166–168] though increased numbers of myonuclei *per* muscle fiber are not consistently reported.[169] Vitamin D_3 supplementation did not affect training-associated changes in muscle fiber proportions or myonuclei content compared to placebo (**Figure 5**).

Irrespective of supplementation arm, the training intervention resulted in 1.14-1.16 fold increases in total RNA *per* unit muscle tissue weight, a proxy marker for ribosomal RNA content that has previously been associated with training-induced changes in muscle growth and strength.[112,170] Similar increases were found for the mature ribosomal species 18s (1.18 fold) and 28s (1.16 fold), in addition to the 45s pre-ribosomal rRNA (1.19 fold) using qPCR. No changes were observed for 5.8s (1.07 fold, p=0.722) or 5s (1.06, p=0.940) following the entire training intervention. Notably, for analyses of total RNA and ribosomal RNA, an additional time point were included in main analyses, i.e. in muscle biopsies sampled after introduction to training (3 ½ weeks, 7 sessions), as early increases in total RNA seem to associate with long-term chronic responses to training, making it a potential hallmark of muscle plasticity.[112] As expected, 3 ½ weeks of training led to marked increases in total RNA (1.10-1.21 fold) and expression of all ribosomal RNA species (1.13-1.27 fold). Whereas these changes corroborates quite well with changes observed in healthy, young persons,[112] with the notable exception of less pronounced relative increases, they contradict previous observations of no resistance training-associated increases in total RNA *per* unit muscle tissue weight in older persons.[171] Vitamin D_3 supplementation did not affect training-associated changes in total RNA or rRNA expression compared to placebo (**Figure 5**, *Secondary outcomes*).

The training intervention led to marked changes in muscle mRNA transcriptome profiles in the two supplementation arms combined, with 499 genes being differentially expressed after 3 ½ weeks of resistance training (post-intro RT; 436 genes↑, 63 genes↓) and 312 genes being

differentially expressed after 13 weeks of resistance training (post-RCT; 255 genes↑, 57 genes↓) (**Paper II**; *Figure 11A, 11B*). VDR was expressed, but unaffected by combined vitamin D_3 supplementation and resistance training, contradicting previous observations of a positive association between supplementation-induced improvements in 25(OH)D status and VDR expression in leukocytes,[172] myoblasts/myotubes[173] and skeletal muscle[174]. GO enrichment analyses revealed increased expression of gene sets associated with extracellular matrix, blood vessel morphogenesis and leukocyte migration at both 3 ½ and 13 weeks (**Paper II**; *Figure 11C*), as well as increased expression of the inflammatory response gene set at 3 ½ weeks. Conversely, decreased expression was observed for gene sets involved in ribosomal functions at both 3 ½ and 13 weeks (**Paper II**; *Figure 11C*). This could be interpreted as contradicting the likely important role of *de novo* ribosomal biogenesis for training-associated muscular adaptations.[112,170] Notably, these analyses were performed using traditional library size-based normalization, which basically provides target gene expression relative to the expression of all other genes.[111] In an alternative set of transcriptome analyses, which rather was performed using a normalization procedure that corrects for muscle sample weight and thus provides gene expression analyses per sample size (tissue-offset normalization),[111] the negative effects of resistance training on ribosomal gene expression was not evident. This was the only major difference between library size and tissue-offset normalization in the present study setting.

Vitamin D_3 supplementation had no effect on training-associated changes in gene expression, neither at 3 ½ weeks (**Paper II**; *Figure 11D*) nor at 13 weeks resistance training (**Paper II**; *Figure 11E*), suggesting that no single gene was differentially affected by combined vitamin D_3 supplementation and resistance training. In contrast to this, enrichment analyses showed traces of vitamin D_3-sensitive changes in expression at both 3 ½ and 13 weeks of resistance training (**Paper II**; *Figure 11F*). At 3 ½ weeks, there was differential expression of gene sets involved in i.e. cell junctions, blood vessel morphogenesis and muscle cell differentiation. These initial responses to resistance training should be interpreted with caution, as they were only evident in one of the two analyses (GSEA or rank-based analyses; **Paper II**, *Figure 11F*). At 13 weeks, the vitamin D_3 arm showed increased expression of gene sets involved in endothelial proliferation and blood vessel morphogenesis compared to placebo (consensus between GSEA and rank-based analyses; **Paper II**, *Figure 11F*). This agrees with the previously observed positive relationship between 25(OH)D-status and endothelial function, potentially interacting through the endothelium-derived vasodilator, nitric oxide.[161] Indeed, this coheres well with a recent study, which showed favorable effects of combined vitamin D_3 supplementation and resistance training for flow-mediated dilation of blood vessels and blood pressure in postmenopausal women.[175] Unfortunately, endothelial function was not assessed in the current study.

Effects of vitamin D_3 on hormones in blood and health-related outcome measures.

Steroid hormones. Vitamin D_3 supplementation did not affect levels of anabolic steroid hormones such as testosterone. This was in discordance with our initial hypothesis, as we presumed a positive association between vitamin D levels (measured as 25(OH)D) and testosterone levels, based on previous observations from vitamin D_3 supplementation studies[55] and cohort studies.[176] However, our finding is in line with several other vitamin D supplementation studies, which has failed to observe any effects on testosterone levels in blood.[177,178] Conversely, vitamin D_3 supplementation seemed to affect serum cortisol levels compared to placebo (Δ48 nmol · L^{-1},

p=0.038), though no main effect of time was observed (i.e. the observed increase in the vitamin D_3 arm was not statistically significant, p=0.374) and there was no statistical difference between supplementation arms at the end of the intervention (p = 0.053).

Lung function. When pooling the data from all study participants, the 28 week long RCT was associated with an undesirable -1.95% reduction in FVC (p=0.006). This was somewhat surprising, as exercise is generally believed to be beneficial for lung functionality, including resistance training,[70,179] but may be due to a general age-related decline, as the magnitude of the changes resemble those seen in corresponding age cohorts over a similar time frame.[180] Notably, other measures of lung function, such as FEV_1, predicted FEV_1 and FEV_1/FVC, were not affected by the intervention *per se*. However, there was a negative effects of vitamin D_3 supplementation on FEV_1/FVC (Δ-2.9 %-points, p = 0.012), which was surprising since previous research has shown beneficial effects of vitamin D supplementation on lung function.[181] The detrimental effect of vitamin D_3 supplementation on FEV_1/FVC showed a clear interaction with study clusters, and as such was only evident in COPD persons in the vitamin D_3 arm, which showed Δ-8.4% reductions compared to placebo. This subgroup analysis was however clearly weakened by the small sample size (COPD, n=9 vs n=11, vitamin D_3 vs placebo). The negative effect of vitamin D_3 on FEV_1/FVC did not interact with pre-RCT levels of FEV_1/FVC, but surprisingly, in another subgroup-analysis including both COPD and Healthy participants, the lowest quartile of pre-RCT 25(OH)D levels in the vitamin D_3 arm was associated with larger decrement in FEV_1/FVC than the corresponding quartile of placebo arm participants (Δ-5.4 %-points, p=0.009). This observation is difficult to explain, as it indirectly opposes the notion that vitamin D deficiency leads to impaired lung functions.[182] More research is clearly needed to elucidate on the consequences of resistance training and vitamin D_3 supplementation for lung functionality.

Bone health. Vitamin D_3 supplementation did not affect bone mineral density (**Figure 5**).

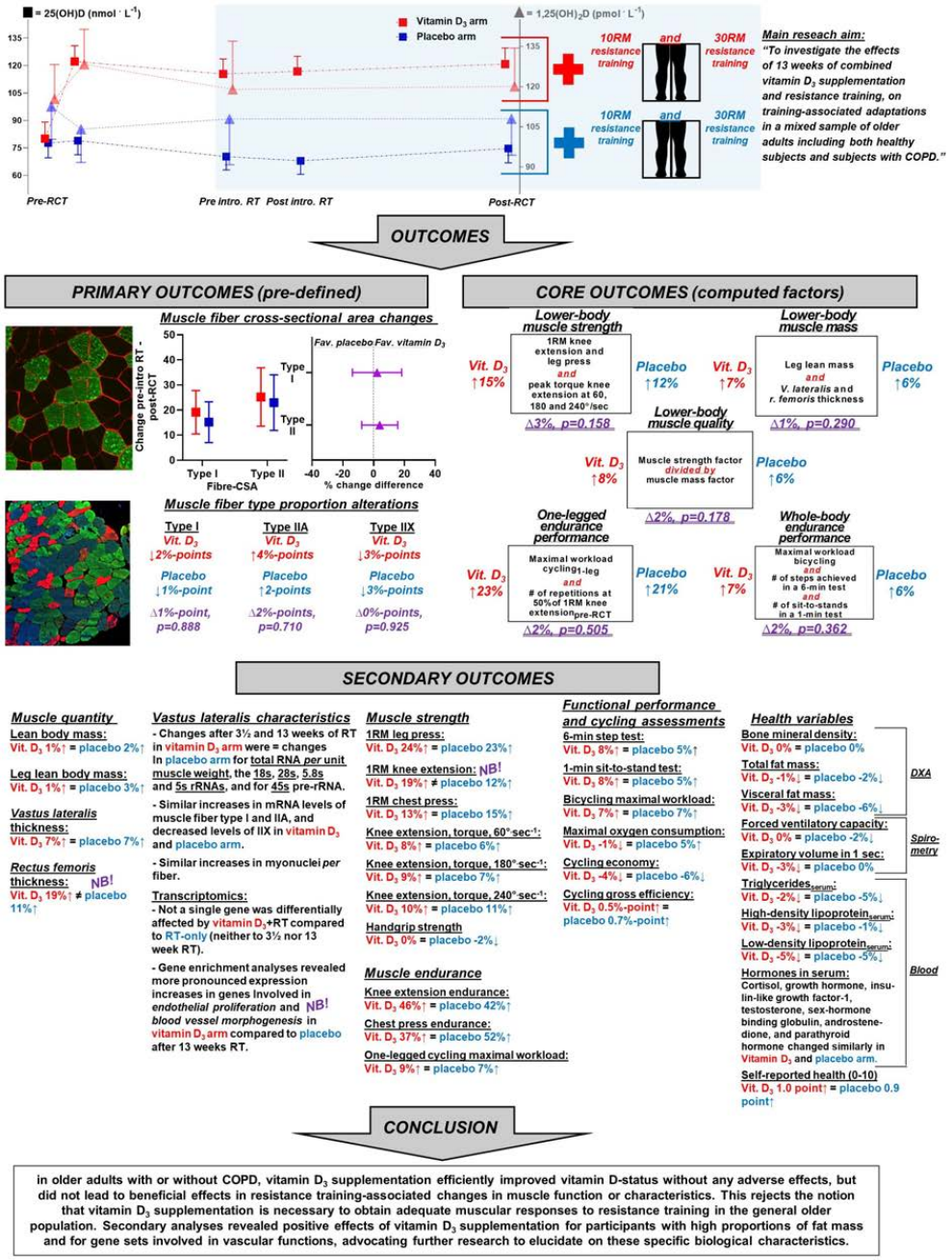

Figure 5. Effects of combined vitamin D_3 supplementation and resistance training in older adults (with and without COPD). In the upper panel, vitamin D status (□, 25-hydroxycholecalciferol (25(OH)D); Δ, 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$) for the two supplementation arms during the RCT, and the two training modalities (high-load and low-load resistance training; 10RM and 30RM, respectively) performed by both supplementation arms during the 13 week training intervention. The training intervention part of the RCT is blue-shaded in the figure. In the middle panel, the effects of combined vitamin D_3 supplementation and resistance training on the study's primary outcomes (*NCT02598830*; changes in muscle fiber cross-sectional area

and fiber type proportions) and core outcome domains (lower-body muscle strength, lower-body muscle mass, lower-body muscle quality, one-legged endurance performance and whole-body endurance performance). For the muscle fiber cross-sectional area-figures, data are presented as means with 95% confidence intervals. For the rest of variables, data are presented as average percent or percent-point changes. Δ, difference in change between supplementation arms (vitamin D_3 – placebo). In the lower panel, an overview of the effects of combined vitamin D_3 supplementation and resistance training on the study's secondary outcomes. Alpha level at $p<0.05$. Red and blue text denotes the vitamin D_3 and placebo arm, respectively. =, $p>0.05$ for comparison of the changes in the vitamin D_3 and placebo arm; ≠, $p<0.05$ for comparison of the changes in the vitamin D_3 and placebo arm.

Remarks on the vitamin D_3 supplementation RCT objective. It seems clear that vitamin D_3 supplementation did not affect muscle functional and biological characteristics in the present study group. This was particularly exemplified in the transcriptome analyses, where not a single gene was found to be vitamin D_3-sensitive after a period of resistance training, which is surprising given the accepted dogma that vitamin D primarily acts as a transcriptional regulator.[58] However, although there was a general lack of effects of vitamin D_3 supplementation, the data set contained a couple of interesting observations. *First*, in the muscle transcriptome data, combined vitamin D_3 supplementation and resistance training had effects on gene sets relating to endothelial and vascular biology. Although speculatively, this may indicate that vitamin D_3 supplementation affects cardiovascular functions and biology, albeit in the current research setting this did not translate into alterations in endurance performance. Possibly, if combining vitamin D_3 supplementation and other training modalities such as endurance training, such changes in vascular gene regulation may be more accompanied by changes in functional improvements. *Second*, in participants with high baseline fat proportions/high body mass index, vitamin D_3 supplementation was associated with increased resistance training-associated changes in muscle strength and muscle quality, but not for other core outcome domains (outlined in **Appendix III**, supplementary material for Paper II). As no such effect was observed for muscle quantity, the potential benefit of vitamin D_3 supplementation for accretion of muscle strength in participants with high proportions of fat may point to improved motoneuron function, and thereby increased muscle activation, as the causal factor. Indeed, motoneuron function has been suggested to be affected by vitamin D supplementation in rodents.[183] These perspectives needs further research.

Despite the arguable success of the vitamin D_3 supplementation protocol, there are still aspects of the vitamin D_3 supplementation that remain unresolved, and that may have affected the conclusions and outcomes of the study. *First*, in skeletal muscle, adequate vitamin D signaling may occur at 25(OH)D levels lower than the defined clinical cutoff (insufficient, <50 $nmol \cdot L^{-1}$).[31] Indeed, studies have suggested that vitamin D insufficiency affects human skeletal muscle in an adverse manner only at concentrations <30 $nmol \cdot L^{-1}$,[184] which was only relevant for one participant in the placebo arm at the onset of the resistance training intervention. In that case, this would leave our 25(OH)D quartile-based analysis (outlined in **Appendix III**, supplementary material for Paper II) with limited biological value. However, in a recent study, no beneficial effects were seen of 12 weeks of vitamin D_3 supplementation (8000 $IU \cdot day^{-1}$) for resistance-training associated changes in lean body mass and a range of muscle strength measures in young vitamin D-deficient male adults (<50 $nmol \cdot L^{-1}$; average at pre-training, 36 $nmol \cdot L^{-1}$; post-training, 142 $nmol \cdot L^{-1}$),[185] suggesting that vitamin D_3 supplementation does not affect muscular functions or trainability in persons with markedly suboptimal baseline 25(OH)D levels. *Second*, serum 25(OH)D levels may be a poor proxy marker for

vitamin D biology as it largely fails to reflect 1,25(OH)$_2$D levels, the metabolically active form of vitamin D.[186] As such, the initial two weeks of high-dosage vitamin D$_3$ supplementation successfully increased the 1,25(OH)$_2$D levels compared to the response in the placebo arm, emphasizing that supplementation is indeed capable of increasing levels of metabolically active vitamin D, at least at high doses and within a short time frame. However, the subsequent 2500 IU·day^{-1} dosage did not result in significant changes compared to pre-RCT levels. Whereas this could be interpreted as a result of insufficient vitamin D$_3$ dosage, this seems unlikely as 25(OH)D levels was clearly elevated, and it is likely rather due to autoregulatory feedback-mechanisms, potentially sustaining 1,25(OH)$_2$D levels within a set and individual physiological range. *Third*, muscle cells may themselves possess the apparatus to convert 25(OH)D into 1,25(OH)$_2$D, as they express the 25-Hydroxyvitamin D 1-alpha-hydroxylase (*CYP27B1*) protein. Indeed, in *in vitro* experiments on murine myoblast and myotubes, 25(OH)D and 1,25(OH)$_2$D treatment seem to lead to similar increases in expression of vitamin D markers such as VDR mRNA, suggesting that peripheral regulation of vitamin D synthesis may be a relevant manner for regulating its biological activity.[173] *Fourth*, while 25(OH)D was assessed as total 25(OH)D levels in the present study, levels of unbound 25(OH)D (i.e. not bound to vitamin D binding protein or albumin; ~0.03%) may represent a more accurate measure of vitamin D status in a clinical setting.[187] Indeed, in mice lacking vitamin D binding protein, and therefore displaying very low total 25(OH)D levels (~8 nmol·L^{-1}), no signs of vitamin D deficiency were seen unless they were put on a vitamin D-deficient diet.[188] *Fifth*, in the present study, the resistance training intervention lasted for only 13 weeks. Speculatively, this may have been too short for vitamin D$_3$ supplementation to manifest its potential benefits for muscle plasticity, despite the presence of a 12-week lead-in supplementation period. Arguably, however, if vitamin D status and signaling are indeed important for muscle biological adaptations to training, even the relatively short intervention should have led to detectable changes in muscle biology, such as its transcriptome. This was not observed, neither in general, nor for specific vitamin D-responsive genes such as the vitamin D receptor.[174] *Sixth*, the study protocol was unavoidably associated with large interindividual variation in responses. This variation may have been related to vitamin D$_3$ supplementation *per se*, resistance training *per se* or to a combination of the two, and may have affected groupwise comparisons. More research is clearly needed to elucidate on all these perspectives. However, for the enrolled study participants with mostly sufficient vitamin D levels at pre-RCT, the conclusion is clear; vitamin D$_3$ supplementation did not affect muscular responses to resistance training, thus rejecting the notion that vitamin D$_3$ supplementation is necessary for obtaining adequate muscular responses to resistance training in the general population of older adults.

4.4 The impact of chronic obstructive pulmonary disease on resistance training-associated adaptations

Muscle strength, muscle mass, muscle quality and one-legged endurance performance. Overall, COPD showed larger training-associated increases in lower-body muscle strength and mass compared to Healthy (the two legs/training modalities combined), measured as relative changes in combined weighted factors from baseline, with no difference being observed between the two study clusters for absolute changes (**Figure 6**, *Core outcomes*). For the singular measures composing the lower-body muscle strength factor (i.e. 1RM knee extension and leg press + knee extension torque executed at

knee angular speeds of 60°, 180° and 240°·sec^{-1}), no differences in either relative or absolute changes were observed between study clusters, with exception of relative change of knee extension torque at 240°·sec^{-1}, which was in favor of the COPD study cluster (**Figure 6**, *Specific outcomes*). Similarly, for the individual measures underlying the muscle mass factor (leg lean mass + *m. vastus lateralis*/*m. rectus femoris* muscle thickness), changes were not significantly different between study clusters, except larger relative change in *m. rectus femoris* thickness in COPD (**Figure 6**, *Specific outcomes*). The COPD and Healthy study clusters showed similarly scaled improvements in muscle quality (Δmuscle strength factor/Δmuscle mass factor) and one-legged endurance performance factors (**Figure 6**, *Specific outcomes*). Notably, COPD showed a larger relative improvement in one-legged cycling maximal workload compared to the response among Healthy counterparts (**Figure 6**, *Specific outcomes*). Taken together, COPD thus showed marked and hitherto largely unrecognized responsiveness to resistance training, contradicting previous suggestions of a negative impact of co-morbidities such as low cardiorespiratory fitness, decreased oxygen levels[20] and chronic low-grade systemic inflammation.[79,189]

Cycling and functional performance. COPD and Healthy showed pronounced and similarly scaled training-associated improvements in whole-body endurance performance, measured as changes from baseline, including 6-min step test performance, 1-min sit-to-stand performance and maximal workload achieved during two-legged cycling (**Figure 6**, *Core outcomes* and *Specific outcomes*). Surprisingly, COPD and Healthy also showed similar changes in performance for these outcome measures in absolute terms, with exception of 6-min step test performance (**Figure 6**, *Specific outcomes*), for which Healthy showed larger improvements (**Figure 6**, *Specific outcomes*), arguably relating to the considerable cardiorespiratory demand of this test, leaving COPD with disease-specific constraints. For other performance indices such as cycling oxygen cost and gross efficiency, which were measured using a one-legged cycling protocol, COPD showed larger relative improvements compared to Healthy (**Figure 6**, *Specific outcomes*).

Together, these observations reiterate on the substantial benefits of resistance training for persons with COPD, even for performance measures that pose large whole-body metabolic demands, which has previously been suggested to be irresponsive to such training.[190] It seems plausible that the observed improvements in 6-min step test performance, 1-min sit-to-stand performance and two-legged cycling were associated with improvements in cycling oxygen cost/gross efficiency and muscle strength, as neither COPD nor Healthy showed training-associated changes in maximal oxygen consumption, with improvements in anaerobic capacity being a potential contributor (not measured).

Muscle characteristics. *Muscle fiber histology.* Whereas COPD and Healthy displayed similar increases in type II fiber CSA in *vastus lateralis* in response to resistance training (**Figure 6**, *Specific outcomes*), only Healthy showed significant increases in type I fiber CSA, with no statistical difference being observed between study clusters (**Figure 6**, *Specific outcomes*). For Healthy, the increase in CSA was accompanied by increased myonuclei·fiber^{-1} in both fiber types (36%/25% for type I/II), leading to decreased myonuclear domain size estimates in type I fibers (-10%). In COPD, no such effects were observed. Despite the lack of difference between the two study clusters for these variables (**Figure 6**, *Specific outcomes*), the data hints at blunted plasticity of type I muscle fibers in COPD only, potentially relating to their altered biological characteristics at baseline (e.g. larger CSA of type I muscle fibers) or to blunted myonuclear accretion.

Both COPD and Healthy displayed training-associated reductions in type IIX muscle fiber proportions. While this reduction was more pronounced in COPD when measured at the protein level (immunohistochemistry), it was more pronounced in Healthy when measured at the mRNA level (**Figure 6**, *Specific outcomes*), suggesting differential orchestration of muscle fiber shifts between study clusters, possibly relating to their inherently different muscle fiber proportions at baseline.

Muscle RNA content. In general, COPD and Healthy showed similar increases in ribosomal RNA abundance *per* unit muscle tissue weight, measured as both total RNA and rRNA expression, and measured after both 3 ½ week (1.19/1.29 and 1.15/1.16 fold increases, total RNA/rRNA abundances) and after finalization of the training intervention (1.13/1.18 and 1.05/1.17 fold increases). While these changes in ribosomal RNA content were generally similar between COPD and Healthy, a few noteworthy differences were evident, including a more robust early increase in 45s pre-rRNA abundance ↑in COPD (**Figure 6**, *Specific outcomes*) and a trend towards reduced changes in response to 13 weeks training in COPD, which was evident by an absence of time effects for all rRNA species. The early increases in ribosomal content seen in both COPD and Healthy resemble those seen after similar interventions in untrained young individuals,[112] and may be important for muscle growth capabilities over the entirety of the study period,[112,170] accommodating increases in protein synthesis capacity, thus potentially contributing to the pronounced muscular responses to resistance training seen in both study clusters.

Even though resistance training led to marked changes in mRNA transcriptome profiles in both COPD and Healthy, no single transcript showed differential responses to training between the two study clusters. This was evident both at 3 ½ weeks and 13 weeks, despite clear differences in transcriptome profiles at baseline (**Figure 4** and **Paper III**, *Figure 3A*). In contrast, enrichment analyses revealed traces of differential changes, with COPD showing more pronounced increases in expression of genes relating to *oxidative phosphorylation* after 3½ weeks (GSEA), and, in particular, more pronounced decreases in genes associated with *myogenesis* after 13 weeks (consensus) (**Figure 6**, *Specific outcomes* and **Paper III**, *Figure 3C*). Interestingly, as these two gene sets represented the most prominent differences between COPD and Healthy at baseline (**Figure 4** and **Paper III**, *Figure 3A-B*), and as resistance training led to directional changes that mitigated these differences, training arguably shifted the COPD phenotype in a healthy direction.

Mitochondrial function. In a subset of study participants (COPD, n=11; Healthy, n=12), mitochondrial measurements were carried out (pre-intro RT and post-RCT, **Figure 2**). Overall, resistance training was associated with beneficial improvements in mitochondrial functions and capacity in the COPD cluster-only. Specifically, in COPD, resistance training led to increased citrate synthase activity (35-43%), thus essentially restoring citrate synthase activity to healthy pre-intro RT levels. In Healthy, no change was observed (p=0.365), yet no statistical difference in resistance training-associated increase in citrate synthase activity was evident between the two study clusters (**Figure 6**, *Specific outcomes*). The increase in citrate synthase activity in COPD contrast a previous study which failed to observe increased citrate synthase activity following a low-load resistance training protocol in COPD,[68] despite applying a higher training frequency than in the current study (three times per week). This may potentially be explained by that the lack of performing the resistance exercises to volitional exhaustion made the exercise effort insufficient to stimulate mitochondrial biogenesis. Furthermore, in COPD, resistance training led to improved mass-specific mitochondrial respiration of fatty acids (13%↑, p=0.033) and total oxidative phosphorylation (9%↑,

p=0.035), and tended to lead to increased complex-I respiration (10%↑, p=0.079), while no significant alterations were observed for leak respiration (7%↑, p=0.340) or electron transfer system capacity (11%↑, p=0.115). In Healthy, significant time effects were lacking for all respiratory states; yet, the changes in the two study clusters were not statistically different (**Appendix V**, supplementary material for Paper IV). In both COPD and Healthy, mRNA levels of mitochondrial genes changed markedly with resistance training (**Appendix V**), but no MitoPathway[191] category and only one mitochondrial gene (*TXNRD2*) were differentially affected by resistance training in COPD compared to Healthy, indicating similar mRNA responses to resistance training for mitochondrial genes in COPD and Healthy. Overall, the results displays that in COPD, resistance training was a potent intervention to increase mass-specific mitochondrial respiration and oxidative enzyme activity. In healthy, mitochondrial function remained unaltered, although mRNA responses to resistance training were largely similar between COPD and Healthy.

Blood and health-related outcomes. Overall, COPD and Healthy showed similar training-associated increases in whole-body and appendicular lean mass (**Figure 6**, *Specific outcomes*). This was accompanied by increased appendicular skeletal muscle mass index relative to the sex-specific mean of young, healthy adults[149] (COPD, from 84% to 86%; Healthy, from 95% to 97%), suggesting that the intervention was effective for reversing age-related decline in muscle mass. For blood variables such as markers of systemic inflammation and hormone, lipid and iron biology, no noteworthy effects were observed of the intervention, nor were any differential changes observed between COPD and Healthy (**Figure 6**, *Specific outcomes*)

Lung function. For COPD overall, the study intervention did not affect any of the lung function variables, evaluated as changes from pre-RCT to post-RCT, implying no effects on the intervention in general on this core epidemiological trait. This seems reasonable given the irreversible nature of the respiratory impairments of COPD, yet contradicting the beneficial effects observed in Hoff *et al*.[70] In contrast, for Healthy, the intervention was associated with reduced FVC and FEV_1 (-2.7% and -1.5%, respectively). Rather than being a consequence of the intervention protocol *per se*, this may be due to a general age-related decline, as the magnitude of the changes resemble those seen in corresponding age cohorts over a similar time frame.[180] Unfortunately, the study was conducted without a negative control group not receiving the intervention protocol, which obviously reduces the interpretations of these analyses. As mentioned previously, subgroup analyses also revealed that vitamin D_3 supplementation in COPD was associated with detrimental effects on FEV_1/FVC (Δ-8.4% reductions compared to placebo). This finding remains difficult to explain as it opposes previous research showing beneficial effects of vitamin D supplementation on lung function,[181] and should also be interpreted with caution as the analysis was clearly weakened by the small sample size (COPD, n=9 vs n=11, vitamin D_3 vs placebo).

Health-related quality of life. For COPD, the intervention was associated with marked improvements in several aspects of health-related quality of life. These included reduced experience of limitations of physical functioning and improved social function and mental health, with only marginal effects being seen in Healthy, but no significant difference in responses between COPD and Healthy (**Paper III**, *Table 6*) While these changes of course may be directly related to the resistance training intervention and the muscle functional improvements, they may also be related to other aspects of the study protocol, such as performing training sessions in a social setting and the close follow-up each participant received from study personnel, as the COPD persons highlighted in the

qualitative interviews conducted during the training period (**Appendix I,** *A qualitative analysis of motivational factors for resistance training in chronic obstructive pulmonary disease: experiences from The Granheim COPD Study*). As the intervention was conducted without a control group (not receiving the intervention protocol), caution is warranted for interpretation of these data.

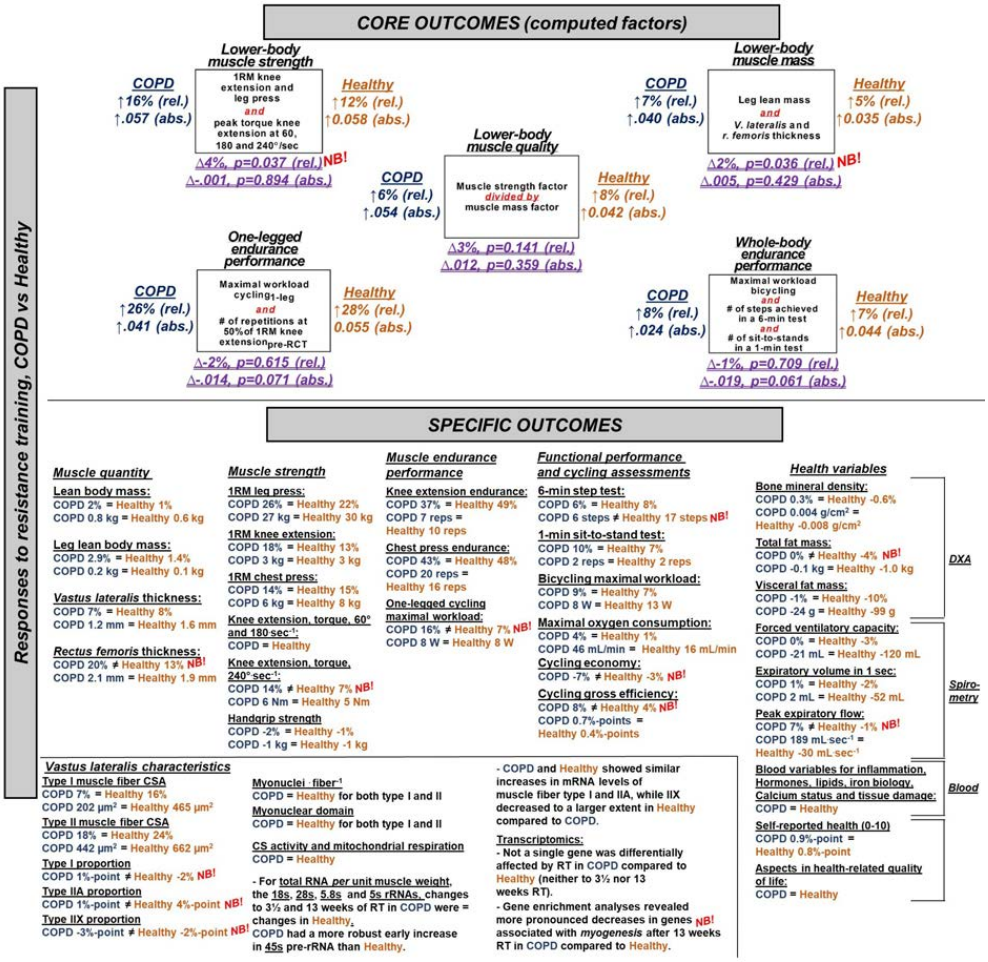

Figure 6. Comparison of the responses to resistance training in COPD and Healthy. In the upper panel, the effects of resistance training in COPD and Healthy on the study's core outcome domains (lower-body muscle strength, lower-body muscle mass, lower-body muscle quality, one-legged endurance performance and whole-body endurance performance) and on singular outcome variables, measured as both relative and absolute change terms. Blue and orange text denotes the COPD and Healthy study cluster, respectively. Δ, difference in change between study clusters (COPD - Healthy). Alpha level at $p<0.05$. =, $p>0.05$ for comparison of the changes in the COPD and Healthy study cluster; ≠, $p<0.05$ for comparison of the changes in the COPD and Healthy study cluster.

Remarks on the COPD vs Healthy objective of the RCT. COPD-related pathophysiologies, such as reduced testosterone,[77] vitamin D[78] and oxygen saturation levels[20,192] in blood, and elevated levels of low-grade inflammation,[79] are generally believed to drive metabolism into a chronic catabolic state.[20,77,80] This has also been suggested to lead to impaired responses to resistance training,[20,193]

which are essential measures for preventing and treating disease-related reductions in skeletal muscle mass and strength in COPD. However, in the RCT study, even though COPD participants displayed clear and well-known disease-related aberrancies compared to Healthy at baseline (**Figure 4A**), resistance training led to improvements in muscle strength, muscle mass, muscle quality and endurance performance that resembled or exceeded those seen in Healthy, contrasting the initial hypothesis. These observations were accompanied by similar alterations in muscle biology, including changes in hallmark traits such as muscle fiber characteristics, rRNA content and transcriptome profiles. Together, these data suggest that COPD-related etiologies and pathophysiologies do not impair responsiveness to resistance training, at least not for skeletal muscle characteristics, and at least not in the enrolled cluster of COPD participants (GOLD grade II-III) and within the time frame of the study.

4.5 The impact of exercise load on resistance training-associated adaptations

For comparisons of the efficacies of 10RM and 30RM resistance training modalities, data from the two study clusters (i.e. COPD and Healthy) were pooled.

Lower-body muscle mass, muscle strength, muscle quality and bone mineral density. For lower-body muscle mass, 30RM resistance training was associated with larger improvements compared to 10RM resistance training (**Figure 7A**). For the individual outcome measures composing this factor (i.e. leg lean mass + *m. vastus lateralis*/*m. rectus femoris* muscle thickness), the average numerical changes also pointed towards favorable gains of 30RM but these changes were not statistically different between 10RM and 30RM resistance training (**Figure 8B**). This reiterates on the potential power of using combined weighted factors based on multiple outcome measures for assessing main outcome domains, as previously described,[194] presumably acting by reducing the methodological variability associated with its singular measurements. Notably, the statistically significant larger response of 30RM training on lower-body muscle mass were not present for study cluster-specific analyses (**Figure 7B-C**), probably due to the lower statistical power associated with such analyses.

For improving lower-body muscle strength, the effects of 10RM and 30RM resistance training were similar (**Figure 7A**). Of note, 10RM resistance training displayed a larger increase in 1RM knee extension compared to 30RM resistance training (**Figure 8A**), but this finding was not confirmed by the results for the other lower-body muscle strength outcome measures (**Figure 8A**), thus no overall effect of 10RM resistance training. For improving muscle quality, i.e. when combining the training modality-specific results for the muscle strength factor with the corresponding change in the muscle mass factor (Δmuscle strength factor/Δmuscle mass factor), 10RM resistance training was associated with a tendency towards a larger effect compared to 30RM resistance training (p=0.075; **Figure 8A**). Notably, the effects observed analyzing the pooled data of all study participants were not evident in study cluster-specific analyses (**Figure 7B-C**).

To maintain bone mineral density in the legs, 10RM resistance training was associated with beneficial effects (p=0.054; **Figure 8C**). This emphasizes the significance of high-load resistance training for delaying the inevitable decrease in bone mineral density with advancing age,[195] thereby reducing the risk of fractures after falling,[196,197] and thus also life expectancy.[198] The effect of resistance training on bone mineral density was as such more evident than the effect of vitamin D_3 supplementation, which indeed showed no such effect, although it previously has been clearly linked to beneficial effects on bone health.[122] Of note, the decrease in bone mineral density observed with

30RM resistance training was probably related to a natural age-related decrease,[195] and not a detrimental effect of this training modality.

Together, these observations suggest that 30RM training is a feasible and efficient resistance training modality for both COPD and healthy older persons, which also offers similar effects on maximal muscle strength and muscle performance, superior effect in terms of muscle mass gains, but less effect on bone mineral density and muscle quality compared to 10RM training. Notably, the two training modalities were associated with similar ratings of perceived exertion, measured by asking the participants how hard the workout was perceived for each leg on the Borg 6-20 scale[199] (10RM, 16.2±1.5; 30RM, 16.3±1.5; p=0.567)), even though the general impression from the participants was that they preferred to perform 10RM over 30RM resistance training.

To the best of my knowledge, this is the first study to compare the effects of low-load and high-load resistance training for muscle functional and gross muscle biological effects in a group of older adults. Indeed, the larger muscle mass accretion associated with low-load resistance training and the similar muscle strength improvements between exercise loads in the RCT study contrasts to a certain extent the training responses commonly seen in young healthy adults, although similar responses also are present.[200] In the younger population, high-load resistance training performed to volitional exhaustion are generally associated with similar[91–93] or greater muscle hypertrophy,[201,202] and larger muscle strength gains compared to low-load resistance training.[21,91,93,201,202] This disparity in responses may be due to age-related changes in skeletal muscle environment and epigenetics,[203,204] leaving young and older persons with dissimilar muscle phenotypes, which possibly can lead to different molecular responses. This may also be related to the lowered ability in older persons to fully activate skeletal muscle during resistance exercise,[102–104] which may even be hypothesized to be differently affected following high- and low-load resistance training in older and young persons. The mechanisms underlying the observed effects clearly needs further study.

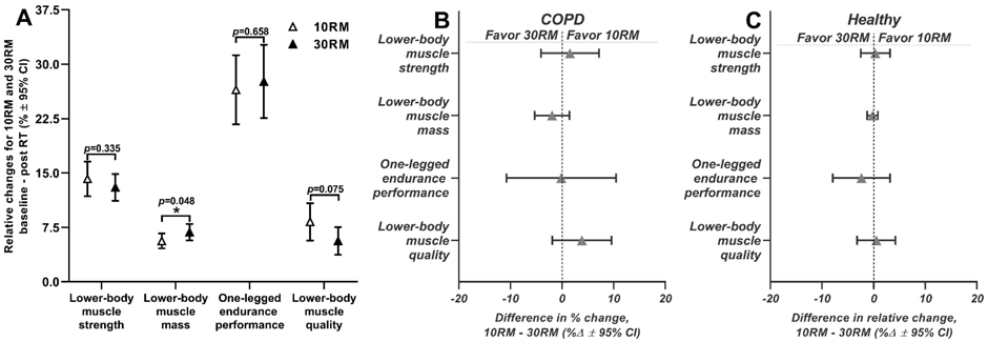

Figure 7. Comparison of changes to high-load (10 repetitions maximum; RM) and low-load (30RM) resistance training on weighted factors of the core outcome domains, i.e. lower-body muscle strength, lower-body muscle mass, one-legged endurance performance and lower-body muscle quality. In (**A**), comparison of the effects of 10RM and 30RM for all study participants combined, whereas in (**B**) and (**C**), the same comparison was performed for COPD-only and Healthy-only, respectively. *P*-values in (A) represents the comparison of change scores between 10RM and 30RM resistance training. Alpha level at p<0.05. *, statistically different response to 10RM and 30RM resistance training.

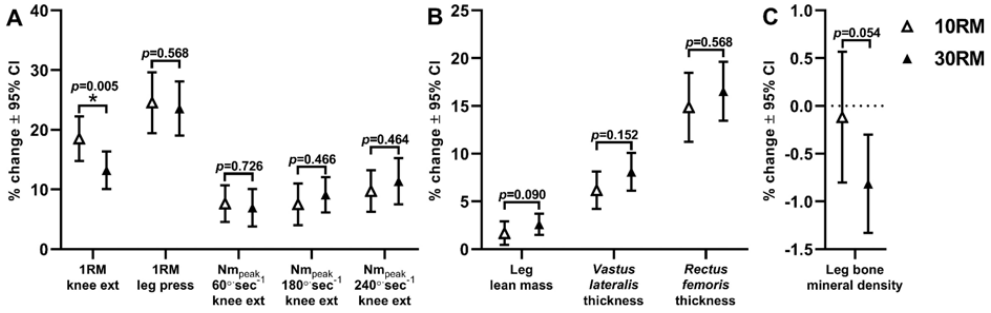

Figure 8. Comparison of changes related to high-load (10 repetitions maximum; RM) and low-load (30RM) resistance training on measures of lower-body unilateral muscle strength (**A**), muscle mass (**B**) and bone mineral density (**C**). *P*-values represents the comparison of change scores between 10RM and 30RM resistance training. Alpha level at $p<0.05$. *, statistically different response to 10RM and 30RM resistance training; 1RM, one repetition maximum; Nm, newton-meters.

One-legged endurance measures. For one-legged endurance performance, the 10RM and 30RM resistance training improvements were similar, both measured as the weighted combined factor (**Figure 8A**), and as each of its containing variables (i.e. one-legged knee extension performance and maximal workload achieved during one-legged cycling; **Figure 9**). This does not resemble with previous findings seen in young healthy persons, where high-load resistance training (3-5RM) was associated with larger improvements in muscular endurance (i.e. repetitions achieved at a load corresponding to 60% of 1RM) compared to low-load training (20-28RM).[21] Furthermore, they observed larger gains in muscle strength and muscle hypertrophy with high-load training compared to low-load training, and as such further emphasized the different training responses in that study sample compared to the responses observed in the RCT study. Of note, the test protocol was also slightly different from the muscular performance test protocol used in the RCT study, where the external load was set to 50% of $1RM_{pre\text{-}RCT}$. The similar improvements between exercise load modalities in maximal workload achieved during one-legged cycling can probably be ascribed different alterations of the underlying performance-determining factors for this measurement; whereas there was a tendency towards larger changes in $\dot{V}O_2max_{one\text{-}legged\ cycling}$ for 30RM resistance training (**Figure 9**), greater improvements in cycling oxygen cost and gross efficiency were observed for 10RM resistance training (**Figure 9**).

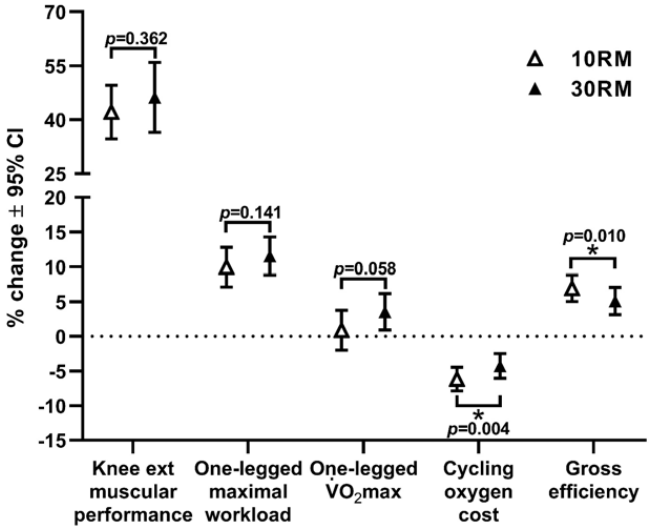

Figure 9. Comparison of changes related to high-load (10 repetitions maximum; RM) and low-load (30RM) resistance training on one-legged endurance measures. Muscular performance was defined as the number of repetitions achieved at 50% of pre-study 1RM in the knee extension exercise. The rest of the variables are collected during maximal (*maximal workload* and *V̇O₂max*) and submaximal (*cycling oxygen cost* and *gross efficiency*) one-legged cycling. *P*-values represents the comparison of change scores between 10RM and 30RM resistance training. Alpha level at $p<0.05$. *, statistically different response to 10RM and 30RM resistance training; V̇O₂peak, maximal oxygen consumption achieved during one-legged cycling.

Muscle fiber cross-sectional area and proportions, and muscle mitochondrial function. For muscle fiber type I and type II cross-sectional area, the two exercise load modalities were associated with similar resistance training-associated changes (**Figure 10A**). However, in study cluster-specific analyses, COPD showed tendencies towards blunted plasticity of type I muscle fibers, with responses to 30RM resistance training almost statistically larger compared to 10RM resistance training (Δ22%, $p=0.060$; (**Paper III**, *Figure 6*). Such study cluster interactions were not observed for muscle fiber type II hypertrophy. For muscle fiber type proportions, 10RM resistance training led to a more pronounced decrease in IIX proportion compared to 30RM resistance training (**Figure 10B**), whereas 10RM and 30RM resistance training altered fiber type I and fiber type IIA proportions in a similar manner (**Figure 10B**). In study cluster-specific analyses, this seemed to be valid for both COPD and Healthy (COPD, Δ-2.6%-points, $p=0.073$; Healthy, Δ-1.7%-points, $p=0.015$). The findings may indicate that 30RM resistance training did not enable to maximally activate the largest motor units, i.e. the type IIX fibers, thus resembling with previous studies showing generally lower mean and peak muscle activation when exercising with a low vs a high resistance training load carried out to muscular failure.[94–97] Of note, neither this nor the lower mechanical tension associated with low-load training[205] translated into impaired muscle fiber hypertrophic responses, measured neither directly using immunohistochemistry nor indirectly using gross measures of muscle mass (dual energy x-ray absorptiometry/ultrasound measures). This emphasizes that other factors as well are of importance for muscle hypertrophy, which indeed may be more altered by low-load than high-load resistance exercise (e.g. total exercise volume,[98] degree of metabolic perturbations,[95,99] and time under tension for low-threshold motor units[100,101]).

At post-RT, muscle mitochondrial quantity (citrate synthase activity) and respiratory capacity were not significantly different between exercise load modalities in neither COPD nor Healthy. Still mentionable, in COPD, 30RM was associated with higher intrinsic oxidative phosphorylation (total oxidative phosphorylation/citrate synthase activity) (Δ11%, *p*=0.065) and intrinsic electron transfer system capacity (electron transfer system capacity/citrate synthase activity) (Δ13%, *p*=0.060) at post-RT. Notably for these analyses, only the 30RM leg biopsies were analyzed pre-RT, which prevented to measure muscle mitochondrial changes for the 10RM leg.

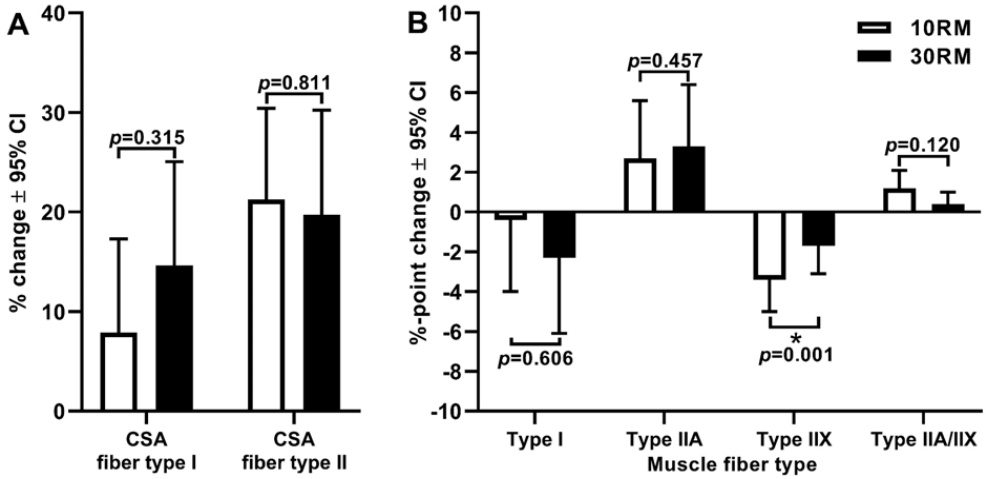

Figure 10. Comparison of the changes related to high-load (10 repetitions maximum; RM) and low-load (30RM) resistance training on *m. vastus lateralis* fiber cross-sectional area (**A**) and fiber type proportions (**B**), measured using immunohistochemistry. *P*-values represents the comparison of change scores between 10RM and 30RM resistance training. Alpha level at *p*<0.05. *, statistically different response to 10RM and 30RM resistance training; CSA, cross-sectional area.

Remarks on the resistance exercise-load objective of the RCT. Traditionally, high-load resistance training has been viewed as necessary to achieve optimal muscle strength and hypertrophy responses in anyone from novices to resistance-trained individuals.[48] This has been claimed based on the postulate that heavy loading is required to fully recruit higher threshold motor units,[90] and consequently it has been reasonable to assume that optimal improvements in muscle strength and hypertrophy only can be achieved through the use of high loads. Recently, this view has been challenged for young healthy individuals, where low-load training has been shown to result in similar,[91–93] or even enhanced,[200] muscle hypertrophic responses compared to high-load resistance training, while high-load resistance training still seems to lead to larger improvements in muscle strength.[21,91,93,201,202]

In the RCT study, we largely verify that low-load resistance training can be a feasible training modality alternative to conventional high-load resistance training also in the general older population. Indeed, when combining the results from all participants in the RCT study, 30RM resistance training executed to volitional exhaustion was associated with generally larger muscle mass gains than 10RM training. However, this did not seem to translate into superior muscle strength or endurance performances for the study participants, although both training modalities

were associated with pronounced improvements for these measures. For some variables, traces towards superior effects for one of the exercise load modalities were observed. This was evident for changes in cycling oxygen cost/gross efficiency (10RM>30RM), $\dot{V}O_2max_{\text{one-legged cycling}}$ (10RM<30RM), change in muscle fiber type IIX proportion (10RM>30RM), and muscle fiber type I size in the COPD cluster (10RM<30RM). However, 10RM resistance training was associated with better abilities to maintain bone mineral density, which emphasizes that resistance training programs for this population as a rule should include elements of high-load resistance training. Of note, analyses of the impact of resistance training load on changes in mRNA transcriptome profiles were not finished at the time this thesis was submitted.

5 Conclusions

The primary findings were:

I. In older adults with moderate COPD (GOLD grade II-III), muscular performance was impaired in two-legged leg press, but not in one-legged leg press. This advocates the use of one-legged resistance exercises for persons with COPD (**Paper I**, *preparatory study*)

II. In older adults with or without COPD, vitamin D_3 supplementation did not lead to beneficial effects in resistance training-associated changes in muscle function or characteristics, although it efficiently improved vitamin D-status without any adverse effects. This rejects the notion that vitamin D_3 supplementation is necessary to obtain adequate muscular responses to resistance training in the general older population. Secondary analyses revealed positive effects of vitamin D_3 supplementation for gene sets involved in vascular functions and for muscle strength improvement for participants with high proportions of fat mass, which advocates further research to elucidate on these specific biological characteristics (**Paper II**, *RCT study*)

III. For the RCT study participants, COPD displayed well-known disease-related pathophysiologies, including elevated levels of systemic low-grade inflammation, reduced muscle mass and functionality, and muscle biological aberrancies. In these persons, the resistance training program led to pronounced improvements for a range of health and muscle functional and biological variables, resembling or exceeding those seen in Healthy. Contrary to our hypothesis, COPD was not associated with impaired responsiveness to resistance exercise training, which rather posed a potent measure to relieve disease-related pathophysiologies (**Paper III**, *RCT study*)

IV. Resistance training was a potent measure to restore muscle mitochondrial quantity and respiratory capacity in COPD (**Paper IV**, *RCT study*)

V. Overall for the RCT study participants, low-load resistance training was associated with larger increases in lower-body muscle mass, while high-load resistance training resulted in a larger decrease in muscle fiber type IIX proportion, larger improvements in cycling economy/gross efficiency, and counteracted decreases in bone mineral density over the course of the intervention. Low-load resistance training performed to volitional failure can be recognized as a feasible and effective alternative to high-load training in the general older population when considering muscle mass/strength/performance enhancement. The slightly diverging and complementing effects of the two training modalities for the range of the outcome measures may advocate that they should be combined in a given training program for older adults to facilitate optimal responses (*RCT study*)

6 Perspectives

As the prevalence of sarcopenia is markedly escalating,[1,206] coinciding with increasing proportions of older adults, efficient lifestyle measures to prevent, treat and reverse sarcopenia are warranted to facilitate elderly to stay healthy, active and independent. With this in mind, the aim of The Granheim COPD Study was to investigate how a combinatorial lifestyle protocol involving both dietary manipulation of vitamin D_3 supplementation and two different resistance-training strategies (high-load and low-load resistance training) would affect indices of muscle function and biology in resistance training-naïve COPD and healthy older persons. Whereas vitamin D_3 supplementation did not lead to beneficial effects on muscle functions or characteristics, resistance training was associated with marked improvements. Thus, resistance training stood out as the most potent measure to alter such variables. The effects of resistance training were in general similar or larger in COPD compared to Healthy, not enhanced by vitamin D_3 supplementation, and not affected by exercise load for the muscle functional measures, albeit training load-specific observations related to alterations in e.g. cycling economy/efficiency, fiber type proportions and bone mineral density. The study also showed that resistance training in COPD can provoke muscle mitochondrial improvements, a feature previously only observed after endurance training for this patient group.

For persons with COPD, there is growing evidence for the use of one-legged exercise protocols for rehabilitation purposes, thus circumventing the cardiorespiratory limitations inherent to the condition, facilitating higher degrees of muscle activation and muscle mass-specific intensities during exercise compared to conventional whole-body exercises for these type of individuals,[74,75,207] which seems to translate into superior functional improvements after both endurance training[208] and resistance training.[67] With such an exercise approach in the RCT study, the training responses seemed to resemble or exceed those seen in Healthy. Training with lower systemic physiological demands is also considered to be beneficial for the emotional perception of training in such patients, as it is associated with lower degrees of dyspnea,[209,210] and thus likely provides a feeling of safety and acts to stimulate long-term motivation for training. Future studies on exercise training rehabilitation of COPD persons should further elucidate on efficient training protocols for this population that can enhance clinically important measures such as well-being, health-related quality of life and level of activities of daily living, and may counteract worsening of the disease and prevent adverse health events. Currently for exercise training rehabilitation of COPD, questions about which persons that should perform exercise training with reduced levels of active muscle, and how exercise training protocols should be organized regarding implementation of resistance training, endurance training, or a combination of these two exercise training modalities remains largely unstudied for different COPD phenotypes.

Within lifestyle therapy, it is an intriguing vision that therapy protocols in the future can be prescriptions, e.g. a prescription of type and dosage of exercise training, which is based on biological characteristics such as an individual's muscle transcriptome, instead of knowledge originated from interventions on whole groups/clusters, such as today. The individual response to a training intervention is largely differing, so also in the current RCT study. If one could successfully link different biological profiles to distinct responses for various types of exercise interventions, it should arguably be possibly to prescribe personalized exercise training therapy. In this regard are the use of unilateral training protocols of particular interest. This enables to study if one intervention is

associated with greater muscle functional and biological improvements compared to another intervention within the same individual, given the presence of the same genetic material for both interventions. Such individual exercise training prescription may be readily available to distinguish responders to 10RM from 30RM and *vice versa* in the current RCT study data set. This data set is also, together with other exercise training intervention data sets, integrated into the general biobank *The Trainome* at INN-Lillehammer, which is created with the same rationale of personalized lifestyle therapy in mind.

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ORIGINAL ARTICLE

WILEY

Muscular performance decreases with increasing complexity of resistance exercises in subjects with chronic obstructive pulmonary disease

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Abstract

Chronic obstructive lung disease (COPD) is associated with impaired muscle functions in addition to the impaired cardiopulmonary capacity inherent to the disease. The purpose of this study was to compare muscular performance between COPD subjects (COPD, n = 11, GOLD grade II/III; FEV_1 = 53 ± 14% predicted; 61 ± 7 years) and healthy controls (HC, n = 12, 66 ± 8 years) in three resistance exercises with different complexity: (a) one-legged knee extension (1KE), and (b) one- and (c) two-legged leg press (1LP and 2LP, respectively). For each exercise, muscular performance was defined as repetitions to exhaustion at 60% of one-repetition maximum or overall exercise volume, calculated as the sum of three exercise sets. In HC, muscular performance increased progressively with increasing physiological complexity: 1KE < 1LP < 2LP. Using 1KE as reference value, muscular performance increased by 1.9 (repetitions) or 4.6-fold (volume) in 1LP and 3.1 or 7.1-fold in 2LP. In COPD, similar increases occurred going from 1KE to 1LP (1.9 or 4.4-fold change), but not from 1LP to 2LP, where no further increase occurred. In conclusion, in COPD, performance is impaired in exercises involving larger amounts of muscle mass (>1LP), advocating utilization of one-legged resistance protocols for rehabilitation purposes.

KEYWORDS

cardiorespiratory capacity, chronic obstructive lung disease, muscular performance, resistance training, strength training, unilateral training

1 | INTRODUCTION

For individuals suffering from chronic obstructive lung disease (COPD), physical exercise is a prerequisite for adequate treatment and rehabilitation. It counteracts the muscle pathophysiology inherent to the disease and improves health-related quality of life and activities of daily living.[1-3] Unfortunately, exercise training is a demanding task for such patients. The accompanying increase in oxygen consumption in working muscles rapidly exceeds the oxygen-delivery capacity of the cardiopulmonary system,[4] leaving muscles in a state of oxygen deficiency. This occurs already at low intensities and

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upon activation of small bulks of muscle (>4 kg), resulting in dyspnea, discomfort, and impaired exercise performance.[5] Accordingly, it is difficult to achieve necessary exercise intensities to provoke muscle cell adaptations,[6,7] which hinders efficient rehabilitative training.[8,9] Despite this, whole-body endurance exercise training, such as cycling or walking, is the most commonly applied exercise modality in pulmonary rehabilitation.[10]

Fortunately, there are ways to solve this issue and to facilitate ergogenic adaptations to exercise training in COPD patients. A readily available solution would be to make use of exercise protocols with lower physiological demands such as resistance exercises, activating smaller amounts of muscle mass.[4] This strategy should ensure maximal muscle activation regardless of blood oxygenation levels, enabling activation of key cellular signaling pathways, and inducing muscle adaptations. In line with this, resistance training has gained momentum in COPD rehabilitation during the last decade, counteracting the muscle dysfunctions accompanying the disease, improving muscle strength and endurance, and increasing muscle mass.[11-13] However, the magnitude of these effects remains equivocal, with available studies displaying a large span of variation in training adaptations, ranging from negligible or trivial[14,15] to substantial and highly relevant.[16,17] Indeed, many patients do not respond to training at all.[8,9] To date, this heterogeneity has been ascribed pathophysiologies accompanying the disease, such as a low-grade systemic inflammation,[18,19] though this is unlikely to explain the between-studies variation. Rather, the heterogeneous response patterns may result from differences in study design, including differences in resistance training protocols. Indeed, the cardiopulmonary limitations of COPD patients may call for specific modifications to resistance training exercises in order to further reduce the physiological demand.[20] At present, we know little about this perspective, with only a handful of studies investigating the efficacy of different resistance exercise modalities.[21-23]

Conventional resistance training of the legs typically involves two-legged exercises. In moderate to severe COPD, this is likely to involve too much muscle mass to allow for optimal activation (and arguably adaptation).[20,24] Intuitively, this is readily solvable by using one-legged resistance exercises, which naturally reduces the amount of active muscle mass. In a recent study, unilateral resistance exercises resulted in superior exercise workloads using elastic bands compared to bilateral exercises in severe to very severe COPD (GOLD grade III/IV), but not in healthy subjects,[22,23] though analysis of interaction effect for difference in exercise workload^{-leg} from single- to two-limb exercises and group (COPD vs healthy) was not performed. This complicates to examine if COPD patients show progressively lowered muscular performance in resistance exercises with increasing complexity compared to healthy subjects. It also remains unknown if this applies to COPD of less severity (GOLD grade II/III), and if it is applicable to isolated resistance exercises performed in apparatus, perhaps exacerbated by increasing physiological complexities of exercises. For endurance exercises, such unilateral training seems to translate into superior training adaptations for COPD subjects.[25,26]

The purpose of this study was to compare muscular performance in three resistance exercises of the legs involving different degrees of active muscle mass in COPD and healthy control subjects (one-legged knee extension, and one- and two-legged leg press). We hypothesized that muscular performance in COPD patients would be increasingly impaired with increasing amount of active muscle mass compared to healthy subjects. Muscular performance was defined as repetitions to exhaustion at 60% of 1RM or overall exercise volume, both calculated as the sum of three sets for each exercise.

2 | METHODS

The study was approved by the Regional Ethics Committee of the Norwegian Research Council for Science and the Humanities as a part of "The Granheim COPD Study" (reference nr: 2013/1094) and was preregistered at clinicaltrials.gov (NCT02598830). All subjects signed informed consent. The study was conducted according to the Declaration of Helsinki.

2.1 | Subjects

Twelve subjects with COPD and 11 healthy control subjects participated in the study. For background variables, see Table 1. COPD subjects were recruited from a pulmonary rehabilitation center (Granheim Lung Hospital), while healthy controls were recruited through acquaintances. All subjects were >55 years of age. COPD subjects had GOLD stage II-III (FEV_1 predicted <80 to >30% and FEV1/FVC <70%) and did not smoke at the time of inclusion and throughout the test period. Healthy controls had normal lung function (FEV_1 predicted >80% and FEV_1/FVC >70%). Exclusion criteria were unstable cardiac disorders and comorbidities that could impair the ability to perform lifts with the lower limbs. COPD subjects received medication as prescribed by their medical doctor (Table 1). None of the subjects utilized supplemental oxygen regularly. Subject characteristics unrelated to muscle strength and performance were similar between groups, except for lung function, oxygen saturation of hemoglobin (SpO_2), and medication use (Table 1).

2.2 | Experimental design

All subjects attended 7 days of performance testing, distributed over a period of 4 weeks. Test days were separated by

TABLE 1 Subject characteristics

	COPD subjects (n = 11)	HC subjects (n = 12)	*P*
Sex (♂/♀)	5/6	5/7	.86
Age	65.5 ± 8.1	61.8 ± 6.7	.24
Height (cm)	165 ± 12	173 ± 10	.11
Weight (kg)	70.1 ± 14.5	76.4 ± 11.5	.26
BMI	25.6 ± 5.1	25.5 ± 2.6	.93
SpO_2 at rest	94 ± 4%	98 ± 1%	.01
Lung function			
FVC (L)	2.7 ± 1.1	4.1 ± 0.8	.00
FEV_1/FVC (%)	49 ± 13	72 ± 6	.00
FEV_1 (% predicted)	53 ± 14	117 ± 12	.00
PEF (L/s)	4.7 ± 1.9	8.1 ± 1.7	.00
GOLD II/III	7/4	—	—
Medication			
B_2-agonists	10	—	—
Muscarinic antagonists	1	—	—
Corticosteroids	1	—	—
4-min step-test (steps)	92 ± 25	137 ± 25	.00

Note: Values are numbers or mean ± standard deviations.
Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; HC, healthy control; PEF, peak expiratory flow; SpO_2, oxygen saturation of hemoglobin.

at least 48 hours. On day 1, subjects performed spirometry testing, anthropometric measurements, 4-minute step-test, and familiarization to one-repetition maximum (1RM) tests in one-legged knee extension (1KE), one-legged leg press (1LP), and two-legged leg press (2LP). On days 2-3, subjects performed 1RM tests. These data were subsequently utilized to calculate relative workload for tests of muscular performance (60% of 1RM), which were performed on days 4-7 (two test days for the one-legged exercises and two test days for the two-legged exercise). All tests were supervised by the same physical training instructor, except for spirometry tests, which were conducted by the same nurse specialist. Apparatus settings were adjusted to the needs and were utilized for all tests.

2.3 | Test protocols

2.3.1 | Spirometry and anthropometry

Spirometry testing (Jaeger MasterScreen PFT; Carefusion) was conducted before the other physical tests. The protocol followed guidelines from the American Thoracic Society and the European Respiratory Society.[27] COPD patients were tested before and after inhalation of two bronchodilators (salbutamol, 0.2 mg and ipratropiumbromid, 20 μg). See Table 1 for values on lung function after optimal bronchodilation.

2.3.2 | Fitness test

Subjects performed a 4-minute step-test to evaluate the subjects' general fitness level. A 20-cm high step box with a non-slip rubber surface (Reebok Step; Reebok) was used. Subjects were asked to perform as many steps as possible within four minutes, placing both legs on the box with the hip fully extended during each step up. Moderate verbal motivation was given throughout the test. Data are presented in Table 1.

2.3.3 | Muscular strength

Muscular strength was measured as 1RM in one-legged knee extension (Technogym, Technogym SpA), one- and two-legged leg press (Gym80 Sygnum Legpress, Gym80 mbH). Warm-up consisted of 5 minutes of low-intensity bicycling on a bicycle ergometer, followed by three sets of 12, 8, and 6 repetitions with low, increasing workloads. Subsequently, a maximum of five 1RM attempts were conducted for each exercise. All three exercises were tested in two separate sessions, and the best result was used for further analysis. One-legged muscle strength was tested on both legs, with one leg performing 1RM in one-legged knee extension and the other leg performing 1RM in one-legged leg press, allocated to the two legs in a randomized manner. On the two test days, subjects alternated between starting with one-legged exercises (1KE and 1LP) and two-legged exercise (2LP), giving each subject an attempt for each exercise modality with fully rested lower limbs. In one-legged knee extension, the 1RM attempt was approved if the knee angle exceeded 170°. In one- and two-legged leg press, the 1RM attempt was approved if the knee angle reached 90° in the eccentric phase, with subsequent full extension of the knee joint in the concentric phase.

2.3.4 | Muscular performance

Muscular performance was assessed in one-legged knee extension, one- and two-legged leg press, and was defined as the number of repetitions achieved at 60% of 1RM. Repetitions were quantified as the total number of repetitions achieved over the course of three sets, with 2 minutes of rest in-between. Each of the three exercise performance tests was conducted twice during the test period, on separate days. One-legged muscular performance tests (1LP and 1KE) were conducted within the same session, with one leg performing one-legged knee extension and the other leg performing one-legged leg press, allocated to the two legs in accordance with 1RM testing. The relative order

of one-legged and two-legged test days was randomized between subjects; half the subjects started with one-legged testing and half the subjects started with two-legged testing. The session following one-legged testing was always two-legged testing and vice versa. For each of the three muscular performance tests, the best result was used for further analyses.

Exercises were performed as previously described. Warm-up consisted of 5 minutes of low-intensity cycling on a cycle ergometer, followed by two sets of 12 and 8 repetitions at loads corresponding to 15% and 30% of 1RM, respectively. During muscular performance tests, subjects were instructed to lift at a composed and controlled pace, with no rest longer than 1 second in the lower or upper position. Moderate verbal motivation was given to all subjects. Blood lactate concentration (Lactate Pro, ARKRAY Inc) and SpO_2 (CMS 50F Oximeter, Innovo Medical) were measured at rest and after tests. Rating of dyspnea (Borg CR10)[28] was registered immediately after the test.

2.4 | Statistical analysis

Differences between groups (COPD vs healthy control subjects) were assessed using unpaired Student's *t*-tests for numeric data and Pearson's chi-squared test for nominal data (sex). Differences between independent groups with repeated measures were assessed using mixed-design ANOVAs with groups (ie, COPD and healthy control subjects) as between-factor and type of exercise (1KE, 1LP, and 2LP) as within-group factors. When a significant *F* value occurred, a Sidak post hoc test was used to determine differences between and within groups. The relationship between percent difference in muscular performance between one-legged knee extension and two-legged leg press and lung function was tested by Pearson's correlation. Statistical significance was set at $P < .05$, and data are expressed as means ± standard deviation in text and means ± 95% confidence intervals in figures. Statistical analyses were performed using IBM SPSS Statistics package (version 24) and figures made using Prism Software (GraphPad 8).

3 | RESULTS

3.1 | Maximal strength

In general, COPD showed lower 1RM strength than healthy controls ($F_{1,21} = 5.7$, $P = .027$; Figure 1). In one-legged knee extension, COPD and healthy controls achieved 33 ± 12 and 42 ± 9 kg, respectively ($P = .052$). In one- and two-legged leg press, corresponding values were 75 ± 22 and 98 ± 18 kg ($P = .012$), and 78 ± 21 and 93 ± 17 kg ($P = .091$, measured as $1RM^{-leg}$), respectively. Within each of the groups, no difference was seen between 1RM-1LP and $1RM^{-leg}$-2LP performance (COPD, $P = .656$; healthy controls, $P = .137$).

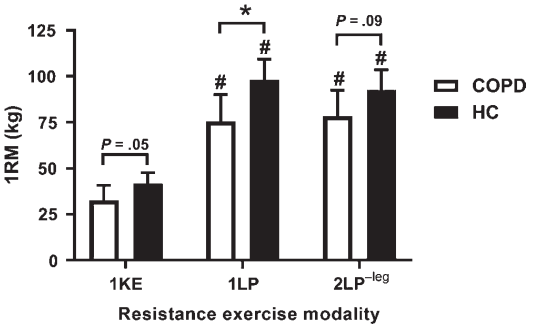

FIGURE 1 Maximal strength per leg for healthy control and COPD subjects. Data are means with 95% confidence levels. 1KE, one-legged knee extension; 1LP, one-legged leg press; $2LP^{-leg}$, two-legged leg press divided by two; COPD, chronic obstructive pulmonary disease; HC, healthy control. *Significant difference between groups ($P < .05$); #significant different from 1KE ($P < .05$)

3.2 | Muscular performance in resistance exercises

There was an interaction effect for groups and exercises on muscular performance, measured as both total number of repetitions achieved during three sets of resistance exercises at 60% of 1RM ($F_{2,42} = 7.3$, $P = .002$; Figure 2A) and as exercise volume ($F_{2,42} = 8.3$, $P = .001$; Figure 2C). In all three exercises, healthy controls generally managed to conduct more repetitions and higher exercise volumes than COPD, except for in one-legged leg press, where there was no difference in repetition to exhaustion between groups ($P = .10$). For healthy controls, muscular performance increased progressively with increasing complexity and physiological demand of the exercise: 1KE < 1LP < 2LP ($P < .05$; Figure 2A,C). For COPD, a similar increase was seen going from one-legged knee extension to one-legged leg press ($P = .004$, repetitions to exhaustion; $P < .001$, exercise volume), but not from one- to two-legged leg press, where no increase occurred ($P = .932$, repetitions to exhaustion; $P = .852$, exercise volume; Figure 2A,C). This progressive increase was highlighted in a subset of analysis where we calculated one- and two-legged leg press performance as relative performance to one-legged knee extension (Figure 2B,D). In this subanalysis, there was a significant interaction effect for groups and exercises for both repetitions to exhaustion ($F_{1,21} = 9.2$, $P = .006$) and exercise volume ($F_{1,21} = 5.5$, $P = .029$), highlighting that muscular performance was impaired during two-legged leg press in COPD compared to healthy controls. In healthy controls, muscular performance in one-legged leg press was 1.9 ± 0.7 fold (repetitions; Figure 2B) and 4.6 ± 1.8 (volume;

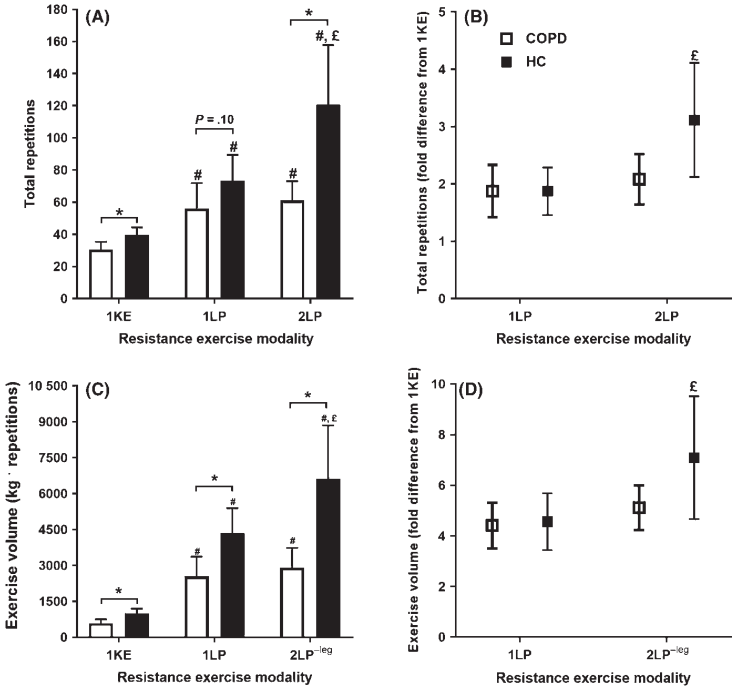

FIGURE 2 Exercise performance in resistance exercises for healthy control and COPD subjects performed as three sets to exhaustion at 60% of 1RM. Exercise performance was measured as A, total number of repetitions to exhaustion, B, number of repetitions to exhaustion in 1LP and 2LP relative to 1KE, C, total exercise volume (kg · repetitions) per leg and D, total exercise volume for 1LP and $2LP^{-leg}$ relative to 1KE. Data are means with 95% confidence levels. 1KE, one-legged knee extension; 1LP, one-legged leg press; 2LP, two-legged leg press; $2LP^{-leg}$, two-legged leg press divided by two; COPD, chronic obstructive pulmonary disease; HC, healthy control. *Significant difference between groups ($P < .05$); #significant different from 1KE ($P < .05$); £significant different from 1LP ($P < .05$)

Figure 2D) fold higher than in one-legged knee extension ($P < .001$). A further increase was seen going from one- to two-legged leg press, which was 3.1 ± 1.6 fold (repetitions; Figure 2B) and 7.1 ± 3.8 fold (volume; Figure 2D) higher than in one-legged knee extension ($P < .001$). In COPD, muscular performance increased in a similar manner going from one-legged knee extension to one-legged leg press (1.9 ± 0.7 fold, repetitions; 4.4 ± 1.3 fold, volume; $P < .005$) (Figure 2B,D), with no differences between COPD and healthy controls ($P = .992$, repetitions; $P = .823$, volume). However, in COPD, no further increase was seen going from one-legged to two-legged leg press (2.1 ± 0.7 fold higher than 1KE, repetitions; 5.1 ± 1.3 fold higher than 1KE, volume; $P = .403$ and 0.226, respectively) (Figure 2B,D). This resulted in tendencies to higher performance in two-legged leg press relative to one-legged knee extension in healthy controls compared to COPD subjects (3.1 vs 2.1 fold and 7.1 vs 5.1 fold, $P = .055$ and 0.118, respectively; Figure 2B,D).

Chronic obstructive lung disease and healthy control subjects displayed similar within-session occurrences of muscular fatigue, measured as differences in muscular performance between set 3 and 1 in each exercise (1KE, healthy controls = −18%, COPD = −23%, $P = .874$; 1LP, healthy controls = −15%, COPD = −23%, $P = .720$; 2LP, healthy controls = −23%, COPD = −27%, $P = .144$). In a merged data set encompassing data from both groups, there was a significant correlation between differences in muscular performance of one-legged knee extension and two-legged leg press and predicted FEV_1 (Pearson $r = .49$, $P = .018$). This suggests that impaired lung function was associated with impaired muscular performance during two-legged leg press.

During muscular performance tests, COPD generally displayed greater falls in oxygen saturation ($F_{1,21} = 9.9$, $P = .005$) and higher degrees of dyspnea ($F_{1,21} = 9.5$, $P = .006$) within each of the three different resistance exercises compared to healthy controls (Table 2). In both COPD and healthy control subjects, there was a significant increase in dyspnea with increasing complexity and physiological demands of the exercises (1KE < 1LP < 2LP; $P < .001$). This increase was not evident for oxygen saturation. Healthy controls displayed greater increases in blood lactate concentration from before to after exercises ($F_{1,21} = 5.9$, $P < .05$; Table 2).

4 | DISCUSSION

The primary finding of this study is that patients with moderate to severe COPD (GOLD grade II or III) display lower muscular performance in the legs compared to healthy controls. This difference increases with the complexity of the exercise, that is, the amount of active muscle mass and associated increases in physiological demands. In particular, in COPD, muscular performance was clearly impaired going from one-legged exercises to two-legged leg press, compared to healthy controls. Whereas the overall reduction in muscular performance seen in COPD compared to healthy controls

TABLE 2 Physiological responses to muscular performance tests

	One-legged knee extension			One-legged leg press			Two-legged leg press		
	COPD	Healthy	b/w groups	COPD	Healthy	b/w groups	COPD	Healthy	b/w groups
SpO_2 (% change)	−3.0 ± 2.1	−2.0 ± 1.0	$P = .16$	−3.1 ± 2.0	−1.3 ± 1.0	$P = .01$	−3.6 ± 2.9	−1.4 ± 1.2	$P = .03$
[BLa^-] (% change)	236 ± 101	365 ± 225	$P = .10$	240 ± 108	352 ± 162	$P = .07$	355 ± 83*,**	539 ± 278	$P = .05$
Degree of dyspnea (0-10)	4.5 ± 2.1	2.9 ± 0.8	$P = .02$	5.6 ± 1.6*	3.9 ± 1.2	$P = .01$	6.3 ± 1.6*	4.4 ± 1.6*	$P = .01$

Note: SpO_2 and [BLa^-] values are presented as percentage change from rest. All values presented as means ± standard deviations.
[BLa^-], blood lactate concentration; degree of dyspnea (1-10); b/w, between; SpO_2, oxygen saturation of hemoglobin.
*Significant different from one-legged knee extension ($P < .05$).
**Significant different from one-legged leg press ($P < .05$).

is likely due to suboptimal muscle functionality,[19] the exaggerated reductions seen in COPD in two-legged leg press is likely due to the cardiopulmonary limitations inherent to the disease.[29] This agrees with previous data on endurance-[5] and resistance-like exercises.[22,23] Overall, these data underline the suitability of one-legged resistance exercises in subjects with COPD, advocating their use in rehabilitation programs.

Overall, COPD subjects displayed lower muscular performance in all exercises compared to healthy controls (total repetitions to exhaustion, −23%, −24%, and −49% for 1KE, 1LP, and 2LP, respectively; overall exercise volume, −41%, −42%, and −56% for 1KE, 1LP, and 2LP, respectively). The reduced performance in one-legged knee extension corroborates with previous observations of ~30% reductions in one-legged knee extension performance in subjects with moderate COPD compared to healthy controls.[30,31] For one-legged exercises, the attenuation in muscular performance is likely due to the muscle pathophysiology inherent to the disease, including reduced proportions of type I muscle fibers, increased proportions of type II (specially IIX) fibers, and reduced oxidative capacity.[19,32,33] Furthermore, the previous studies have shown that subjects with moderate to severe COPD (such as the participants in this study) are not limited by ventilatory capacity during one-legged knee extension exercises.[5,34] Our data supports this perspective, with COPD and healthy control subjects showing similar increases in muscular performance going from one-legged knee extension to one-legged leg press. This increase occurred without concomitant increase in lactate concentration, suggesting that oxygen supply was sufficient to fuel the increase in working muscle mass in one-legged leg press.

Chronic obstructive lung disease subjects were unable to increase muscular performance going from one-legged leg press to two-legged leg press. This contrasts data from healthy controls, who displayed 65% and 52% increases in performance (repetitions and volume, respectively), and agrees with data from previous studies.[35-38] In effect, this led to an exaggerated difference between COPD and healthy control subjects in muscular performance in two-legged leg press, which cannot be attributed muscular dysfunctions. Instead, the causative explanation likely resides in the cardiopulmonary limitations inherent to the COPD disease. Unfortunately, we do not have cardiorespiratory measurements to support this view. However, it is logical that the increase in working muscle mass accompanying going from one-legged leg press to two-legged leg press led to oxygen requirements that surpassed the oxygen-delivery capacity of the cardiopulmonary system, hence impairing muscle function and performance. This is supported by data from Nyberg *et al*,[23] who found evidence for ventilatory limitation in COPD patients at workloads corresponding to two-legged knee extension exercise. There, a decrease in muscular performance^{-leg} for COPD subjects was present going from one- to two-limb exercises, but whether this decrease was different from what the healthy subjects experienced was not evaluated. Nyberg *et al*[23] performed their study on COPD patients with more severe pulmonary obstruction (38% vs 53% of predicted FEV_1), which may explain the absence of impaired muscular performance in one-legged leg press in the present data. In our study, the crossing point between exercising with sufficient amounts of oxygen and exercising with insufficient amounts of oxygen occurred around or slightly after activation of muscle mass corresponding to one-legged leg press.

In the present data set, a comparison of 1RM data from healthy subjects and COPD provides an unexpected observation. In healthy controls, $1RM^{-leg}$ in two-legged leg press was 6% lower than 1RM in one-legged leg press (though without reaching statistical significance). This phenomenon is frequently described in the literature and is coined the bilateral deficit.[39] In contrast, in COPD, $1RM^{-leg}$ in two-legged leg press was 5% higher (non-significant) than 1RM in one-legged leg press, suggesting that the bilateral deficit was absent in these patients. This is not common, but has been previously observed in populations such as well-trained individuals.[40,41] This absence of a bilateral deficit in COPD is likely due to underperformance in one-legged leg press 1RM tests (and not overperformance in two-legged leg

press), perhaps related to poor technical performance caused by instability of the exercising limb or psychological factors. Regardless of causation, this phenomenon may have affected muscular performance during one-legged leg press testing, arguably lowering loads corresponding to 60% of 1RM and increasing estimates of muscular performance measured as repetitions to exhaustion,[38] potentially disguising impairing effects of cardiopulmonary limitations. Accordingly, for this exercise, there was no difference between COPD and healthy subjects in repetitions to exhaustion at 60% of 1RM ($P = .10$). This indirectly supports the notion that 1RM estimates for one-legged leg press were too low, as each of the two other exercises revealed clear reductions in muscular performance in COPD compared to healthy controls. Indeed, after taking into account workload (ie, exercise volume), one-legged leg press was also associated with marked reductions in muscular performance in COPD. Importantly, this potential issue does not change the take-home message in our data: muscular performance in COPD subjects is impaired in two-legged leg press, advocating the use of resistance exercises with lower amounts of active muscle mass.

4.1 | Perspectives

We have shown that COPD subjects display impaired muscular performance in resistance exercises compared to healthy controls. This impairment was exacerbated in exercises involving larger amounts of muscle mass (>one-legged leg press), suggesting that performance in such exercises was negatively influenced by the cardiopulmonary limitations inherent to the disease. A similar observation has previously been made in COPD patients with more severe diagnoses,[22,23] but not in the present patient population and not in connection with isolated resistance exercises performed in apparatus. This is also the first study to explicitly show that COPD patients show progressively lowered muscular performance in resistance exercises compared to healthy controls. Our data advocate implementation of resistance exercises targeting smaller amounts of muscle mass into rehabilitation programs for COPD subjects, including one-legged exercises.

Importantly, in healthy adults, one-legged resistance training leads to similar improvements of muscle functions as two-legged training, measured as strength and hypertrophy.[42-44] For COPD patients, there seems to be "a threshold" of muscle mass that can be exercised before muscular performance is limited by the cardiopulmonary capacity. In our study, this threshold seemed to occur around the muscle mass needed to perform one-legged leg press, though this remains circumstantial, as it was beyond the scope of the project to set such a threshold. Adding to this, the threshold is probably of individual character, determined by the subjects' cardiorespiratory capacity and the severity of the disease. Based on our data, we cannot conclude that one-legged resistance training will bring higher efficacy to COPD rehabilitation, which may resolve the seemingly lowered responses to training observed in this population. However, such training may enable COPD patients to perform resistance training on equal terms as healthy individuals, freeing them from the obstructions of cardiopulmonary limitations. Future studies should aim to target this perspective.

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Paper II

ORIGINAL ARTICLE

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Vitamin D_3 supplementation does not enhance the effects of resistance training in older adults

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Abstract

Background Lifestyle therapy with resistance training is a potent measure to counteract age-related loss in muscle strength and mass. Unfortunately, many individuals fail to respond in the expected manner. This phenomenon is particularly common among older adults and those with chronic diseases (e.g. chronic obstructive pulmonary disease, COPD) and may involve endocrine variables such as vitamin D. At present, the effects of vitamin D supplementation on responses to resistance training remain largely unexplored.

Methods Ninety-five male and female participants (healthy, n = 71; COPD, n = 24; age 68 ± 5 years) were randomly assigned to receive either vitamin D_3 or placebo supplementation for 28 weeks in a double-blinded manner (latitude 61°N, September–May). Seventy-eight participants completed the RCT, which was initiated by 12 weeks of supplementation-only (two weeks with 10 000 IU/day, followed by 2000 IU/day), followed by 13 weeks of combined supplementation (2000 IU/day) and supervised whole-body resistance training (twice weekly), interspersed with testing and measurements. Outcome measures included multiple assessments of muscle strength ($n_{variables}$ = 7), endurance performance (n = 6), and muscle mass (n = 3, legs, primary), as well as muscle quality (legs), muscle biology (*m. vastus lateralis*; muscle fibre characteristics, transcriptome), and health-related variables (e.g. visceral fat mass and blood lipid profile). For main outcome domains such as muscle strength and muscle mass, weighted combined factors were calculated from the range of singular assessments.

Results Overall, 13 weeks of resistance training increased muscle strength (13% ± 8%), muscle mass (9% ± 8%), and endurance performance (one-legged, 23% ± 15%; whole-body, 8% ± 7%), assessed as weighted combined factors, and were associated with changes in health variables (e.g. visceral fat, −6% ± 21%; $[LDL]_{serum}$, −4% ± 14%) and muscle tissue characteristics such as fibre type proportions (e.g. IIX, −3% points), myonuclei per fibre (30% ± 65%), total RNA/rRNA abundances (15%/6–19%), and transcriptome profiles (e.g. 312 differentially expressed genes). Vitamin D_3 supplementation did not affect training-associated changes for any of the main outcome domains, despite robust increases in $[25(OH)D]_{serum}$ (Δ49% vs. placebo). No conditional effects were observed for COPD vs. healthy or pre-RCT $[25(OH)D]_{serum}$. In secondary analyses, vitamin D_3 affected expression of gene sets involved in vascular functions in muscle tissue and strength gains in participants with high fat mass, which advocates further study.

Conclusions Vitamin D_3 supplementation did not affect muscular responses to resistance training in older adults with or without COPD.

Keywords Strength training; Cholecalciferol; Muscle plasticity

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Introduction

Aging is associated with progressive loss of muscle strength and mass, accompanied by declines in physical performance. In 2016, this had escalated into ~11 million Europeans (>65 years of age) suffering from sarcopenia,[1] a formally recognized disease characterized by severe loss of muscle quantity and quality.[1] Sarcopenia increases the likelihood of adverse events such as falling, fractures, physical disability, morbidity and mortality,[2,3] further fuelling muscle deterioration, resulting in a spiralling decrease in overall health and health-related quality of life.[4–6] In Europe, the prevalence of sarcopenia is expected to increase to at least ~19 million by 2045,[1] coinciding with increasing proportions of older adults, potentiated by suboptimal nutrition and increasing incidences of causal morbidities such as systemic inflammatory diseases.[7,8] For elderly to stay healthy, active and independent, efficient interventions are warranted for its prevention, treatment and reversal.[7,8] To this end, lifestyle therapy with resistance training is an attractive, low-cost and potent intervention.[9,10] Unfortunately, the benefits of such interventions are not always consistent, especially in the older population, with selected individuals and populations showing impaired abilities to increase muscle strength and mass.[11,12] At present, this training-response-spectrum has an unknown causality, although it interdepends on factors such as genetics,[13,14] epigenetics,[14] and composites of the inner physiological milieu, including nutrition,[15,16] endocrine variables (e.g. vitamin D),[17,18] and hallmarks of health such as low-grade chronic inflammation.[19] There is thus a need for development of combinatorial lifestyle protocols that target and correct these factors alongside resistance training, thereby allowing adequate muscle adaptations to occur.

Over the last two decades, vitamin D has emerged as a potential determinant of muscle functionality and biology.[20] There seems to be a robust relationship between heterogeneity in vitamin D status and traits such as physical performance[21–23] and susceptibility to falling,[24] suggesting a causal association between vitamin D and increased risk of sarcopenia.[25] As such, vitamin D status varies substantially in the human population, both in an annual cycle, and between individuals and groups of individuals.[26,27] Vitamin D insufficiency is particularly prevalent in older adults, measured as 25-hydroxyvitamin D (25(OH)D) levels <50 nmol/L, and especially in older adults living in the Northern Hemisphere,[27,28] where cutaneous vitamin D synthesis is miniscule or absent during winter months.[29] In accordance with this, exogenous vitamin D supplementation is gaining momentum as a potential ergogenic aid for preventing and treating sarcopenia.[25] Unfortunately, the presumed benefits of vitamin D supplementation deduced from crossover studies are not necessary supported by data from interventional studies. While some studies and meta-analyses report favourable effects of vitamin D supplementation *per se* on muscle strength[30–32] and falling,[33,34] with benefits being more pronounced in subjects with low baseline values (<30 nmol/L)[35] and in older subjects,[35] others do not.[36–39] These discrepancies may not be surprising, as resistance training is arguably necessary to provoke changes in muscle functions.[40] However, a similar ambiguity is present in the few studies that have assessed the effects of vitamin D supplementation on outcomes of resistance training.[41–44] While none of these studies report clear benefits of vitamin D supplementation for alterations in muscle strength,[41–44] muscle mass,[42–44] or incidences of falling,[41,43] a recent meta-analysis still concluded that it provides benefits for training-associated changes in lower body muscle strength.[40]

Consequently, we have limited and conflicting knowledge about the combined effects of vitamin D supplementation and resistance training for muscle functions and biology in humans. The present confusion may partly be attributed to methodological uncertainties in available studies, potentially lowering their ecological validity and explaining their lack of coherence with the resulting meta-analysis data. This includes heterogeneous study populations (varying from young adults[42,44] to older adults[44] to elderly[41,43]) with large differences in baseline 25(OH)D levels (average 31 nmol/L[43]–71 nmol/L[44]), large variation in vitamin D dosage (from 400 IU/day[43]–4000 IU/day[42]), lack of familiarization to strength tests,[41,43] suboptimal training protocols[41,43] (failing to comply to current guidelines, advocating resistance training with controlled maximal effort[45,46]), low compliance to training,[41,43] and a lack of dietary assessment during the intervention.[41,43,44] Also, neither of the studies included a period of vitamin D supplementation prior to resistance training, which may be necessary to prime muscle cells for adaptations, potentially acting by changing epigenetic traits, which has been observed in other cell types, such as T-cells[47] and oral squamous cell carcinoma cells.[48] Furthermore, the effects of vitamin D supplementation on muscle fibre characteristics and biology remain poorly understood and unclear.[49] In theory, vitamin D may potentiate muscle fibre responsiveness in two ways. Either directly by acting through vitamin D receptors in muscle fibres or progenitor cells, perhaps inducing intramuscular signalling pathways such as the p38 mitogen-activated protein kinase pathway,[50,51] or indirectly by interacting with systemic signalling event, perhaps inducing testosterone signalling[52] and thereby facilitating muscle plasticity. Our lack of insight is underlined by the longstanding uncertainty of the presence

of vitamin D receptors in muscle tissue,[53] although several indications advocate its expression. First, there seems to be associations between mutations in the vitamin D receptor and muscle weakness in both humans and mice.[54,55] Second, muscle-specific knock-out of the vitamin D receptor in mice deteriorates muscle strength and mass in a manner that resemble sarcopenia.[56,57] The prevailing uncertainty is fuelled by a seeming lack of effects of vitamin D supplementation *per se* on the muscle transcriptome in vitamin D-insufficient frail elderly, although also in that study the vitamin D dosage was relatively low (400 IU/day).[58] To date, a mere single study has assessed the effects of vitamin D supplementations on resistance training-induced muscle biological adaptations in humans, and as such assessing only a limited selection of traits and failing to disclose conclusive findings.[44]

The aim of the present study was to investigate the effects of 12 weeks of vitamin D_3 supplementation only (the initial two weeks with 10 000 international units (IU)/day, succeeded by 10 weeks with 2000 IU/day), followed by 13 weeks of combined vitamin D_3 supplementation (2000 IU/day) and resistance training, on training-associated adaptations in a mixed population of older subjects. The RCT thus allowed assessment of responses to both vitamin D_3 supplementation-only and combined vitamin D_3 supplementation and resistance training. The study population included individuals that were either at risk of developing sarcopenia (age or disease, i.e. COPD patients)[59,60] or showed diagnostic indications of sarcopenia (16.4% of the participants had appendicular lean mass (kg)/m^2 greater than two standard deviations below the sex-specific means of young adults).[61] Outcome measures included a large range of muscle strength and endurance performance tests, multiple assessments of muscle mass, muscle quality, in-depth analyses of muscle biology including muscle fibre characteristics and analyses of the muscle transcriptome, and a range of health-related measures including body composition, blood variables and self-reported health variables.

Methods

Study ethics and participants

The study was approved by the Regional Committee for Medical and Health Research Ethics - South-East Norway (reference no: 2013/1094) and was preregistered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT02598830). All participants were informed about the potential risks and discomforts associated with the study and gave their informed consent prior to study enrolment. The study was conducted according to the Declaration of Helsinki.

Ninety-five male and female participants (age 68 ± 5 years, range 56–77) were enrolled into the study (*Figure* 1). Eligibility criteria were consumption of less than 400 international units (IU) of vitamin D_3 per day for the two months leading up to the study, and either normal lung function or medical diagnosis of COPD (GOLD[62] grade II or III, FEV_1 predicted between 80% - 30%, $FEV_1/FVC < 70\%$ after reversibility testing with inhalation of salbutamol and ipratropiumbromid). Exclusion criteria were unstable cardiovascular disease, chronic granulomatous disease, known active malignancy within the last five years, serious psychiatric comorbidity, steroid use the previous two months and musculoskeletal disorders preventing the participant from participating in the resistance training programme. Initially, all participants were screened using spirometry and a medical questionnaire. For healthy participants, this formed the basis for inclusion. For COPD participants and participants with unclear disease status, the initial screening was followed by consultation with a medical doctor to ensure that they met diagnostic criteria corresponding to GOLD grade II or III, followed by inclusion. All participants were recreationally active, but none had partaken in systematic resistance training for the 12 months leading up to the study. During study conduct, all participants were instructed to restrict vitamin D intake from food sources to $<$400 IU/day and to abstain from solarium and travels to southern and/or sunny areas.

Participants were randomly assigned into one of the two study arms (vitamin D_3 vs. placebo) using concealed allocation, stratified by sex and health status (COPD vs. non-COPD) (*Figure* 1 and *Table* 1). An off-site third party performed the randomization. During the initial two weeks of the study, the vitamin D_3 arm consumed 10 000 IU vitamin D_3/day, followed by 2000 IU/day for the remainder of the study period. Placebo capsules contained cold-pressed olive oil and were identical in appearance to vitamin D_3 capsules. Pharma Nord ApS (Vejle, Denmark) produced the two supplements, complying with Good Manufacturing Practice requirements. All participants consumed 500 mg calcium/day (Nycoplus, Takeda AS, Asker, Norway). Vitamin D status was primarily assessed as 25(OH)D levels in blood (*Figure* 2), corroborating with previous studies,[63] and secondarily as 1,25 dihydroxycholecalciferol ($1,25(OH)_2D$; the biologically active form). 25(OH)D is accepted to be the most reliable measure of vitamin D status,[64] as it is unaffected by parathyroid hormone (PTH) activity, and is more stable and represents more accurate measurements compared with $1,25(OH)_2D$.[64]

Of the 95 participants included in the study, one withdrew from the study prior to onset on supplementation, 12 withdrew prior to onset of resistance training (vitamin D_3 arm, n = 9; placebo arm, n = 3), and 4 participants withdrew during the resistance training period (vitamin D_3 arm, n = 3; placebo arm, n = 1) (*Figure* 1). In summary, 78 participants completed the study; 58 healthy participants and 20 COPD participants. For participant characteristics, see *Table* 1.

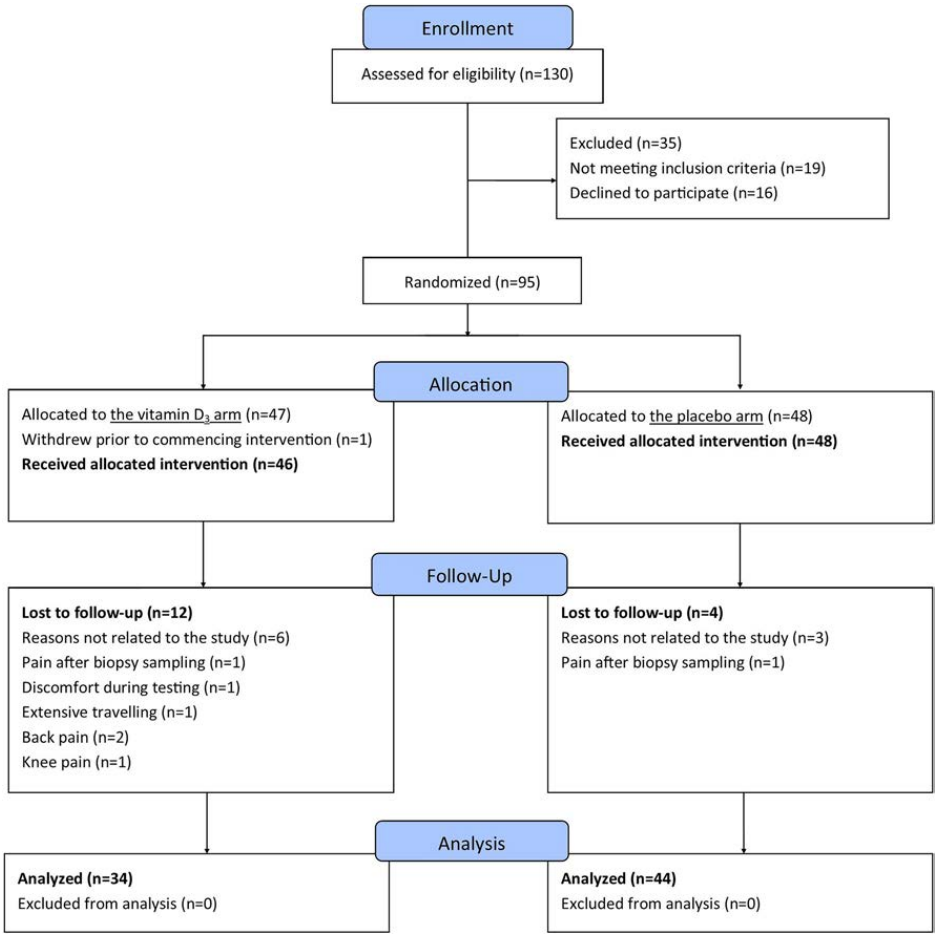

Figure 1 CONSORT flow chart of the study.

Study conduct

The study was conducted as a double-blind randomized clinical trial (RCT), consisting of an initial 12 weeks of supplementation-only (in average, 3333 IU vitamin D_3/day or placebo; 14 days of 10 000 IU vitamin D_3/day, 10 weeks of 2000 IU/day), followed by 13 weeks of combined supplementation (2000 IU vitamin D_3/day or placebo) and resistance training (*Figure* 2). During study conduct, supplement allocation was blinded for both participants and investigators. Unblinding was performed after completion of primary outcome measure clean-up and analyses. The intervention was conducted at Lillehammer, Norway (latitude 61°N) from September to May, ensuring low or no natural vitamin D synthesis by the skin from sunlight UVB radiation.[29] Prior to onset of the supplementation protocol (i.e. pre-RCT), participants undertook two weeks of baseline testing and tissue/blood sampling (*Figure* 2, Weeks −2 and −1), including testing of unilateral strength and muscle performance (tested twice, separated by at least 48 h; the first test was performed at ~95% of maximal effort), lung function, and collection of fasting blood and rested-state muscle biopsy, sampled from *m. vastus lateralis* of the dominant leg using the microbiopsy technique (Bard Magnum, Bard, Covington, GA, USA). Thereafter, participants were randomized to the two supplementation arms. After two weeks of supplementation, a second blood sample was collected (*Figure* 2, Week 2) to validate the efficacy of vitamin D_3 supplementation for blood 25 (OH)D and 1,25$(OH)_2$D levels. Prior to introduction to resistance training, the participants conducted repeated

Table 1 Participant characteristics

	Vitamin D_3 arm	Placebo arm
Participants (*n*)	46	48
Females (*n*)	24	27
COPD subjects (*n*)	12	12
Age (years ± SD)	69 ± 5	67 ± 4
Weight (kg ± SD)	75 ± 17	75 ± 16
Lean mass (kg ± SD)	48 ± 11	48 ± 9
Fat percentage (% ± SD)	35 ± 6	34 ± 9
Body mass index (kg/m^2 ± SD)	26 ± 5	26 ± 5
1RM knee extension (kg ± SD)	18 ± 8	18 ± 7
1RM chest press (kg ± SD)	47 ± 17	45 ± 16
Withdrawn prior to intro. RT (*n*)	9	3
Withdrawn after intro. RT (*n*)	3	1
Renal function		
Creatinine (μmol/L)	78 ± 18	80 ± 22
Est. GFR (mL/min/1.73 m^2)	80 ± 15	79 ± 15
CKD stage 3, i.e. est. GFR of 30–59 (*n*)	2	3
Lung function		
FVC (L ± SD)	3.4 ± 0.8	3.6 ± 0.9
FEV_1/FVC (% ± SD)	67 ± 15	69 ± 14
FEV_1 (% predicted ± SD)	87 ± 24	94 ± 26
PEF (L/s ± SD)	6.9 ± 2.4	7.1 ± 2.1
Habitual dietary data		
Kilocalories/day ± SD	1777 ± 529	1985 ± 611
Protein (g/kg/day ± SD)	1.26 ± 0.40	1.27 ± 0.36
Fat (g/kg/day ± SD)	0.99 ± 0.47	1.05 ± 0.38
Carbohydrates (g/kg/day ± SD)	2.46 ± 1.05	2.88 ± 1.03
Alcohol (units/day ± SD)	0.76 ± 0.92	0.67 ± 1.04
Vitamin D (IU/day ± SD)	281 ± 235	331 ± 260
Other vitamin D exposures		
Number of hours outdoors per week	8.8 ± 6.0	8.9 ± 6.4
Fish for dinner per week	1.9 ± 0.8	1.8 ± 0.7
Fish for other meals per week	2.0 ± 1.7	1.6 ± 1.1
Cod liver oil (teaspoons per week)	1.2 ± 3.8	1.6 ± 3.4
Cod liver oil (capsules per week)	1.5 ± 3.8	2.0 ± 3.8
Number of eggs eaten per week	3.2 ± 1.8	2.9 ± 2.2
Adherence		
Adherence to supplementation plan (%)	99 (91–100)	99 (93–100)
Adherence to the training protocol (%)	98 (81–100)	98 (81–100)

Training volume (kg x repetitions)	Leg press	Knee extension	RPE	Leg press	Knee extension	RPE
Training week 1 (Introduction period, week 1)	4074 (1741)	298 (143)	15.4 (1.4)	4307 (1737)	360 (206)	15.4 (1.5)
Training week 4 (Training period, week 1)	5117 (2199)	364 (187)	15.9 (1.4)	5393 (2247)	407 (201)	16.0 (1.3)
Training week 8 (Training period, week 5)	6071 (2710)	446 (233)	16.5 (1.5)	6200 (2638)	495 (255)	16.6 (1.3)
Training week 13 (Training period, week 10)	6698 (3183)	489 (255)	17.0 (1.3)	6706 (2598)	550 (293)	17.1 (1.2)

1RM, one repetition maximum; CKD, chronic kidney disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GFR, glomerular filtration rate (calculated using the *Modification of Diet in Renal Disease study* equation; IU, international units; PEF, peak expiratory flow; RT, resistance training; RPE, rating of perceived exertion (6–20).

performance tests at several occasions (*Figure* 2, Week −2–Week 13), including unilateral maximal strength and muscular performance, isokinetic unilateral knee-extension torque, measures of functional capacity (i.e. 6-min step and 1-min sit-to-stand test), submaximal and maximal one-legged cycling, and maximal bicycling. During the last week before introduction to resistance training (*Figure* 2, Week 13), bilateral rested-state biopsies and a fasted blood sample were collected, muscle thickness of *m. vastus lateralis* and *m. rectus femoris* were measured using ultrasound (SmartUs EXT-1 M; Telemed, Vilnius, Lithuania), and body composition was measured using dual-energy X-ray absorptiometry scan (DXA; Lunar Prodigy, GE Healthcare, Chicago, IL, USA).

The training intervention consisted of 13 weeks of two weekly whole-body resistance training sessions (*Figure* 2, Week 14–27). Leg exercises were performed unilaterally to allow within-participant differentiation of resistance training load. Accordingly, for each participant, the two legs were randomly assigned to perform either three sets with 10 repetitions to exhaustion (high-load resistance exercise) or three sets with 30 repetitions to exhaustion (low-load resistance exercise); that is, each participant performed both protocols

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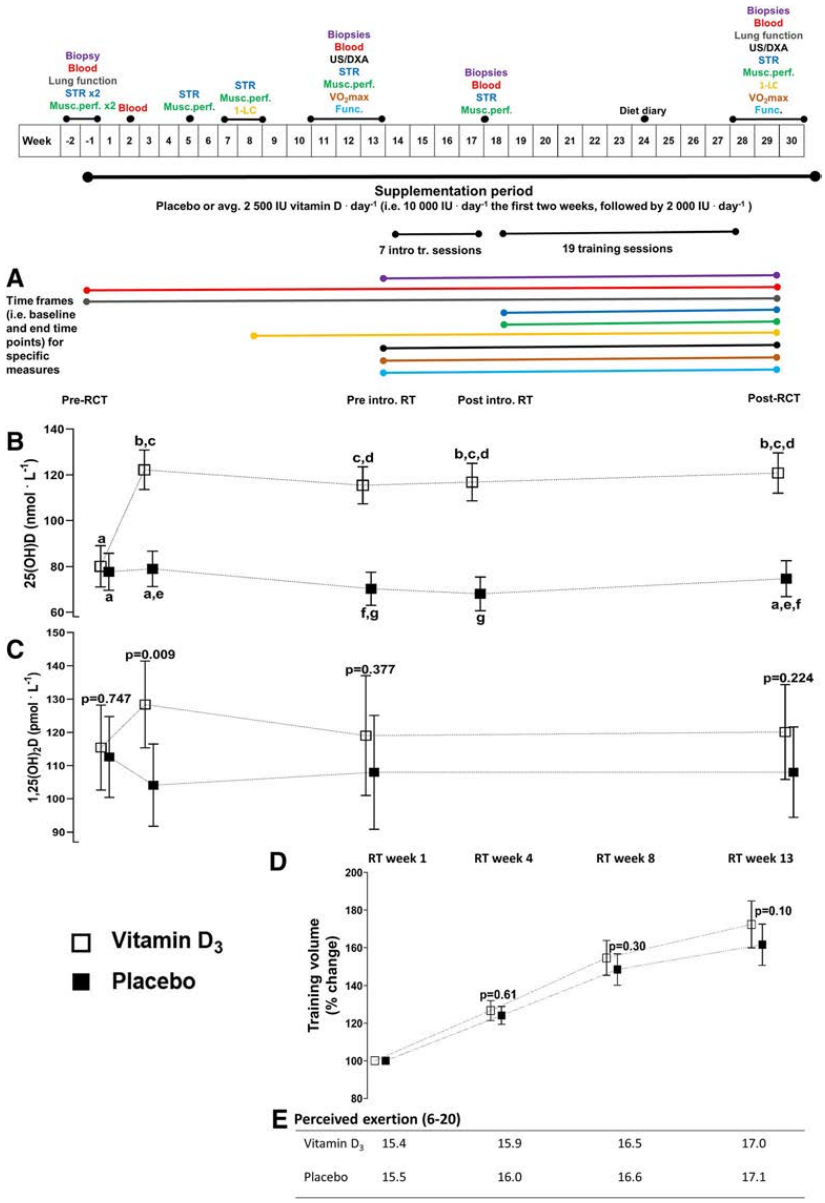

Figure 2 Schematic overview of the study protocol. Pre-defined main time frames (baseline and end time points) for specific outcome measures (the color lines represents the measurement marked with the same color at the top of the figure; *(A)*, vitamin D-status (25-hydroxyvitamin D levels, *(B)* and 1,25 dihydroxyvitamin D levels *(C)* during the RCT, training volume during the resistance training intervention *(D)*, and perceived exertion (Borg RPE, 6–20) reported after training sessions *(E)*. The training volume was calculated as average increase in volume (kg · repetitions) in leg press and knee extension from the first week of training. STR, maximal strength test; Musc.perf., test of muscular performance; 1-LC, one-legged cycling test; Func., test of functional capacity (6-min step test and 1-min sit-to-stand test); US, ultrasound measures of muscle thickness; DXA, Dual-energy X-ray Absorptiometry; $\dot{V}O_{2max}$, maximal oxygen consumption; IU, international units; RT, resistance training; 25(OH)D, 25-hydroxyvitamin D. In *(B)*, statistical differences between time points and supplementation arms are denoted by letters: different letter indicates $P < 0.05$, that is, all time point measures denoted with the same letter are statistically similar ($P > 0.05$). Data for 25(OH)D and training volume are presented as means with 95% confidence intervals.

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in each session. For the upper-body, resistance exercises were performed bilaterally, consisting of two sets of 10 repetitions to exhaustion. After seven training sessions (i.e. after 3.5 weeks of training; post-introduction to resistance training), participants performed a selected battery of tests and measurements (*Figure* 2, i.e. Week 17–18), including rested-state bilateral muscle biopsies, a fasted blood sample, and measures of muscle strength, performance and torque. These tests were conducted for two reasons i) to assess the initial response to resistance training and ii) to reduce the impact of neural adaptations for training-associated increases in performance (i.e. Week 17–18 was defined as baseline for these performance measurements). After the training intervention (post-RCT), the complete battery of tests and measurements were repeated (*Figure* 2, i.e. Week 28–30). During week 24, participants conducted a dietary registration, in which they logged their dietary intake for 3 days, including one weekend day (*Table* 1). Throughout the entirety of the study, participants completed a weekly health survey every Sunday evening, which included information about supplementation compliance, self-reported health and potential discomforts caused by the nutritional supplement, such as digestive issues, sleep issues, issues with the urinary system, issues with the vestibular system, and dermal irritations. Moderate verbal motivation was given to all participants during all performance tests.

Resistance-exercise training protocol

All participants performed the same whole-body resistance-exercise training programme, consisting of the following exercises (listed in order of conductance): unilateral leg press, unilateral knee extension, unilateral knee flexion, chest press, and lat pulldown. Leg exercises were performed as three series of 10 repetitions (high-load) and 30 repetitions (low-load) to exhaustion (10RM and 30RM, respectively), and upper-body exercises were performed as two series of 10 repetitions (high-load) to exhaustion, as previously described. Exercises and sets were separated by 2 min of rest. For leg exercises, all three sets for one leg were conducted before the other leg was exercised. The order in which the two legs were exercised was switched between each session. For all exercises, training loads were adjusted from session to session, i.e. when participants managed to perform more than 12 or 35 repetitions per set for high- and low-load training, respectively. All sessions were supervised by qualified personnel to ensure correct technical execution and to ensure maximal efforts through verbal encouragement. To aid recovery and to ensure adequate protein intake after training, participants ingested half a protein bar immediately after each training session (~15 g protein; Big 100, Proteinfabrikken, Sandefjord, Norway).

Spirometry

Spirometry testing was performed using either the Oxycon Pro™ with the TripleV digital volume sensor (Carefusion GmbH, Höchberg, Germany) or the Spirare SPS320 ultrasonic spirometer (Diagnostica AS, Oslo, Norway) following guidelines from the American Thoracic Society and the European Respiratory Society.[65] Importantly, for each particular participant, all spirometry tests were performed using the same system. Participants with COPD were tested before and after inhalation of two bronchodilators (salbutamol, 0.2 mg and ipratropiumbromid, 20 μg).

Muscle strength and performance

Maximal muscle strength was assessed as one repetition maximum (1RM) in unilateral knee extension and leg press (Technogym, Cesena, Italy) and bilateral chest press (Panatta, Apiro, Italy). Each test started with specific warm-up, consisting of 10, 6, and 3 repetitions at 40%, 70%, and 85% of the anticipated maximum. Thereafter, 1RM was found by increasing the resistance progressively until the weight could not be lifted through the full range of motion. Loads were increased in intervals of 1.25, 2.5, and 1.25 kg for knee extension, leg press, and chest press, respectively. Two minutes of rest was provided between attempts. Maximal handgrip strength was measured for the dominant hand using a hand-held dynamometer (Baseline®, Fabrication Enterprises, Inc., Elmsford, NY, USA). Each test session consisted of three attempts, and the average score was used in further analyses.

Muscle performance was defined as the maximal number of repetitions achieved at 50% of pre-RCT 1RM and was assessed in unilateral knee extension and bilateral chest press. Participants were instructed to lift at a composed and controlled pace, with <1 s breaks in the lower and upper position. Whenever this requirement was not met, or participants failed to lift the weight through the full range of motion, the test was aborted.

Isokinetic unilateral knee-extension torque was assessed using a dynamometer (Humac Norm, CSMi, Stoughton, MA, USA). Participants were seated and secured with the knee joint aligned with the rotation axis of the dynamometer. Maximal isokinetic torque was tested at three angular speeds (60°, 120°, and 240° per second) with 2 min of rest provided between each of them. Prior to each test session, participants were familiarized with the test protocol by performing three submaximal efforts at each angular speed. Participants were given three attempts performed in immediate succession. The highest value was used in further analyses.

For all tests of unilateral strength and performance, the dominant leg was tested first. Seat position and general

settings for each test were noted for each participant and reproduced at each time-point.

One-legged cycling and bicycling performance

Participants conducted one-legged cycling tests (Excalibur Sport, Lode BV, Groningen, the Netherlands) to assess O_2-costs of submaximal cycling, and maximal one-legged oxygen consumption ($\dot{V}O_{2max}$) and power output (W_{max}). Each test was initiated by 2 × 5 min submaximal workloads at 30 and 40 watts (healthy), respectively, or 20 and 30 watts (COPD) with a cadence of 60 revolutions per minute. Loads were individually adjusted if the predefined workload was higher than 50% of the W_{max} achieved during the familiarization session. Thereafter, a maximal step-wise incremental protocol was conducted (10 and 5 watts/min for healthy and COPD participants, respectively). Starting loads were individually adjusted to elicit exhaustion after 6–10 min of cycling, based on results from the familiarization session. The cadence was freely chosen (>50 rpm). The test was terminated when cadence fell below 50 rpm. For all participants, submaximal and maximal performance on the dominant leg was tested first. After testing of the first leg, participants were allowed 20 min rest and/or low-intensity cycling, before testing of the other leg. During one-legged cycling tests, a 10 kg counterweight was attached to the contralateral ergometer crank to facilitate smooth cycling. The foot of the non-exercising leg was rested on a chair placed in front of the subject. Breath-to-breath measurements of pulmonary oxygen consumption and ventilation (JAEGER Oxycon PRO™; Carefusion GmbH, Höchberg, Germany) and heart rate (Polar Electro Oy, Kempele, Finland) was monitored continuously during all tests. The average oxygen consumption during the last 2 min of each submaximal workload was defined as the O_2-cost, while $\dot{V}O_{2max}$ was defined as the highest average oxygen consumption measured over a period of 30-s. Measurement of capillary lactate concentration (Biosen C-line, EKF Diagnostics, Barleben, Germany) was performed after finalization of tests.

Testing of maximal bilateral cycling $\dot{V}O_{2max}$ and W_{max} was performed on a separate day. A step-wise incremental protocol (20 and 15 watts/min for healthy men and women, respectively; 10 watts/min for participants with COPD) was conducted. Oxygen consumption was measured continuously using a computerized metabolic system with mixing chamber (JAEGER Oxycon PRO™; Carefusion GmbH, Höchberg, Germany). Prior to each cycling test, the gas analyser was calibrated using certified calibration gases with known concentrations, and the flow turbine (TripleV; JAEGER, Carefusion GmbH, Höchberg, Germany) was calibrated using the metabolic system's automatic volume calibration, or a 3 L, 5530 series calibration syringe (Hans Rudolph Inc., Kansas City, MO, USA), for one-legged and bicycling tests, respectively.

Functional performance

One-minute sit-to-stand and 6-min step tests were conducted in consecutive order on the same test day. Each test session was initiated with 10 min warm-up of low-intensity bicycling. Briefly, during the 1-min sit-to-stand tests, participants were instructed to fold their arms and sit/stand up for as many times possible during a 1-min period. The seat was 45 cm from the floor. Sit-to-stand repetitions were approved if both knees and hip joints were fully extended after each seating. Three minutes after the 1-min sit-to-stand test, the 6-min step test was conducted. Briefly, participants were instructed to perform as many steps as possible onto a 20 cm high step box with a non-slip rubber surface within 6 min (Reebok Step; Boston, MA, USA). During each step, participants were instructed to place both legs on the box, with the hip fully extended.

Muscle thickness by ultrasound and dual-energy X-ray absorptiometry-derived body mass measures

Prior to measurements of muscle thickness and DXA measurements, the participants were instructed to attend an overnight fast and avoid heavy physical activity for the last 24 h leading up to the event.

Muscle thickness of *m. vastus lateralis* and *m. rectus femoris* were measured using B-mode ultrasonography (SmartUs EXT-1 M, Telemed, Vilnius, Lithuania) with a 39 mm 12 MHz, linear array probe. Transverse images were obtained ~60% distally from the *trochanter major* towards the femoral lateral epicondyle. Three images were captured for each muscle, where the probe was relocated to the same position between each image. The position of the probe was marked on the skin and subsequently marked on a soft transparent plastic sheet superimposed on the thigh. Landmarks such as moles and scars were also marked on the plastic sheet for relocation of the scanned areas during post-training measurements. During analysis, pre and post images from the same participant were analysed consecutively using the Fiji software[66] and by two independent researchers. The average muscle thickness of the three images captured per muscle was used for further analyses.

Body composition was determined using DXA (Lunar Prodigy, GE Healthcare, Madison, WI, USA) and was analysed using the manufacturer's software, in accordance with the manufacturer's protocol. Leg lean mass was defined as the region distally of *collum femoris*. Care was taken to match the region of interest on pre and post images. Analyses of both muscle thickness and body composition were performed in a blinded manner regarding participant identity and time point of the measurement.

Blood sampling and measurements, and muscle biopsy sampling

Prior to collection of blood and muscle biopsies, participants were instructed to attend an overnight fast and to avoid heavy physical activity for the last 48 h leading up to the event. All blood samples and muscle biopsies were collected between 08:00 and 11:00 a.m. Blood samples were collected from an antecubital vein into serum-separating tubes and kept at room temperature for 30 min before centrifugation (2600 *g*, 15 min). Serum was aliquoted and stored at −80°C until further processing. Serum concentrations of total testosterone, cortisol, growth hormone, insulin-like growth-factor 1 (IGF-1), sex-hormone binding globulin (SHBG) and androstenedione were measured using an Immulite 2000 analyser with kits from the Immulite Immunoassay System menu (Siemens Medical Solutions Diagnostics, Malvern, PA, USA). Serum 25(OH)D, parathyroid hormone, calcium, albumin, creatinine, creatine kinase, aspartate aminotransferase, C-reactive protein, triglycerides, low-density lipoprotein, high-density lipoprotein, thyroid hormones and iron metabolism variables were measured using a Roche Cobas 6000 analyser and kits from Roche (Roche Diagnostics, Rotkreuz, Switzerland). In a subset of participants, $1,25(OH)_2D$ levels in serum were measured at Week −1, Week 2, Week 13 and Week 28 (vitamin D_3 arm, n = 19; placebo arm, n = 21) using enzyme immunoassays with kits from Immunodiagnostic Systems (IDS, Boldon, Tyne & Wear, UK).

Muscle biopsies were sampled from *m. vastus lateralis* under local anaesthesia (Lidocaine, 10 mg/mL, AstraZenaca AS, Oslo, Norway) using a 12-gauge needle (Universal Plus, Medax, San Possidonio, Italy) operated with a spring-loaded biopsy instrument (Bard Magnum, Bard, Covington, GA, USA), as previously described.[67] Biopsies were sampled at 1/3 of the distance from the patella to the *anterior superior iliac spine*. The tissue was quickly dissected free of blood and visible connective tissue in ice-cold sterile saline solution (0.9% NaCl). Samples for immunohistochemistry were transferred to a 4% formalin solution for fixation for 24–72 h, before further preparation. Samples for RNA analyses were blotted dry, snap-frozen in isopentane (−80°C) and stored at −80°C until further processing.

Immunohistochemistry

Formalin-fixed muscle biopsies were processed rapidly using a Shandon Excelsior ES (Thermo Fisher Scientific, Waltham, MA, USA), whereupon biopsies were paraffin-embedded and sectioned into transverse sections (4 μm). Antigen retrieval was performed at 97°C for 20 min in a target retrieval solution (cat. no. DM828, Agilent Dako, Santa Clara, CA, USA) using a PT link (PT 200, Agilent Dako, Santa Clara, CA, USA). Staining was performed using a DAKO Autostainer Link 48 (Agilent Dako, Santa Clara, CA, USA). For determination of muscle fibre types, cross-sections were first treated with protease 2 (cat. no. 760–2019, Roche Diagnostics, Rotkreuz, Switzerland), before they were triple-stained using 2.5 μg/mL BA-F8, BF-35 and 6H1 (all from Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA; BA-F8 and BF-35 deposited by Schiaffino, S., Uni. of Padova, Italy; 6H1 deposited by Lucas, C., Uni. of Sydney, Australia). Visualization of the primary antibodies was achieved by incubation of appropriate secondary antibodies, diluted 1:400: goat anti-mouse Alexa Fluor (Thermo Fisher Scientific, Waltham, MA, USA) 350 (IgGγ2b, cat. no. A21140), 488 (IgGγ1, cat. no. A21121) and 594 (IgM H + L, cat. no. A21044) for BA-F8, BF-35 and 6H1, respectively.

For determination of muscle fibre cross-sectional area (CSA) and numbers of myonuclei per muscle fibre type, a different tissue cross-section was double-stained using primary antibodies against muscle fibre membrane (dystrophin, diluted 1:100, cat. no. PA1–21011; Thermo Fisher Scientific, Waltham, MA, USA) and myosin heavy chain I (diluted 1:2000, cat. no. M8421, Sigma-Aldrich, Saint-Louis, MO, USA). Visualization was achieved using the secondary antibodies Alexa Fluor 594 (IgG H + L, diluted 1:400, cat. no. A11037) and 488 (IgG1γ1, diluted 1:400, cat. no. A21121), respectively (Thermo Fisher Scientific, Waltham, MA, USA). Muscle sections were then covered with a coverslip and glued with EverBrite™ Hardset Mounting Medium with DAPI (cat. no. 23004, Biotium Inc., Fremont, CA, USA), to visualize cell nuclei.

Images of stained cross-sections were captured using a high-resolution camera (Axiocam, Zeiss, Oberkochen, Germany) mounted on a light microscope (Axioskop-2, Zeiss, Oberkochen, Germany), with a fluorescent light source (X-Cite 120, EXFO Photonic Solutions Inc., Mississauga, Canada). Multiple images were taken using 20× objectives to capture the entirety of each cross-section. For representative images, see *Figure* 3. All analyses of muscle fibre characteristics were performed using automated procedures, ensuring unbiased quantification.

Analyses of muscle fibre type proportions were performed using the Cell Counter function in the Fiji software,[66] whereby muscle fibres were categorized as either type I, type IIA, type IIX or hybrid fibres type IIA/IIX. Sections and/or images with insufficient staining to distinguish between fibre types were excluded. Muscle fibre type-specific CSA (type I or type II) were calculated using the TEMA software (CheckVision, Hadsund, Denmark). Myonuclei were counted using the CellProfiler software.[68]

Total RNA extraction and qPCR

Approximately 10–20 mg of wet muscle tissue (average 13 ± 4 mg, range 3–26 mg) was homogenized in a total

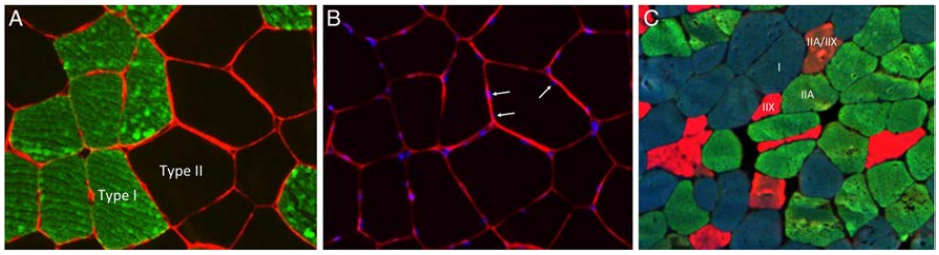

Figure 3 Representative immunohistochemistry images of *(A)* myosin heavy chain I (green) and cell membrane (red), *(B)* myonuclei (blue) and cell membrane (dystrophin, red), and *(C)* myosin heavy chain I (blue), IIA (green), IIX (red), and IIA/IIX hybrids (orange). Images in *(A)* and *(B)* are from the same tissue cross-section: triple-staining myosin heavy chain I, dystrophin and cell nuclei.

volume of 1 mL TRIzol reagent (Invitrogen, Carlsbad, CA, USA) using 0.5 mm RNase-free zirconium oxide beads and a bead homogenizer (Bullet Blender, Next Advance, Averill Park, NY, USA), as previously described.[67] To enable analysis of target gene expression per unit tissue weight, an exogenous RNA control (λ polyA External Standard Kit, Takara Bio Inc., Shiga, Japan) was added at a fixed amount (0.04 ng/mL of Trizol reagent) per extraction prior to homogenization, as previously described.[69,70] Following phase separation, 450 μL of the upper phase was transferred to a new tube and RNA was precipitated using isopropanol. The resulting RNA pellet was washed three times with 75% ethanol, eluted in 30 μL TE buffer, and diluted to 100 ng RNA/μL, following quantification of total RNA concentration using μDrop plate and the Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was assessed using capillary electrophoresis (Experion Automated Electrophoresis Station using RNA StdSens Assay, Bio-Rad, Hercules, CA, USA) with average integrity score (RNA quality indicator; RQI): 8.9 ± 0.8.

Five hundred nanograms of RNA were reverse transcribed using anchored oligo-dT (Thermo Fisher Scientific, Waltham, MA, USA), random hexamer primers (Thermo Fisher Scientific, Waltham, MA, USA) and Super-Script IV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA), according to manufacturers' instructions. All samples were reverse transcribed in duplicates and diluted 1:50 prior to quantitative real-time polymerase chain reaction (qPCR). qPCR reactions were conducted using a fast-cycling real-time detection system (Applied Biosystems 7500 fast Real-Time PCR Systems, Life Technologies AS), with total volumes of 10 μL, containing 2 μL cDNA (1:25 dilutions), target gene-specific primers (final concentration 0.5 μM) and a commercial master mix (2× SYBR Select Master Mix, Applied Biosystems, Life Technologies Corp., Carlsbad, CA, USA). qPCR reactions consisted of 40 cycles (3 s 95°C denaturing and 30 s 60°C annealing). Melt-curve analyses were performed for all reactions to verify single-product amplification. Gene-specific primers were designed using Primer3Plus[71] and synthesized by Thermo Scientific, except for the external RNA control, for which primers were supplied with the kit (λ polyA External Standard Kit, Takara Bio Inc., Shiga, Japan). Raw fluorescence data were exported from the platform-specific software and amplification curves were modelled using a best-fit sigmoidal model using the qpcR-package[72] written for R.[73] Threshold cycles (Ct) were estimated from the models by the second-derivate maximum method with technical duplicates modelled independently. Amplification efficiencies were estimated for every reaction.[74] For every primer pair, mean amplification efficiencies (E) were utilized to transform data to the linear scale using E^{-Ct}. Primer sequences and primer characteristics (i.e. average primer efficiencies and Ct values) are presented in Supporting Information, *Table* S1. Gene expression data were log-transformed prior to statistical analysis. As Ct values, but not primer efficiencies depend on RNA integrity,[75] RQI scores were used as a random variable on a per-target basis to control for potential degradation during statistical analyses (see below).

RNA sequencing

RNA sequencing was performed on pairwise muscle samples collected before the RCT (vitamin D_3, n = 11; placebo, n = 13), after 12 weeks of supplementation-only (vitamin D_3, n = 24; placebo, n = 29), after 3.5 weeks of introduction to resistance training (vitamin D_3, n = 23; placebo, n = 28), and after 13 weeks of resistance training (vitamin D_3 arm, n = 24; placebo arm, n = 29). Samples was selected based on quality of total RNA samples (RQI > 7.0, avg 9.0 ± 0.5). Participants with complete sets of muscle biopsies were prioritized. For each muscle sample, mRNA sequencing libraries were prepared from 1000 ng of total RNA using TruSeq Stranded Total RNA Library Prep (Illumina, San Diego, CA, USA). Paired-end sequencing (150 bp) was performed using an Illumina HiSeq

3000 (Illumina, San Diego, CA, USA) at the Norwegian Sequencing Centre, Oslo, Norway.

Data analyses and statistics

As defined in the pre-registration of the study protocol (ClinicalTrials.gov Identifier: NCT02598830), the effects of vitamin D_3 supplementation for different outcome measures were evaluated using different baseline time points (outlined in *Figure* 2). For transparency, statistical comparisons of all outcome measures and all relevant time points are presented in Supporting Information, *Tables* S2 and S3. These tables also specify the statistical models used for each specific variable and analysis. In general, for continuous variables, the effects of vitamin D_3 supplementation (compared with placebo) were investigated using linear mixed-effects models with the relative change from baseline being defined as the dependent variable and the supplementation arms being defined as the fixed effect. The two different training loads (high- and low-load) were added to the model as repeated measures/observations (for unilateral outcome measures), and baseline values were used as co-variates. For all participants, random intercepts were specified. For all unilateral leg variables, interaction effects were explored between the fixed effect and health status (COPD vs. non-COPD) and training loads. For other variables, interactions were investigated between the fixed effect (vitamin D_3 vs. placebo) and health status, with the exception for blood variables, for which the interaction with sex was also examined. For all statistical analyses of immunohistochemical variables (muscle fibre CSA, fibre type proportion, and myonuclei per fibre), the models were weighted for the number of counted fibres per biopsy. This was carried out to account for the reduced reliability accompanying fewer observations/fibres (see Supporting Information, *Figure* S2). For non-continuous variables, a different statistical approach was used to investigate the effects of the vitamin D_3 supplementation. For fibre type proportions (immunohistochemistry) and variables from the weekly health survey, a generalized linear mixed model (GLMM) with binomial error distribution and link function was used to examine differences in changes between supplementation arms (time*supplementation arm interactions). For gene family-based analyses of myosin heavy-chain mRNA data,[76] a GLMM with negative binomial distribution/link function (log-link) was used following transformation to transcript counts.[77] Target gene mRNA abundance, expressed as per unit muscle weight using the external reference gene, were analysed using mixed linear models with within-model normalization through the addition of random effects of technical replicates. To allow for gene-specific variances, variance functions were specified per strata (per gene). RQI scores were included in the model on a per target basis to control for RNA degradation. The number of observations per statistical analysis is presented in Supporting Information, *Table* S2. For most outcome measures, the main effect of time was examined using mixed modelling, using absolute values for the dependent variable and time points as repeated measures/observations with random intercepts for each subject (Supporting Information, *Table* S2 for complete overview).

During transcriptome analyses, gene counts were modelled using negative binomial GLMM with the total library size modelled as a fixed effect[78] together with sex and study conditions (time point and supplementation arms). The effect of resistance training on gene counts was assessed as i) the effect of time and ii) its interaction with supplementation arm (vitamin D_3 and placebo supplementation). For analyses of the effect of time, differential expression was evaluated using GLMMs containing only the time factor, combining all data irrespective of supplementation arm. For analyses of the effect of supplementation over time, differential expression was evaluated using GLMMs containing the interaction between time and supplementation arm. The supplementation-only period was modelled independently of the training period. In all models, a single random effect was used, giving each participant an individual intercept. Models were iteratively fitted using glmmTMB.[79] Model adequacy was tested for each model fit by assessing uniformity of simulated residuals.[80] A total of 15 093 genes were included in the RNA-seq data set after initial filtering, and 0.4–3.7% of these were subsequently removed due to violation of the uniformity assumption ($P < 0.05$). Genes were identified as differentially expressed when the absolute $\log_2$ fold-change was greater than 0.5 and the adjusted *P*-value (false discovery rate adjusted per model coefficient) was below 5%. Enrichment analyses of gene ontology (GO) gene sets were performed using two approaches. First, a non-parametric rank test[81,82] was performed based on gene-specific minimum significant differences (MSD). MSD was defined as the lower limit of the 95% confidence interval (CI, based on estimated standard errors) around the log fold-change (FC) when log (FC) > 0 and the negative inverse of the upper 95% CI when log (FC) < 0. Genes with MSD < 0 were further ranked based on *P*-values. The rank test assessed non-directional changes in gene sets. Second, gene set enrichment analysis (GSEA)[83] was performed to quantify directional regulation of the gene set. GSEA was performed using the fgsea package,[84] with $-\log_{10}$(*P*-values) *$\log_2$(fold-change) acting as the gene level metric.[85] Consensus results between the two analyses were given higher importance. GO gene sets (biological process, cellular component and molecular function), as well as Hallmark and KEGG gene sets were retrieved from the molecular signature database (version 7.1).[86] Overview of enrichment analyses with exact *P*-values are presented in Supporting Information, *Tables* S5, S6, and S8–S10.

To achieve reliable assessment of the main outcome domains muscle strength, muscle mass, one-legged endurance

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performance and whole-body endurance performance, and thus to lower the risk of statistical errors, combined factors were calculated for outcome measures. For complete overview over the composition of each factor, see Supporting Information, *Table* S4. During factor calculation, each of the underlying variables were normalized to the participant with the highest value recorded during the RCT, resulting in individual scores ≤ 1. Thereafter, outcome domain factors were calculated as the mean of the normalized values for each variable for each subject (e.g. the muscle mass factor of the legs included muscle thickness, leg lean mass, and muscle fibre CSA). To evaluate the biological coherence of these factors, a factor analysis was performed to ensure correlation between the combined factors and their underlying outcome variables (Supporting Information, *Table* S4).[87] To assess the effect of vitamin D_3 supplementation for changes in these combined factors, linear mixed-effects models were used, as previously described. In addition, these factors were used to investigate the influence of pre-RCT levels of 25(OH)D, body fat proportions and body mass index on the effects of vitamin D_3 supplementation. To perform these analyses, each of the two supplementation arms were divided into quartiles, defined by baseline 25(OH)D, body fat percentage and body mass index levels, respectively (quartile 1, lowest, … quartile 4, highest). For each of the calculated factors, the effect of quartile and the interaction between quartile and supplementation arm was examined using mixed modelling.

Statistical significance was set to $P < 0.05$. In the text, data are presented as means ± standard deviation. In figures, data are shown as adjusted, estimated marginal means of relative changes and differences in relative changes between supplementation arms, with 95% confidence intervals, unless otherwise stated. Statistical analyses were performed using SPSS Statistics package version 24 (IBM, Chicago, IL, USA) and R software.[73] Figures were made using Prism Software (GraphPad 8, San Diego, CA, USA) and R software.[73]

Results and discussion

Effects of vitamin D_3 supplementation on 25(OH)D and 1,25(OH)$_2$D in blood

At pre-RCT, participants in vitamin D_3 and placebo intervention arms had similar [25(OH)D] levels in serum (80 nmol/L vs. 78 nmol/L, range: 24–144 nmol/L, *Figure* 2). [25(OH)D] levels did not differ between participants with different health status (i.e. with or without COPD diagnosis). In the vitamin D_3 arm, the study was initiated by 14 days of high-dosage vitamin D_3 intake (10 000 IU per day), which led to 42 nmol/L increases in [25(OH)D] (to 122 ± 24 nmol/L; range = 82–175 nmol/L; $P < 0.001$), with no change in the placebo arm (79 ± 31 nmol/L; range = 36–167 nmol/L) (*Figure* 2). During the remainder of the study (weeks 3–30), vitamin D_3 was ingested at 2000 IU per day, which led to stabilization of [25(OH)D] at elevated levels compared with the placebo arm (Week 13, Δ45 nmol/L; Week 17, Δ49 nmol/L; Week 29, Δ46 nmol/L; *Figure* 2), resembling the efficacy of previous studies with comparable study protocols (~2500 IU per day).[88,89] Conversely, in the placebo arm, [25(OH)D] either declined or was similar to pre-RCT levels (Week 13, −8 nmol/L; Week 17, −11 nmol/L; Week 29, −6 nmol/L; *Figure* 2), corroborating with changes typically seen in Northern populations during winter months,[27] with the notable observation that values were slightly higher than expected.[28]

After the initial 14 days of supplementation-only, the marked increases in 25(OH)D in the vitamin D_3 arm were accompanied by robust increases in [1,25(OH)$_2$D] compared with the placebo arm (vitamin D_3, +17 pmol/L; placebo, −7 pmol/L; Δ24 pmol/L, $P = 0.004$; *Figure* 2). During this time frame, change scores for [1,25(OH)$_2$D] were correlated with change scores for [25(OH)D] ($r = 0.429$, $P = 0.006$; data not shown). At Week 13 and 29, the statistical difference in changes in [1,25(OH)$_2$D] between supplementation arms had disappeared (Δ11 pmol/L, $P = 0.377$, and Δ12 pmol/L, $P = 0.224$; *Figure* 2), and the correlation between changes in [1,25(OH)$_2$D] and [25(OH)D] was no longer evident ($r = 0.169$–0.243, $P = 0.131$–0.298; data not shown). The initial period of high-dosage vitamin D_3 supplementation thus led to rapid elevations in 1,25(OH)$_2$D levels, which was subsequently reversed towards baseline levels during the follow-up period with maintenance intake (2000 IU/day), although vitamin D_3 supplementation was still associated with increased numerically values and the levels of individual variation was large. In all but three samples, measures of [1,25(OH)$_2$D] were within the normal range for adults (39–193 pmol/L), as defined by the manufacturer,[90] with all deviating samples being >193 pmol/L (vitamin D_3, $n = 2$; placebo, $n = 1$).

At the onset of introduction to training (Week 13) and throughout the training intervention (Week 17, Week 29), participants in the vitamin D_3 arm were all vitamin D-sufficient, as classified by the National Academy of Medicine ([25(OH)D] > 50 nmol/L),[27] while in the placebo arm, 13 (Week 13), 12 (Week 17) and 5 (Week 29) participants were vitamin D-insufficient. In both supplementation arms, calcium was ingested at 500 mg/day throughout the intervention. Despite this, no changes were seen in calcium or albumin-corrected calcium levels in blood at any time point (Supporting Information, *Table* S11). Levels of the parathyroid hormone decreased throughout the intervention ($P = 0.035$; Supporting Information, *Table* S11), most likely caused by an autoregulatory response to increased calcium intake.[91] Vitamin D_3 supplementation did not alter this response. Compliance to the supplementation protocol was high in both intervention arms (vitamin D_3, 99.3%; placebo, 99.3%;

P = 0.998). Together, these observations suggest that vitamin D_3 supplementation led to improved vitamin D-status during the intervention, measured as 25(OH)D, whereas placebo led to reduced or maintained levels, with approximately 1/3rd of placebo-receiving participants showing levels associated with impaired muscle functionality (<50 nmol/L) at the onset of resistance training.[21,22,92]

Effects of vitamin D_3 supplementation on resistance training-associated changes in myofibre cross-sectional area and proportions (primary objectives)

In contrast to our main hypotheses, vitamin D_3 supplementation did not enhance resistance training-associated increases in muscle fibre cross-sectional area or changes in muscle fibre proportions (*Figure* 4; pre-defined as primary objectives of the study), despite clear improvements in vitamin D status (25(OH)D). The results are presented in more detail in later sections (*Effects of vitamin D_3 supplementation on training-associated changes in maximal muscle strength and lower-limb muscle mass* and *Effects of vitamin D_3 supplementation on training-associated changes in muscle fibre characteristics and transcriptomics)*.

Effects of 12 weeks of vitamin D_3-supplementation only (weeks 1–12) on muscle strength, performance and characteristics

The main purpose of the initial 12 weeks of vitamin D_3 supplementation-only was to ensure physiologically elevated [25(OH)D] for a prolonged period prior to onset of resistance training, thus potentially priming muscle cells for plasticity. Vitamin D_3 supplementation itself had no effect on upper- and lower-body muscle strength and performance, muscle fibre area and characteristics (*m. vastus lateralis*), or hormone concentrations in blood compared with placebo (Supporting Information, *Figure* S1 and *Table* S2), showing no interaction with health status. Surprisingly, the only exception was 1RM knee extension, for which vitamin D_3 led to negative changes compared with placebo (Δ−8.4%; *P* = 0.008), opposing the seemingly accepted dogma that vitamin D supplementation *per se* exerts positive effects on leg muscle strength.[35,93] Notably, for all muscle strength and muscular performance variables, the initial 12 week supplementation period was

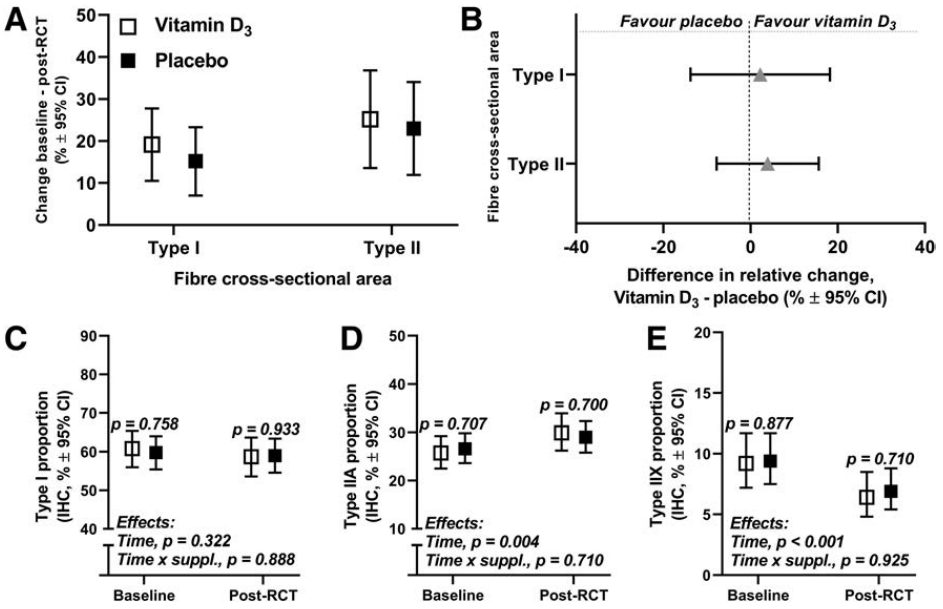

Figure 4 Primary outcome objectives of the study; effects of combined vitamin D_3 supplementation and resistance training on changes in muscle fibre cross-sectional area *(A, B)* and fibre type proportions *(C–E)* in older adults. Alpha level at $P < 0.05$. Data are presented as means with 95% confidence intervals.

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associated with improved performance in all performance tests (5–71%; for details, see Supporting Information, *Figure* S1). These improvements occurred without any apparent changes in muscle cell characteristics in thigh muscle, including muscle fibre CSA (type I, 4%, $P = 0.573$; type II, 9%, $P = 0.312$), muscle fibre type proportions ($P = 0.127$–0.901), and total RNA/rRNA expression ($P = 0.604$–1.000) (Supporting Information, *Figure* S1). They were hence likely caused by technical, psychological and neural learning effects,[94] effectuated by repeated exposure to testing prior to and during the supplementation period (Supporting Information, *Figure* S1), as is typically seen in older subjects.[95] Indeed, dynamic exercises like knee extension and chest press are associated with lower intra-rater reliability than the grip strength test,[94] which remains unaffected by test–retest,[94] as was likely the case in the present study.

Overall, the 12-weeks supplementation-only period did not lead to marked changes in mRNA transcriptome profiles in the two supplementation arms combined (vitamin D_3, $n = 11$; placebo, $n = 13$). Vitamin D_3 supplementation was, however, associated with differential changes in the expression of a selected genes compared with placebo; 27 genes ↑ and 27 genes ↓ (*Figure* 5A and Supporting Information, *Table* S7). This included increased expression of B-cell lymphoma 6 and prolyl 4-hydroxylase subunit alpha-1 (*BCL6* and *P4HA1*; *Figure* 5A), both of which are known to oppose accumulation of reactive oxygen species (ROS),[96–98] and decreased expression of angiopoietin-like protein 4 (*ANGPTL4*; *Figure* 5A), which is closely correlated with levels of mitochondrial respiration.[99] These findings were reaffirmed by gene enrichment analyses, which showed a general reduction in the expression of gene sets relating to both oxidative and glycolytic metabolism in the vitamin D_3 arm (*Figure* 5B and Supporting Information, *Tables* S5–S6). This is in line with previous observations whereby vitamin D has been shown to counteract ROS and mitochondrial oxidative stress.[100] The seemingly negative effect of vitamin D_3 supplementation for expression of mitochondrial genes may thus be due to reduced mitochondrial turnover. Of note, expression of the vitamin D receptor (VDR) was observed in the data set, but was not affected by supplementation.

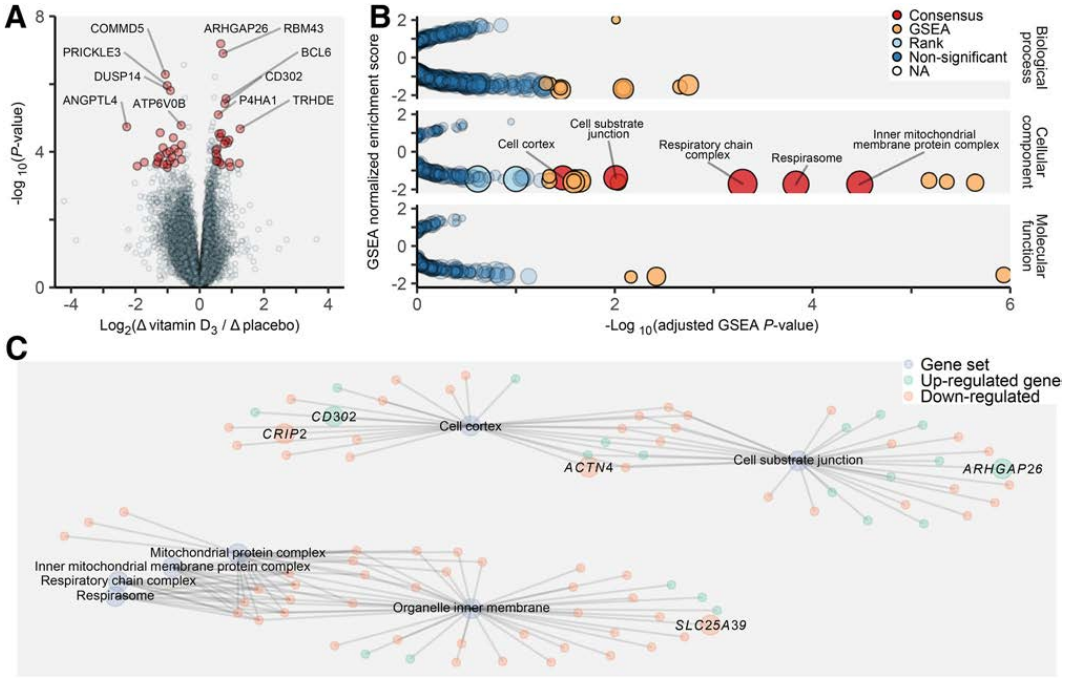

Figure 5 Effects of 12 weeks of vitamin D_3 supplementation-only on whole-genome transcriptome profiles in *m. vastus lateralis* of older adults. After 12 weeks of supplementation-only, numerous genes were differentially expressed between the vitamin D_3 and the placebo arm *(A)*; Δ, pre-introduction to resistance training/pre-RCT). Gene ontology (GO) enrichment analyses showed that these genes were primarily related to mitochondrial function and cell cortex/cell-substrate junction *(B)*; positive/negative GSEA-normalized enrichment scores indicates higher/lower expression of gene sets in the vitamin D_3 arm compared with the placebo arm). The seven differentially expressed gene sets were clustered into two distinct groups of genes *(C)*.

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Introductory observations on the quality and general efficacy of the resistance training protocol (weeks 13–28)

Before assessing the effects of combined vitamin D_3 supplementation and resistance training, it is vital to reaffirm that the protocols and methods held sufficient validity and reliability, including a general assessment of the efficacy of the resistance training intervention. All training sessions were supervised by qualified personnel, as suggested by others,[46] which likely contributed to the very low drop-out rate (n = 4 during the training period, ~5%, *Table* 1), and ensured high adherence to the protocol (98%, range 81–100%, *Table* 1) and appropriate training progression throughout the intervention (*Figure* 2). Training volume (repetitions x kg) increased by 20% (knee extension) and 30% (leg press) from Week 14 (the first week of training) to Week 18 (the 4th week of training), by 48% and 54% to Week 22 (the 8th week of training) and by 65% and 68% to Week 27 (the last week of training) (*Figure* 2). This resembles or exceeds training progression seen in similar studies on previously untrained participants[101,102] and was accompanied by progressive increases in perceived exercise intensities (using the Borg RPE-scale[103]) (*Figure* 2). For these training characteristics, no differences were observed between supplementation arms (P = 0.897–0.980). The arguably successful completion of the resistance training intervention was accompanied by marked functional and biological adaptations in the participants, including increased muscle strength and performance (e.g. 22% and 72% increases in 1RM and muscular performance in knee extension, respectively, $P < 0.05$, Supporting Information, *Figure* S1), increased muscle mass (e.g. 16–24% increases in muscle fibre CSA for *m. vastus lateralis*, $P < 0.05$, Supporting Information, *Figure* S1), increases in myonuclei number per fibre (30–37%, $P < 0.05$, Supporting Information, *Figure* S1), alterations in muscle fibre proportions (e.g. type IIX fibre proportions changed from 10% to 7%, $P < 0.05$, Supporting Information, *Figure* S1), and robust alterations in muscle transcriptome profiles (499 and 312 differentially expressed genes at post-introduction resistance training and post-RCT, compared with pre-introduction to resistance training, *Figure* 11A,B). Importantly, neither of these muscle fibre characteristics changed from pre-RCT to before onset of resistance training (Week 13), suggesting that muscle biopsies sampled before and after the supplement-only period could be regarded as a sampling-resampling event (Supporting Information, *Figure* S1). For muscle strength, the intervention had relative efficiencies of 0.86% (knee extension) and 1.43% (leg press) increase per session, which resemble or exceeds expectations based on previous studies of untrained older adults (0.5–1.0% per session).[104–106]

Analytical measures to increase the validity of vitamin D_3-based analyses

To ensure valid analyses of the effects of vitamin D_3 supplementation on muscle-related features, two precautionary measures were deemed to be necessary. First, for muscle strength and muscle performance (apparatus exercises), we defined baseline levels to be equivalent to values collected after 3.5 weeks of introduction to resistance training (main analyses, *Figure* 2), rather than values collected before its onset, as noted in the preregistration of the study (NCT02598830). At this time point, initial adaptations to training were likely to have occurred, preferably non-hypertrophic effects relating to technical, psychological and neural learning effects,[94] phenomena that are particularly prominent in older subjects.[95] Using this time point as baseline arguably strengthens the association between changes in muscle strength and muscle mass, which was the main perspective of our vitamin D_3-based analyses. For other outcome measures, baseline levels were either defined as values obtained at the onset of introduction to resistance training (*Figure* 2, Week 13; muscle biological data, muscle thickness, body composition, endurance-related outcome measures) or as values obtained pre-RCT (Week −1, *Figure* 2; self-reported health, blood variables, lung function).

To further minimize the confounding effects of non-hypertrophic increases in strength and performance, all participants conducted a series of repeated tests prior to baseline tests, including five repeated 1RM and muscular performance tests in knee extension and chest press (Supporting Information, *Figure* S1a,b,e,f), respectively, four of which was conducted prior to onset of introduction to training. As expected, this led to marked and progressive increases in strength/performance levels for all test procedures compared with pre-RCT values (e.g. 4–8 – 14% for 1RM knee extension, 3–5 – 13% for 1RM bench press; the first test was conducted at ~95% of maximal effort and was thus removed from analyses) (Supporting Information, *Figure* S1). For leg press, three tests were performed prior to the defined baseline test at post-introduction to resistance training, resulting in similarly scaled improvements as observed for knee extension and chest press (Supporting Information, *Figure* S1, 14%; the first test was conducted at ~95% of maximal effort and was thus removed from analyses). These improvements occurred without any apparent hypertrophy in *m. vastus lateralis* of the dominant leg, measured as muscle fibre CSA (pre-RCT vs. pre-introduction to resistance training; type I, P = 0.573; type II, P = 0.312), as previously presented (Supporting Information, *Figure* S1g), strengthening the notion that the improvements were due to other factors. After adopting the post-introduction-to-training time point as baseline for the strength outcome measures, the efficiency of the intervention

on muscle strength was still somewhat higher than expected based on previous observations[104–106] (1RM knee extension, 0.8% per session; 1RM leg press, 1.3% per session). Notably, while these former studies contained less extensive measures to ensure reproducibility, they reported low test–retest variability, which does not concur with our results.[104–106]

Second, for analyses of the effects of vitamin D_3 supplementation on changes in muscle mass, we found it necessary to reconsider our choice of using changes in muscle fibre CSA and fibre type proportions in *m. vastus lateralis* as the primary objective of the study. These data were associated with large degrees of sampling-to-resampling variation, as evaluated using repeated muscle biopsies from the dominant leg, sampled at weeks −1 and 13, i.e. prior to introduction to resistance training (Supporting Information, *Figure* S2). Similar issues have been previously reported for such analyses,[107] although not in all studies[108,109] and are likely exacerbated in older adults, for whom larger spatial heterogeneity are present in muscle fibre characteristics compared with young adults,[110] possibly relating to the age-related remodeling of motor units.[111] Despite these issues, the data provided sufficient resolution to disclose marked increases in muscle fibre CSA and changes in muscle fibre proportions over the entirety of the training intervention, as previously presented (*Figure* 4 and Supporting Information, *Figure* S1).

In order to achieve reliable assessment of changes in muscle mass, we thus had to take on a different approach. Instead of relying on muscle fibre CSA data alone, we developed a combined muscle mass factor, in which change scores from a collection of muscle mass-related outcome measures were combined in a weighted manner (Supporting Information, *Table* S4). This factor included data on muscle fibre CSA, leg lean mass (DXA) and muscle thickness (*m. rectus* femoris, *m. vastus lateralis*; ultrasound), all of which are known to correlate.[112–114] Careful investigation of the computed muscle mass factor suggested that it increased the biological value of muscle mass-related analyses (for more information, see Supporting Information, *Table* S4). As such, it changed markedly from baseline to post-RCT (9%, $P < 0.001$, Supporting Information, *Table* S4). Following this logic, combined factors were also computed for other core outcome domains, including maximal muscle strength and one-legged and whole-body endurance performance (Supporting Information, *Table* S4).

Effects of vitamin D_3 supplementation on training-associated changes in maximal muscle strength and lower-limb muscle mass

Participants in both vitamin D_3 and placebo arms showed increases for every measure of muscle strength and mass, assessed from baseline to after finalization of the resistance training intervention: 12–25% for upper- and lower body 1RM muscle strength, 6–11% for leg muscle torque, 7–26% for muscle fibre CSA and muscle thickness and 1–3% for leg lean mass (*Figures* 6 and 7). Unsurprisingly, after combining these measures into weighted muscle strength and muscle mass factors, similarly scaled increases were observed (13% ± 8% and 9% ± 8%, respectively; *Figures* 6 and 7), which was also the case for a calculated score of relative muscle quality (Δmuscle strength factor/Δmuscle mass factor; 4% ± 10%, *Figure* 7).

Overall, vitamin D_3 supplementation did not affect these outcome measures compared with placebo in the participants, primarily evaluated as changes in muscle strength and muscle mass factors (strength, Δ2.5% (95% CI, −1.0, 6.0), $P = 0.194$; mass, Δ0.4% (95% CI, −3.5, 4.3), $P = 0.940$, *Figures* 6 and 7), and secondarily as changes in each of the underlying outcome measures (i.e. seven measures of muscle strength and three measures of muscle mass; *Figures* 6 and 7). This lack of a beneficial effect was also evident for changes in relative muscle quality (Δ1.9% (95% CI, −3.0, 6.8), $P = 0.415$; *Figure* 7). Vitamin D_3 supplementation thus had no main effect on training-associated changes in muscle functionality or gross muscle biology. While this conclusion coheres with the few comparable studies assessing the effect of combined vitamin D_3 intake and resistance training,[40,42–44] it contrasts the conclusion drawn in the only available meta-analysis on this subject, wherein vitamin D_3 supplementation was associated with augmented increases in muscle strength in older adults.[41] Notably, among the selection of ten specific outcome measures, two did not conform with the main finding. Vitamin D_3 was associated with beneficial effects for changes in 1RM knee extension (Δ6.8% (95% CI, 1.3, 12.3), $P = 0.016$; *Figure* 6) and muscle thickness of *m. rectus femoris* (Δ7.5% (95% CI, 1.8, 13.2), $P = 0.011$; *Figure* 7). For 1RM knee extension, the effect was interrelated with the negative development seen from pre-RCT to pre-introduction to training in the vitamin D_3 arm (Supporting Information, *Figure* S1). Indeed, when assessing the effect of vitamin D_3 on 1RM knee extension from pre- to post-RCT (rather than from baseline at post-introduction to training), no beneficial effect was observed compared with placebo (Δ−2% (95% CI, −12, 7), $P = 0.628$; Supporting Information, *Table* S2). As for muscle thickness in *m. rectus femoris*, we did not collect data pre-RCT and can thus not deduce if this variable followed the same pattern as 1RM knee extension. The observed benefits of vitamin D_3 supplementation for changes in *m. rectus femoris* thickness contrasts observations made for *m. vastus lateralis* thickness (Δ−0.3%, $P = 0.838$), and even oppose those made for lean mass of the legs, which tended to increase less in the vitamin D_3 arm compared with the placebo arm (Δ−1.8%, $P = 0.090$).

So far, analyses have focused on the main effect of vitamin D_3 supplementation for training-induced development of muscle strength and mass, and have thus neglected potential interactions with other independent variables such

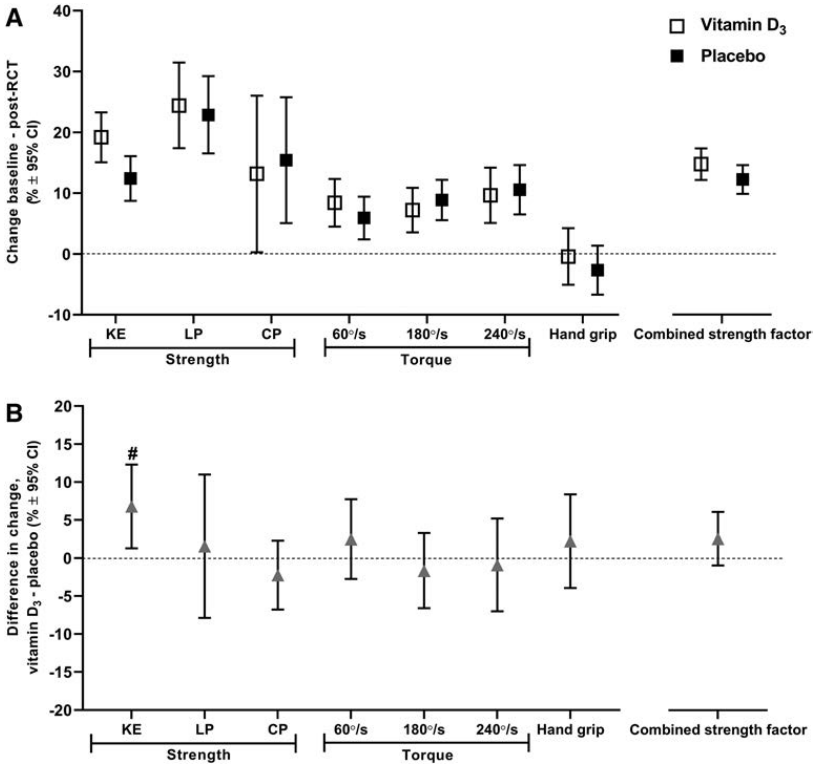

Figure 6 Effects of combined vitamin D_3 supplementation and resistance training on maximal muscle strength in older adults. Changes in muscle strength from baseline (after three weeks of introduction to resistance training) to post-RCT *(A)*, and differences in changes between vitamin D_3 and placebo arms *(B)*. KE, one-legged knee extension; LP, one-legged leg press; CP, chest press; maximal torque measured using one-legged knee extension at three velocities; 60, 180, and 240° per second; #, significant difference between vitamin D_3 and placebo arms; combined strength factor, weighted combined strength factor of unilateral strength measures (one-repetition maximum in KE and LP, and KE torque at 60, 180, and 240° per second). Alpha level at $P < 0.05$. Data are presented as means with 95% confidence intervals.

as pre-RCT levels of 25(OH)D, health status (COPD vs. non-COPD) or training modality (high-load, 10RM, vs. low-load, 30RM). The benefits of vitamin D_3 supplementation were expected to be more pronounced in participants with low baseline levels of 25(OH)D (ClinicalTrials.gov Identifier: NCT02598830). This hypothesis was based on observations made in cohort studies, wherein subjects with levels <30–50 nmol/L are more likely to show adverse muscle phenotypes.[21–23] To investigate this perspective, participants in each supplementation arm were divided into quartiles based on pre-RCT 25(OH)D levels in blood (Supporting Information, *Figure* S3). This resulted in two lower quartiles, one for the vitamin D_3 arm (vitamin $D3_{low}$, $[25(OH)D]_{mean}$ = 49.5 nmol/L, n = 8), and one for the placebo arm ($placebo_{low}$, $[25(OH)D]_{mean}$ = 47.4 nmol/L, n = 12) (Supporting Information, *Figure* S3). At the onset of introduction to resistance training, 25(OH)D levels in vitamin $D3_{low}$ had increased to 103.3 nmol/L (range 76–138), with all participants being classified as sufficient (>50 nmol/L),[17] whereas 25(OH)D levels in $placebo_{low}$ remained unchanged (45.5 nmol/L, range 22–71), with 9 out of 12 participants being classified as insufficient (<50 nmol/L). Within each of the pre-RCT 25(OH)D quartiles, the effect of vitamin D_3 and placebo supplementation on training-induced changes in muscle strength and mass (using the combined factors) were assessed. With exception of one quartile (muscle strength factor, quartile 3, P = 0.048; Supporting Information, *Figure* S3), no beneficial effects of vitamin D_3 supplementation were observed in any quartile (e.g. vitamin $D3_{low}$ vs. $placebo_{low}$, muscle strength, Δ−2.0% (95% CI, −8.0, 3.9, P = 0.496) (Supporting Information, *Figure* S3). Instead, in vitamin $D3_{low}$, training-associated changes in muscle mass were reduced compared with $placebo_{low}$ (Δ−6.5% (95% CI, −12.7, −0.27), P = 0.041; Supporting Information, *Figure* S3), suggesting that vitamin

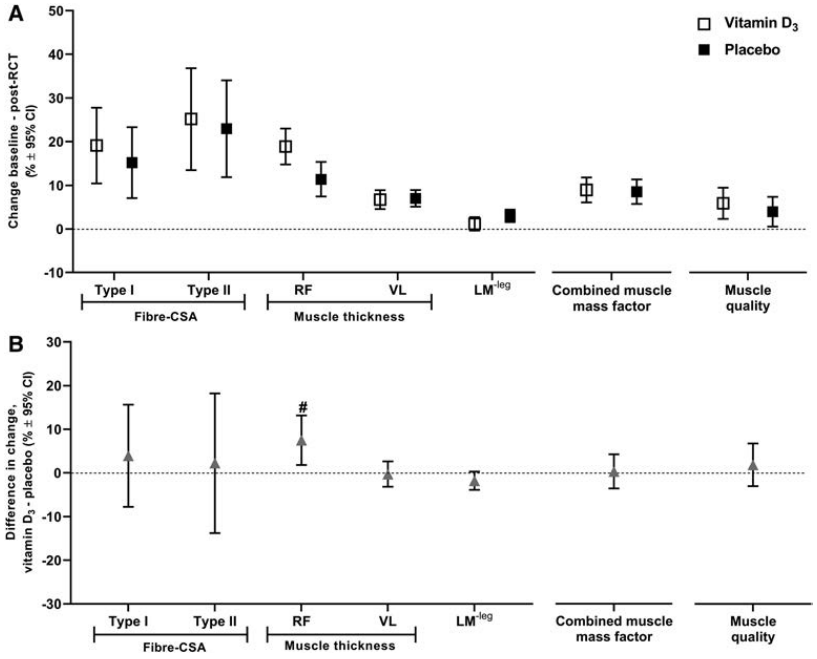

Figure 7 Effects of combined vitamin D_3 supplementation and resistance training on lower-limb muscle mass in older adults. Changes in lower-limb muscle mass from baseline (before introduction to resistance training) to post-RCT *(A)*, and differences in changes between vitamin D_3 and placebo arms *(B)*. CSA, cross-sectional area (also presented in Figure 4); RF, *m. rectusfemoris*; VL, *m. vastus lateralis*; LM per leg, leg lean mass per leg; #, significant difference between vitamin D_3 and placebo arms; combined muscle mass factor, weighted combined muscle mass factor including fibre cross-sectional area (type I and type II), muscle thickness (RF and VL) and LM per leg; muscle quality, muscle strength factor/muscle mass factor. Alpha level at $P < 0.05$. Data are presented as means with 95% confidence intervals.

D_3 supplementation may even have compromised training adaptations in subjects with low pre-RCT 25(OH)D levels. Adding to this, participants in the entire spectre of quartiles responded quite similarly to resistance training, irrespective of supplementation arms, evident as no interaction between 25(OH)D quartiles/supplementation arm and changes in muscle strength ($P = 0.237$) or muscle mass ($P = 0.159$). Arguably, the statistical power of these analyses were not sufficiently high to conclude on this perspective.

The impact of vitamin D_3 supplementation for training-associated changes in muscle strength and muscle mass factors did not interact with health status (COPD vs. non-COPD) or training modality (10RM vs. 30RM) (Supporting Information, *Table* S2). However, it should be noted that for selected specific outcome measures, interactions were found with both of these independent variables (summarized in Supporting Information, *Table* S2), including an interaction between changes in type II-fibre CSA and COPD/non-COPD, and between changes in 1RM knee extension/vastus lateralis thickness and 10RM/30RM. In addition to these interaction analyses, we also investigated the potential relation between the effects of vitamin D_3 supplementation and baseline body fat proportions, as overweight and obese have been shown to have decreased bioavailability of vitamin D due to deposition of 25(OH)D in body fat compartments (while concomitantly showing attenuated anabolic response to resistance exercise[115]).[116] To this end, we performed quartile-based analyses, as previously described. These analyses did not reveal an effect of baseline body fat proportions for changes in [25(OH)D] (fat percentage, $P = 0.432$; BMI, $P = 0.369$) or muscle mass factor (fat percentage, $P = 0.355$; BMI, $P = 0.293$) (Supporting Information, *Figure* S4). However, it did have an effect on changes in the muscle strength factor (fat percentage, $P = 0.016$; BMI, $P = 0.706$), that is, in $\text{quartile}_{\text{high fat percentage}}$, vitamin D_3 supplementation was associated with larger increases in muscle strength compared with placebo (fat percentage, Δ 5.8% (95% CI, 0.5, 11.0), $P = 0.032$; BMI, Δ7.8% (95% CI, 2.5, 13.1), $P = 0.005$; Supporting Information, *Figure* S4 and *Table* S2), suggesting beneficial effects of vitamin D_3 supplementations in subjects with high proportions of body fat, opposing our initial expectations.

Effects of vitamin D_3 supplementation on training-associated changes in one-legged and whole-body endurance performance

Participants in both vitamin D_3 and placebo arms showed improvements in one-legged and whole-body endurance performance over the course of the resistance training intervention: 42–74% increases in one-legged muscular performance (*Figure* 8), 7–9% increases in peak power output (W_{max}) in one- and two-legged cycling (*Figure* 8), 3–5% reductions in O_2 costs of submaximal one-legged cycling (Supporting Information, *Table* S2), and 6–10% increases in functional performance (1-min sit-to-stand test and 6-min step test, *Figure* 8). In accordance with this, marked increases were observed in weighted one-legged and whole-body endurance performance factors (one-legged, vitamin D_3 25% ± 19%, placebo 22% ± 11%; whole-body, vitamin D_3 9% ± 8%, placebo 7% ± 6%; *Figure* 8). These effects cohere well with previously observed benefits of resistance training for endurance variables in older adults.[117–119]

Vitamin D_3 supplementation had no effect for any of these outcome measures compared with placebo, neither for weighted endurance performance factors (one-legged, Δ2% (95% CI, −5, 10), P = 0.773; two-legged, Δ2% (95% CI, −2, 6), P = 0.636; *Figure* 8), nor for any of the specific outcome measures (*Figure* 8). For combined endurance factors, there was no interaction between baseline 25(OH)D quartiles and effects of vitamin D_3 supplementation (one-legged, P = 0.950; whole-body, P = 0.266; Supporting Information, *Figure* S3 and *Table* S2), nor was there any interactions with health status (one-legged, P = 0.747, whole-body, P = 0.129, Supporting Information, *Table* S2) or training modality (one-legged, P = 0.719, Supporting Information, *Table* S2).

Effects of vitamin D_3 supplementation on training-associated changes in muscle fibre characteristics and transcriptomics

Participants in both vitamin D_3 and placebo arms showed marked changes in muscle fibre characteristics over the course of the training intervention. These included decreased type IIX muscle fibre proportions from 10% to 7% (*Figure* 9), increased type IIA proportions from 26% to 29% (*Figure* 9),

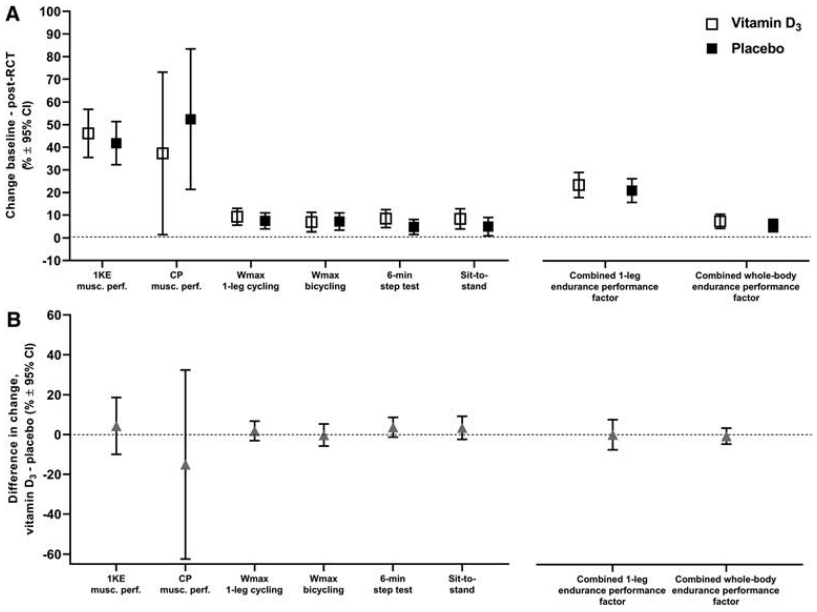

Figure 8 Effects of combined vitamin D_3 supplementation and resistance training on one-legged and whole-body endurance performance in older adults. Changes in endurance performance from baseline (before introduction to resistance training) to post-RCT *(A)*, and differences in changes between vitamin D_3 and placebo arms *(B)*. 1KE, repetitions to failure in one-legged knee extension (50% of pre-intervention 1RM); CP, repetitions to failure in chest press (50% of pre-intervention 1RM); W_{max}, maximal power output; 6-min step test, maximal number of steps achieved during 6 min; Sit-to-stand, maximal number of sit-to-stands achieved during 1 min; combined 1-leg endurance performance factor, weighted combined one-legged endurance factor including 1KE muscular performance and one-legged cycling W_{max}; weighted combined whole-body endurance factor including W_{max} bicycling, 6-min step test and sit-to-stand test. Alpha level at $P < 0.05$. Data are presented as means with 95% confidence intervals.

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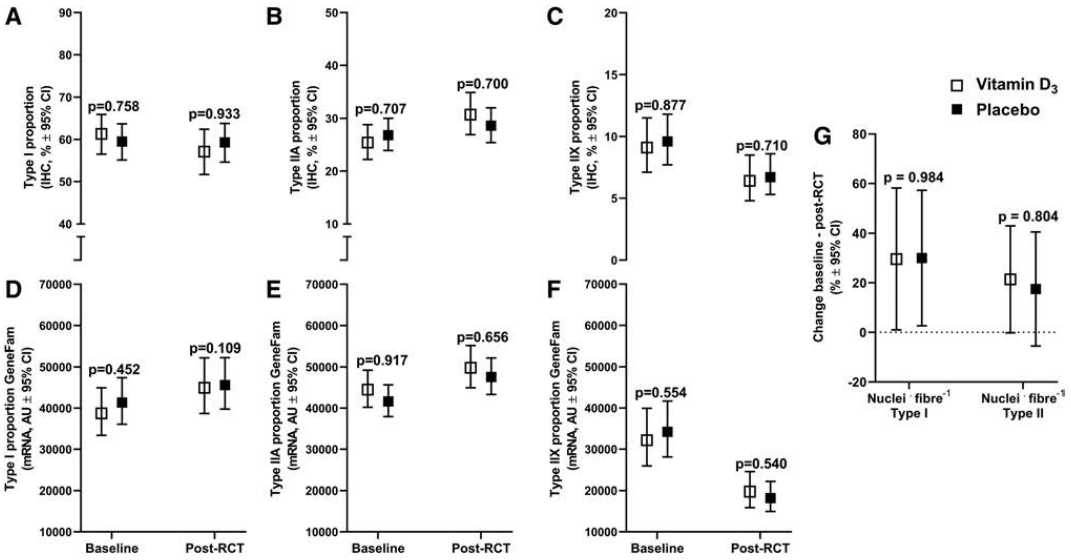

Figure 9 Effects of combined vitamin D_3 supplementation and resistance training on muscle fibre type proportions and myonuclei per fibre in *m. vastus lateralis* of older adults. Muscle fibre type proportions *(A–F)* at baseline (before introduction to resistance training) and post-RCT measured using immunohistochemistry *(A–C)* and qPCR (gene family profiling (GeneFam)-normalized myosin heavy chain mRNA expression, *(D–F)*, and changes in myonuclei count per type I and type II fibre from baseline to post-RCT *(G)*. Significant changes were observed for fibre type IIA and IIX using both methods (significant increase and decrease, respectively; $P < 0.05$). For fibre type I, an increased expression was present using qPCR ($P < 0.05$), but no change was observed for immunohistochemistry ($P = 0.322$). *P*-values denotes the statistical difference between the supplementation arms. RT, resistance training. Data are presented as means with 95% confidence intervals.

increased type IIA/IIX hybrid fibres abundances from 2.6% to 3.2% (Supporting Information, *Table* S2), and 25–48% increases in myonuclei number per muscle fibre (*Figure* 9). Changes in IIX and IIA proportions were verified using qPCR, showing decreased levels of type IIX mRNA abundance and increased levels of type IIA (*Figure* 9), calculated using the gene family-profiling approach.[76] These analyses also revealed increased proportions of type I mRNA after the training intervention (*Figure* 9), potentially caused by increased type I protein turnover. The observed changes in muscle fibre-type characteristics corroborate well with previous studies in older adults,[120–122] although increased numbers of myonuclei per muscle fibre are not consistently reported.[123] Vitamin D_3 supplementation had no effect on training-associated changes in muscle fibre proportions or myonuclei content compared with placebo (*Figure* 9).

The training intervention resulted in 1.14- to 1.16-fold increases in total RNA per unit muscle tissue weight (*Figure* 10), a proxy marker for ribosomal RNA content that has previously been associated with training-induced changes in muscle growth and strength.[67,124] Similar increases were found for the mature ribosomal species 18 s (1.18-fold) and 28 s (1.16-fold), in addition to the 45 s pre-ribosomal rRNA (1.19-fold) using qPCR (*Figure* 10). No changes were observed for 5.8 s (1.07-fold, $P = 0.722$) or 5 s (1.06, $P = 0.940$) following the entire training intervention. Notably, for analyses of total RNA and ribosomal RNA, an additional time point were included in main analyses, i.e. in muscle biopsies sampled after introduction to training (3.5 weeks, 7 sessions), as early increases in total RNA seem to associate with long-term chronic responses to training, making it a potential hallmark of muscle plasticity.[67] As expected, 3.5 weeks of training led to marked increases in total RNA (1.10- to 1.21-fold) and expression of all ribosomal RNA species (1.13- to 1.27-fold) (*Figure* 10). Whereas these changes corroborates quite well with changes observed in healthy, young subjects,[67] although with a notable reduction in the relative increase, they contradict previous observations of no resistance training-associated increases in total RNA per unit muscle tissue weight in older subjects.[125] Vitamin D_3 supplementation had no effect on training-associated changes in total RNA or rRNA expression compared with placebo.

The training intervention led to marked changes in muscle mRNA transcriptome profiles in the two supplementation arms combined, with 499 genes being differentially expressed (DE) after 3.5 weeks of resistance training

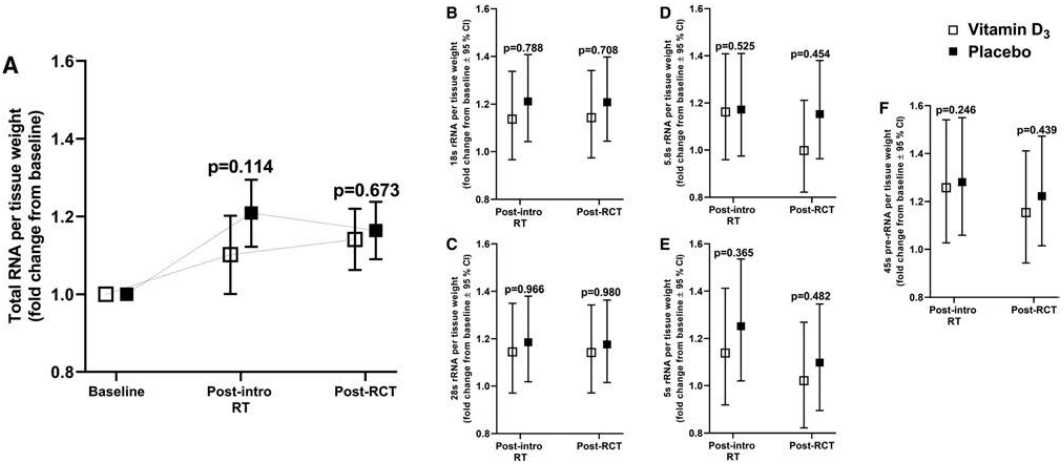

Figure 10 Effects of combined vitamin D_3 supplementation and resistance training on total RNA abundances and rRNA expression in *m. vastus lateralis* of older adults. Total RNA *(A)*, 18 s rRNA *(B)*, 28 s rRNA *(C)*, 5.8 s rRNA *(D)*, 5 s rRNA *(E)*, and 45 s pre-rRNA *(F)* abundances at baseline (before introduction to resistance training) and post-RCT. Significant increases from baseline–post-introduction to resistance training were present for all variables ($P < 0.05$). From baseline–post-RCT significant increases were present for all variables ($P < 0.05$), with the exception of 5.8 s rRNA ($P = 0.722$) and 5 s rRNA ($P = 0.940$). RT, resistance training. *P*-values denotes the statistical difference between the supplementation arms. Alpha level at $P < 0.05$. Data are presented relative to amounts of tissue weight. Data are presented as means with 95% confidence intervals.

(post-intro RT; 436 genes ↑, 63 genes ↓, *Figure* 11A) and 312 genes being DE after 13 weeks of resistance training (post-RCT; 255 genes ↑, 57 genes ↓) (*Figure* 11A,B). VDR was expressed, but unaffected by combined vitamin D_3 supplementation and resistance training, contradicting previous observations of a positive association between supplementation-induced improvements in 25(OH)D status and leukocyte,[126] myoblast/myotube[127] and skeletal muscle[128] VDR expression. GO enrichment analyses revealed increased expression of gene sets associated with extracellular matrix, blood vessel morphogenesis and leukocyte migration at both 3.5 and 13 weeks (*Figure* 11C, Supporting Information, *Table* S8), as well as increased expression of the inflammatory response gene set at 3.5 weeks (Supporting Information, *Table* S8). Conversely, decreased expression was observed for gene sets involved in ribosomal functions at both 3.5 and 13 weeks (*Figure* 11C). This could be interpreted as contradicting the likely important role of *de novo* ribosomal biogenesis for training-associated muscular adaptations.[67,124] Notably, as these analyses were performed using traditional library size-based normalization, which basically provided target gene expression relative to the expression of all other genes.[129] In an alternative set of transcriptome analyses, which rather included normalization that corrected for muscle sample weight and thus provided gene expression analyses per sample size (tissue-offset normalization),[129] the negative effects of resistance training on ribosomal gene expression was not evident (data not shown). This was the only major difference between library size and tissue-offset normalization in the present study setting.

Vitamin D_3 supplementation had no effect on training-associated changes in gene expression, neither at 3.5 weeks (*Figure* 11D) nor at 13 weeks (*Figure* 11E), suggesting that no single gene was differentially affected by combined vitamin D_3 supplementation and resistance training and resistance training-only. In contrast to this, enrichment analyses showed traces of vitamin D_3-sensitive changes in expression at both 3.5 and 13 weeks of resistance training (*Figure* 11F and Supporting Information, *Tables* S9–S10). After 3.5 weeks of training, there was differential expression of gene sets involved in cell junctions, blood vessel morphogenesis and muscle cell differentiation. These initial responses to resistance training should be interpreted with caution, as they were only evident in one of the two analyses (GSEA or rank-based analyses; *Figure* 11F and Supporting Information, *Tables* S9–S10). After 13 weeks of resistance training, the vitamin D_3 arm showed increased expression of gene sets involved in endothelial proliferation and blood vessel morphogenesis compared with placebo (*Figure* 11F). This agrees with the previously observed positive relationship between 25(OH)D-status and endothelial function, potentially interacting through the endothelium-derived vasodilator, nitric oxide.[100] Indeed, this coheres well with a recent study, which showed favorable effects of combined vitamin D_3 supplementation and resistance

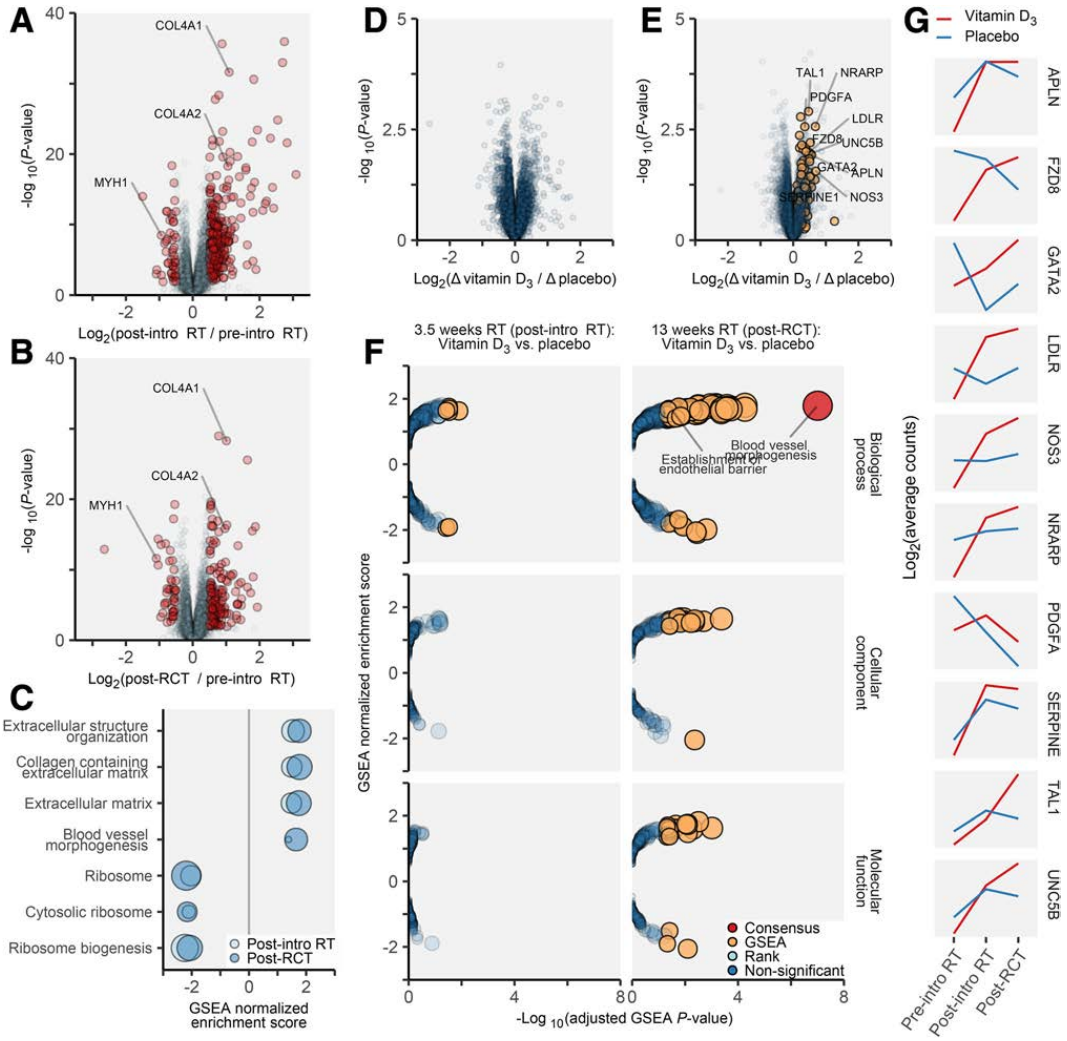

Figure 11 Effects of 3.5/13 weeks of resistance training-only *(A–C)* and 3.5/13 weeks of combined vitamin D_3 supplementation and resistance training *(D–G)* on mRNA transcriptome profiles in *m. vastus lateralis* of older adults. Resistance training-only led to robust changes in gene expression at both 3.5 weeks (*A*; post-intro resistance training – pre-intro resistance training) and 13 weeks (*B*; post-RCT – pre-intro resistance training), including increased expression of collagen type IV α1 and α2 genes (*COL4A1* and *COL4A2*, respectively) and decreased expression of the myosin heavy chain IIX gene (*MYH1*). The three most enriched gene sets with increased and decreased expression, in addition to the 'blood vessel morphogenesis' gene set are shown in *C* (light blue, 3.5 weeks; dark blue, 13 weeks; according to the GSEA enrichment score). Combined vitamin D_3 supplementation and resistance training did not lead to differential changes in expression for a singular gene compared with placebo at neither 3.5 weeks (*D*; Δ, post-introduction to resistance training - pre-introduction to resistance training) nor 13 weeks of resistance training (*E*; Δ, post-RCT - pre-introduction to resistance training; orange dots/genes denotes leading edge genes from the 'blood vessel morphogenesis' GO gene set, that is, the most highly enriched gene set between supplementation arms after 13 weeks of resistance training). GO enrichment analyses of differentially regulated gene sets between the vitamin D_3 and the placebo arms following 3.5 weeks (left panel, *F*) and 13 weeks of resistance training (right panel, *F*; positive/negative GSEA-normalized enrichment scores indicates higher/lower expression of gene sets in the vitamin D_3 arm compared with the placebo arm). (*G*) Timeline for the 10 most affected genes between vitamin D_3 and placebo arms belonging to the 'blood vessel morphogenesis' GO gene set. RT, resistance training; Consensus, when both the non-directional rank-based enrichment test and the directional gene-set enrichment analysis (GSEA) turned out significant. In *Figure* 11*C,F*, circle sizes of gene sets are relative to *P*-values, i.e. larger circles indicate lower *P*-values (see Supporting Information, *Tables S5–S10* for exact *P*-values).

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training on flow-mediated dilation of blood vessels and blood pressure in postmenopausal women.[130] Unfortunately, endothelial function was not assessed in the current study.

Effects of vitamin D_3 on hormones in blood and health-related outcome measures

In general, the intervention was associated with beneficial changes for several health-related variables, including reduced levels of lipids (triglycerides and low-density lipoprotein/LDL), reduced levels of fat mass (total and visceral fat) and improved self-reported health (Supporting Information, *Table* S11). Conversely, a small but undesirable decrease was observed in lung capacity, measured as forced ventilatory capacity (FVC) (Supporting Information, *Table* S2). The intervention was not associated with changes in whole-body bone mineral density or changes in serum levels of hormones, except for decreased levels of parathyroid hormone (Supporting Information, *Table* S11), as previously presented. For most of the health variables, there was no effect of vitamin D_3 supplementation (Supporting Information, *Tables* S2 and S11), with exception of cortisol levels in blood, which increased more in the vitamin D_3 arm (*Table* S11), and lung function measured as FEV_1/FVC-ratios, which declined in subjects with COPD in the vitamin D_3 arm (Supporting Information, *Table* S2).

Sarcopenia

The intervention proved effective for treating age-related loss in muscle mass, leading to 1.4% increases in total lean body mass ($P < 0.001$) (Supporting Information, *Table* S11). This reduced the number of participants that could be defined as sarcopenic from 16% (11 subjects) to 12% (8 subjects), with sarcopenia being defined as appendicular lean mass $(kg)/m^2$ greater than two standard deviations below the sex-specific means of young adults.[61] Speculatively, the increase in total lean mass was supported by increased levels of serum creatinine in both supplementation arms (+6%; Supporting Information, *Table* S11). Although serum creatinine is generally used for evaluation of renal function,[131] creatinine production and levels also increases with increases in total muscle mass.[131,132]

Steroid hormones

Vitamin D_3 supplementation did not affect levels of anabolic steroid hormones such as testosterone. This was in discordance with our initial hypothesis, as we presumed a positive association between vitamin D levels (measured as 25(OH)D) and testosterone levels, based on previous observations from vitamin D_3 supplementation studies[52] and cohort studies.[133] Despite this, our finding is in line with several other vitamin D supplementation studies, which has reported no effect on testosterone in blood.[134,135] Conversely, vitamin D_3 supplementation seemed to affect serum cortisol levels compared with placebo (Δ48 nmol/L, $P = 0.038$; Supporting Information, *Table* S11), although no main effect of time was observed (i.e. the observed increase in the vitamin D_3 arm was not statistically significant, $P = 0.374$) and there was no statistical difference between supplementation arms at the end of the intervention ($P = 0.053$).

Lung function

The small -1.95% reduction in FVC seen after the 28 week long RCT ($P = 0.006$; Supporting Information, *Table* S2) was surprising, as exercise is generally accepted to be beneficial for lung functionality, including resistance training.[136,137] Notably, other measures of lung function, such as forced ventilatory volume in one second (FEV_1 and predicted FEV_1) and FEV_1/FVC, were not affected by the intervention (Supporting Information, *Table* S2).

The negative effects of vitamin D_3 on lung function, measured as FEV_1/FVC ($\Delta -2.9\%$ points, $P = 0.012$; Supporting Information, *Table* S2), were also surprising. This effect showed a clear interaction with health status, and as such was only evident in COPD patients in the vitamin D_3 arm, which showed $\Delta -8.4\%$ reductions compared with placebo (Supporting Information, *Table* S2). This subgroup analysis was however clearly weakened by the small sample size (COPD, $n = 9$ vs. $n = 11$, vitamin D_3 vs. placebo). The negative effect of vitamin D_3 on FEV_1/FVC did not interact with pre-RCT levels of FEV_1/FVC, but surprisingly, in another subgroup-analysis, the pre-RCT 25(OH)D vitamin $D3_{low}$ quartile was associated with larger decrement in FEV_1/FVC than $placebo_{low}$ ($\Delta -5.4\%$ points, $P = 0.009$; data not shown). This observation is difficult to explain, as it indirectly opposes the notion that vitamin D deficiency leads to impaired lung functions.[138] More research is clearly needed to elucidate on the consequences of resistance training and vitamin D_3 supplementation for lung functionality.

Adverse effects of the intervention

Overall, neither vitamin D_3 supplementation nor resistance training was associated with adverse effects or events during the intervention, with potential exception of certain aspects of lung function, as previously discussed, and iron biology (see Supporting Information, *Table* S11).

Primarily, a health survey was administered to the participants on a weekly basis. This included rating of 11 potential discomforts relating to digestion problems, sleep problems, issues with the urinary system, issues with the vestibular system and dermal irritations (Supporting Information, *Table* S2). No effect of vitamin D_3 supplementation was found for any of these variables. In the health survey, participants were also asked to rate their experienced health on a point-scale from 0–10. This self-reported conception of health improved from 6.3 ± 1.6 to 7.1 ± 1.6 ($P < 0.001$, Supporting Information, *Table* S2), with no difference between

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supplementation arms (P = 0.433, Supporting Information, *Table* S11).

The intervention was not associated with training-associated injuries, with only five participants (6%) reporting discomforts with training towards the end of the intervention and only four participants (5%) withdrawing from study during the resistance training intervention, neither of which were due to injuries associated with the training. As such, serum levels of markers of muscle tissue damage (creatine kinase and aspartate aminotransferase) even decreased during the intervention, with no effects of vitamin D_3 supplementation (Supporting Information, *Table* S11). Supervised resistance training can safely be advocated for both healthy older adults and persons with COPD.

Concluding remarks

The study was conducted as a double-blinded RCT, addressing the effects of 12 weeks of vitamin D_3 supplementation only (i.e. two weeks of 10 000 IU/day, followed by ten weeks of 2000 IU/day), and 13 weeks of combined vitamin D_3 (2000 IU/day) and resistance training on functional measures, health markers and muscle biology in a mixed population of older adults. Vitamin D_3 supplementation is often hailed as an ergogenic aid for optimizing the outcome of resistance training, and is recommended for a variety of human populations, ranging from healthy subjects to athletes and chronically diseased subjects.[7,20] Vitamin D is thus presumed to play an important role in training-associated muscle plasticity. Despite this, its importance for humans remains largely elusive, with current knowledge stemming predominantly from animal research,[55] and the few existing human studies providing limited, uncertain and contradicting results.[41–44] Indeed, the present data do not support a role for vitamin D in training-associated muscle plasticity and functionality, at least not in older adults (with and without moderate COPD) with suboptimal to adequate baseline levels of 25(OH)D. More precisely, vitamin D_3 supplementation had no effect on core outcome domains such as changes in muscle strength, muscle mass, endurance performance and general muscle cell characteristics, and its effects on the muscle transcriptome was largely limited to gene sets relating to endothelial and cardiovascular functions. The validity of this insight is fortified by the thorough methodological and analytical approach. This included accounting for previous methodological issues such as a lack of a pre-training supplementation period, low vitamin D dosages, and neglecting to standardize test/training routines such as supervision of training sessions, test–retest analyses of functional and biological outcome measures, familiarization to training and a low reproducibility of singular outcome measures. The analytical approach also accounted for the potential confounding effects of the heterogeneity of the study population, as no interaction was found between effects of vitamin D_3 supplementation and disease status (healthy vs. COPD), or differences in pre-RCT vitamin D status, as all $[25(OH)D]_{baseline}$ quartiles responded in similar manners.

Despite our substantial efforts to strengthen the ecological value of the data set, there are aspects of vitamin D biology that remain unresolved, and that may have affected the conclusions and outcomes of the study. First, in skeletal muscle, adequate vitamin D signaling may occur at 25(OH)D levels lower than the defined cutoff (insufficient, $<$50 nmol/L).[27] Speculatively, all participants in the placebo arm may thus have been vitamin D-sufficient at the onset of resistance training, leaving our quartile-based analyses with limited biological value. Indeed, studies have suggested that vitamin D insufficiency will affect human muscle in an adverse manner only at concentrations $<$30 nmol/L.[139] Second, although serum 25(OH)D level is widely regarded as an adequate measure of vitamin D status,[63] it may be a poor proxy marker for vitamin D biology, as it largely fails to reflect $1,25(OH)_2D$ levels, the metabolically active form of vitamin D.[140] In line with this, in the present study, [25(OH)D] was not correlated with $[1,25(OH)_2D]$ at baseline (data not shown) and was not increased by long-term vitamin D_3 supplementation (at weeks 13 and 29). Such decoupling of 25(OH)D and $1,25(OH)_2D$ levels have several potential explanations. These include feedback-mediated regulation of vitamin D biology, which is largely affected by PTH levels,[141] as well as impaired $25(OH)D \rightarrow 1,25(OH)_2D$ conversion in individuals with pathophysiological indications such as renal dysfunction.[142] The latter is unlikely to explain the lack of increases in $[1,25(OH)_2D]$ in the present study, as only two participants were indicated with renal dysfunction (estimated based on levels of creatinine in serum; *Table* 1). Rather, the initial two weeks of high-dosage vitamin D_3 supplementation did lead to marked increases in $[1,25(OH)_2D]$, emphasizing that supplementation is indeed capable of increasing levels of metabolically active vitamin D, at least at high doses and within a short time frame. At weeks 13 and 29 were the PTH levels suppressed for both supplementation arms compared with pre-RCT levels. This was possibly related to the calcium supplement, and may have contributed to the unaltered $1,25(OH)_2D$ levels at these time points. Third, muscle cells may themselves possess the apparatus to convert 25(OH)D into $1,25(OH)_2D$, as they express the 25-Hydroxyvitamin D 1-alpha-hydroxylase (*CYP27B1*) protein. Indeed, in *in vitro* experiments on murine myoblast and myotubes, 25(OH)D and $1,25(OH)_2D$ treatment seem to lead to similar increases in the expression of vitamin D markers such as VDR, suggesting that peripheral regulation of vitamin D biology is a biological opportunity.[127] Fourth, while 25(OH)D was assessed as $[25(OH)D]_{total}$ in the present study, levels of unbound 25(OH)D (i.e. not bound to vitamin D binding protein or albumin; ~0.03%) may represent a more accurate measure of vitamin D status in a clinical setting.[143] Indeed,

in mice lacking vitamin D binding protein, and therefore displaying very low $[25(OH)D]_{total}$ (~8 nmol/L), no signs of vitamin D deficiency are seen unless they are put on a vitamin D deficient diet.[144] Fifth, in the present study, the resistance training intervention lasted for only 13 weeks. Speculatively, this may have been too short for vitamin D_3 supplementation to manifest its potential benefits for muscle plasticity, despite the presence of a 12-week lead-in supplementation period. Arguably, however, if vitamin D status and signaling is indeed important of muscle biological adaptations to training, even shorter interventions should lead to detectable changes in muscle biology, such as its transcriptome. This was not observed, neither in general, nor for specific vitamin D-responsive genes such as VDR.[128] Sixth, the study protocol was unavoidably associated with large interindividual variation in responses. This variation may have been related to vitamin D_3 supplementation *per se*, resistance training *per se* or to a combination of both, and may have affected groupwise comparisons. More research is clearly needed to elucidate on these perspectives.

Despite these uncertainties, it seems clear that vitamin D_3 supplementation did not affect muscle biological characteristics in the present study, particularly those measured using RNA-seq. Indeed, in our transcriptome analyses, not a single gene was found to be vitamin D_3-sensitive after a period of resistance training, which is surprising given the accepted dogma that vitamin D primarily acts as a transcriptional regulator,[55] and that the VDR was rather highly expressed in the data set, although it did not change with vitamin D_3 supplementation. Moreover, gene sets that were identified as vitamin D_3-sensitive in gene enrichment analyses were largely associated with vascular function rather than muscle cell biology.

Despite the general lack of effects of vitamin D_3 supplementation on muscle mass and phenotype (primary objectives of the study), as well as the lack of effects on other muscle functional and biological traits, the data set contained a couple of interesting observations. First, in the muscle transcriptome data, the effects of vitamin D_3 supplementation *per se* on expression of mitochondrial genes and the effects of combined vitamin D_3 supplementation and resistance training on biomarkers of endothelial and vascular biology calls for further study. Arguably, these biological features would be more decisive for adaptations to endurance-like training, posing the intriguing possibility that vitamin D_3 supplementation may be beneficial for the outcome of such training. Second, in participants with high baseline fat proportions/high BMI, vitamin D_3 supplementation led to increased training-associated changes in muscle strength. In these participants, the bioavailability of vitamin D may have been compromised by the high fat content (in the placebo arm, although they did not exhibit lowered 25(OH)D levels), corroborating with previous observation of interactions between vitamin D biology and fat mass.[116] While this may indicate that vitamin D exerts direct effects on muscle biology, as muscle strength is predominately defined by muscle mass,[145] this still seems unlikely as no such vitamin D_3-effect was seen for other muscle-specific outcome measures (e.g. muscle mass and phenotype). The causality may thus involve other physiological adaptations such as motoneuron function,[146] which has indeed been suggested to be affected by vitamin D supplementation in rodents.[147]

In retrospect, the pre-identified primary objectives of the current study were not ideal (i.e. the effects of vitamin D_3 supplementation on muscle fibre CSA and proportions). The underlying rationale behind this choice was to investigate the effects of vitamin D_3 supplementation on a set of unbiased biological variables (not prone to test–retest fluctuations), adhering to the existing notion that vitamin D may affect muscle fibre size and fibre type proportions (e.g. elucidated in the review from Ceglia, 2009[148]). This clearly underestimating the reliability issues associated with histological measures, which were indeed evident in the data set (Supporting Information, *Figure* S2). Importantly, vitamin D_3 supplementation was not associated with beneficial effects for any of the investigated primary or secondary outcome measures, hence leaving the overall conclusion as unambiguous.

In conclusion, in older adults with or without COPD, vitamin D_3 supplementation efficiently improved vitamin D-status without any adverse effects, but did not lead to beneficial effects in resistance training-associated changes in muscle function or characteristics. This rejects the notion that vitamin D_3 supplementation is necessary to obtain adequate muscular responses to resistance training in the general older population. Secondary analyses revealed positive effects of vitamin D_3 supplementation for participants with high proportions of fat mass and for gene sets involved in vascular functions, advocating further research to elucidate on these specific biological characteristics. Finally, the training programme was well-tolerated and associated with pronounced effects for a variety of health variables, emphasizing the potency of resistance training for relieving sarcopenia and maintaining functional capacity in older adults with and without COPD.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. qPCR primer sequences and performance.
Table S2. Statistical summary table.
Table S3. Statistical summary table, qPCR data.
Table S4. Computed factors for main outcome domains.
Table S5. Gene ontology analyses, effects of vitamin D_3 supplementation.
Table S6. KEGG and Hallmark analyses, effects of vitamin D_3 supplementation.
Table S7. Differentially expressed genes, effects of vitamin D_3 supplementation.
Table S8. Gene ontology analyses, effects of resistance training.
Table S9. Gene ontology analyses, effects of combined vitamin D_3 supplementation and resistance training.
Table S10. KEGG and Hallmark analyses, effects of combined vitamin D_3 supplementation and resistance training.
Table S11. Blood and health variables.
Figure S1. General efficacy of the RCT.
Figure S2. Sample-resample reliability measures of immunohistochemical assessments.
Figure S3. Baseline vitamin D-status and the interaction with the study's main outcomes.
Figure S4. Baseline body fat proportions/body mass index and the interaction with the study's main outcomes.

Conflict of interest

None declared. Pharma Nord ApS procured supplements but was not in any way involved in data collection, analyses or interpretations.

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Paper III

Chronic Obstructive Pulmonary Disease Does Not Impair Responses to Resistance Training

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4 ***Running head***

5 Resistance training adaptations in COPD subjects

Abstract

Background. Subjects with chronic obstructive pulmonary disease (COPD) are prone to accelerated decay of muscle strength and mass with advancing age. This is believed to be driven by disease-inherent systemic pathophysiologies, which are also assumed to drive muscle cells into a state of anabolic resistance, leading to impaired abilities to adapt to resistance exercise training. Currently, this phenomenon remains largely unstudied. In this study, we aimed to investigate the assumed negative effects of COPD for health- and muscle-related responsiveness to resistance training using a healthy control-based translational approach.

Methods. Subjects with COPD (n=20, GOLD II-III, $FEV_{1predicted}$ 57±11%, age 69±5) and healthy controls (Healthy, n=58, $FEV_{1predicted}$ 112±16%, age 67±4) conducted identical whole-body resistance training interventions for 13 weeks, consisting of two weekly supervised training sessions. Leg exercises were performed unilaterally, with one leg conducting high-load training (10RM) and the contralateral leg conducting low-load training (30RM). Measurements included muscle strength ($n_{variables}$=7), endurance performance ($n_{variables}$=6), muscle mass ($n_{variables}$=3), muscle quality, muscle biology (*vastus lateralis*; muscle fiber characteristics, RNA content including transcriptome) and health variables (body composition, blood). For core outcome domains, weighted combined factors were calculated from the range of singular assessments. Differences in responses to resistance training between COPD and Healthy were assessed using mixed-effects models.

Results. COPD displayed well-known pathophysiologies at baseline, including elevated levels of systemic low-grade inflammation ([c-reactive protein]), reduced muscle mass and functionality, and muscle biological aberrancies. Despite this, resistance training led to

improved lower-limb muscle strength (15±8%), muscle mass (7±5%), muscle quality (8±8%) and lower-limb/whole-body endurance performance (26±12%/8±9%) in COPD, resembling or exceeded responses in Healthy, measured as both relative and absolute change terms. This was accompanied by similar changes in hallmarks of muscle biology such as rRNA-content↑, muscle fiber cross-sectional area↑, type IIX proportions↓, and changes in mRNA transcriptomics. Neither of the core outcome domains were differentially affected by resistance training load.

Conclusions. COPD showed hitherto largely unrecognized responsiveness to resistance training, rejecting the notion of disease-related impairments and rather advocating such training as a potent measure to relieve pathophysiologies.

Trial registration. ClinicalTrials.gov ID: NCT02598830. Registered November 6th 2015, https://clinicaltrials.gov/ct2/show/NCT02598830

KEYWORDS. anabolic resistance, COPD, pathophysiology, skeletal muscle, strength training, training load

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Introduction

Chronic obstructive pulmonary disease (COPD) is associated with impaired cardiorespiratory fitness and decreased skeletal muscle mass and strength, leading to reduced levels of daily activity and reduced quality of life (1,2). This deterioration is accompanied by systemic co-morbidities such as reduced levels of testosterone (3), vitamin D (4,5) and oxygen saturation levels (6), and elevated levels of low-grade inflammation (7), which arguably leaves COPD subjects in a state of anabolic resistance (8), resulting in impaired abilities to adapt to exercise training (9–11). In particular, these pathophysiologies are believed to impair adaptations to resistance training, which represent the most potent intervention for improving muscle functions (12–15) and preventing escalation into late-stage morbidities such as pulmonary cachexia (16). Despite this general belief, the presence of anabolic resistance in COPD subjects and its consequences for responses to resistance training remain circumstantial. A mere single study has compared functional and biological adaptations to resistance training between COPD and healthy controls (ISRCTN ID: 22764439) (17–19), and as such was limited by a relatively short training intervention (8 weeks), a rather untraditional training protocol with little clinical and practical relevance, and a limited selection of outcome variables. Whereas the study failed to disclose COPD-related impairments in muscle strength and growth responses, it seems premature to dismiss the notion that COPD pathophysiologies may impair training responsiveness (20), and there is clearly need for further study.

The primary aim of the present study was to investigate the assumed negative effects of COPD pathophysiologies on physiological responses to 13 weeks of resistance training, with emphasis on a broad range of muscle functional and biological outcome measures. The

secondary aim was to investigate inherent differences between COPD and Healthy, and to investigate the interaction between two different resistance training modalities and training responsiveness (high-load vs. low-load resistance training; 10 vs 30 repetitions maximum, RM).

Methods

For in-depth description of study protocols and methods, including description of a placebo-controlled vitamin D_3 supplementation protocol (randomized clinical trial), see Figure 1-2 and clinicaltrial.gov (ClinicalTrials.gov Identifier: NCT02598830). The study was designed and scaled to allow elucidation of the effects of vitamin D_3 supplementation for adaptations to resistance training, as well as to compare training responsiveness between COPD and Healthy. The vitamin D_3 perspective is covered in detail elsewhere (21).

Study ethics and participants. The study was approved by the Regional Committee for Medical and Health Research Ethics (reference no. 2013/1094), preregistered at clinicaltrials.gov (NCT02598830), and conducted according to the Declaration of Helsinki. All participants were informed about the potential risks and discomforts associated with the study and gave their informed consent prior to study enrolment.

Persons with either medical diagnosis of stable COPD (GOLD grade II-III (22), predicted forced expiratory volume in first second (FEV_1) between 80%-30%, FEV_1/forced vital capacity (FVC) <70% after reversibility testing, n=24, age 70±5) or normal lung function (n=70, age 67±5) were recruited to the study. For study flow chart, see Figure 1. For baseline characteristics, see Table 1.

Insert Figure 1 and Table 1 around here

Study conduct. COPD and Healthy conducted identical 13-week resistance training protocols, consisting of two weekly full-body training sessions (Figure 2). Leg exercises were performed unilaterally, with one of the legs of each participant being randomly assigned to perform three sets of 10RM (high-load) and the contralateral leg to perform three sets of 30RM (low-load). All sessions were supervised by qualified personnel. The effectiveness of the training intervention was assessed as a wide range of outcome measures (Figure 2), including multiple assessments of endurance performance, muscle strength and mass, measures of work economy/efficiency, and collection of blood and *vastus lateralis* biopsies (both legs) (Figure 2).

Insert Figure 2 around here

Blood and muscle measurements. Prior to collection of blood and muscle biopsies, participants were instructed to attend an overnight fast and to avoid heavy physical activity for the last 48 h. Blood samples were analyzed for serum concentrations of hormones, lipids, and markers of iron metabolism and tissue damage, as previously described (21). Muscle biopsies were analyzed for muscle fiber type proportions, myonuclei content, muscle fiber cross-sectional area (CSA), and rRNA and mRNA content (total RNA, rRNA subspecies, myosin heavy chain isoforms I, IIA and IIX, and whole-genome transcriptome), as previously described (21,23,24). Transcriptome analysis was restricted to a subset of participants (COPD, n=19; Healthy, n=34).

Data analyses and statistics. For continuous variables, linear mixed-effects models were used to examine differences between COPD and Healthy, both at baseline and as responses to resistance training. For the latter, relative and absolute changes from baseline were defined as dependent variables, with COPD/Healthy being defined as the fixed effect. Analyses

included evaluation of interaction effects with training load (repeated measures/observations from the high- and low-load training leg were added to the model for unilateral outcome measures) and sex. The effects of sex were implemented into the models. Time effects were examined using mixed modelling, with the dependent variable and time points being defined as repeated measures/observations.

For non-continuous variables (fiber type proportions, rRNA/mRNA content), generalized linear mixed-effects models were used. In transcriptome analyses, genes were regarded as differentially expressed when the absolute $\log_2$ fold-change/difference were greater than 0.5 and the adjusted *p*-value (false discovery rate adjusted *per* model coefficient) was below 5% (23). Moreover, enrichment analyses were performed on hallmark, KEGG and gene ontology gene sets, using two approaches. First, a non-parametric rank test was performed based on gene-specific minimum significant differences. Second, gene set enrichment analysis (GSEA) was performed to quantify directional regulation of the gene set. Consensus results were interpreted as having larger biological meaning, while Hallmark was providing the most meaningful stand-alone interpretation, as it reduces the analytical noise by taking into account genes that overlap between gene sets (25). All gene sets were retrieved using the molecular signature database (version 7.1.) (26). Overview of gene enrichment analyses with exact *p*-values are presented in Supplementary Table 3.

For all immunohistochemical variables, statistical models were weighted for numbers of counted fibers *per* biopsy. This was done to account for the reduced reliability accompanying fewer observations/fibers (21).

To achieve reliable assessment of core outcome domains, and thus to lower the risk of statistical errors, combined factors were calculated for outcome measures relating to *lower-body muscle strength* (composed of values from the variables 1RM knee extension and

leg press (I), and peak torque for knee extension at 60, 180 and 240°/sec (II)), *lower-body muscle mass* (leg lean mass (I) and *vastus lateralis* and *rectus femoris* thickness (II)), *one-legged endurance performance* (maximal workload achieved during one-legged cycling (I) and number of repetitions at 50% of 1RM knee extension at pre-study (II)) and *whole-body endurance performance* (maximal workload achieved during bicycling (I), maximal number of steps achieved in a 6-min test (II), and maximal number of sit-to-stands in a 1-min test (III)), as previously described (21). During factor calculation, each of the underlying variables were normalized to the participant with the highest value recorded during the RCT, resulting in individual scores ≤1. Thereafter, outcome domain factors were calculated as the mean of the normalized values for each variable for each participant. For details, see Supplementary Table 1.

Statistical significance was set to $p<0.05$. In both text and figures, data are presented as adjusted, marginal means, with or without 95% confidence intervals, unless otherwise stated. Statistical analyses were performed using SPSS Statistics package version 24 (IBM, Chicago, IL, USA) and R software (27). Figures were made using Prism Software (GraphPad 8, San Diego, CA, USA) and R software (27).

Results and discussion

Baseline characteristics: COPD vs Healthy

Exercise capacity, body composition and muscle and blood biology. At baseline, COPD displayed impaired exercise capacity compared to Healthy, as expected from previous studies (2,17,19,28). This was evident as impaired whole-body performance (range: -41% to -54%, Table 1), and lower-body unilateral muscle strength and endurance performance (-17% to -30%, Table 1), reflecting the cardiorespiratory and muscular limitations inherent to the

condition (20). In accordance with this, COPD had less lean body mass than Healthy (Δ-13%, Table 1), with 45% of COPD showing signs of sarcopenia, as defined by Baumgartner *et al.* (29). This difference was unlikely to be due to the miniscule age difference between COPD and Healthy (-2 years; Table 1), as this would have implied an annual loss of ~2.6 kg lean mass *per* year, markedly deviating from the expected loss in this age group (~0.5 kg *per* year) (30). The negative effects of COPD for muscle mass was underlined by -9%/-24% smaller *vastus lateralis/rectus femoris* thicknesses (Table 1), corresponding well with difference in leg-specific lean mass (-16%; Table 1), offering potential explanations for the impaired maximal leg muscle strength. The general impaired exercise capacity in COPD was presumably decoupled from differences in habitual physical activity patterns prior to the study intervention (COPD, 4266 ± 4035 kcals · week^{-1} (average ± standard deviation); Healthy, 4520 ± 2837 kcals · week^{-1}; p=0.760).

The two study clusters also differed at the muscle biological level, with COPD showing greater proportions of type IIA and IIX muscle fibers in *vastus lateralis* compared to Healthy (32%/23% vs 13%/9%, respectively), with concomitant lowering of proportions of type I fibers, corroborating with previous studies (31,32). For type I fibers, COPD showed larger CSA (12%, Table 2) and larger myonuclear domain (CSA *per* myonuclei) (Δ20%, Table 2), with no difference being observed for type II fibers. This contrasts previous studies, who have reported smaller or similar CSA in type I fibers in COPD compared to Healthy (28,33,34), but may point to a compensatory mechanism for the likely loss of motor units in COPD subjects (35), whereby reduced quantities of muscle fibers are compensated for by increased sizes of remaining fibers, as previously reported in rodents (36). Furthermore, COPD also affected whole-genome transcriptome profiles and displayed differential expression of 227 genes compared to Healthy (151↑ and 76↓; Figure 3a and Supplementary Table 2). Hallmark

enrichment analysis revealed lower expression of genes involved in *oxidative phosphorylation* (consensus), corroborating with the lower type I proportion, and greater expression of genes involved in regulation of *myogenesis* (Rank) (Figure 3a-b, Table 3; findings confirmed in gene ontology analysis, Supplementary Table 3), which may be related to the pathophysiological elevation of protein turnover in COPD (37,38).

For other muscle characteristics, such as the content of total RNA and rRNA *per* amount of muscle tissue, no differences were observed between COPD and Healthy at baseline (Table 2).

For blood variables, the COPD cluster showed elevated levels of low-grade inflammation, measured as c-reactive protein levels, at pre-study compared to Healthy (5.0 vs 1.6 $mg \cdot L^{-1}$) and tended to differ at baseline (p=0.053; Table 4), as expected from previous studies (7). For other characteristics, such as hormonal status in blood (e.g. testosterone), no differences were observed between COPD and Healthy at baseline (Table 4).

Insert Figure 3, Table 2 and Table 3 around here

The efficacy of the resistance training intervention: COPD vs Healthy

For both COPD and Healthy, the training intervention was associated with low drop-out rates (n=4, ~5%; COPD, n=2), high adherence to the protocol (COPD, 97%; Healthy, 98%), progressive increases in training volume (Figure 2), and robust increases in muscle strength *per* training session (e.g. 1RM knee extension, 0.9% · $session^{-1}$/0.8% · $session^{-1}$, COPD/Healthy; 1RM leg press, 1.4% · $session^{-1}$/1.3% · $session^{-1}$). The habitual dietary intake was similar between COPD and Healthy, with protein intake being 1.2 ± 0.3 (average ± standard devation) and 1.3 ± 0.4 $g \cdot kg^{-1} \cdot day^{-1}$, respectively, complying with current guidelines (39). The vitamin D_3 supplementation RCT of the project did not enhance or affect training-associated changes for any of the primary or secondary outcome measures (21).

Muscle strength, muscle mass, muscle quality and one-legged endurance performance. Overall, COPD showed larger training-associated increases in lower-body muscle strength and mass compared to Healthy (the two legs/training modalities combined), measured as relative changes in combined factors from baseline (Figure 4A), with no difference being observed for absolute changes (Figure 4A). COPD and Healthy showed similarly scaled improvements in muscle quality and one-legged endurance performance (Figure 4A). Notably, neither of these four core outcome domains were differentially affected by resistance training load (neither in COPD nor in Healthy), suggesting that 30RM training is an effective alternative to 10RM training in older individuals (Figure 4B-C). COPD thus showed marked and hitherto unrecognized responsiveness to resistance training, contradicting previous suggestions of a negative impact of co-morbidities such as low cardiorespiratory fitness and chronic low-grade systemic inflammation (7,40).

Insert Figure 4 around here

Cycling and functional performance. COPD and Healthy showed pronounced and similarly scaled training-associated improvements in whole-body endurance performance, measured as changes from baseline, including 6-min step test performance, 1-min sit-to-stand performance and maximal workload achieved during two-legged cycling (Figure 5). Surprisingly, COPD and Healthy also showed similar changes in performance for these outcome measures as absolute terms, with exception of 6-min step test performance (Δ-11 steps, Figure 5), for which Healthy showed larger improvements, arguably related to the considerable cardiorespiratory demand of this test, leaving COPD with morbidity-specific restraints. For other performance indices such as cycling economy and gross efficiency, which were measured using a one-legged cycling protocol, COPD showed larger relative improvements compared to Healthy (Δ4%, Figure 5). For these outcome measures, COPD,

but not Healthy, displayed benefits of 10RM compared to 30RM training (Figure 5), corresponding to previously observed effects of heavy resistance training in healthy, young individuals (41).

Together, these observations reiterate on the substantial benefits of resistance training for subjects with COPD, even for performance measures that pose large whole-body metabolic demands, which has previously been suggested to be irresponsive to such training (42). As such, it seems plausible that the observed improvements in 6-min step test performance, 1-min sit-to-stand performance and two-legged cycling were associated with improvements in work economy/gross efficiency and muscle strength, as neither COPD nor Healthy showed training-associated changes in maximal oxygen consumption (Figure 5), with improvements in anaerobic capacity being a potential contributor (not measured).

Insert Figure 5 around here

Muscle fiber characteristics. Whereas COPD and Healthy displayed similar increases in type II fiber CSA in *vastus lateralis* in response to resistance training (Δ-6%, p=0.438; Figure 6, upper panel), only Healthy showed increases in type I fiber CSA (16%), with no statistical difference being observed between study clusters. For Healthy, the increase in CSA was accompanied by increased myonuclei·fiber^{-1} in both fiber types (36%/25% for type I/II; Figure 7), leading to decreased myonuclear domain size estimates in type I fibers (-10%, Figure 7). For COPD, no such effects were observed (Figure 7). Despite the lack of difference between the two study clusters for these variables, the data hints at blunted plasticity of type I muscle fibers in COPD only, potentially relating to their altered biological characteristics at baseline or to blunted myonuclear accretion. Interestingly, in sub-analyses, the blunted type I responses in COPD seemed to be specific to 10RM training, with a tendency towards

superior responses to 30RM training (Δ22%, p=0.060; Figure 6, middle panel). Such a phenomenon is supported by previous observations in responses to blood-flow-restricted low-load training (43), which arguably is mimicked by COPD subjects during low-load training, as they display inherent lowering of oxygen saturation in blood.

Both COPD and Healthy displayed training-associated reductions in type IIX muscle fiber proportions (Figure 7). While this reduction was more pronounced in COPD when measured at the protein level (immunohistochemistry), it was more pronounced in Healthy when measured at the mRNA level, suggesting differential orchestration of muscle fiber shifts between study clusters, possibly relating to their inherently different muscle fiber proportions at baseline.

Insert Figures 6 and 7 around here

Muscle RNA content. In general, COPD and Healthy showed similar increases in ribosomal RNA abundance *per* unit muscle tissue weight, measured as both total RNA and rRNA expression, and measured after both 3½ week (1.19/1.29 and 1.15/1.16 fold increases, total RNA/rRNA abundances) and after finalization of the training intervention (1.13/1.18 and 1.05/1.17 fold increases) (Figure 8). While these changes in ribosomal RNA content were generally similar between COPD and Healthy, a few noteworthy differences were evident, including a more robust early increase in 45s pre-rRNA abundance in COPD (Figure 8) and a trend towards reduced changes in response to 13 weeks training in COPD, which led to the absence of time effects for all rRNA species. The early increases in ribosomal content seen in both COPD and Healthy resemble those typically seen after similar interventions in untrained young individuals (24), and may be important for muscle growth capabilities over the entirety of the study period (24,44), accommodating increases in protein synthesis capacity,

thus potentially contributing to the pronounced muscular responses to resistance training seen in both study clusters.

Insert Figure 8 around here

In both COPD and Healthy, resistance training led to marked changes in mRNA transcriptome profiles, with 499 and 312 differentially expressed genes being observed after 3½ and 13 weeks of resistance training, respectively (for general information about transcriptomic responses, see Mølmen *et al*. (21)). Overall, at the single-gene level, no transcripts showed differential responses to training between the two study clusters, neither at 3½ weeks nor at 13 weeks, despite clear differences in transcriptome profiles at baseline (Figure 3a and Supplementary Table 2). In contrast, enrichment analyses revealed traces of differential changes (Figure 3C, Table 3 and Supplementary Table 3), with COPD showing more pronounces increases in expression of genes relating to *oxidative phosphorylation* after 3½ weeks (GSEA), and, in particular, more pronounced decreases in genes associated with *myogenesis* after 13 weeks (consensus) (Figure 3C, Table 3). Interestingly, as these two gene sets represented the most prominent differences between COPD and Healthy at baseline (Figure 3A-B), and as resistance training led to directional changes that mitigated these differences, training arguably shifted the COPD phenotype in a healthy direction.

Blood and health-related outcomes. Overall, COPD and Healthy showed similar training-associated increases in whole-body and appendicular lean mass (Table 4). This was accompanied by increased appendicular skeletal muscle mass index relative to the sex-specific mean of young, healthy adults (COPD, from 84% to 86%; Healthy, from 95% to 97%), suggesting that the intervention was effective for reversing age-related decline in muscle mass. For blood variables such as markers of systemic inflammation and hormone, lipid and

iron biology, no noteworthy effects were observed of the intervention, nor were any differential changes observed between COPD and Healthy (Table 4).

Insert Table 4 around here

Lung function. For COPD, the training intervention did not affect any of the lung function variables (Table 5), implying no effects on this core epidemiological trait. This seems reasonable given the irreversible nature of the respiratory impairments of COPD, contradicting the beneficial effects observed in Hoff *et al*. (13) In contrast, for Healthy, the intervention was associated with reduced FVC and FEV_1 (-2.7% and -1.5%, respectively). Rather than being a consequence of the intervention protocol *per se*, this may be due to a general age-related decline, as the magnitude of the changes resemble those seen in corresponding age cohorts over a similar time frame (45).

Insert Table 5 around here

Health-related quality of life. For COPD, the intervention was associated with marked improvements in several aspects of health-related quality of life (Table 6). These included reduced experience of limitations of physical functioning and improved social function and mental health, with only marginal effects being seen in Healthy. While these changes of course may be directly related to the resistance training intervention, they may also be related to other aspects of the study protocol, such as performing training sessions in a social setting and the close follow-up each participant received from study personnel. As the intervention was conducted without a control group (not receiving the intervention protocol), caution is warranted for interpretation of these data.

Insert Table 6 around here

Concluding remarks

COPD-related pathophysiologies, such as reduced testosterone (3), vitamin D (4) and oxygen saturation levels (6,46) in blood, and elevated levels of low-grade inflammation (7), are generally believed to drive metabolism into a chronic catabolic state (3,6,8). This has been suggested to lead to impaired responses to lifestyle interventions such as resistance training (6,47), which are essential measures for preventing and treating disease-related reductions in skeletal muscle mass and strength, counteracting escalation into serious conditions such as pulmonary cachexia (16). Despite this general belief, the presence of impaired training responsiveness in COPD is not backed by experimental data, and there is limited *de facto* evidence for such impairments. To date, a mere single study has compared responses between COPD and healthy control subjects (17–19), and as such failing to lend support to the prevailing view, though being limited by a relatively short time span (8 weeks) and a restricted selection of outcome variables. In the present study, we largely disavow the myth of impaired responsiveness to training in COPD, measured as responses to a 13-week whole-body resistance training intervention, conducted using an exhaustive follow-up and testing protocol, which included extensive test-retest validations (for details, see Mølmen *et al*. (21)). Whereas COPD participants displayed clear and well-known disease-related aberrancies compared to Healthy at baseline, including altered skeletal muscle characteristics and elevated levels of systemic inflammation, they showed similar or superior improvements for virtually every measure of health, performance and biology. Specifically, COPD showed greater relative improvements in core outcome domains such as lower-body muscle strength and mass, and similar relative improvements in muscle quality, one-legged endurance performance and whole-body endurance performance. These similarities were

also evident in absolute change terms, suggesting that the improvements seen in COPD was decoupled from the compromised levels at baseline. These observations were accompanied by similar alterations in muscle biology, including changes in hallmark traits such as muscle fiber characteristics, rRNA content and transcriptome profiles. Together, these data suggest that COPD-related etiologies and pathophysiologies do not impair responsiveness to resistance training, at least not for skeletal muscle characteristics, and at least not in the enrolled cluster of COPD participants (GOLD grade II-III) and within the time frame of the study.

During planning of the study protocol, two strategies were implemented to resolve the hypothesized, albeit rejected, negative impact of COPD-specific pathophysiologies for the efficacy of resistance training. *First*, as vitamin D insufficiency is common among COPD subjects (4), and has been suggested to contribute to development of anabolic resistance (48), dietary habits were manipulated to investigate the effects of vitamin D_3 supplementation. Contrary to our hypothesis, vitamin D_3 did not enhance responses to resistance training for any of the outcome variables (21).

Second, the resistance training protocol was conducted using two different training modalities, 10RM and 30RM resistance training, performed in a contralateral manner. The efficacies of these training modalities were initially hypothesized to be dissimilarly affected by COPD-related pathophysiologies, as they convey muscular adaptations through different signaling cues in the cellular environment (i.e. mechanical tension vs metabolic perturbation) (49), and may thus well be differentially affected by extracellular signaling such as inflammation and oxygen availability. While this hypothesis was rejected for all core outcome domains, with no differences being observed between training modalities and no evidence being found for the presence of impaired training responsiveness, a noteworthy

observation was made for muscle fiber-specific traits. Specifically, in COPD, 10RM training was associated with blunted growth of type I muscle fiber CSA, a phenomenon that was not observed for responses to 30RM training, suggesting that 30RM offers benefits for muscle fiber type I hypertrophy. In addition to this, 10RM was associated with greater improvements in cycling economy and gross efficiency in COPD. These observations warrant further study.

In conclusion, 13-week resistance training program was well-tolerated by subjects with COPD and led to pronounced improvements for a range of health and muscle functional and biological variables, resembling or exceeding those seen in Healthy. COPD was thus not associated with impaired responsiveness to exercise training, which rather posed a potent measure to relieve disease-related pathophysiologies.

Additional information

Supplementary information. This article has an online data supplement.

Abbreviations. COPD, chronic obstructive pulmonary disease; RM, repetition(s) maximum; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; CSA, cross-sectional area; GSEA, gene set enrichment analysis

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Authors' contributions. KSM and SE developed the project, with input from GSF, TJR, BRR and TR. KSM led the study intervention, including coordination and conduction of exercise training and testing, with aid from DH, GSF, BRR and SE. MG and TJR planned, organized and conducted participant recruitment and performed medical screening. BM and RL planned, organized and conducted lung spirometry and DXA measurements. KSM, DH, LK, MH, YK, RA and SE planned and performed muscle biological analyses. KSM, DH and SE planned and performed data analyses, with input from YK and RA. KSM, TR and SE drafted the manuscript. All authors provided useful input to data interpretation and contributed to

drafting and finalizing the manuscript. All authors have read and approved the final manuscript.

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Consent for publication. Not applicable.

Competing interests. The authors have no conflicts of interest to disclose.

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Figure legends/captions

Figure 1. CONSORT flow chart of the study. The study was conducted as a double-blind randomized clinical trial, with the primary aim of investigating the effects of vitamin D_3 supplementation on resistance training-associated adaptations in a mixed population of older subjects, including both COPD and healthy control subjects (COPD and Healthy, respectively) (ClinicalTrials.gov Identifier: NCT02598830). Vitamin D_3 supplementation did not affect any primary or secondary outcome, and no conditional effects were observed for COPD vs Healthy in that context (21). In the present study, the main purpose was to compare the effects of resistance training between COPD and Healthy participants (COPD, n=20; Healthy, n=58).

Figure 2. Schematic overview of the study protocol, including its time line (A; ‡ indicates the defined baseline measurement for the specific outcome measure), training volumes during the resistance training (RT) intervention (B), perceived exertion (Borg RPE, 6-20) reported after training sessions (C), and relative training loads (% of 1RM) during the training period (D). Training volume is presented as average increases in per-session for lower-body appendices from the first week of training (kg · repetitions; high-load (10RM) and low-load (30RM) leg press and knee extension combined). COPD, participants diagnosed with chronic obstructive pulmonary disease; Healthy, healthy control participants; *, statistical different from 1th training week; #, statistical difference between COPD and Healthy. Data are presented as means with 95% confidence limits. Methodological notes on retrieval of outcome measures: **i)** Lung function. Spirometry testing was performed following the guidelines from the American Thoracic Society and the European Respiratory Society (50). Participants with COPD were tested before and after inhalation of two bronchodilators

(salbutamol/ipratropiumbromid). **ii)** Muscle strength and performance (STR and Musc. perf). Muscle strength was assessed as one-repetition maximum (1RM) in unilateral knee extension and leg press, bilateral chest press, and handgrip. Muscle performance was defined as the number of repetitions achieved at 50% of pre-study 1RM and was assessed using unilateral knee extension and bilateral chest press. Isokinetic unilateral knee-extension torque was tested at three angular speeds (60°, 120° and 240° $\cdot$ sec^{-1}; Humac Norm, CSMi, Stoughton, MA, USA). **iii)** One-legged cycling and bicycling performance (1-LC and VO_2max). Participants conducted one-legged cycling tests (Excalibur Sport, Lode BV, Groningen, the Netherlands) to assess O_2-costs and mechanical efficiency (51) during submaximal cycling, and maximal one-legged oxygen consumption ($\dot{V}O_2$max) and maximal workload. Maximal two-legged cycling $\dot{V}O_2$max and workload were tested on a separate day. Oxygen consumption was measured using the JAEGER Oxycon Pro^{TM} system (Carefusion GmbH, Höchberg, Germany). **iv)** Functional performance (Func.). Functional tests were conducted as the maximal number of sit-to-stands during one minute (seat height: 45 cm) and as the number of steps onto a 20 cm step box during 6 minutes. **v)** Health-related quality of life (SF-36 and CAT). All participants completed the Short Form (36-item) Health Survey (SF-36). COPD participants also completed the COPD Assessment Test (CAT) questionnaire. **vi)** Muscle thickness and body mass composition (US/DXA). Muscle thickness of *m. vastus lateralis* and *m. rectus femoris* were measured using B-mode ultrasonography (SmartUs EXT-1M, Telemed, Vilnius, Lithuania). Body mass composition was measured using dual-energy X-ray absorptiometry (DXA; Lunar Prodigy, GE Healthcare, Madison, WI, USA).

Figure 3. Whole-genome transcriptome analyses of *m. vastus lateralis* in COPD and Healthy. At baseline, numerous genes were differentially expressed between COPD and Healthy. In

(A), differences in gene expression between COPD and Healthy are presented with leading edge genes (i.e. genes that contributes to the enrichment score) from two gene sets identified as differentially expressed between COPD and Healthy from gene enrichment analyses (*oxidative phosphorylation* and *myogenesis*; see Table 3). In (B), average fold differences (COPD - Healthy) of genes contributing to baseline differences in *oxidative phosphorylation* and *myogenesis* gene sets are shown as individual data points, and violin plots shows the distribution of all leading edge genes from each gene set. (C) displays the average development of each gene set over time, where the dotted line indicates the mean fold change of all genes contributing to the differential change over time between COPD and Healthy. COPD displayed larger increases in expression of genes relating to *oxidative phosphorylation* after 3½ weeks of training, and more pronounced decreases in genes associated with *myogenesis* to after the training intervention (Post-RT; see Table 3). FDR, false discovery rate-adjusted *p*-value.

Figure 4. Effects of the resistance training intervention on lower-body muscle strength, lower-body muscle mass, one-legged endurance performance and lower-body muscle quality in COPD and Healthy. Each outcome domain is represented by a combined factor, computed from various performance assessments, as defined in the upper panel of the figure and previously described (21). (A) presents comparison of overall training effects between COPD and Healthy, measured as relative changes from baseline to after the resistance training intervention (per study cluster; left panel) and as relative and absolute differences in change scores between study clusters (right panels). In these analyses, high- and low-load resistance training (10RM and 30RM, respectively) were combined, warranted by the lack of differences between training load conditions in (B, C). COPD showed greater relative changes in muscle strength and muscle mass than Healthy. (B, C) presents

comparison of effects of 10RM and 30RM resistance training in COPD (B) and Healthy (C) (i.e. per study cluster), measured as relative changes from baseline to after the intervention (left panels) and as relative and absolute differences in change scores between load conditions (right panels). #, statistically different effects of resistance training between COPD and Healthy. Data are presented as means with 95% confidence limits.

Figure 5. Comparison of the effects of the resistance training intervention on whole-body endurance performance in COPD and Healthy, presented as relative changes from baseline (per study cluster; A) and as relative and absolute differences in change scores between study clusters (B and C, respectively). Endurance measures included maximal oxygen consumption ($\dot{V}O_2max$, $cl \cdot min^{-1}$) and maximal workload (watts) achieved during two-legged cycling, cycling economy ($cl \cdot min^{-1}$) and gross efficiency measured during submaximal one-legged cycling, the number of steps achieved during 6-min step test, and the number of sit-to-stands achieved during a 1-min sit-to-stand test. COPD showed greater relative improvements in cycling economy and gross efficiency. For these outcome measures, COPD, but not Healthy, displayed benefits of high-load training (10RM) compared to low-load training (30RM) (D and E). Healthy showed greater absolute improvement in the number of steps achieved during the 6-min step test. COPD and Healthy showed similar relative and absolute training-associated changes in the whole-body endurance performance factor. #, statistically different response to resistance training between study clusters. ‡, statistically different response to 10RM and 30RM resistance training in study cluster. Data are presented as means with 95% confidence limits.

Figure 6. Effects of the resistance training intervention on cross-sectional area of muscle fiber types I and II in *m. vastus lateralis* in COPD and Healthy. (A) presents comparison of

overall training effects on fiber CSA between COPD and Healthy, measured as relative changes from baseline to after the training intervention (per study cluster; left panel) and as relative differences in change scores between study clusters (right panel). In these analyses, high- and low-load resistance training (10RM and 30RM, respectively) were combined, warranted by the lack of significant differences between training load conditions in (B, C), though COPD tended to show higher efficacy of 30RM resistance training for changes in fiber type I CSA. (B, C) presents comparisons of effects of 10RM and 30RM resistance training on fiber CSA in COPD (B) and Healthy (C) (i.e. per study cluster), measured as relative changes from baseline to after the training intervention (left panels) and as relative and absolute differences in change scores between load conditions (right panels). Data are presented as means with 95% confidence limits.

Figure 7. Comparisons of the effects of the resistance training intervention on changes in myonuclei *per* fiber and myonuclei domain in muscle fiber types I and II (A, B), and on changes in muscle fiber type proportions in COPD and Healthy, measured using immunohistochemistry (C-E) and qPCR (gene family profiling-normalized myosin heavy chain mRNA expression, F-H), as previously described (24,52). Myonuclei domain was calculated as mean fiber cross-sectional area divided by myonuclei *per* fiber. For myonuclei *per* fiber and myonuclei domain in muscle fiber types I and II, comparisons are presented as relative changes from baseline to after the training intervention (per study cluster; A) and as relative differences in change scores between study clusters (B). For muscle fiber type proportions, data are presented as adjusted values at baseline and after the training intervention (Post RT), and results are presented as the effect of the training intervention for the study clusters combined and its interaction with study clusters (C-H). For myonuclei variables, no training-

associated differences were observed between study clusters. Both COPD and Healthy displayed training-associated reductions in proportions of type IIX muscle fibers, measured using both immunohistochemistry and qPCR. Intriguingly, while this reduction was greater in COPD when measured at the protein level (immunohistochemistry), it was greater in Healthy when measured at the mRNA level (qPCR), indicating differentially regulated muscle fiber shifting in COPD and Healthy. Data are presented as means with 95% confidence limits.

Figure 8. Effects of the resistance training intervention on total RNA content (A) and rRNA expression (B-F) in *m. vastus lateralis* of COPD and Healthy. Data are presented as fold changes from baseline to Week 3½ (Post-intro RT; seven training sessions) and to after the training intervention (Post RT; 26 training sessions). Total RNA (A), 18s rRNA (B), 28s rRNA (C), 5.8s rRNA (D) 5s rRNA (E) and 45s pre-rRNA (F) abundances. Total RNA- and qPCR-analyses were assessed as per-amounts of tissue weight, as previously described (21,24). *#,* statistical difference in fold change between COPD and Healthy (alpha level, $p<0.05$). Data are presented as means with 95% confidence limits.

Tables

Table 1. Characteristics of the participants completing the study

General	COPD	Healthy	Sex-adjusted estimated difference COPD – Healthy (95% CI)	*P*-value
Participants, completing (no. ♂/♀) / dropouts† (no.)	20 (12/8) / 2	58 (21/37) / 2	-	-
Age (years)	69 ± 5 (range, 60-79)	67 ± 4 (range, 57-78)	2 (0, 5)	0.049*
Height (cm)	171 (10)	170 (10)	-3 (-6, 0)	0.056
Body mass (kg)	73 (18)	76 (16)	-7 (-14, 0)	0.061
Body mass index (kg · m^2)	25 (5)	26 (5)	-2 (-4, 1)	0.237
Pack-years (no.)	30 (16)	6 (10)	23 (17, 29)	< 0.001*
GOLD grade (no. of grade II/III)	15/5	-	-	-
COPD Assessment Test™ score (0-40)	16.6 (6.8)	-	-	-
Self-reported conception of health (0-10)	4.9 (1.2)	6.7 (1.6)	-1.7 (-2.5, -0.7)	0.001*
Pulmonary function				
FVC (L)	3.2 (0.9)	3.6 (0.9)	-0.7 (-1.0, -0.4)	<0.001*
FVC (% predicted)	97 (19)	112 (16)	-13 (-22, -4)	0.003*
FEV_1 (L · sec^{-1})	1.5 (0.4)	2.7 (0.7)	-1.4 (-1.6, -1.2)	< 0.001*
FEV_1 (% predicted)	57 (11)	104 (16)	-47 (-55, -39)	< 0.001*
FEV_1/FVC (%)	47 (8)	75 (6)	-28 (-31, -24)	< 0.001*
PEF (L · sec^{-1})	5.0 (1.6)	7.7 (2.1)	-3.4 (-4.1, -2.7)	< 0.001*
Medication				
B_2-agonists (no.)	17/20	-	-	-
Muscarinic agonists (no.)	15/20	-	-	-
Combined b_2-agonist and corticosteroid (no.)	10/20	-	-	-
Body composition				
Total lean mass (kg)	♂, 53 (4); ♀, 36 (6)	♂, 60 (5); ♀, 41 (4)	-6 (-9, -4)	< 0.001*
Whole-body bone mineral density (g · cm^2)	♂, 1.2 (0.1); ♀, 1.0 (0.2)	♂, 1.3 (0.1); ♀, 1.1 (0.1)	-0.1 (-0.2, -0.0)	0.007*
Total fat mass (kg)	♂, 26 (10); ♀, 27 (15)	♂, 26 (9); ♀, 25 (10)	1 (-5, 7)	0.703
Visceral fat (kg)	♂, 1.9 (1.3); ♀, 1.0 (0.7)	♂, 1.7 (1.0); ♀, 0.8 (0.7)	0.2 (-0.3, 0.7)	0.412
Lower-body muscle strength				
1RM leg press (kg)	♂, 121 (35); ♀, 82 (21)	♂, 152 (27); ♀, 124 (25)	-36 (-47, -26)	< 0.001*
1RM knee extension (kg)	♂, 21 (4); ♀, 11 (4)	♂, 31 (5); ♀, 16 (3)	-7 (-9, -5)	< 0.001*
Peak torque knee extension 60° · sec^{-1} (Nm)	♂, 127 (34); ♀, 80 (25)	♂, 160 32); ♀, 101 (16)	-27 (-36, -17)	< 0.001*
Peak torque knee extension 180° · sec^{-1} (Nm)	♂, 83 (25); ♀, 47 (17)	♂, 102 (23); ♀, 62 (11)	-19 (-28, -9)	< 0.001*
Peak torque knee extension 240° · sec^{-1} (Nm)	♂, 68 (20); ♀, 38 (14)	♂, 84 (20); ♀, 50 (9)	-15 (-20, -9)	< 0.001*
Lower-body muscle strength factor (AU)	♂, 0.5 (0.1); ♀, 0.3 (0.1)	♂, 0.6 (0.1); ♀, 0.4 (0.1)	**-0.1 (-0.2, -0.1)**	**< 0.001***
Lower-body muscle mass measures				
Leg lean mass (kg)	♂, 18 (2); ♀, 12 (3)	♂, 20 (2); ♀, 14 (2)	-3 (-4, -2)	< 0.001*
M. vastus lateralis thickness (mm)	♂, 20 (3); ♀, 18 (5)	♂, 22 (3); ♀, 20 (3)	-2 (-3, -1)	0.002*
M. rectus femoris thickness (mm)	♂, 13 (4); ♀, 10 (3)	♂, 16 (4); ♀, 15 (4)	-4 (-5, -2)	< 0.001*
Lower-body muscle mass factor (AU)	**♂, 0.6 (0.1); ♀, 0.5 (0.1)**	**♂, 0.7 (0.1); ♀, 0.6 (0.1)**	**-0.1 (-0.2, -0.1)**	**< 0.001***
Endurance measures				
Maximal power output one-legged cycling (W)	♂, 73 (13); ♀, 48 (17)	♂, 148 (28); ♀, 108 (21)	-67 (-77, -58)	< 0.001*
Maximal power output two-legged cycling (W)	♂, 118 (38); ♀, 75 (32)	♂, 252 (48); ♀, 167 (32)	-113 (-134, -92)	< 0.001*
Maximal oxygen consumption (mL O_2 · kg^{-1} · min^{-1})	♂, 20 (5); ♀, 16 (5)	♂, 35 (7); ♀, 28 (6)	-14 (-18, -10)	< 0.001*
6-min step test (maximal number of steps)	♂, 123 (35); ♀, 115 (44)	♂, 208 (41); ♀, 196 (38)	-83 (-105, -61)	< 0.001*
1-min sit-to-stand test (maximal number)	♂, 21 (5); ♀, 21 (6)	♂, 30 (5); ♀, 29 (5)	-9 (-12, -6)	< 0.001*
$n_{repetitions}$ at 50% of 1RM knee $extension_{pre\ study}$	♂, 19 (5); ♀, 17 (5)	♂, 23 (6); ♀, 20 (7)	-4 (-6, -1)	0.005*
One-legged endurance performance factor (AU)	**♂, 0.2 (0.0); ♀, 0.2 (0.0)**	**♂, 0.4 (0.1); ♀, 0.3 (0.1)**	**-0.2 (-0.2, -0.1)**	**< 0.001***
Whole-body endurance performance factor (AU)	**♂, 0.4 (0.1); ♀, 0.3 (0.1)**	**♂, 0.7 (0.1); ♀, 0.6 (0.1)**	**-0.3 (-0.3, -0.2)**	**< 0.001***

COPD, participants diagnosed with chronic obstructive pulmonary disease; Healthy, healthy control participants; ♂, males; ♀, females; †, dropouts during the training period; *, study clusters are significantly different from each other (p<0.05); GOLD, Global Initiative for Chronic Obstructive Lung Disease; pack-years, (number of cigarettes smoked per day/20) × number of years smoked; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; PEF, peak expiratory flow; 1RM, one repetition maximum; Nm, newton-meter; AU, arbitrary units. Data mainly presented as mean (SD), and sex-adjusted estimated mean differences between study clusters (95% CI). Computed factors for core outcome domains, i.e. lower-body muscle strength, lower-body muscle mass, one-legged endurance performance and whole-body endurance performance, are indicated in bold text. Briefly, each factor was calculated using multiple singular outcome measures, where each of these variables were normalized to the participant with the highest value recorded during the study, resulting in individual scores ≤1. Thereafter, outcome domain factors were calculated as the mean of the normalized values for each variable for each subject (see Supplementary Table 1 for complete overview over calculations and composition of each factor).

Table 2. Baseline characteristics of m. vastus lateralis for COPD and Healthy

Cross-sectional area (μm²)	COPD	Healthy	Sex-adjusted estimated difference COPD – Healthy (95% CI)	*P*-value
Type I	4614 (1088)	3720 (951)	449 (70, 827)	0.020*
Type II	3639 (1235)	3059 (1121)	182 (-118, 482)	0.232
Myonuclei per fiber				
Type I	2.2 (0.9)	2.1 (0.9)	-0.1 (-0.4, 0.2)	0.357
Type II	2.1 (0.7)	1.9 (0.7)	-0.1 (-0.3, 0.2)	0.504
Myonuclear domain (cross sectional area/nuclei per fiber)				
Type I	2292 (585)	1928 (1030)	360 (107, 613)	0.006*
Type II	1775 (529)	1740 (1049)	-62 (-316, 191)	0.628
Fiber type proportion (%)				
Type I	52 (15)	65 (14)	-16 (-24, -9)	< 0.001*
Type IIA	32 (12)	23 (11)	10 (4, 16)	0.001*
Type IIX	13 (7)	9 (6)	5 (1, 9)	0.007*
Type IIA/IIX	3 (2)	2 (2)	0.7 (-0.4, 1.9)	0.159
Total RNA (ng/ml)	403 (86)	432 (92)	-24 (-57, 10)	0.168

COPD, participants diagnosed with chronic obstructive pulmonary disease; Healthy, healthy control participants. Data presented as mean (SD), and sex-adjusted estimated mean differences between study clusters (95% CI). Alpha level at $p<0.05$.

Table 3. Comparison of Hallmark gene sets identified in whole-genome transcriptome data between COPD and Healthy, assessed at baseline and as resistance training-associated changes.

Comparison	Gene set	Significance category*	Set size†	Rank *P*-value‡	% MSD > 0§	GSEA *P*-value‖	NES	LE**	Log_2 fold difference in LE (95% CI)
Baseline: COPD vs. Healthy	Oxidative phosphorylation	Consensus	190 (200)	0.007	36.8%	<0.001	-2.10	70 (94.3%)	-0.24 (-0.45, -0.13)
	Myogenesis	Rank	163 (200)	<0.001	33.7%	0.417	1.21	45 (75.6%)	0.46 (0.19, 1.5)
3½ weeks of training: ΔCOPD vs ΔHealthy	Allograft rejection	GSEA	115 (200)	0.956	7.8%	0.014	1.71	20 (35%)	0.39 (0.13, 0.76)
	Oxidative phosphorylation	GSEA	190 (200)	0.999	1.1%	0.009	1.69	83 (2.4%)	0.11 (0.05, 0.39)
	Pancreas beta cells	GSEA	15 (40)	0.969	6.7%	0.028	1.71	3 (33.3%)	0.35 (0.08, 0.54)
Post-RT (13 weeks of training): ΔCOPD vs ΔHealthy	Myogenesis	Consensus	163 (200)	<0.001	42.3%	<0.001	-1.52	68 (85.3%)	-0.5 (-1.13, -0.26)

*, Consensus significance indicates agreement between directional (GSEA) and non-directional (Rank) hypothesis test of overrepresentation (see methods for details). † Indicates number of identified genes in the gene set and total number of genes in the gene set in parentheses. ‡ Rank-based enrichment test, based on minimum significant difference (MSD), identifies gene sets that are overrepresented among top-ranked genes without a directional hypothesis. § Fraction of genes in gene set with unadjusted 95% CI not spanning zero, i.e. MSD > 0. ‖ Gene-set enrichment analysis (GSEA) tests for overrepresentation among top and bottom genes based on Log_2 fold differences or changes × $-log_{10}$(*P*-values) in comparing differences at baseline or changes from baseline between COPD and Healthy. A positive normalized enrichment score (NES) indicate gene set with higher expression in COPD than Healthy; negative NES indicate gene set with lower expression at respective time-points. ** Number of genes in leading edge (LE, genes that contributes to the enrichment score) with the fraction of leading edge genes with unadjusted 95% CI not spanning zero. Δ, change score.

Table 4. Effects of the training intervention on body composition and blood variables in COPD and Healthy, assessed as changes from baseline to after completion of the study (per study cluster) and as differential changes between study cluster.

	COPD			Healthy			Δ COPD vs Δ Healthy
	Baseline	Post RT	Time effect (*P*<0.05)	Baseline	Post RT	Time effect (*P*<0.05)	(*P* value)
Dual-energy x-ray absorptiometry							
Whole-body bone mineral density (g · cm^2)	1.13 (0.21)	1.13 (0.21)	No	1.15 (0.16)	1.14 (0.15)	No	0.119
Total lean mass (kg)	46.7 (9.9)	47.6 (10.2)	Yes ↑	48.1 (10.0)	48.6 (10.0)	Yes ↑	0.395
Appendicular lean mass (kg)	20.3 (5.3)	20.9 (5.5)	Yes ↑	21.6 (5.0)	21.9 (5.0)	Yes ↑	0.166
Total fat mass (kg)	26.4 (11.7)	26.3 (11.5)	No	25.3 (9.3)	24.4 (9.2)	Yes ↓	0.068
Visceral fat (kg)	1.59 (1.18)	1.56 (1.21)	No	1.12 (0.98)	1.01 (0.81)	Yes ↓	0.138
Inflammation							
C-reactive protein (mg · L^{-1})	3.4 (5.0)	3.6 (4.0)	No	1.7 (2.5)	1.8 (3.5)	No	0.934
Hormones							
Cortisol (nmol · L^{-1}) *	307 (130)	310 (109)	No	369 (88)	372 (99)	No	0.861
Growth hormone (µg · L^{-1})	1.4 (2.8)	1.4 (3.1)	No	1.1 (1.7)	1.3 (1.6)	No	0.837
IGF-1 (nmol · L^{-1})	15.7 (4.2)	15.0 (4.5)	No	14.4 (3.2)	13.6 (3.1)	Yes ↓	0.977
Testosterone (nmol · L^{-1})†	11.2 (4.4)	11.4 (4.2)	No	11.9 (3.3)	12.4 (4.2)	No	0.938
Sex-hormone binding globulin (nmol · L^{-1})	60 (33)	60 (34)	No	60 (22)	60 (21)	No	0.488
Androstenedione (nmol · L^{-1})	3.3 (2.4)	3.3 (2.4)	No	3.8 (2.7)	3.8 (2.4)	No	0.984
Parathyroid hormone (pmol · L^{-1})	5.7 (2.6)	6.0 (3.3)	No	5.0 (2.2)	5.2 (1.9)	No	0.870
Lipid profile variables							
Triglycerides (mmol · L^{-1})	1.2 (0.5)	1.1 (0.5)	No	1.2 (0.5)	1.1 (0.6)	Yes ↓	0.661
HDL (mmol · L^{-1})	1.6 (0.6)	1.7 (0.5)	No	1.7 (0.5)	1.7 (0.5)	No	0.523
LDL (mmol · L^{-1}) *	2.8 (1.0)	2.8 (1.0)	No	3.4 (1.0)	3.3 (0.8)	No	0.775
Iron biology variables							
Fe^{2+} (µmol L^{-1})	18 (7)	18 (6)	No	18 (6)	18 (5)	No	0.410
Transferrin (g · L^{-1}) *	2.66 (0.44)	2.67 (0.45)	No	2.41 (0.27)	2.38 (0.29)	No	0.563
Ferritin (µg · L^{-1})	113 (92)	90 (81)	Yes ↓	139 (79)	133 (68)	No	0.089
Calcium status							
Calcium (mmol · L^{-1})	2.4 (0.1)	2.4 (0.1)	No	2.4 (0.1)	2.4 (0.1)	No	0.865
Albumin-corrected calcium (mmol · L^{-1})	2.3 (0.1)	2.3 (0.1)	No	2.3 (0.1)	2.3 (0.1)	No	0.802
Tissue damage variables							
Aspartate transaminase (units · L^{-1})	27 (9)	24 (6)	No	26 (21)	26 (7)	No	0.807
Creatine kinase (units · L^{-1})	112 (69)	123 (71)	No	95 (47)	125 (72)	Yes ↑	0.523

*, significant difference between COPD and Healthy at baseline; †, only men were included in testosterone analysis; ↓, significant decrease from baseline to post RT (after 13 weeks of resistance training); ↑, significant increase from baseline to post RT. Alpha level at $p<0.05$. Data are presented as means (SD).

Table 5. Effects of the training intervention on lung function in COPD and Healthy, assessed as changes from baseline to after completion of the study (per study cluster) and as differential changes between study clusters.

	COPD			Healthy			
	Baseline	**Post RT**	**Time effect p<0.05)**	**Baseline**	**Post RT**	**Time effect (p<0.05)**	**Δ COPD vs Δ healthy (*p*-value)**
FVC (L)	3.3 ± 0.9	3.2 ± 0.9	No	3.6 ± 0.9	3.5 ± 0.8	Yes ↓	0.189
FEV_1 ($L \cdot sec^{-1}$)	1.5 ± 0.4	1.5 ± 0.4	No	2.7 ± 0.7	2.7 ± 0.6	Yes ↓	0.243
FEV_1 (% predicted)	56 ± 11	58 ± 13	No	103 ± 16	103 ± 16	No	0.138
FEV_1/FVC (%)	47 ± 8	48 ± 10	No	75 ± 6	76 ± 6	No	0.714
PEF ($L \cdot sec^{-1}$)	5.0 ± 1.6	5.1 ± 1.6	No	7.8 ± 2.1	7.6 ± 2.2	No	0.238

FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; PEF, peak expiratory flow; Δ, change score. Alpha level at $p<0.05$. Values are means with standard deviation.

Table 6. Effects of the training intervention on health-related quality of life in COPD and Healthy, measured using COPD Assessment Test (CAT; COPD-only) and the 36-item Short Form Health Survey (SF-36; all participants), and assessed as changes from baseline to after completion of the study (per study cluster; CAT and SF-36) and as differential changes between study clusters (SF-36).

	COPD			Healthy			Δ COPD vs Δ Healthy (*P* value)
	Baseline	Post RT	Time effect *P*<0.05)	Baseline	Post RT	Time effect (*P*<0.05)	
COPD assessment Test™ score (0-40)	16.6 ± 6.8	16.4 ± 6.8	No	-	-	-	-
Short Form (36) Health Survey (0-100)							
Physical function *	63 ± 19	67 ± 18	No	90 ± 14	92 ± 12	No	0.321
Role physical *	43 ± 34	59 ± 37	Yes ↑	87 ± 25	94 ± 18	No	0.226
Bodily pain	71 ± 27	82 ± 19	Yes ↑	79 ± 21	80 ± 19	No	0.070
General health *	48 ± 20	56 ± 19	No	75 ± 18	80 ± 12	No	0.208
Vitality *	52 ± 16	57 ± 13	No	72 ± 18	78 ± 11	Yes ↑	0.509
Social function *	74 ± 23	84 ± 16	Yes ↑	90 ± 18	94 ± 13	No	0.280
Role emotional *	65 ± 39	84 ± 26	Yes ↑	93 ± 19	96 ± 15	No	0.059
Mental health *	77 ± 13	84 ± 13	Yes ↑	86 ± 11	89 ± 8	Yes ↑	0.196

*, difference between COPD and Healthy at baseline; ↑, significant increase from baseline to after the training intervention (Post RT). Alpha level at $p<0.05$. Values are means with standard deviation.

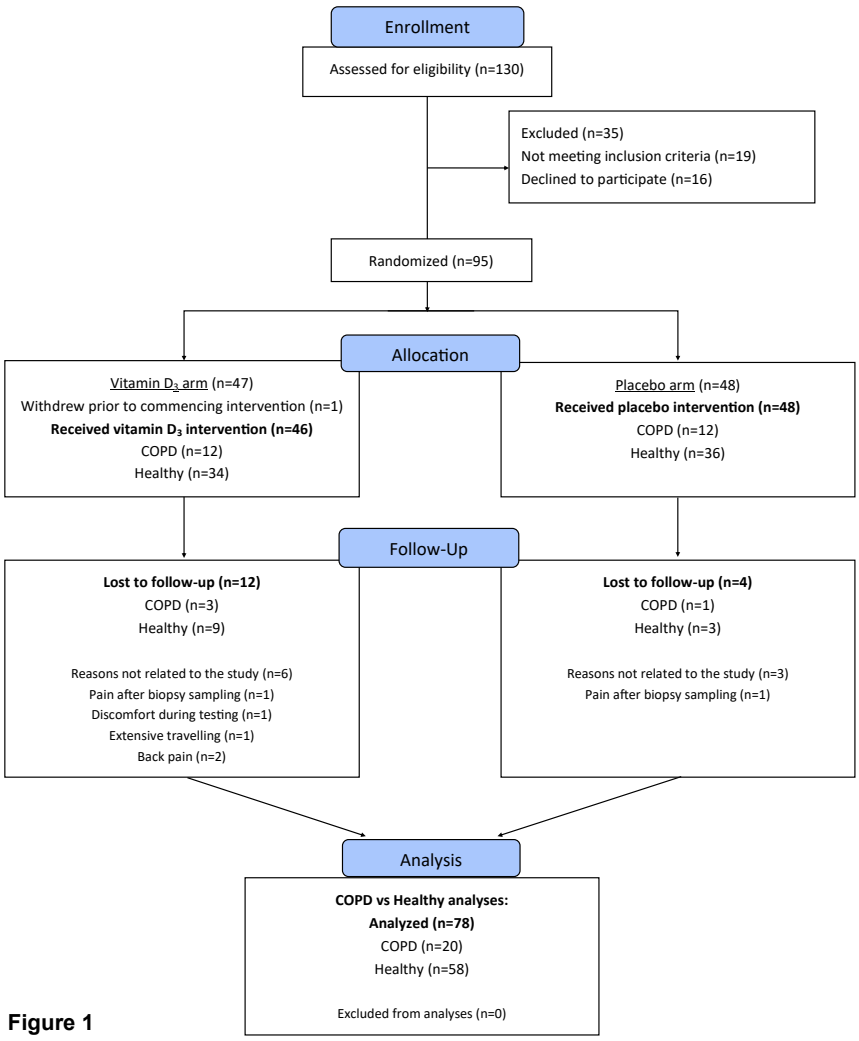
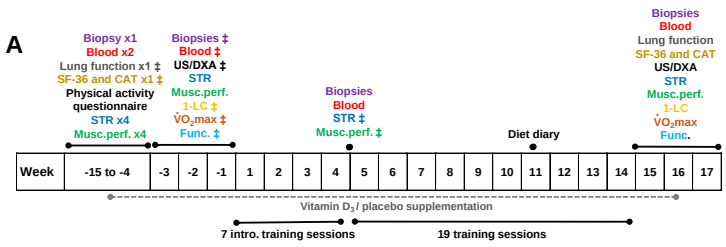
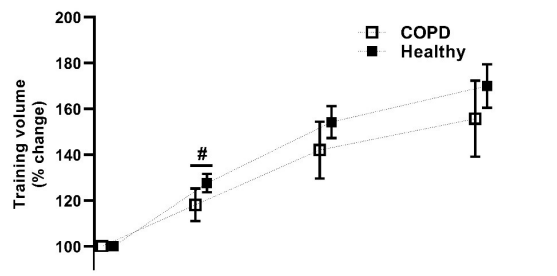

Enrollment
Assessed for eligibility (n=130)
Excluded (n=35)
Not meeting inclusion criteria (n=19)
Declined to participate (n=16)
Randomized (n=95)
Allocation
Vitamin D3 arm (n=47)
Withdrew prior to commencing intervention (n=1)
Received vitamin D3 intervention (n=46)
COPD (n=12)
Healthy (n=34)
Placebo arm (n=48)
Received placebo intervention (n=48)
COPD (n=12)
Healthy (n=36)
Follow-Up
Lost to follow-up (n=12)
COPD (n=3)
Healthy (n=9)
Reasons not related to the study (n=6)
Pain after biopsy sampling (n=1)
Discomfort during testing (n=1)
Extensive travelling (n=1)
Back pain (n=2)
Lost to follow-up (n=4)
COPD (n=1)
Healthy (n=3)
Reasons not related to the study (n=3)
Pain after biopsy sampling (n=1)
Analysis
COPD vs Healthy analyses:
Analyzed (n=78)
COPD (n=20)
Healthy (n=58)
Excluded from analyses (n=0)

Figure 1

A



B



C

Perceived exertion (6-20)				
COPD	15.2 ± 1.0	15.7 ± 1.1	16.4 ± 1.0 *	16.8 ± 1.2 *
Healthy	15.5 ± 1.6	16.1 ± 1.4 *	16.6 ± 1.5 *	17.1 ± 1.2 *

D

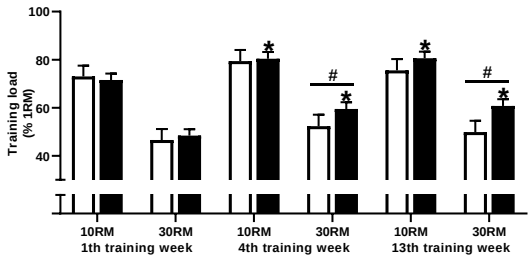


Figure 2

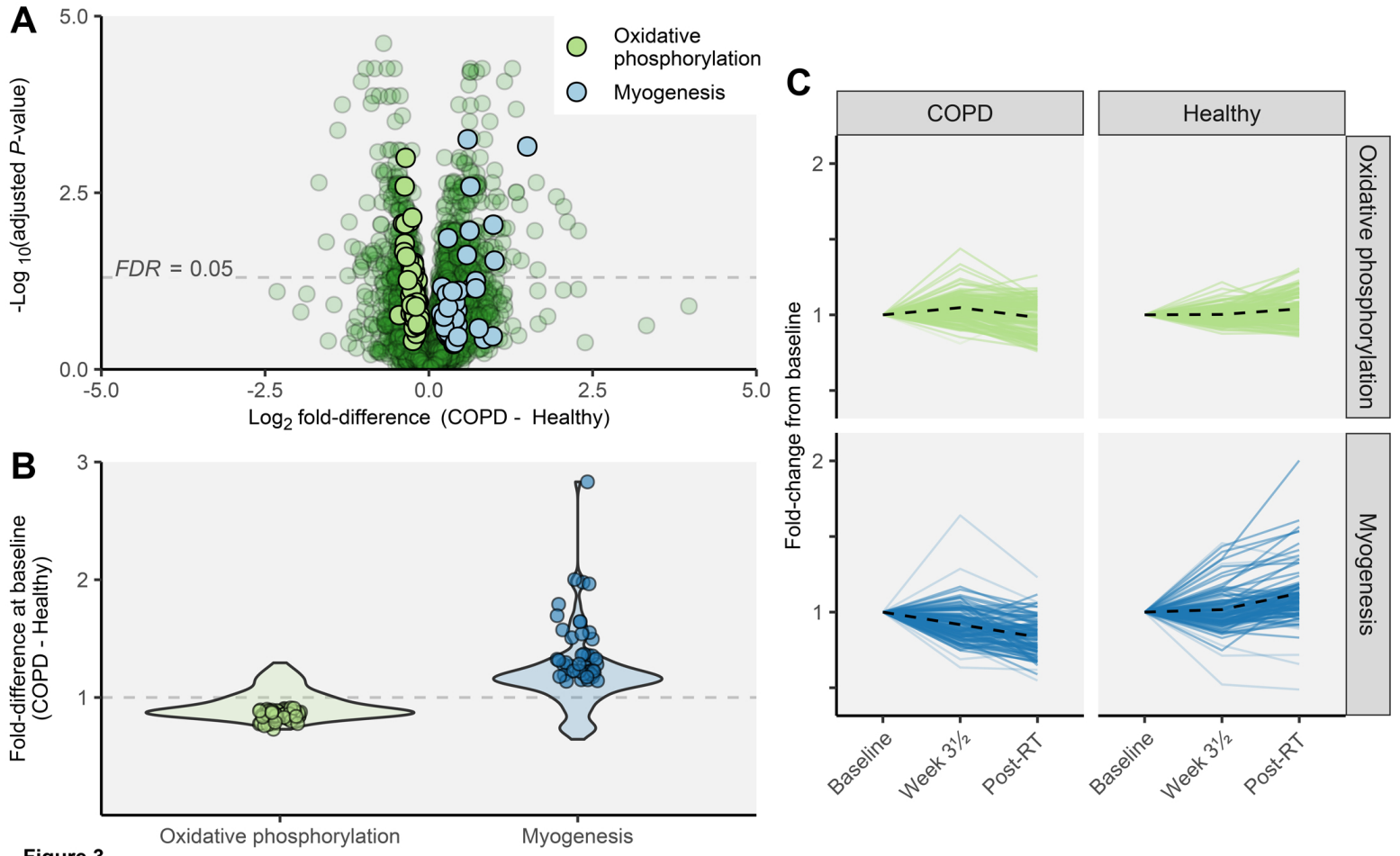

A
5.0
2.5
0.0
-Log 10(adjusted P-value)
FDR = 0.05
-5.0
-2.5
0.0
2.5
5.0
Log2 fold-difference (COPD - Healthy)
Oxidative phosphorylation
Myogenesis
B
3
2
1
Fold-difference at baseline (COPD - Healthy)
Oxidative phosphorylation
Myogenesis
C
COPD
Healthy
Oxidative phosphorylation
Myogenesis
Fold-change from baseline
2
1
Baseline
Week 3½
Post-RT

Figure 3

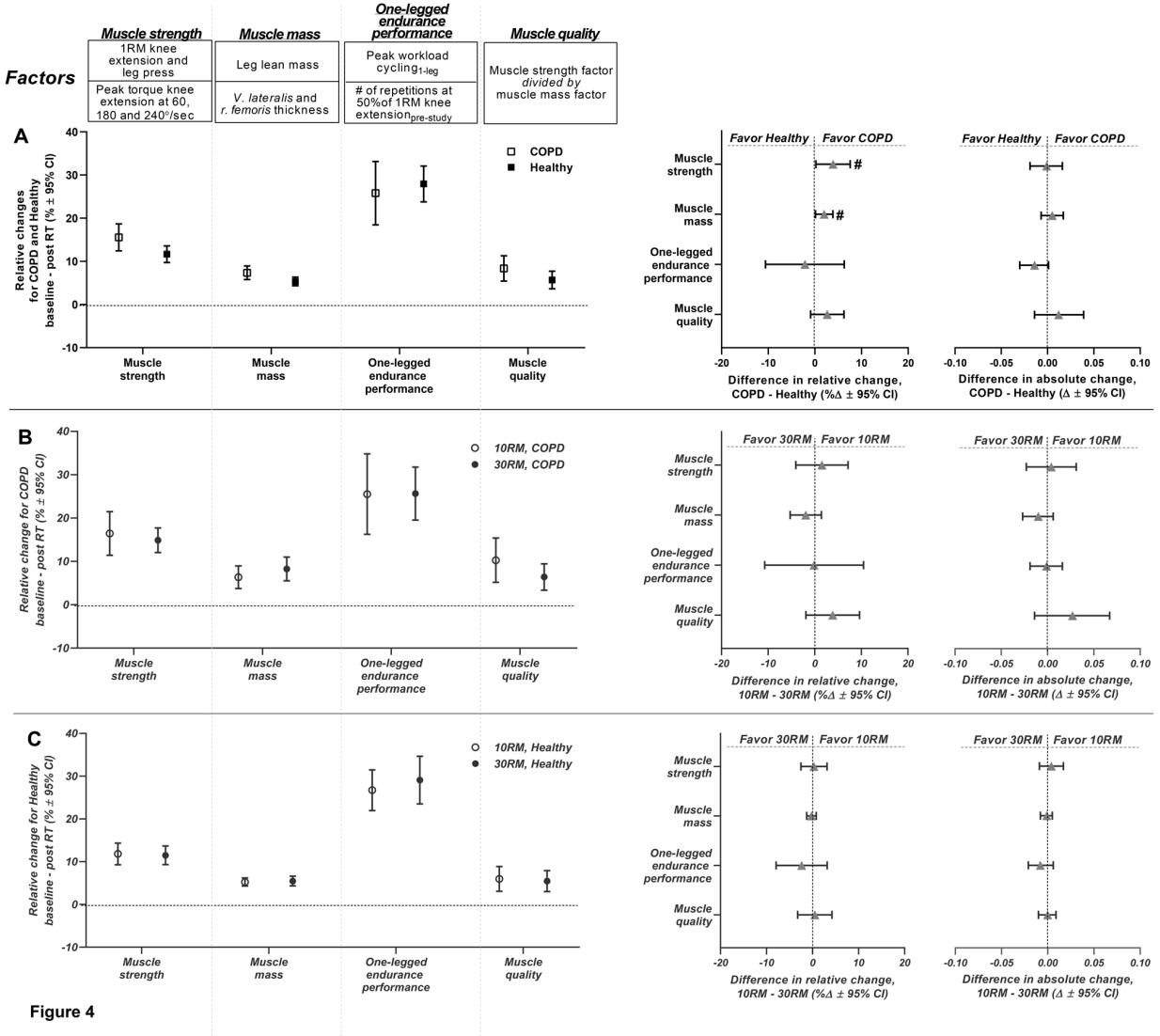

Factors
Muscle strength
1RM knee extension and leg press
Peak torque knee extension at 60, 180 and 240°/sec
Muscle mass
Leg lean mass
V. lateralis and r. femoris thickness
One-legged endurance performance
Peak workload cycling1-leg
# of repetitions at 50%of 1RM knee extensionpre-study
Muscle quality
Muscle strength factor divided by muscle mass factor
A
Relative changes for COPD and Healthy baseline - post RT (% ± 95% CI)
COPD
Healthy
Muscle strength
Muscle mass
One-legged endurance performance
Muscle quality
Favor Healthy
Favor COPD
Difference in relative change, COPD - Healthy (%Δ ± 95% CI)
Difference in absolute change, COPD - Healthy (Δ ± 95% CI)
B
Relative change for COPD baseline - post RT (% ± 95% CI)
10RM, COPD
30RM, COPD
Favor 30RM
Favor 10RM
Difference in relative change, 10RM - 30RM (%Δ ± 95% CI)
Difference in absolute change, 10RM - 30RM (Δ ± 95% CI)
C
Relative change for Healthy baseline - post RT (% ± 95% CI)
10RM, Healthy
30RM, Healthy

Figure 4

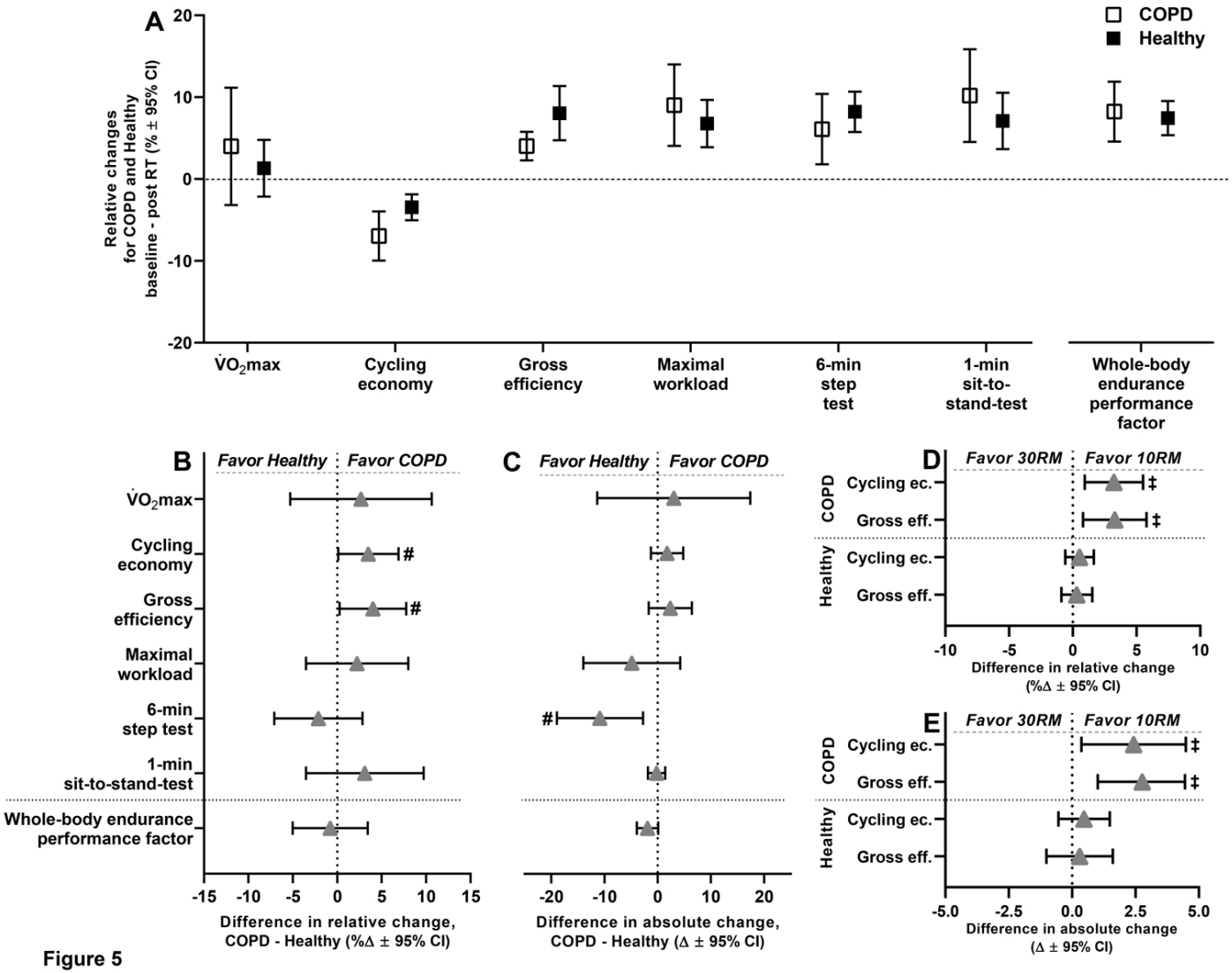

A
COPD
Healthy
Relative changes for COPD and Healthy baseline - post RT (% ± 95% CI)
20
10
0
-10
-20
V̇O2max
Cycling economy
Gross efficiency
Maximal workload
6-min step test
1-min sit-to-stand-test
Whole-body endurance performance factor
B
Favor Healthy
Favor COPD
V̇O2max
Cycling economy
Gross efficiency
Maximal workload
6-min step test
1-min sit-to-stand-test
Whole-body endurance performance factor
#
#
-15 -10 -5 0 5 10 15
Difference in relative change, COPD - Healthy (%Δ ± 95% CI)
C
Favor Healthy
Favor COPD
#
-20 -10 0 10 20
Difference in absolute change, COPD - Healthy (Δ ± 95% CI)
D
Favor 30RM
Favor 10RM
COPD
Cycling ec.
Gross eff.
Healthy
Cycling ec.
Gross eff.
‡
‡
-10 -5 0 5 10
Difference in relative change (%Δ ± 95% CI)
E
Favor 30RM
Favor 10RM
COPD
Cycling ec.
Gross eff.
Healthy
Cycling ec.
Gross eff.
‡
‡
-5.0 -2.5 0.0 2.5 5.0
Difference in absolute change (Δ ± 95% CI)

Figure 5

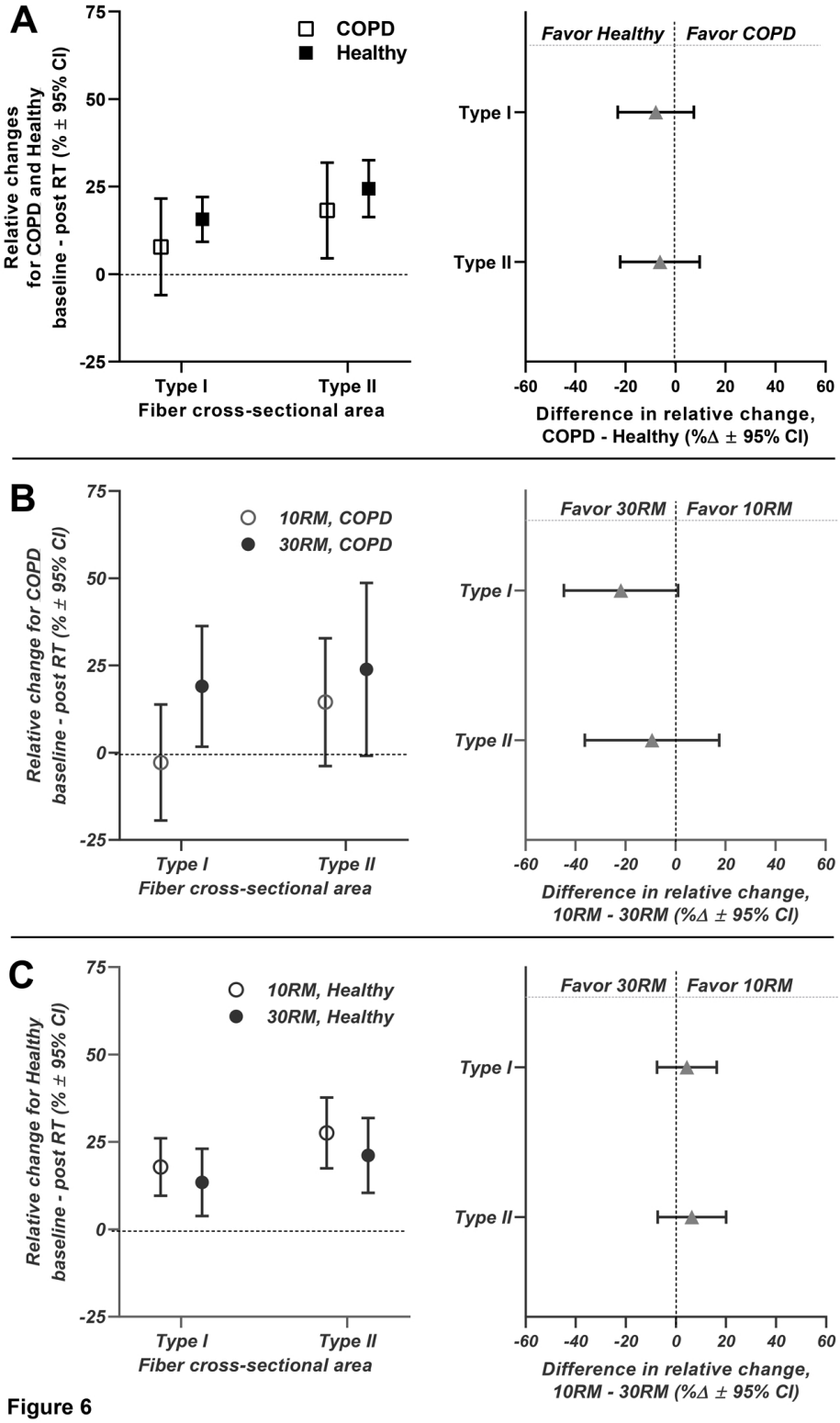

A
75
50
25
0
-25
COPD
Healthy
Relative changes for COPD and Healthy baseline - post RT (% ± 95% CI)
Type I
Type II
Fiber cross-sectional area
Favor Healthy
Favor COPD
Type I
Type II
-60 -40 -20 0 20 40 60
Difference in relative change, COPD - Healthy (%Δ ± 95% CI)
B
10RM, COPD
30RM, COPD
Relative change for COPD baseline - post RT (% ± 95% CI)
Type I
Type II
Fiber cross-sectional area
Favor 30RM
Favor 10RM
Type I
Type II
-60 -40 -20 0 20 40 60
Difference in relative change, 10RM - 30RM (%Δ ± 95% CI)
C
10RM, Healthy
30RM, Healthy
Relative change for Healthy baseline - post RT (% ± 95% CI)
Type I
Type II
Fiber cross-sectional area
Favor 30RM
Favor 10RM
Type I
Type II
-60 -40 -20 0 20 40 60
Difference in relative change, 10RM - 30RM (%Δ ± 95% CI)

Figure 6

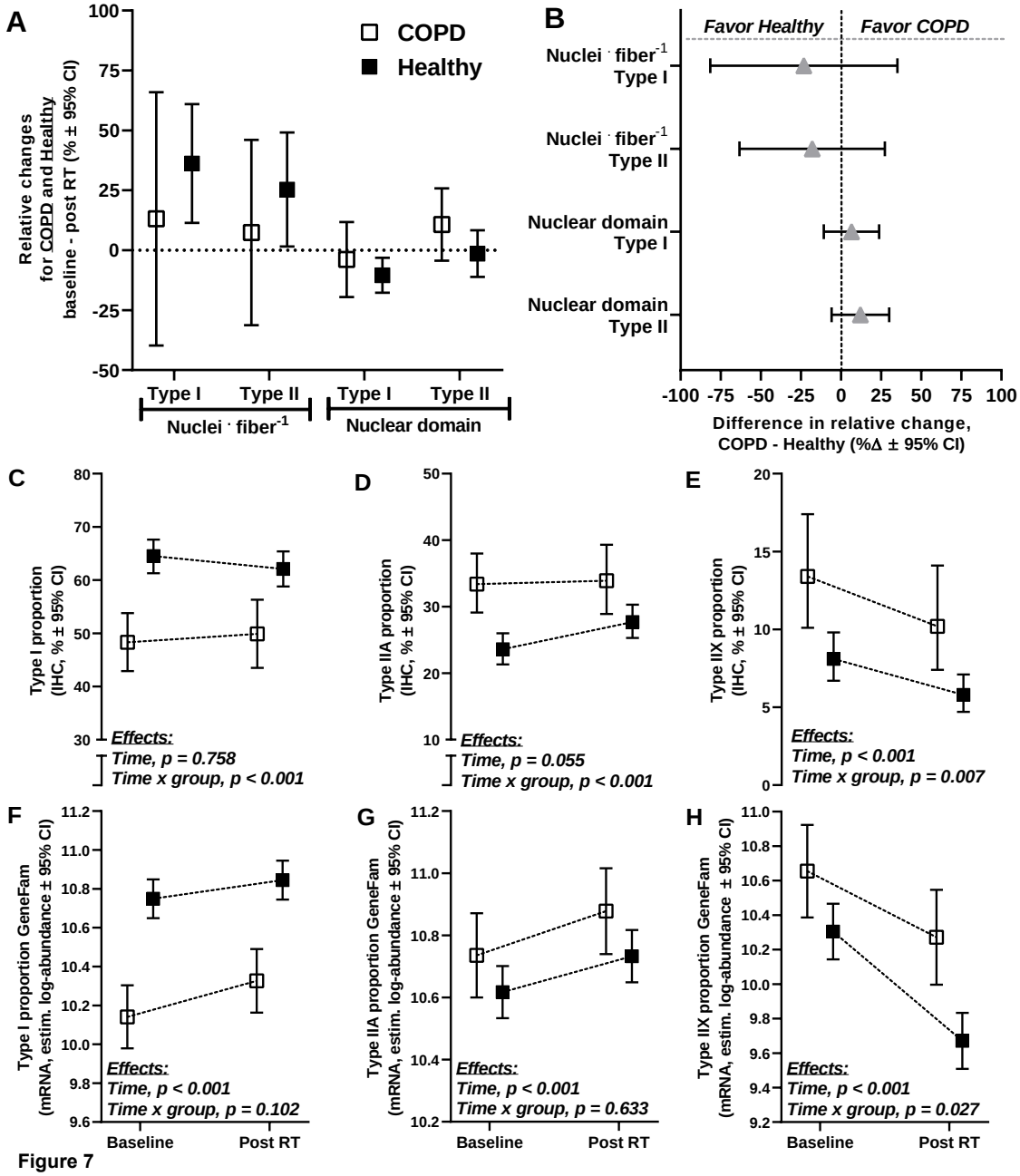

A
COPD
Healthy
Relative changes for COPD and Healthy baseline - post RT (% ± 95% CI)
Type I
Type II
Type I
Type II
Nuclei · fiber-1
Nuclear domain
B
Favor Healthy
Favor COPD
Nuclei · fiber-1 Type I
Nuclei · fiber-1 Type II
Nuclear domain Type I
Nuclear domain Type II
Difference in relative change, COPD - Healthy (%Δ ± 95% CI)
C
Type I proportion (IHC, % ± 95% CI)
Effects:
Time, p = 0.758
Time x group, p < 0.001
D
Type IIA proportion (IHC, % ± 95% CI)
Effects:
Time, p = 0.055
Time x group, p < 0.001
E
Type IIX proportion (IHC, % ± 95% CI)
Effects:
Time, p < 0.001
Time x group, p = 0.007
F
Type I proportion GeneFam (mRNA, estim. log-abundance ± 95% CI)
Effects:
Time, p < 0.001
Time x group, p = 0.102
Baseline
Post RT
G
Type IIA proportion GeneFam (mRNA, estim. log-abundance ± 95% CI)
Effects:
Time, p < 0.001
Time x group, p = 0.633
Baseline
Post RT
H
Type IIX proportion GeneFam (mRNA, estim. log-abundance ± 95% CI)
Effects:
Time, p < 0.001
Time x group, p = 0.027
Baseline
Post RT

Figure 7

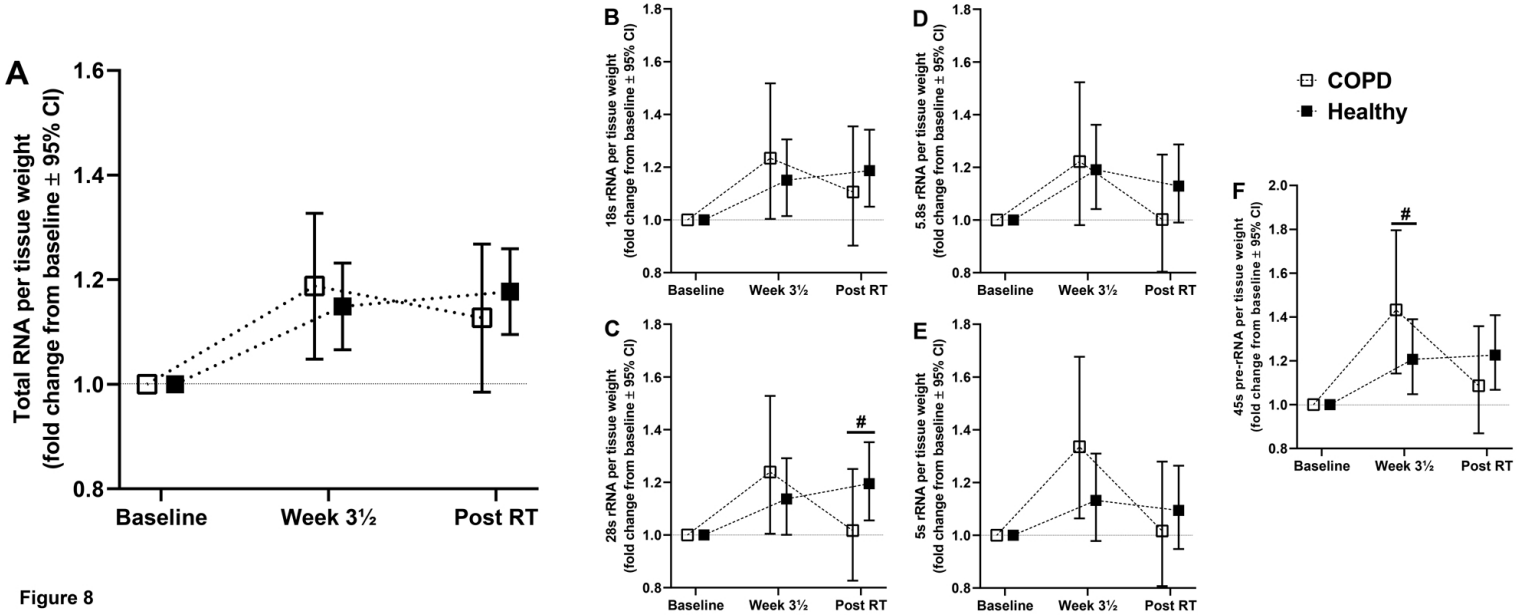

A
Total RNA per tissue weight
(fold change from baseline ± 95% CI)
1.6
1.4
1.2
1.0
0.8
Baseline
Week 3½
Post RT
B
18s rRNA per tissue weight
(fold change from baseline ± 95% CI)
C
28s rRNA per tissue weight
(fold change from baseline ± 95% CI)
#
D
5.8s rRNA per tissue weight
(fold change from baseline ± 95% CI)
E
5s rRNA per tissue weight
(fold change from baseline ± 95% CI)
F
45s pre-rRNA per tissue weight
(fold change from baseline ± 95% CI)
2.0
1.8
COPD
Healthy

Figure 8

Paper IV

Resistance exercise training increases skeletal muscle mitochondrial respiration in chronic obstructive pulmonary disease

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Tables and figures: 7 (8)

References: 34 (40)

The manuscript includes an online data supplement

Abstract

Background: Chronic obstructive pulmonary disease (COPD) is associated with skeletal muscle mitochondrial dysfunction. Resistance exercise training (RT) is a training modality with minimal pulmonary involvement which has been shown to increase skeletal muscle oxidative enzyme activity in COPD. Whether RT also improves mitochondrial respiratory capacity in COPD is yet to be established.

Methods: This study investigated the effects of 13 weeks of RT on m. vastus lateralis mitochondrial capacity in 11 persons with moderate COPD (age: 69 ± 4 years (mean ± SD)) and 12 healthy controls (age: 66 ± 5 years). RT was performed supervised and 2x·week^{-1}. Leg exercises included leg press, knee extension and knee flexion and were performed unilaterally with one leg conducting high-load training (10RM) and the other leg conducting low-load training (30RM). Along one-legged muscle mass, muscle strength and endurance performance, mitochondrial respiratory capacity, citrate synthase (CS) activity, a marker for mitochondrial volume density, and mRNA expression of mitochondrial genes were assessed prior to and after the RT period.

Results: RT led to similar improvements in one-legged muscle mass, muscle strength and endurance performance in COPD and healthy individuals. Mitochondrial fatty acid oxidation capacity and oxidative phosphorylation increased following RT in COPD (+13 ± 22%, p=0.033 and +9 ± 23%, p=0.035, respectively). Marked increases were also seen for mitochondrial volume density (CS activity, +39 ± 35%, p=0.001), which increased more than mitochondrial respiration, leading to lowered intrinsic mitochondrial function (respiration/CS activity) for complex-1-supported respiration (-12 ± 43%, p=0.033), oxidative phosphorylation (-10 ± 42%, p=0.037), and electron transfer system capacity (-6 ± 52%, p=0.027) in COPD. No differences were observed between 10RM and 30RM RT, nor were there any adaptations in mitochondrial function following RT in controls. Transcriptome

analysis revealed differential expression of numerous mitochondrial genes after RT while these changes were similar in COPD and healthy controls.

Conclusions: 13 weeks of RT resulted in augmented mitochondrial respiratory capacity in COPD driven by an increase in mitochondrial quantity and not an improved mitochondrial quality.

Abstract word count: 328 (400)

Key words: Resistance exercise training; Muscle plasticity; Chronic obstructive pulmonary disease; Mitochondrial function

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by persistent airflow limitations that are manifested as dyspnoea and chronic cough [1]. As a consequence, a key pathology of COPD is a reduced aerobic exercise capacity to which in fact also deteriorated skeletal muscle function contributes [2]. Indeed, the reduced whole-body maximal oxygen uptake (VO_{2max}) and the shorter distance covered during a 6 min walking test are partially explained by an attenuated skeletal muscle function [3].

Specifically, reduced quadriceps muscular strength and endurance, as well as increased fatiguability are frequent in COPD [4]. Furthermore, phenotypic traits commonly observed with COPD include lower thigh muscle cross-sectional area, reduced m. vastus lateralis fibre type I and increased fibre type IIx proportion [3, 4]. Skeletal muscle oxidative capacity is diminished, exemplified by decreased m. vastus lateralis oxidative enzyme activity and mitochondrial function [5, 6]. Whereas these traits limit aerobic exercise capacity, they are also known to be improved following exercise training interventions [7, 8], making exercise training highly relevant for COPD rehabilitation [9].

However, due to their pulmonary limitations, individuals with COPD have limited ability to perform whole-body aerobic exercise training at intensities that are required to achieve skeletal muscle adaptations [10]. In accordance with this, more accentuated physiological adaptations were observed when individuals with COPD performed single-limb versus two-limb cycling training, which arguably is related to the lower systemic physiological demands of one-legged exercise, activating less muscle mass [11, 12]. This makes resistance exercise training (RT) a particularly relevant training modality for improving limb muscle function [13]. Indeed, RT allows targeted and maximal exercise of isolated muscle groups without posing large demands on pulmonary ventilation and as such, is more tolerable for persons with COPD [14]. While RT may not be intuitively associated with improvements in aerobic metabolism, some studies have demonstrated a positive effect of RT on

skeletal muscle mitochondrial adaptations in healthy individuals [15]. Moreover, in individuals with COPD, increased citrate synthase (CS) activity and hydroxyacyl coenzyme A dehydrogenase protein levels were reported following eight weeks of low-load high-repetition RT [13]. Thus, RT may provide a stimulus to augment oxidative capacity of skeletal muscles in COPD. Whether that is also reflected in increased skeletal muscle mitochondrial respiration and whether the response is specific to low-load high-repetition RT remains to be elucidated.

The purpose of this study was to determine the effects of 13 weeks of RT on mitochondrial respiratory capacity in m. vastus lateralis (VL) in persons with COPD and to investigate the potential influence of the RT load (low vs. high). Briefly, leg exercises were performed unilaterally, with one leg conducting high-load training and the contralateral leg conducting low-load training. Healthy controls of similar age were included to compare the RT responses between the two populations. We hypothesized that RT would increase mitochondrial respiration in COPD and controls.

Materials and methods

The study was approved by the Regional Committee for Medical and Health Research Ethics - South-East Norway (reference nr. 2013/1094), pre-registered on clinicaltrials.gov (NCT02598830) and conducted according to the guidelines of the Declaration of Helsinki. The present article reports mitochondrial function which was pre-registered as a secondary outcome of The Granheim COPD double-blind randomized clinical trial (NCT02598830). For a thorough description of the study intervention and the assessment of muscle mass, strength and endurance performance, as well as the results for the primary objective of the study, the reader is referred to the main article [16].

Study participants and design

Participants comprised a subset of the individuals enrolled in The Granheim COPD study whose primary objective was to investigate the effects of vitamin D_3 supplementation in combination with RT for RT-associated adaptations [16]. Due to the lack of response to vitamin D_3 supplementation in

general [16], and for mitochondrial parameters in particular (Supplemental figure 1), the vitamin D_3 and placebo group are presented pooled for the purpose of the herein presented analysis. Eleven persons with a clinical diagnosis of stable, moderate COPD (Global Initiative for Obstructive Lung Disease (GOLD) stages II (n=10) and III (n=1), predicted forced expiratory volume in 1 s (FEV_1) between 30%-80% and FEV_1/forced vital capacity <70% after reversibility testing [1]) were included (Table 1). Exclusion criteria were unstable cardiovascular diseases, physically disabling musculoskeletal diseases and intake of steroids. Three patients were current smokers (<10 cigarettes/day). Twelve healthy non-smoking participants of similar age with normal pulmonary function (predicted FEV_1 >80%) served as controls. All participants completed the study in accordance with the study protocol, except for two patients. One withdrew for personal reasons and one was excluded from the analyses due to non-adherence to the RT prescription. All individuals completed a physical activity log during a regular week prior to the intervention and weekly-spent kilocalories were calculated thereof to assess physical activity levels. All measurements were undertaken prior to and following the RT intervention. Study participants received oral and verbal information about the study and provided informed consent prior to participation.

Resistance exercise training

Participants underwent 13 weeks of RT with two supervised sessions·week^{-1} as detailed in [16]. RT consisted of two upper (lat pulldown and chest press) and three lower body resistance exercises (leg press, knee extension and knee flexion) (Technogym, Italy). Lower body exercises were conducted unilaterally, with one leg exercising with low loads (30 repetitions maximum, 30RM) and the contralateral leg exercising with high loads (10 repetitions maximum, 10RM) to volatile exhaustion. Loads were increased from session to session, i.e. when participants managed to perform more than 12 or 35 repetitions per set for 10RM and 30RM, respectively, and were randomly assigned to each leg. The 10RM and 30RM loads were allocated to the same leg during the entire RT period.

Leg lean mass and muscle thickness

Leg lean mass was determined using dual-energy X-ray absorptiometry (Lunar Prodigy; GE Healthcare, USA) and was defined as the region distally of the collum femoris. M. rectus femoris and VL thickness were assessed using B-mode ultrasonography (SmartUs EXT-1M; Telemed, Lithuania) with a 39 mm 12MHz linear array probe as detailed in [16].

One-legged muscle strength and endurance performance, and bicycling aerobic capacity

Maximal muscle strength was determined as one repetition maximum (1RM) in unilateral knee extension (KE) and leg press (Technogym Italy), and KE performance was assessed as the number of repetitions that could be conducted at 50% of baseline 1RM. Unilateral maximal isokinetic KE torque was tested with a dynamometer (Humac Norm; CSMi, USA) at three angular speeds (60°, 120° and 240°·s^{-1}). A one-legged incremental cycling test to exhaustion (Excalibur Sport; Lode BV, The Netherlands) was performed to assess maximal minute power output (W_{max}) and VO_{2max} (JAEGER Oxycon PRO 280; Carefusion GmbH, Germany) for each leg, while one-legged exercise economy was assessed as O_2 cost of submaximal cycling at a constant load. Two-legged W_{max} and VO_{2max} were determined by an incremental bicycling test [16].

Skeletal muscle biopsy

VL biopsies were obtained under local anaesthesia (1% lidocaine) from the 30RM leg at baseline and from both legs after RT using the micro-biopsy procedure [17]. Muscle tissue was dissected free of fat and connective tissue and divided into two parts. One part was immediately placed in ice-cold biopsy preservation medium (BIOPS) [18] for *ex vivo* measurements of mitochondrial respiration. The second part was snap-frozen in isopentane and stored at -80°C for later analysis of CS activity and the transcriptome profile.

High-resolution respirometry

148 The fresh muscle tissue was mechanically dissected and chemically permeabilized as described in
149 [18]. One to four milligrams of permeabilized fibres were added to each respirometer chamber
150 (Oxygraph-2k; Oroboros Instruments Austria) that contained mitochondrial respiration medium 05
151 plus 20 mM creatine and 280 $U \cdot mL^{-1}$ catalase. Chamber oxygen concentration ($nmol \cdot ml^{-1}$) and oxygen
152 flux [$pmol \cdot (s \cdot mg\ wet\ weight)^{-1}$] were recorded (DatLab; Oroboros Austria) at 37°C with the titration of
153 various substrates at saturating concentrations (Table 2). Respiratory states were normalized to CS
154 activity to assess mitochondrial intrinsic respiratory capacity. Samples were analysed in duplicate in
155 hyper-oxygenated chambers ($[O_2]$ ~200-450 $nmol \cdot ml^{-1}$). Prior to the experiment, respirometers were
156 calibrated for instrumental and chemical background oxygen flux [18].

157 *Citrate synthase activity*

158 Muscle samples (0.4 – 5 mg dry weight) were homogenized as detailed elsewhere [19]. Total protein
159 concentrations were determined by BCA assay (Thermo Scientific Pierce, USA). CS activity was
160 assayed in lysates using an assay kit (C3260; Sigma-Aldrich USA). All activities were normalized to mg
161 of protein.

162 *Transcriptome analysis*

163 mRNA transcriptome analysis was performed on a larger number of participants (COPD, n=19;
164 controls, n=34) from the Granheim COPD study [16], as previously described [20]. For these analyses,
165 biopsies taken after 3½ weeks of RT were also included. The Mitocarta v3.0 data set was used to
166 highlight mitochondrial genes [21].

167 *Data analyses and statistics*

168 For a detailed description, see online data supplement. Prior to analyses, data were evaluated for
169 normality and homogeneity of variance and were log-transformed if required. Baseline differences
170 between controls and COPD were examined using linear regression analysis with sex as a covariate.

For one-legged muscle mass, strength and endurance performance, combined factors were computed from singular outcome measures [16]. To address the RT effects on exercise factors and mitochondrial function, linear mixed-effects models were applied. Statistical analysis was performed using the IBM SPSS version 22 (IBM SPSS, Chicaco, IL) and R software (see [20] for packages). Figures were made using Prism Software (GraphPad 8, San Diego, CA, USA). Statistical significance was set to a two-tailed p-value <0.05. Data are presented as mean ± SD.

Results

General characteristics

Six of the controls and four of the individuals with COPD were supplemented with vitamin D_3. The 12-week vitamin D_3 supplementation-only period prior to RT did not affect baseline mitochondrial function (Supplemental figure 1, legend). Likewise, combined vitamin D_3 supplementation and RT did not induce differential alterations in mitochondrial function compared to placebo and RT. There were no differences in age, body mass, body mass index and physical activity level between COPD and controls prior to the intervention (Table 1). Per definition, individuals with COPD showed marked impairments in pulmonary function and displayed lower aerobic exercise capacity compared to the healthy controls.

Muscle mass, strength, and endurance performance

At baseline, one-legged muscle strength and endurance performance were lower in COPD than in controls, while muscle mass tended to be lower (Table 3). Briefly, RT led to similar improvements in muscle mass, strength, and endurance performance in controls and COPD, and the RT mode (10RM vs. 30RM) did not modify these improvements. One-legged cycling VO_{2max} remained similar following RT in controls but tended to be improved in COPD, while the O_2 cost during steady-state one-legged cycling decreased in controls and COPD (Supplemental figure 2). The RT mode did not affect the changes in one-legged VO_{2max} and O_2 cost.

Citrate synthase activity

At baseline, CS activity was 28% lower (p=0.005) in COPD (142.7 ± 36.8 mlU·mg protein^{-1}) than in controls (197.2 ± 40.0 mlU·mg protein^{-1}) (Figure 1). CS activity was not altered following RT in controls (10RM: 220.8 ± 60.0 mlU·mg protein^{-1}, 30RM: 211.4 ± 37.9 mlU·mg protein^{-1}, p=0.365). In COPD, RT led to increased CS activity by 35-43% (10RM: 185.3 ± 30.0 mlU·mg protein^{-1}, 30RM: 197.4 ± 20.6 mlU·mg protein^{-1}, p=0.001), restoring CS activity to healthy levels, though the increase in CS activity in COPD was not significantly higher than the RT-induced changes in CS activity in the controls. The RT mode did not modify the alterations in CS activity in controls or COPD.

Mitochondrial respiratory capacity

In COPD, baseline mass-specific fatty acid oxidation (P_{FAO}), complex-I respiration (P_{CI}), and oxidative phosphorylation (P) were lower (-18%, p=0.022, -20%, p=0.020, and -21%, p=0.018, respectively) and electron transfer system capacity (ETS) tended to be lower (-18%, p=0.056) than in controls, whereas leak respiration (L_N) was similar (-4%, p=0.794) (Figure 2, Supplemental table 1). When respiration was normalized to CS activity (intrinsic mitochondrial function), baseline differences between controls and COPD disappeared, except for a tendency towards higher L_N per CS activity (+20%, p=0.098) in COPD. Also, mitochondrial efficiency to oxidize fatty acids (LCR_{FAO}) was similar (p=0.311) and remained unaltered with RT in COPD and controls (Figure 3, Supplemental table 1). Following RT, L_N, P_{FAO}, P_{CI}, P and ETS, mass-specific or expressed per CS activity, remained unaltered in controls. In COPD, RT led to increased P_{FAO} (+13%, p=0.033) and P (+9%, p=0.035), and tended to lead to increased P_{CI} (+10%, p=0.079) with no differences being evident between RT modes. No alterations were observed for L_N (+7%, p=0.340) and ETS (+11%, p=0.115). Furthermore, in COPD, RT led to reduced mitochondrial respiration/CS activity for P_{CI} (-12%, p=0.033), P (-10%, p=0.037) and ETS (-6%, p=0.027) following RT. RT mode tended to impact this reduction, evident as lower intrinsic P (-11%, p=0.065) and ETS (-13%, p=0.060) in the 10RM leg compared to the 30RM leg after RT.

Mitochondrial genes

At baseline, 78 mitochondrial genes were differentially expressed between controls and COPD (Supplemental table 2); mostly genes associated with cellular metabolism [22]. Specifically, COPD showed lower expression of genes related to carbohydrate, fat and protein metabolism (Table 4). When combining controls and COPD, RT led to marked changes in mRNA levels of mitochondrial genes, with 225 (116 ↑, 109 ↓) and 228 (117↑, 111↓) differentially expressed genes being observed after 3½ and 13 weeks of RT, respectively (Supplemental table 3). However, only one mitochondrial gene, TXNRD2, was differentially affected by 13 weeks of RT between controls and COPD (Supplemental table 2) and no MitoPathway categories were differentially changed, indicating similar mRNA responses to RT in controls and COPD.

Discussion

The main and novel finding of the present study is that m.vastus lateralis mass-specific mitochondrial respiration and oxidative enzyme activity were augmented after 13 weeks of supervised RT in COPD, while remaining unaltered in healthy individuals.

In line with previous studies, we found diminished rates of VL mitochondrial respiration in COPD [5, 7]. Specifically, P_{FAO}, P_{CI}, P, and ETS were 18-21% lower in COPD than in controls, accompanied by decreased mRNA expression of genes involved in fatty acid oxidation and carbohydrate metabolism. Moreover, CS activity was reduced by 28% in COPD, which is also in accordance with previous studies [5, 6, 23, 24]. CS activity is frequently used as a proxy measure for mitochondrial volume density ($Mito_{VD}$), and is also valid for pre-post comparisons following interventions [19]. When expressed per CS activity, the difference in baseline mitochondrial respiration between controls and COPD disappeared. This confirms that intrinsic mitochondrial function is not compromised by COPD, and that the lowered VL respiratory capacity largely results from reduced $Mito_{VD}$, i.e. reduced mitochondrial quantity rather than quality [5, 7]. In support of an intact mitochondrial quality, the

mitochondrial efficiency to oxidize fatty acids was similar in COPD and controls. Intriguingly, previous research has shown that $Mito_{VD}$ is similar between activity-level matched COPD and healthy individuals suggesting that physical inactivity causes the mitochondrial phenotype in COPD [10]. This has recently been challenged [25] and indeed, in the present dataset, the lower $Mito_{VD}$ in COPD could not readily be explained by activity levels, as COPD and controls reported similar physical activity levels prior to RT. This rather indicates that the lowered CS activity was a result of disease-related mechanisms that could involve the long-term exposure of the mitochondria to cellular hypoxia [26], the augmented skeletal muscle oxidative stress, as well as the increased peripheral inflammatory state in COPD [27]. Nonetheless, our results confirm that mass-specific mitochondrial respiratory capacity and oxidative enzyme activity are reduced in COPD.

In COPD, RT successfully normalized CS activity to healthy levels (controls pre: 194.4 ± 42.3 mlU·mg protein^{-1} vs. COPD post: 191.3 ± 25.3 mlU·mg protein^{-1}; 10RM and 30RM pooled), corresponding to a 39% increase from pre to post RT. This increase is similarly scaled to observations made in healthy individuals undergoing endurance exercise training [19, 28]. As such, $Mito_{VD}$ shows responsiveness to chronic exercise training stimuli in COPD [7, 13, 29], although this is not a consistent finding [30, 31]. A previous study failed to observe higher CS content following a low-load high-repetition RT regimen in COPD [30]. With the study population being similar, the differences in the methodology to determine CS (activity versus content) may explain the discrepancy. Lastly, in the present study, RT mode did not affect changes in CS activity in COPD (10RM vs. 30RM POST: - 12.1 ± 22.8 mlU·mg protein^{-1}, p=0.341), which is comparable to previous findings in healthy individuals [32].

The most prominent finding was the increased mass-specific P_{FAO} and P following RT in COPD, as well as the tendency towards increased P_{CI}. The observed 9-13% improvement in mitochondrial respiration was, however, lower than the ~25% increase commonly observed after endurance exercise training in healthy individuals [29], and the 40% increment in P_{CI} previously observed after endurance-like high-intensity KE training in COPD [7]. It could thus be argued that the aerobic

stimulus is more accentuated with endurance-like KE training than with RT. However, we did not observe differences in RT-induced mitochondrial adaptations between 10RM and 30RM training; the latter arguably approximating endurance-like exercise training. Furthermore, there was no effect of RT on CS activity and mitochondrial respiration in the healthy controls, despite the substantial mitochondrial reprogramming implied by changes in the mRNA transcriptome. Hence, although there are indications on the mRNA level that RT may potentially elicit mitochondrial adaptations, this did not translate into improved mitochondrial respiration in the controls. A common view is that RT-induced muscular hypertrophy is more pronounced than the mitochondrial biogenesis with RT which may thus "dilute" the mitochondrial adaptations [15]. In line with this, the observed increase in VL thickness in COPD and control individuals in the present study corresponded to 10% and 9%, respectively, arguably masking an even greater increase in total VL mitochondrial capacity. Importantly, the augmented mitochondrial respiratory capacity in COPD was accompanied by functional improvements induced by RT, e.g. enhanced one-legged muscle endurance performance and reduced submaximal O_2 cost. Whereas these improvements were also present in the controls, the unaltered mitochondrial respiration suggests other mechanisms underlying the enhanced muscle endurance performance in the healthy individuals.

Lastly, when expressed per CS activity, P_{CI}, P and ETS were reduced after RT in COPD, indicating lowered intrinsic mitochondrial function. This is not a unique phenomenon and lowered mitochondrial quality has previously been shown after 2-6 weeks of exercise training in healthy individuals [19, 33]. Altogether, the present findings suggest that in COPD, $Mito_{VD}$ is a key determinant of the increased mass-specific respiratory capacity observed after exercise training, with the increase in CS activity being more pronounced than the increase in mitochondrial respiratory capacity.

Methodological considerations

We did not include a non-exercising COPD control group and disease progression may, theoretically, have complicated the attempt to find more accentuated increases in oxidative capacity. However, this is unlikely as pulmonary function and the score of the COPD assessment test were preserved from before to after the intervention [22]. Furthermore, small sample size and lack of biopsy sampling from the 10RM leg prior to RT reduced the statistical power to find the favourable RT mode. Though, it is important to emphasize that 10RM and 30RM training was randomized to the two legs, ensuring equal distribution of the dominant leg between the RT modes.

Conclusions

The presented evidence suggests that RT is a potent intervention to restore mitochondrial function in COPD in whom the improvement in mitochondrial respiratory capacity was determined by an increased CS activity and not by an augmented quality of the mitochondrion. RT is a well-tolerated, time-efficient and efficacious exercise training mode that induces beneficial alterations in VL oxidative capacity in COPD.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Figure legends

Figure 1. Citrate synthase (CS) activity prior to (PRE) and following 10RM and 30RM (POST) resistance exercise training. Dots illustrate individual values and lines represent mean ± SD. ** p<0.01 between CONTROLS and COPD at PRE, $$ p<0.01 effect of time (PRE vs. 10RM/30RM POST pooled) in COPD. n=8 for CONTROLS, n=6 for COPD.

Figure 2. Mitochondrial respiratory capacity prior to (PRE) and following 10RM and 30RM (POST) resistance exercise training. Mitochondrial O_2 flux per mg of vastus lateralis muscle tissue with titration of malate and octanoyl carnitine (L_N), ADP (P_{FAO}), glutamate and pyruvate (P_{CI}), succinate (P), FCCP (ETS) in CONTROLS and COPD patients (shaded). Dots illustrate individual values and lines represent mean ± SD. * p<0.05 between CONTROLS and COPD at PRE 30RM, $ p<0.05 effect of time in COPD. n=10 for CONTROLS, n=8 for COPD.

Figure 3. Leak control ratio for fatty acid oxidation (LCR_{FAO}) prior to (PRE) and following 10RM and 30RM (POST) resistance exercise training. Dots illustrate individual values and lines represent mean ± SD. No differences were observed between CONTROLS and COPD, nor was there any effect of time. n=10 for CONTROLS, n=8 for COPD.

Tables

Table 1. Baseline characteristics

	CONTROLS	COPD	p-value
Participants (n, females/males)	12 (9/3)	11 (5/6)	
Age (y)	66 ± 5	69 ± 4	0.104
Body mass (kg)	70 ± 12	71 ± 20	0.740
BMI ($kg \cdot m^{-2}$)	24.5 ± 3.4	24.3 ± 6.1	0.946
FEV_1 (L)	2.78 ± 0.66	1.48 ± 0.32	**<0.001**
Predicted FEV_1 (%)	110 ± 16	56 ± 7	**<0.001**
FVC (L)	3.65 ± 0.75	3.08 ± 0.73	0.079
FEV_1 / FVC (%)	76 ± 6	49 ± 7	**<0.001**
VO_{2max} ($L \cdot min^{-1}$)	2.38 ± 0.67	1.54 ± 0.35	**<0.001**
W_{max} (W)	199 ± 46	98 ± 35	**<0.001**
Physical activity level ($kcal \cdot week^{-1}$)	4855 ± 3137	4666 ± 4694	**0.687**

Body mass index (BMI), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), bicycling maximal oxygen uptake (VO_{2max}), maximal minute power output (W_{max}). Data are presented as mean ± SD.

Table 2. Substrate uncoupler titration protocol

Step	Substrate (concentration)	Inner mitochondrial membrane process
1	Malate (2 mM) and Octanoyl carnitine (250 µM)	**L_N**: leak respiration
2	ADP (5 mM)	**P_{FAO}**: fatty acid oxidation
3	Pyruvate (5 mM) and Glutamate (10 mM)	**P_{CI}**: complex-1 linked respiration
4	Succinate (10 mM)	**P**: total oxidative phosphorylation
5	Cytochrome c (10 µM)	Inner mitochondrial membrane integrity
6	FCCP (0.5 - 1 µM steps)	**ETS**: Electron transfer system

(1) Malate and octanoyl carnitine were titrated into the chambers to induce leak respiration through electron entry in absence of ADP and ATP (L_N), (2) ADP to assess mitochondrial capacity to couple electron transport through electron-transferring flavoprotein to the phosphorylation of ADP to ATP (P_{FAO}), (3) pyruvate and glutamate as substrates of complex I to stimulate complex-I-linked respiration (P_{CI}), (4) succinate to determine total oxidative phosphorylation capacity (P) and (5) cytochrome c to test for the integrity of the mitochondrial membrane. Respiratory data which exhibited >10% increase in oxygen flux following cytochrome c titration were not included in data analysis (9.6% of all measurements). (6) Maximal electron transfer system capacity (ETS) was determined with the addition of the uncoupler carbonylcyanide p-trifluoromethoxyphenyl-hydrazone (FCCP). The leak control ratio for fatty acid oxidation (LCR_{FAO}) was computed as L_N/P_{FAO} indicating mitochondrial efficiency to oxidize fat.

Table 3. Muscle mass, strength, and endurance performance prior to (PRE) and following (POST) resistance exercise training in CONTROLS (n=12) and COPD (n=11)

	PRE				Condition	POST				CONTROLS Time	COPD Time	Time x Condition
	CONTROLS		COPD		P-value	CONTROLS		COPD		P-value	P-value	P-value
	10RM	30RM	10RM	30RM		10RM	30RM	10RM	30RM			
Leg lean mass (kg)	7.63±2.48	7.47±2.41	7.32±2.13	7.28±2.10		7.41±2.10	7.37±2.26	7.34±2.19	7.37±2.24			
M. vastus lateralis thickness (mm)	19.50±2.26	19.85±3.42	18.65±3.02	19.94±4.88		21.07±3.29	21.71±3.02	20.20±2.92	21.94±4.94			
M. rectus femoris thickness (mm)	13.01±2.73	13.13±2.72	10.61±2.36	11.06±3.33		14.20±3.38	14.30±2.91	11.62±1.83	12.89±3.66			
One-legged muscle mass factor (AU)	**0.59±0.14**		**0.54±0.12**		**0.052**	**0.62±0.15**		**0.57±0.12**		**0.010**	**0.001**	**0.404**
1RM KE (kg)	19±7	20±7	15±7	15±7		22±7	22±8	20±8	19±8			
1RM leg press (kg)	111±25	107±27	96±42	97±40		159±56	165±51	146±59	139±62			
Peak torque during KE (Nm)												
60 °·s^{-1}	113±30	119±33	101±31	101±41		121±34	126±35	111±42	114±39			
180 °·s^{-1}	73±23	69±21	63±20	64±26		78±21	77±19	68±27	74±26			
240 °·s^{-1}	62±18	58±18	55±19	59±24		63±18	63±16	59±20	62±23			
One-legged muscle strength factor (AU)	**0.46±0.13**		**0.40±0.14**		**0.001**	**0.52±0.13**		**0.46±0.17**		**<0.001**	**<0.001**	**0.577**
One-legged W_{max} (W)	126±29	127±31	60±20	60±20		131±29	135±30	70±23	70±19			
KE performance (Rep)	16±3	16±3	14±5	15±5		28±7	28±9	15±5	23±7			
One-legged endurance performance factor (AU)	**0.35±0.07**		**0.19±0.05**		**<0.001**	**0.40±0.08**		**0.24±0.06**		**<0.001**	**<0.001**	**0.860**
One-legged VO_{2max} (L·min^{-1})	1.92±0.46	1.95±0.50	1.31±0.29	1.24±0.25	**<0.001**	1.95±0.50	2.01±0.49	1.32±0.27	1.33±0.25	0.233	0.079	0.873
Δ% One-legged O_2 cost						-5±6	-7±6	-13±4	-9±4	**<0.001**	**<0.001**	0.122

The factor *One-legged muscle mass* is composed of leg lean mass, m. vastus lateralis and m. rectus femoris thickness. *One-legged muscle strength* includes 1RM KE and leg press and the peak torque for knee extension at 60, 180 and 240° s^{-1}. The factor *One-legged endurance performance* is composed of values from maximal workload achieved during one-legged cycling and the number of repetitions at 50% of baseline 1RM KE. For each singular outcome measure, each participants' values (pre and post) were normalized to the participant with the highest value recorded during the study, resulting in individual scores ≤1. Thereafter, outcome domain factors were calculated as the mean of the normalized values for each variable for each participant and averaged between both legs (10RM and 30RM). One-legged VO_{2max} and O_2 cost were not included in any factors and are displayed individually. Workload to assess O_2 cost was defined as 50% W_{max} and was therefore different between individuals, but constant for each individual PRE and POST; CONTROLS: 50 ± 10 W, COPD: 26 ± 2 W. Arbitrary unit (AU), knee extension (KE), one repetition maximum (1RM), repetitions (Rep), maximal oxygen uptake (VO_{2max}), maximal minute power output (W_{max}). Data are presented as mean ± SD.

Table 4. MitoPathway analysis of genome-wide transcriptome data comparing CONTROLS (n=34) and COPD (n=19) at baseline

MitoPathway	Significance category[a]	Rank P-value[b]	GSEA P-value[c]	NES
Carbohydrate metabolism	Consensus	0.070	0.033	-1.66
Fatty acid oxidation		0.070	0.005	-1.77
Amino acid metabolism	GSEA	0.141	0.087	-1.58
Branched-chain amino acid metabolism		0.229	0.050	-1.64
Metabolism		0.229	0.007	-1.54
Metals and cofactors		0.563	0.087	-1.53
Oxidative phosphorylation		0.960	0.087	-1.50

[a] Consensus indicates agreement between directional (GSEA) and non-directional (Rank) hypothesis test of over-representation (when p-values <0.1 for both analyses; see online supplemental data for details). [b] Rank-based enrichment tests identify MitoPathways that are over-represented among top-ranked genes without a directional hypothesis. [c] Gene-set enrichment analysis (GSEA) tests for over-representation among top and bottom genes based on $\log_2$ fold-differences × $-\log_{10}$(p-values) when comparing baseline differences between COPD and CONTROLS. A negative normalized enrichment score (NES) indicates a MitoPathway with lower expression in COPD compared to CONTROLS. P-values are adjusted for false discovery rate.

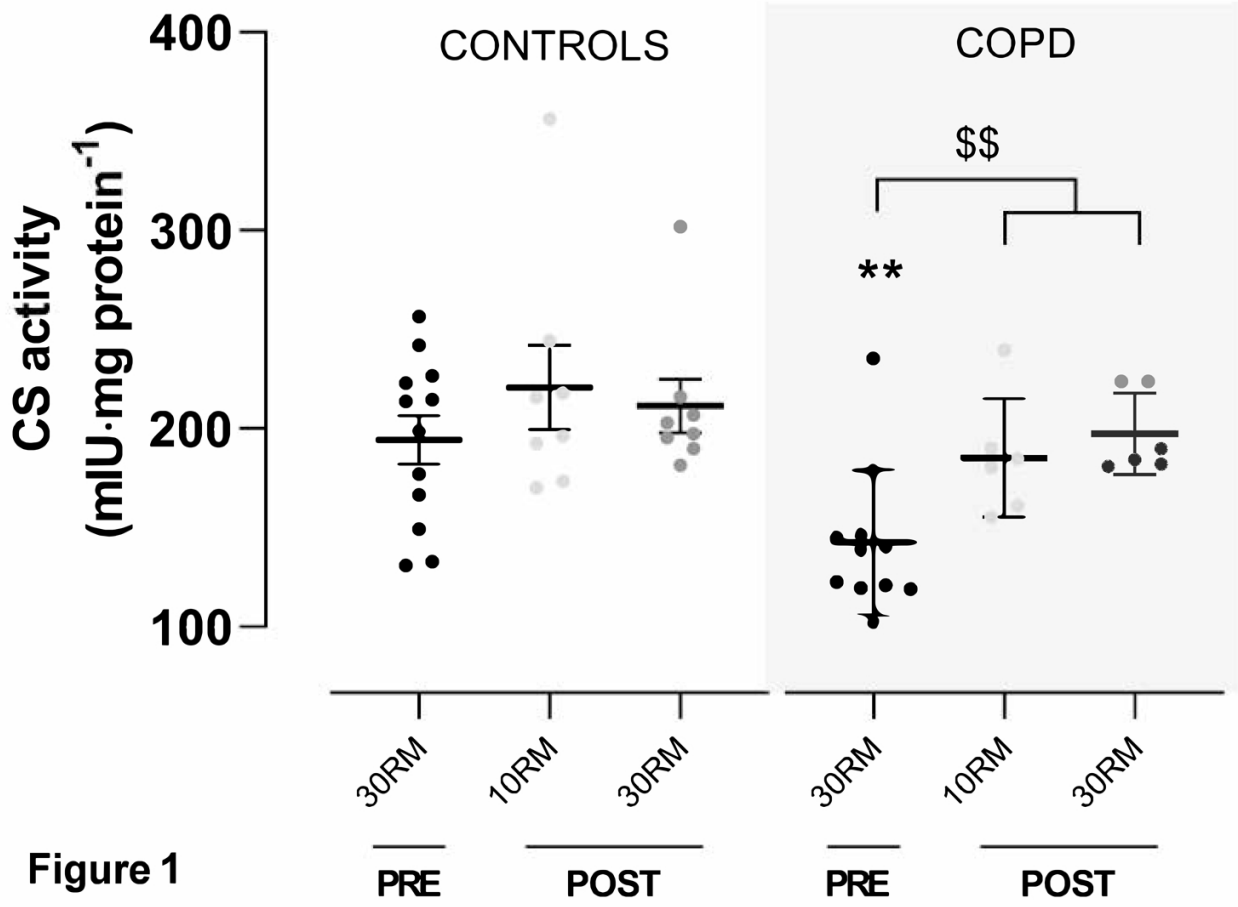
400
300
200
100
CS activity
(mIU·mg protein-1)
CONTROLS
COPD
$$
**
30RM
10RM
30RM
30RM
10RM
30RM
PRE
POST
PRE
POST

Figure 1

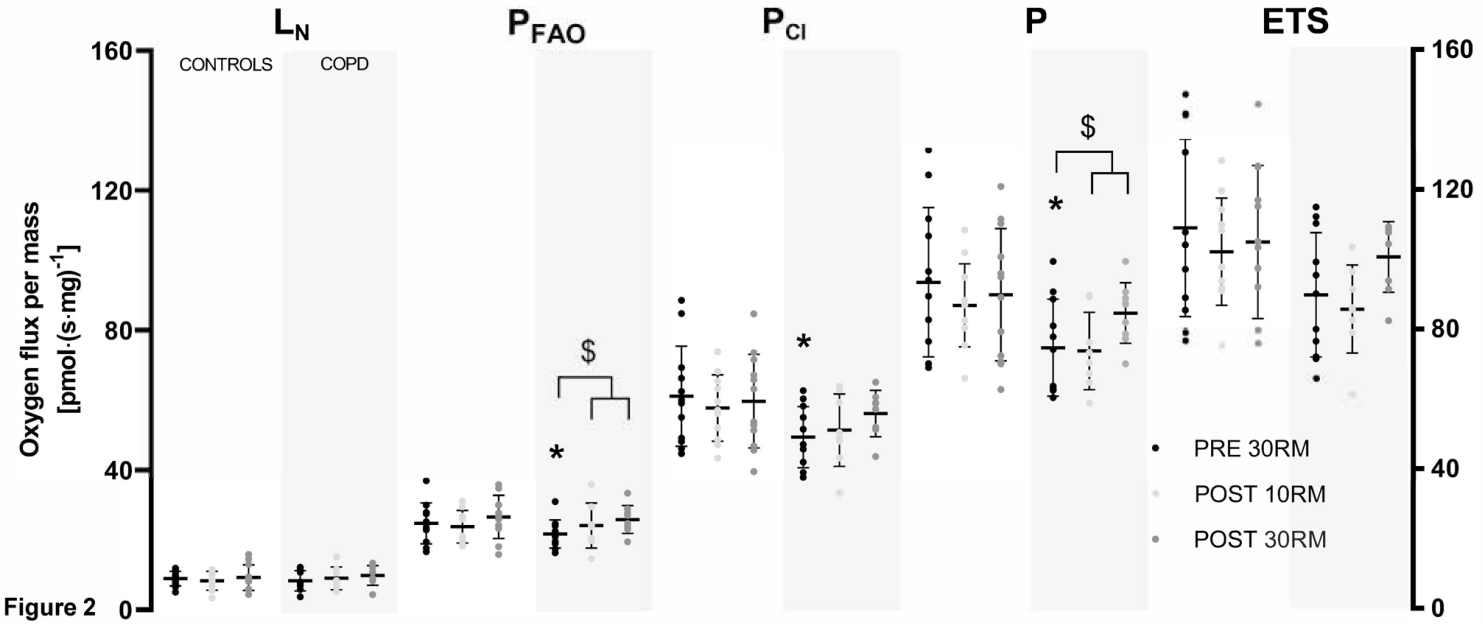
L_N
P_{FAO}
P_{CI}
P
ETS
CONTROLS
COPD
Oxygen flux per mass [pmol·(s·mg)$^{-1}$]
160
120
80
40
0
*
$
PRE 30RM
POST 10RM
POST 30RM

Figure 2

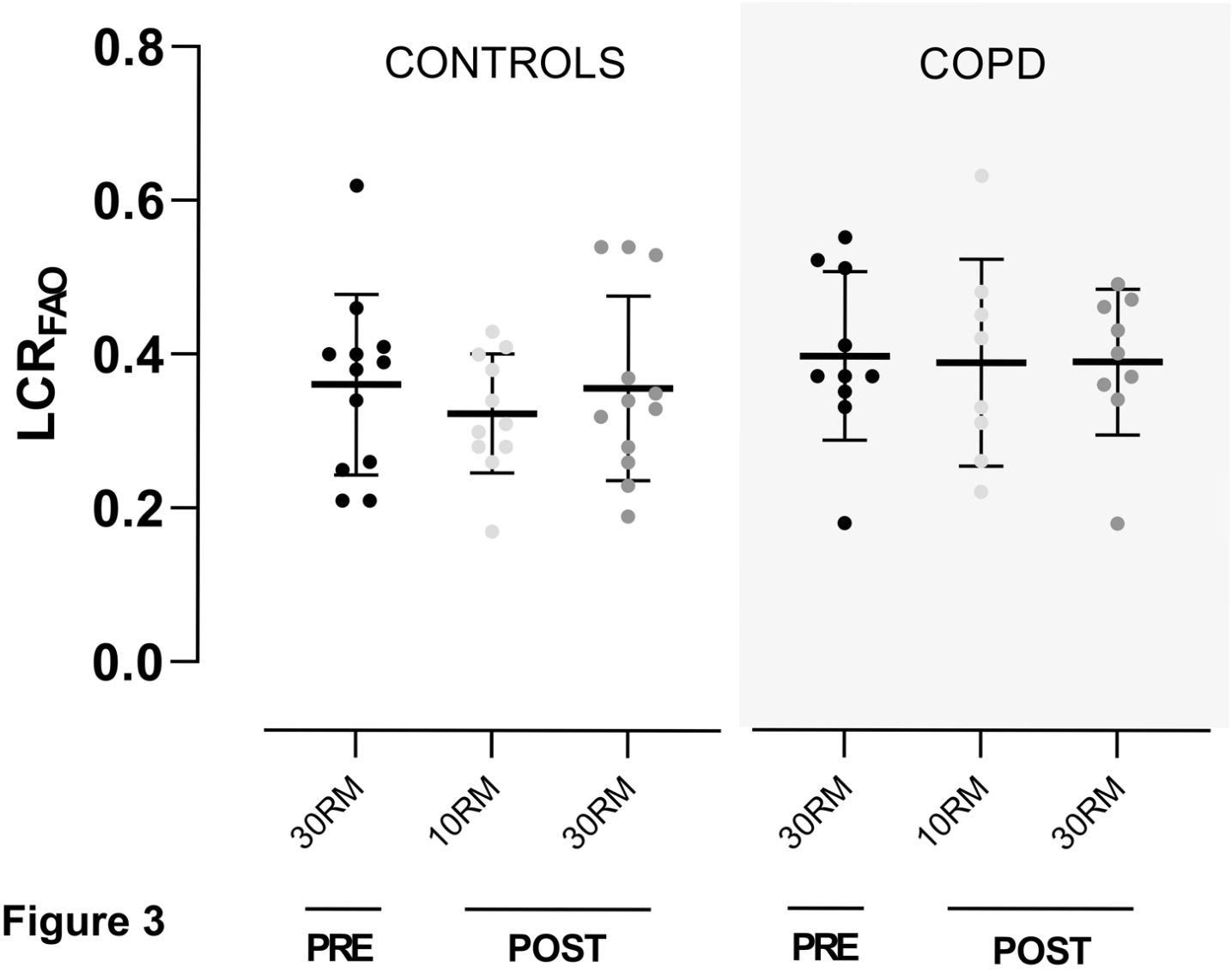
0.8
0.6
0.4
0.2
0.0
LCR$_{FAO}$
CONTROLS
COPD
30RM
10RM
30RM
30RM
10RM
30RM
PRE
POST
PRE
POST

Figure 3

Appendix I

A qualitative paper (manuscript in Norwegian)

En kvalitativ analyse av motivasjonsfaktorer for styrketrening ved kronisk obstruktiv lungesykdom: erfaringer fra The Granheim COPD Study

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Sammendrag

Bakgrunn: Regelmessig styrketrening gir gunstige helseeffekter for personer med kronisk obstruktiv lungesykdom (KOLS), og er derfor en naturlig del av lungerehabiliteringen. Pasienters opplevelse av slik trening er imidlertid lite studert. Hensikten med denne kvalitative studien var å kartlegge erfaringer med styrketrening hos deltakere i The Granheim COPD Study.

Materiale og metode: Åtte av 24 prosjektdeltakere med KOLS (kvinner/menn, n=3/n=5; KOLS grad II/III, n=4/n=4; alder, 64-79 år) gjennomførte semistrukturerte, kvalitative intervjuer i den femte av totalt 13 uker med styrketrening.

Resultater: Samlet for alle prosjektdeltakerne med KOLS var treningsadherensen høy (97%) og frafallet lavt (n=2 av 22) under treningsperioden. Informantene som gjennomførte kvalitative intervjuer opplevde nokså høy og stigende grad av motivasjon for å trene under intervensjonen. Dette var knyttet til tett personlig oppfølging fra treningskyndig personell og opplevelse av trygghet under treningsøktene, samt økt mestringsfølelse og økt kompetanse.

Fortolkning: Personlig veiledning fra treningskyndig personell var en avgjørende faktor for å øke treningsmotivasjonen blant studiedeltakerne med KOLS. Individuell tilrettelegging og oppfølging ser derfor ut til å være en forutsetning for å oppnå gode aktivitetsvaner hos personer med KOLS.

Abstract

Background: Regular resistance exercise provides beneficial health effects for people with chronic obstructive pulmonary disease (COPD), and constitutes a natural component of lung rehabilitation programs. However, the patients' personal experience with such training remains largely unstudied. The purpose of this qualitative study was to map experiences with resistance training in participants enrolled in The Granheim COPD Study.

Material and method: Eight out of 24 study participants with COPD (women/men, n=3/n=5; COPD grade II/III, n=4/n=4; age, 64-79 years) conducted semi-structured, qualitative interviews during the 5th week of a total 13 weeks of resistance training.

Results: Overall, for all study participants with COPD, the training adherence was high (97%), with a concomitant low dropout rate (n=2 of 22) during the training period. The informants experienced a fairly high and increasing level of motivation for exercise training during the intervention. This was related to close personal follow-up from experienced personnel and a sense of security during training sessions, as well as an increased feeling of self-efficacy and competence.

Interpretation: In subjects with COPD, the increasing motivation for conducting resistance training was closely associated with personal guidance from experienced supervisors during training sessions. Close follow-up from qualified personnel thus seems to be a prerequisite for achieving beneficial physical activity habits among such patients.

Introduksjon

Kronisk obstruktiv lungesykdom (KOLS) er en økende folkehelseutfordring, både i Norge og i verden forøvrig. Sykdommen er av de vanligste årsakene til sykehusinnleggelser og død[1] og er assosiert med store menneskelige og sosioøkonomiske konsekvenser, deriblant høyt arbeidsfravær og tidlig pensjonering.[2] Diagnostiseringen av KOLS baserer seg på lungefunksjonsmål,[3] men KOLS er også knyttet til en rekke tilleggslidelser som blant annet større risiko for å utvikle fysiske lidelser som overvekt, diabetes, hjertesvikt og koronare hjertesykdommer,[4] og mentale lidelser som angst og depresjon.[4] I sum bidrar dette til at KOLS-rammede har lavere livskvalitet enn friske, jevnaldrende personer.[5,6]

Til tross for at det er sterk evidens for at fysisk aktivitet og trening er gunstig for personer med KOLS, er fysisk inaktivitet vanligere hos KOLS-rammede enn hos friske personer.[7] Fysisk aktivitet og trening er den eneste rehabiliteringsformen som kan bedre prognosen. Det reduserer tungpustethet, bedrer arbeidsevnen og følelse av at man har kontroll på sykdommen, samt forbedrer andre aspekter knyttet til sykdommen som for eksempel emosjonelle funksjoner og helserelatert livskvalitet.[8,9] Viktigheten av gode aktivitetsvaner har blitt tydeligere de siste årene. Dette har medført at interesseorganisasjonene European Respiratory Society og American Thoracic Society har definert fysisk trening som «grunnsteinen innen lungerehabilitering».[10] Likevel viser det seg å være vanskelig å endre aktivitetsvanene til KOLS-rammede i retning av en mer aktiv livsstil. Årsakene til dette kan se ut til å være relatert til begrenset erfaring med slik type aktivitet, og at man av den grunn kan oppleve fysisk trening som ukjent og uviktig.[11] Manglende informasjon om fysisk aktivitet kan også være en potensiell utfordring, noe som ble trukket frem som hovedårsaken til at KOLS-rammede ikke var tilstrekkelig fysisk aktive i en dansk studie.[12]

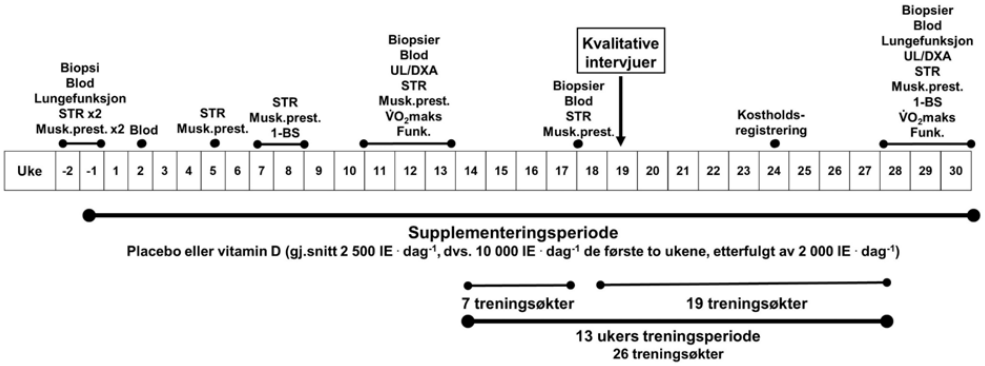
I The Granheim COPD Study ble 24 personer med KOLS forespurt å delta i en kombinert kostsupplementerings- og treningsintervensjon, hvor hovedformålet var å undersøke de funksjonelle og biologiske effektene av styrketrening med og uten daglig tilskudd av vitamin D. For et utvalg av disse studiedeltakerne ble det gjennomført kvalitative intervjuer for å undersøke hvilke motivasjonsfaktorer som var avgjørende for at de meldte sin interesse for studien og hva som påvirket treningsmotivasjonen underveis i intervensjonen.

Materiale og metode

For detaljert beskrivelse av studieprotokollen og metoder benyttet i The Granheim COPD Study, samt resultatene knyttet til vitamin D-perspektivet i studien, se Mølmen m.fl.[13] For en oversikt over de funksjonelle og biologiske treningseffektene til de KOLS-rammede sammenlignet med de lungefriske kontrollene i studien, se Mølmen m.fl.[14] Studien var godkjent av Regional etisk komité – sørøst (referansenr. 2013/1094), forhåndsregistrert hos clinicaltrials.gov (ClinicalTrials.gov ID: NCT02598830) og ble gjennomført i henhold til *Helsinkideklarasjonen*.

Deltakerne i studien ble rekruttert via oppslag på Granheim Lungesykehus og lokale legekontorer, nyhetsartikler i lokalavisen (Gudbrandsdølen Dagningen) og annonser på Høgskolen i Innlandets digitale plattformer. Totalt ble 95 personer inkludert i studien (KOLS-

rammede, n=24; lungefriske kontroller, n=71), hvorav 78 fullførte studien (KOLS-rammede, n=20; lungefriske kontroller, n=58). Samtlige KOLS-rammede bodde innenfor en radius på 55 km fra Høgskolen i Innlandet – studiested Lillehammer, hvor intervensjonen og datainnsamlingen ble gjennomført. For grov oversikt over studieprotokollen, se Figur 1.



Figur 1. Studieprotokoll for The Granheim COPD Study. Kvalitative intervjuer ble gjennomført i uke 19, sammenfallende med deltakernes 9. treningsøkt. STR, test av maksimal styrke; Musk.prest., test av muskulær prestasjon; 1-BS, ettbeins sykkeltest; Funk., test av funksjonell kapasitet (6-minutts step-test og 1-minutts sit-to-stand-test); UL, ultralydmåling av muskeltykkelse; DXA, test av kroppssammensetning; $\dot{V}O_2$maks, test av maksimalt oksygenopptak; IE, internasjonale enheter.

Kvalitative intervju. Semistrukturerte, kvalitative intervjuer ble gjennomført på åtte KOLS-rammede studiedeltakere (Tabell 1). Dette representerte samtlige personer med KOLS som på tidspunktet for de kvalitative intervjuene (26. - 27. februar 2018) var i studiens treningsperiode. Intervjuene varte i ca. 30 minutter og ble gjennomført før eller etter den 9. treningsøkten i studien. Alle intervjuene ble gjennomført av den samme rutinerte intervjueren. Vedkommende var ikke tilknyttet prosjektet på annet vis og kjente ikke informantene fra før. Alle intervjuene fulgte den samme intervjuguiden (se vedlegg), men rekkefølgen på spørsmålene var ikke konsekvent. Spørsmålene ble stilt på en slik måte at det oppmuntret informantene til å reflektere, samt gi fyldige kommentarer. Det ble ikke gjort notater under intervjuene, men det ble gjort lydopptak som i ettertid ble transkribert. Intervjuene ble analysert ved hjelp av systematisk tekstkondensering.[15] Alle lydopptak, transkribert materiale og kodede analyser ble slettet i etterkant av analyse.

Treningsintervensjonen. Treningsintervensjonen i The Granheim COPD Study ble gjennomført ved Høgskolen i Innlandet – studiested Lillehammer. Den varte i totalt 13 uker (Figur 1, uke 14 – 27) og bestod av to ukentlige treningsøkter. All trening ble gjennomført under veiledning fra studenter under utdanning i bachelorprogrammet i helse og treningsfysiologi ved Høgskolen i Innlandet. Treningsinstruktørene hadde oppfølging av én eller to deltakere samtidig. Instruktørene ble rullert mellom deltakerne, slik at ingen deltakere skulle ha den samme treningsinstruktøren på alle sine treningsøkter. Treningen var identisk for KOLS-rammede og de lungefriske kontrollene, og bestod av et helkropps styrketreningsprogram, der samtlige øvelser ble gjennomført med høy grad av anstrengelse. For å redusere tungpustethet ble beinøvelsene gjennomført med ett bein av gangen. Treningsprogrammet var definert i forkant, men enkelte individuelle hensyn ble tatt. Disse inkluderte justering av

treningsbelastning underveis i økter og på tvers av økter, pauselengde mellom treningssett og -øvelser, teknikktilbakemeldinger og grad av oppmuntring. Det var et uttalt fokusområde i studien å etablere en sosial og hyggelig ramme rundt treningssituasjonen. Dette inkluderte blant annet å tilrettelegge for at ektepar og venner kunne trene sammen, at flere deltakere trente samtidig i lokalet, at flere treningsinstruktører var tilstede under hver treningsøkt, og etablering av en møteplass før og etter treningsøkter, der deltakerne og treningsinstruktørene kunne ta seg en kaffekopp og en matbit før hjemreise.

Resultater

Bakgrunnsinformasjon om informantene og årsaker til at de meldte sin interesse for studien. Seks av informantene var pensjonister, mens to var i fast arbeid under studieintervensjonen. Samtlige av informantene hadde tidligere røyket sigaretter på daglig basis (Tabell 1), men alle utenom én hadde sluttet fullstendig. Vedkommende som fremdeles røyket sigaretter, hadde likevel redusert tobakksforbruket kraftig de siste årene. Ingen av informantene hadde særlig erfaring med styrketrening før inklusjon i studien, og benyttet seg ei heller av andre treningstilbud på jevnlig basis.

Tabell 1. Deskriptiv informasjon om informantene

Kvinner/menn (antall)	3/5
Alder (gj.snitt år ± SD)	71 ± 5 (64 – 79)
KMI (gj.snitt kg · m^{-2} ± SD)	26 ± 4 (19 – 32)
Forventet FEV$_1$ (gj.snitt % ± SD)	51 ± 9 (36 – 61)
FEV$_1$/FVC (gj.snitt % ± SD)	45 ± 10 (35 – 58)
Pakkeår (gj.snitt ± SD)	33 ± 16 (8 – 59)

I parentes, variasjonsbredden. SD, standardavvik; KMI, kroppsmasseindeks; FEV$_1$, forsert ekspiratorisk volum på ett sekund; FVC, forsert vitalkapasitet; pakkeår, ett pakkeår tilsvarer 20 sigaretter · dag^{-1} i ett år.

Samtlige informanter rapporterte at KOLS-sykdommen hadde utviklet seg gradvis, og at de hadde tilpasset seg sykdommen etter hvert som symptomene meldte seg. Disse inkluderte livsstilsendringer som ble ansett som hensiktsmessige for å holde sykdommen under kontroll, herunder røykeslutt/redusert tobakksforbruk og regelmessig fysisk aktivitet. Samtlige informanter hadde dermed en positiv innfallsvinkel til fysisk aktivitet, og flertallet var i fysisk aktivitet daglig (f.eks. hus- og hagearbeid og gåturer). De fleste meldte sin interesse for The Granheim COPD Study fordi de anså studieintervensjonen som et fornuftig tiltak for å nå målet om fortsatt selvstendighet i eget hjem og daglige aktiviteter. Disse observasjonene står i kontrast til tidligere kvalitative studier, som fremhever at KOLS-rammede anser fysisk aktivitet og trening som uviktig og et lite effektivt middel for å forbedre helsen.[11,16] Det er derfor rimelig å anta at informantene i studien ikke var representative for alle KOLS-rammede, men at de var godt informert om de positive effektene av trening, og i tillegg opplevde det som meningsfullt å benyttes seg av dette tilbudet.

Frafall, adherens og treningseffekter. Av de 24 KOLS-rammede som var innrullert i The Granheim COPD Study, fullførte 20 deltakere hele studieintervensjonen (83%). To deltakere trakk seg før treningsintervensjonen startet (Figur 1, før uke 18), mens ytterligere to trakk seg underveis i treningsintervensjonen. Årsakene til frafallene var henholdsvis ikke-relatert til studien (n=2), smerter etter muskelbiopsi (n=1) og for lang reisevei (n=1). Samtlige deltakere som deltok i kvalitative intervjuer fullførte studien.

De 20 deltakerne med KOLS som fullførte studien utførte treningsprogrammet som forespeilet. De gjennomførte 97% av alle treningsøktene (for informantene, 99,5%) og viste solide effekter av treningen. Dette var synlig som større eller sammenlignbar økning i muskelstyrke, muskelmasse, funksjonsmål og andre helsemål sammenlignet med lungefriske kontroller.[14]

Faktorer som påvirket treningsmotivasjonen

Viktigheten av fagkompetanse hos treningsinstruktører og organiseringen av studien. Informantene var opptatt av hvordan studien var organisert, og la vekt på at trening med personlig instruktør var en avgjørende årsak til at de meldte sin interesse for studien. Det ble fremhevet som viktig at det var rom for individuelle tilpasninger, til tross for at alle deltakerne fulgte det samme treningsopplegget, samt at det var viktig med individuelle, konstruktive tilbakemeldinger under hver treningsøkt. Det ble trukket frem at instruktørene var gode til å tilpasse opplegget hvis informantene hadde dårlige dager. Én av informantene fortalte om at engstelsen for å presse seg og oppleve tungpustethet ble redusert gjennom individuelle tilbakemeldinger og støtte fra treningsinstruktør. Informantene følte at treningsinstruktørene hadde gode kunnskaper om styrketreningsfaget og at de derfor fikk svar på det de lurte på av faglig interesse. I tillegg ble det trukket frem at treningsinstruktørene hadde gode relasjonelle ferdigheter og fremsto som hyggelige mennesker, noe som var medvirkende til at informantene opprettholdt motivasjonen til å gjennomføre treningen.

> *«Studentene er motiverende og spør om jeg klarer én repetisjon til. De tar hensyn hele tiden, men de ser på deg om du greier mer eller ikke. De er flinke sånn.»*

Studien innebar mye testing og mange muligheter til å betrakte endringer i egne prestasjoner over tid. Dette ble trukket fram som positivt for motivasjonen. Muskelbiopsiene som ble tatt underveis i prosjektet opplevdes som ubehagelige og vonde for enkelte av informantene, og ble trukket frem som det de likte minst i prosjektet. Det var likevel forståelse for at dette var en viktig del av studien.

> *«Det er veldig artig å se hvor man ligger. Jeg synes testing er interessant for å se om det faktisk er fremgang, og ikke bare hva jeg synes selv.»*

Flere av informantene fortalte at de følte en viss forpliktelse til å møte til trening og testing. De opplevde at de som deltakere var en viktig del av studien, og at det alltid var instruktører som ventet på dem eller forventet at de skulle komme. Dersom man ikke møtte opp, visste deltakerne at man ville bli oppringt og etterspurt. Det ble påpekt at man med et

mindre forpliktende opplegg mest sannsynlig ville hatt større frafall og lavere adherens til treningen.

«Det går greit når jeg har fått et bestemt klokkeslett for trening, men hvis jeg ikke har det utsetter jeg det til i morgen, og når morgendagen kommer utsetter jeg til dagen derpå.»

Studien innebar ikke særskilt tilrettelegging for KOLS-rammede deltakere. Deltakerne ble ikke på noe vis informert om hvem som hadde KOLS eller hvem som var lungefriske. Informantene opplevde dette som positivt, og de følte seg behandlet som «vanlige mennesker» istedenfor pasienter. De fortalte at de ikke ønsket å bli identifisert som «han eller hun med KOLS». Informantene fremhevet også at det var lite snakk om sykdom i prosjektet, og at dette var behagelig, uten at det gikk på bekostning av tilrettelegging. Prosjektets omgivelser ved Høgskolen i Innlandet – studiested Lillehammer ble også trukket frem som positivt, med gode kollektivtransportalternativer og parkeringsmuligheter for bil. At prosjektet ble gjennomført på en høgskole istedenfor et sykehus ble omtalt som gunstig, siden sykehus generelt minnet informantene om «diagnoser, behandling, sykdom og dårlige nyheter».

Informantene beskrev det sosiale miljøet i studien som godt. Det var rom for å snakke, le og spøke sammen, og studiens daglige leder og instruktører hadde alltid tid til å slå av en prat. Informantene var trygge i styrketreningssettingen og var ikke redde for hverken å gjøre feil, trene hardt eller tyne sine fysiske grenser. Det opplevdes som meningsfullt å være med i prosjektet.

«Det er viktig å ha veileder; hvis du trener bare for deg selv er det ikke sikkert at du gidder å ta de to siste repetisjonene.»

Mestringsopplevelser ved styrketrening. Det å holde sykdommen under kontroll var informantenes hovedmotivasjon for å melde seg til The Granheim COPD Study. Likevel ble det fremhevet at de var usikre på om de kunne forvente seg særlige forbedringer i egen helse. De fortalte at motivasjonen for å trene ikke var så fremtredende ved oppstart av studien, men at den ble større etterhvert som de opplevde fremgang. Informantene oppgav både kvantifiserbare og opplevde kroppslige forbedringene som viktige motivasjonsfaktorer for å fortsette å trene i treningsperioden.

«Jeg kan gjøre ting nå som jeg ikke kunne før, for eksempel å gå opp ei trapp med ti trappetrinn. Det er motivasjon.»

Informantene oppgav at det å oppleve god utførelse av treningsøvelsene ga god mestringsopplevelse og følelse av økt kompetanse. Disse opplevelsene ble forsterket gjennom positive tilbakemeldinger fra treningsinstruktører. Til tross for dette varierte motivasjonen for å trene fra dag til dag, og informantene fortalte at de noen ganger hadde lyst til å holde seg hjemme. De møtte likevel opp på grunn av forpliktelsen de følte til studien, samt vissheten om at treningsøkten kunne justeres etter dagsform. Informantene opplevde at det var god balansegang mellom det å bli støttet i sine plager og utfordringer og det å bli utfordret til å ta et steg videre.

«Det er hardere å trene her enn på andre treningssteder fordi de (instruktørene) presser oss til vi ikke klarer mer, men jeg synes det er meget positivt.»

Alle informantene fortalte at de ikke hadde klart å trene like hardt og med like høy intensitet på egen hånd. Dette var først og fremst på grunn av tilstedeværelse av instruktør som kunne motivere til å ta i litt ekstra og som ga følelse av trygghet under treningen, og i mindre grad på grunn av mangel på egnet utstyr. Flere av informantene var skeptiske til om de ville klare å fortsette å trene strukturert etter studien, mest fordi treningen ikke ville bli sett på som like forpliktende.

«For meg er det lettere å trene sammen med flere og til bestemte klokkeslett. Når du driver på aleine tar du det når det passer deg, og da er det ikke sikkert du gjør det hver dag. Her er det godt organisert.»

Diskusjon

Studien viser at fagkompetansen til treningspersonellet var viktig for at studiedeltakerne med KOLS skulle føle seg trygge nok til å utfordre sine egne fysiske grenser under styrketreningsøktene. Dette var en forutsetning for de positive mestringsopplevelse og den økte kompetansen som informantene opplevde underveis i treningsperioden. I sum bidro dette til at de økte sin treningsmotivasjon.

Informantene meldte seg til studien fordi de anså fysisk aktivitet og trening som et gunstig middel for å holde sykdommen under kontroll og fortsatt være selvstendig i eget hjem og daglige aktiviteter. Motivasjonen for å være med var dermed primært knyttet til målet om å løse personlige utfordringer. Forankret i *selvbestemmelsesteorien*,[17] noe som kan antyde at motivasjonen var *ytre, identifisert regulert*,[18] siden de betraktet deltakelse i The Granheim COPD Study som et fordelaktig tiltak for å nå sitt eget, personlige mål. De hadde sågar en historikk med andre personlige livsstilstiltak for å begrense negativ utvikling av sykdommen, herunder gjennomføring av livsstilsendringer som for eksempel røykeslutt/redusert tobakksforbruk og regelmessig fysisk aktivitet. Dette støtter oppunder at informantene hadde en identifisert-regulert motivasjon, men også at informantene hadde høy grad av *opplevelse av sammenheng* (engelsk, *sense of coherence*), definert som opplevelse av deres situasjon som forståelig og forklarlig (*comprehensible*), med tro på at de hadde ressurser til å finne løsninger på problemer som oppstod (*manageable*), og opplevelse av at det var meningsfullt å forsøke å finne disse løsningene (*meaningful*).[19] Informantenes nivå av forståelse for situasjonen og endringsvilje skiller dem fra KOLS-rammede i tidligere studier, som snarere anså fysisk aktivitet og trening som et lite effektivt behandlingstiltak.[11,16] Det er dermed betimelig å spørre seg om utvalget av KOLS-rammede og betydningen av dataene er valide for hele sykdomspopulasjonen, eller om betydningen er begrenset til '*KOLS-rammede med selvbestemt treningsmotivasjon'*. Dette må imidlertid sies å være et generelt kjennetegn for treningsintervensjoner, siden umotiverte eller typisk eksternt-regulert motiverte personer vil ha større risiko for frafall fra slike programmer og oppleve større vanskeligheter med å opprettholde atferd.[20,21]

Informantene ga uttrykk for at de utviklet en mer mangfoldig motivasjon i løpet av den relativt korte treningsperioden frem til intervju (~5 uker). Denne bar preg av økt selvfølelse, mestringsfølelse, kompetanseheving og personlig tilfredstillelse, drevet frem av positive erfaringer og opplevelse av kvantifiserbare, kroppslige forbedringer, i tillegg til

opplevelse av forpliktelser ovenfor forskningsprosjektet. Dette understøttes av den betydelige forbedringen i kvantitativ helserelatert livskvalitet observert hos de KOLS-rammede deltakerne i løpet av studieperioden for kategoriene *generell* og *mental helse*, samt *emosjonell, sosial* og *fysisk funksjon*.[14] Motivasjonen utviklet seg dermed fra å handle om å unngå uønskede konsekvenser av sykdommen, til å i større grad handle om tilfredsstillelse i form av positive opplevelser fra studieintervensjonen. Styrketrening under tett oppfølging førte simpelthen med seg opplevelser og endringer som i seg selv var motiverende. I et teoretisk perspektiv innebærer den observerte endringen i motivasjon en dreining mot mer internalisert ytre motivasjon og sågar større grad av indre motivasjon. Det er imidlertid lite som tyder på at informantene nådde den mest autonome formen for motivasjon. Denne kjennetegnes av at aktiviteten har blitt en vane, er engasjerende, og en viktig del av personens identitet, og er sannsynligvis gunstig for å klare å opprettholde atferd i et lengre tidsperspektiv.[21]

Informantene rapporterte at fem uker med styrketrening hadde selvforsterkende effekter på motivasjon for å drive denne typen aktiviteter. Dette kunne primært knyttes til to hovedmomenter: i) tett individuell oppfølging fra treningskyndig personell under alle treningsøkter, og ii) opplevelse av mestring, både knyttet til utførelse av treningsarbeidet og hverdagslivet forøvrig. Dette er effekter som også er rapportert tidligere. Sosial støtte og personlig oppfølging har vist seg å ikke bare ha stor betydning for å oppnå høy treningsadherens og unngå frafall,[22] men også føre til større funksjonelle forbedringer enn ikke-veiledet trening.[23] Årsakene til den forbedrede motivasjonen er flere, men informantene trakk blant annet frem det sosiale miljøet i prosjektet som positivt. De følte seg trygge og ivaretatt, samtidig som det føltes som meningsfullt å være med i prosjektet. Instruktørene, i kraft av å være treningsfysiologer under utdanning, med god kompetanse innen både styrketreningsteori og å utvikle og motivere deltakere gjennom relasjonelle ferdigheter, viste seg å ha en avgjørende rolle. De klarte å ivareta de grunnleggende psykologiske behovene, som ifølge selvbestemmelsesteorien er gunstig for å forbedre motivasjonen,[18] noe som medførte at informantene følte seg trygge i treningssettingen. Dette la grunnlaget for at de kunne utfordre seg selv, og dermed oppleve økt mestringstro og kompetanse i form av at de i økende grad mestret øvelser, nye situasjoner og nye ting i hverdagen (f.eks. gå opp en trapp uten pauser).[18,24]

Informantene opplevde imidlertid studieintervensjonen som forpliktende ovenfor instruktørene og studien som helhet, og pekte på at dette var medvirkende for den gode treningsadherensen. Flere av informantene reflekterte rundt at det ville bli vanskelig å opprettholde treningsrutinene etter studien, siden treningen da ikke ville bli sett på som like forpliktende. Dette tyder på at informantene til en viss grad også var ytre, introjekt-regulerte i sin motivasjon, noe som ikke blir sett på som gunstig for langvarig opprettholdelse av motivasjon.[18] Oppfølgingssamtaler av informantene i etterkant av studien kan også tyde på at motivasjonen og atferden ikke var tilstrekkelig internalisert og integrert ved studiens slutt. I oppfølgingssamtaler gjennomført 2-3 år etter intervensjon rapporterte ingen av informantene at de hadde fortsatt med regelmessig styrketrening. Årsakene til dette kan være at treningsintervensjonen var for kort til å oppnå varige atferdsendringer. Fire ukers lungerehabiliteringsprogram har tidligere konkludert i samme retning.[25] En annen årsak kan være at de kontrollerte betingelsene ved et slikt forskningsprosjekt knyttet til den fastsatte

protokollen som må gjennomføres, kan tenkes å gå på bekostning av den optimale individuelle tilnærmingen for å tilrettelegge for selvstendig, internalisert og integrert opprettholdelse av endret adferd.[26] Dette antyder, iallfall for denne gruppen personer, at regelmessig, personlig oppfølging også etter en slik type livsstilsintervensjon er gunstig og nødvendig for å opprettholde ønsket atferd og motivasjon.

Konklusjon. Tett, individuell oppfølging fra treningskyndig personell la til rette for at studiedeltakere med KOLS følte seg trygge og kunne utfordre seg selv under styrketreningsøktene. Dette la grunnlaget for positive mestringsopplevelser, økt kompetanse, forbedret mestringstro og mer internalisert motivasjon.

Hovedfunn. Fysisk inaktivitet og lav treningsmotivasjon er vanlig blant KOLS-rammede. Dette fører til økt forekomst av tilleggslidelser og redusert livskvalitet. Tett, individuell oppfølging fra treningskyndig personell viste seg å ha en gunstig innvirkning på faktorer bestemmende for treningsmotivasjon.

Denne artikkelen har ett vedlegg:

- Intervjuguide

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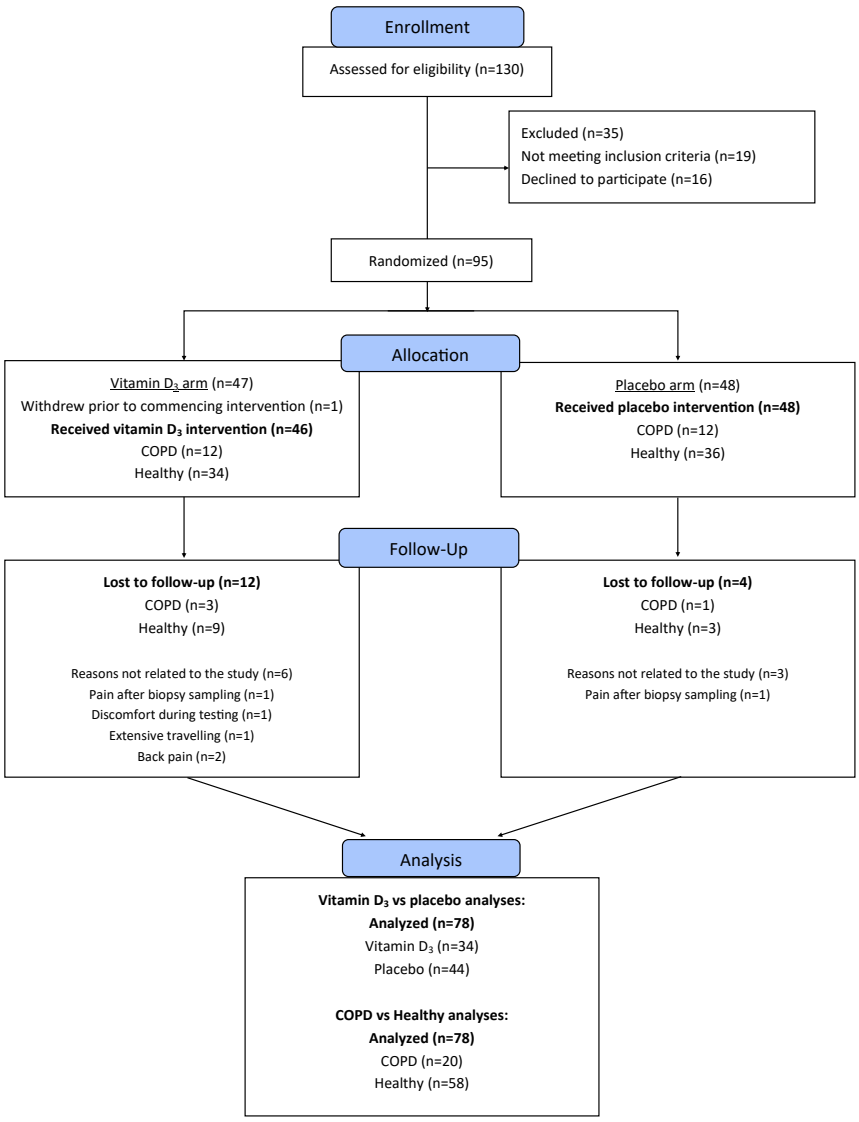
Appendix II

CONSORT flow chart of the RCT study


Enrollment
Assessed for eligibility (n=130)
Excluded (n=35)
Not meeting inclusion criteria (n=19)
Declined to participate (n=16)
Randomized (n=95)
Allocation
Vitamin D3 arm (n=47)
Withdrew prior to commencing intervention (n=1)
Received vitamin D3 intervention (n=46)
COPD (n=12)
Healthy (n=34)
Placebo arm (n=48)
Received placebo intervention (n=48)
COPD (n=12)
Healthy (n=36)
Follow-Up
Lost to follow-up (n=12)
COPD (n=3)
Healthy (n=9)
Reasons not related to the study (n=6)
Pain after biopsy sampling (n=1)
Discomfort during testing (n=1)
Extensive travelling (n=1)
Back pain (n=2)
Lost to follow-up (n=4)
COPD (n=1)
Healthy (n=3)
Reasons not related to the study (n=3)
Pain after biopsy sampling (n=1)
Analysis
Vitamin D3 vs placebo analyses:
Analyzed (n=78)
Vitamin D3 (n=34)
Placebo (n=44)
COPD vs Healthy analyses:
Analyzed (n=78)
COPD (n=20)
Healthy (n=58)

Appendix III

Supplementary material for Paper II

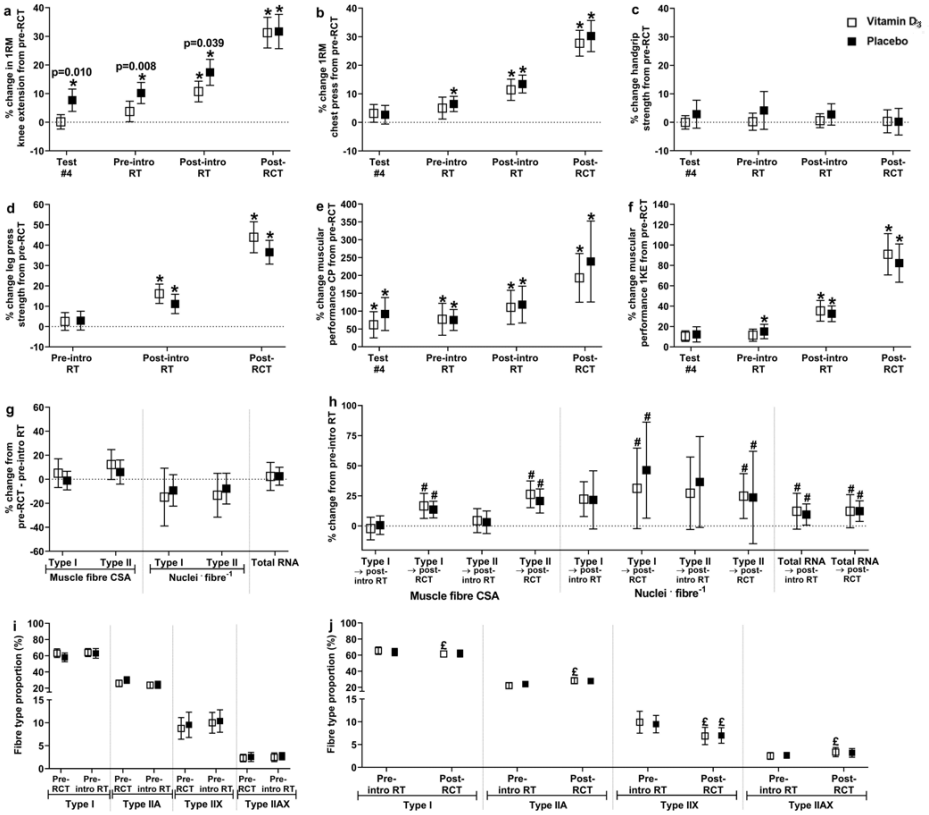


Figure S1 General efficacy of the RCT measured as changes in one repetition maximum one-legged knee extension (a), one repetition maximum chest press (b), grip strength (c), one repetition maximum one-legged leg press (d), muscular performance chest press (number of repetitions at 50 % of pre-RCT one repetition maximum; e), muscular performance one-legged knee extension (number of repetitions at 50 % of pre-RCT one repetition maximum; f), muscle fibre cross-sectional area (CSA; type I and type II fibres), nuclei per fibre and total RNA in m. vastus lateralis (from pre-RCT to baseline/pre-introduction resistance training, dominant leg only, g; from baseline/pre-introduction resistance training to post-introduction resistance training and post-RCT, h), and muscle fibre type proportions (immunohistochemistry; pre-RCT and pre-introduction resistance training/baseline, dominant leg only, i; pre-introduction resistance training/baseline and post-RCT, both legs, j). Test 4, test performed in Week 8 (see Figure 2); *, significant change from pre-RCT; #, significant change from baseline/pre-introduction resistance training; £, significant different from baseline/pre-introduction resistance training. Significant differences between supplementation arms are marked with p-values.

For the initial 12 weeks of supplementation-only was all muscle strength and performance measures associated with improvements (1RM knee extension, 5 %; 1RM chest press 8 %; muscular performance knee extension, 13 %; muscular performance chest press, 71 %; $p < 0.05$), with the only exception being handgrip strength ($p = 0.805$). This occurred without any apparent changes in muscle cell characteristics in thigh muscle, including muscle fibre CSA, muscle fibre type proportions, and total RNA/rRNA expression. The repeated testing of performance indices conducted prior to baseline testing (post-intro RT) was associated with marked and progressive improvements. E.g. for 1RM knee extension, this was evident as 4 % (test #4, after 8 weeks of supplementation), 8 % (pre-introduction to resistance training) and 14 % (post-introduction to resistance training/baseline) increases, while 1RM chest press improved 3 %, 5 % and 13 %, respectively (notably the third test was conducted at ~95 % of maximal effort and was omitted from these analyses). For leg press, three tests were performed prior to the baseline test, resulting in similar improvements as observed for knee extension and chest press (14 %) The subsequent 13 weeks training period was accompanied by marked functional and biological adaptations for the participants, including increased muscle strength and performance (e.g. 22 % and 72 % increases in 1RM and muscular performance in knee extension, respectively, $p < 0.05$), increased muscle mass (e.g. 16 - 24 % increases in muscle fibre CSA for m. vastus lateralis, $p < 0.05$), increases in myonuclei number per fibre (30 – 37 %, $p < 0.05$), and alterations in muscle fibre proportions (e.g. type IIX fibre proportions changed from 10 % to 7 %, $p < 0.05$).

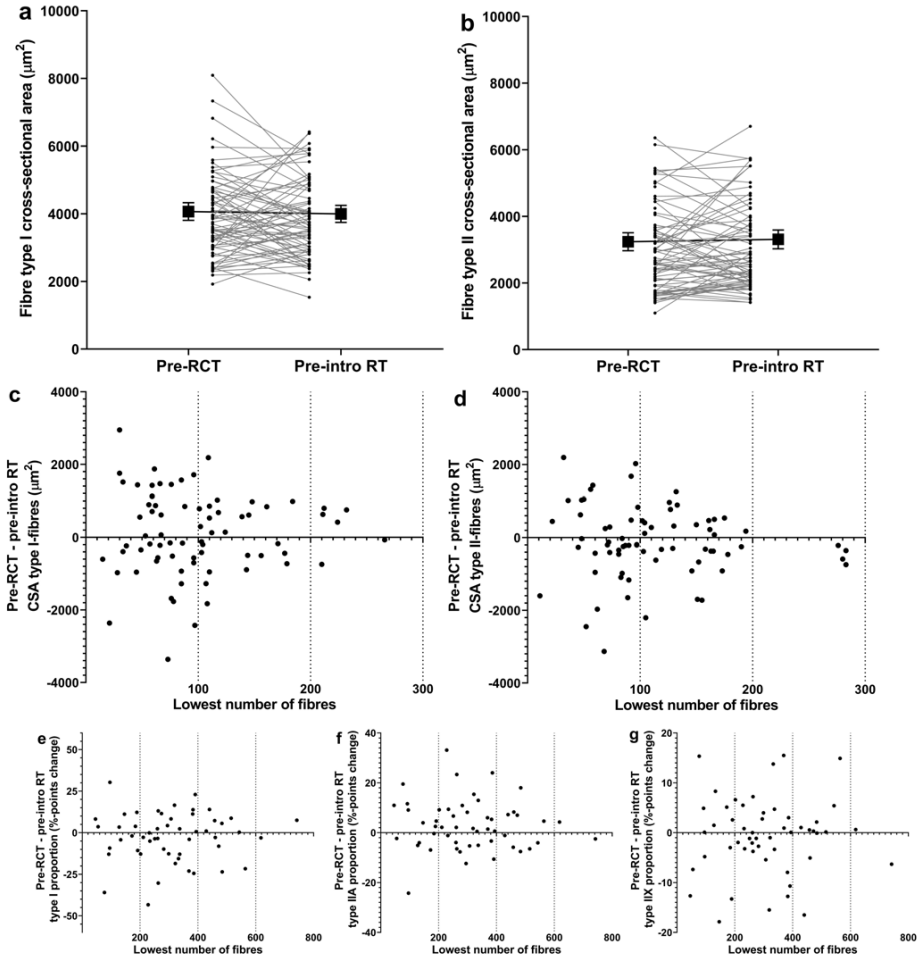


Figure S2 Sample-resample reliability measures of immunohistochemical assessments of muscle fibre cross-sectional area (a-d) and muscle fibre proportions (e-g) in m. vastus lateralis sampled at pre-RCT and pre-introduction to resistance training (pre-intro RT). In a-b, data are presented as means with 95 % confidence limits. In c-g, data are presented as individual values in p-plots, emphasizing the relationship between differences in muscle fibre characteristics measured at the two time points and the lowest number of fibres counted at any time point. In general, these data display increasing differences in sample-resample muscle characteristics with decreasing number of analysed fibres. RT, resistance training. Rough analyses suggested that we would have needed > 250 fibres of each fibre type to achieve reliable assessment of CSA and > 600 fibres to achieve reliable assessment of fibre type proportions, of which our material contained an average of 118 ± 64/137 ± 69 fibres (type I/type II, range 0 – 428/11 - 424) and 462 ± 265 fibres (range 26 - 1982), respectively.

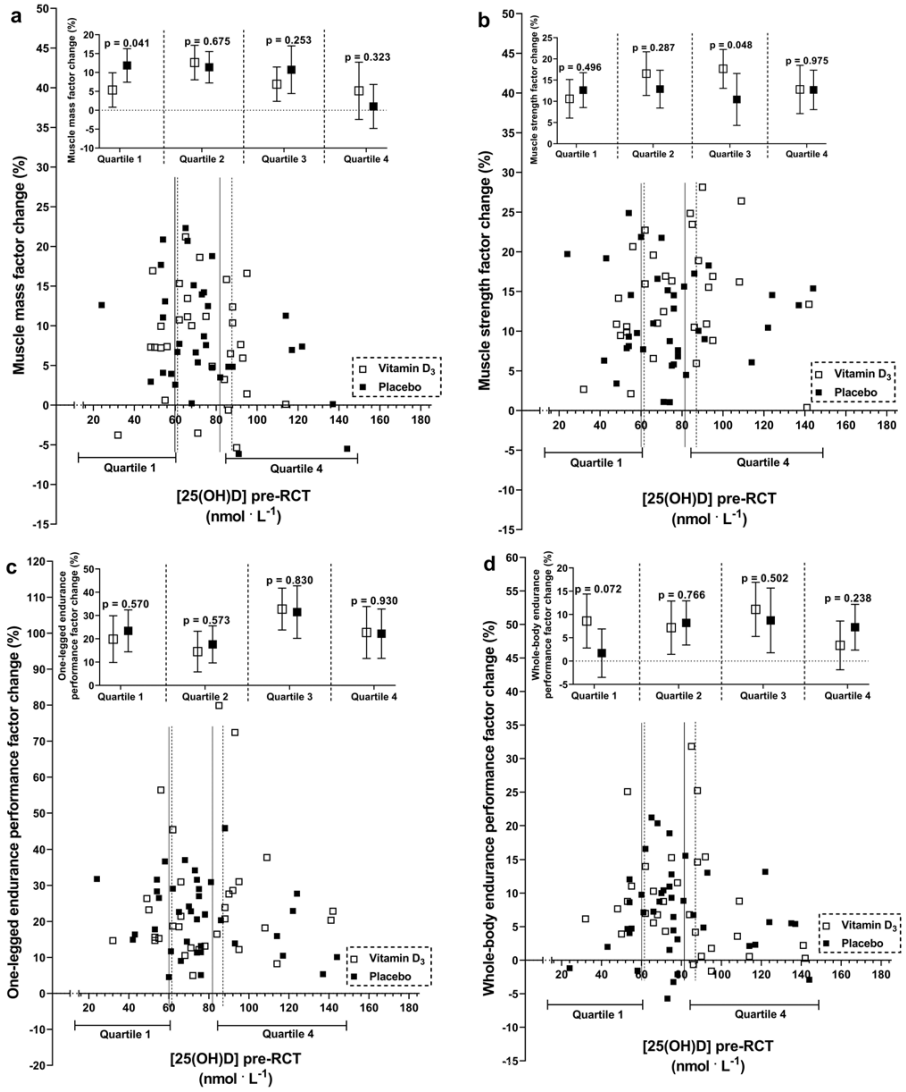


Figure S3 The impact of baseline vitamin D-status ([25(OH)D]) on the effects of combined vitamin D_3 supplementation and resistance training on muscle mass (a), muscle strength (b), one-legged endurance performance (c) and whole-body endurance performance (d). Data are presented as changes in weighted combined factors (means with 95 % confidence limits; upper within-figure panels) and as individual values (lower within-figure panels). For each supplementation arm (vitamin D_3 and placebo), baseline [25(OH)D] quartiles were calculated. Within-quartile comparisons between supplementation arms are shown in the upper panel of each figure. Overall, there was no beneficial effects of vitamin D_3 supplementation in any quartile. Dotted and solid lines in the figure marks the quartile limits for vitamin D_3 and placebo arm, respectively. 25(OH)D, 25-hydroxyvitamin D.

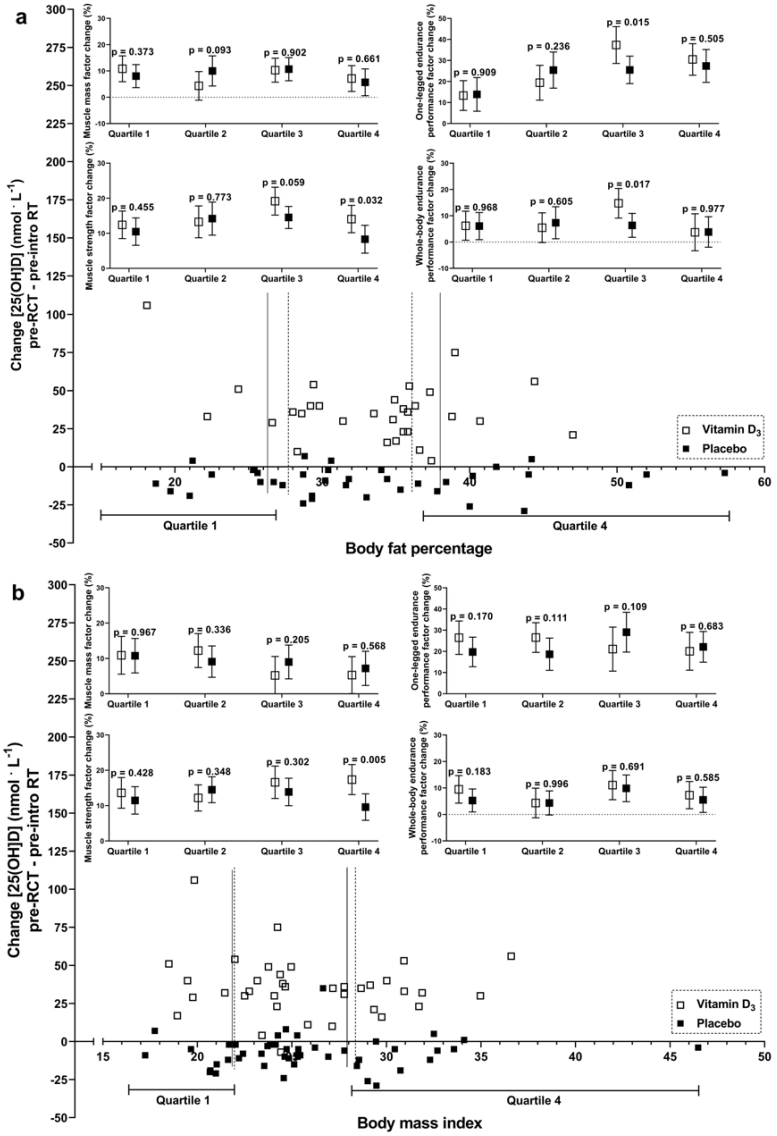


Figure S4 The impact of baseline body fat proportions and body mass index on the effects of combined vitamin D_3 supplementation and resistance training on changes in [25(OH)D] (a-b; lower within-figure panels), muscle mass (a-b; upper within-figure panels), muscle strength (a-b; upper within-figure panels), one-legged endurance performance (a-b; upper within-figure panels) and whole-body endurance performance (d; upper within-figure panels). Data are presented as changes in weighted combined factors (means with 95 % confidence limits; upper within-figure panels) and as individual values (lower within-figure panels). For each supplementation arm (vitamin D_3 and placebo), baseline fat proportion/body mass index quartiles were calculated. Within-quartile comparisons of changes in muscle/performance characteristics between supplementation arms are shown in the upper panel of each figure. Overall, there was no association between quartiles and benefits of vitamin D_3 supplementation for changes in [25(OH)D], muscle mass, one-legged performance and whole-body performance. In the highest quartiles, vitamin D_3 supplementation was associated with more pronounced increases in muscle strength. Dotted and solid lines in the figure marks the quartile limits for vitamin D_3 and placebo arm, respectively. 25(OH)D, 25-hydroxyvitamin D; RT, resistance training.

Table S1 Primer sequences and performance

Gene (symbol)	Primer sequence (forward and reverse)	Ct mean (SD)	*E*
Myosin heavy chain 1 (MYH7)	5′-AGGAGCTCACCTACCAGACG-3′ 5′-TGCAGCTTGTCTACCAGGTC-3′	20.4 (2.0)	1.88
Myosin heavy chain 2A (MYH2)	5′-AACATGAGAGGCGAGTGAAG-3′ 5′-GTGTTGGATTGTTCCTCAGC-3′	19.6 (1.8)	1.82
Myosin heavy chain 2X (MYH1)	5′-TGGTGGACAAACTGCAAGC-3′ 5′-TTGTTCCTCCGCTTCTTCAG-3′	21.7 (2.9)	1.89
5.8S ribosomal RNA (rRNA5.8s)	5′-ACTCTTAGCGGTGGATCACTC-3′ 5′-GTGTCGATGATCAATGTGTCCTG-3′	14.4 (2.3)	1.87
28S ribosomal RNA (rRNA28s)	5′-TGACGCGATGTGATTTCTGC-3′ 5′-TAGATGACGAGGCATTTGGC-3′	9.5 (1.9)	1.84
18S ribosomal RNA (rRNA18s)	5′-TGCATGGCCGTTCTTAGTTG-3′ 5′-AACGCCACTTGTCCCTCTAAG-3′	10.0 (2.3)	1.93
5S ribosomal RNA (rRNA5s)	5′-TACGGCCATACCACCCTGAAC-3′ 5′-GGTCTCCCATCCAAGTACTAACC-3′	16.4 (1.4)	1.82
45S pre-ribosomal RNA (rRNA45s)	5′-GCCTTCTCTAGCGATCTGAGAG-3′ 5′-CCATAACGGAGGCAGAGACA-3′	21.2 (1.7)	1.87
External Standard Kit (λ polyA)	Proprietary sequences	23.2 (1.7)	1.86

Average threshold cycles (Ct) and priming efficiencies (*E*) were calculated from all qPCR reactions

Table S2

Statistical summary table

Manuscript Title: Vitamin D_3 supplementation does not enhance the effects of resistance training in older adults

Authors: Mølmen KS, Hammarström D, Pedersen K, Lian Lie AC, Steile RB, Nygaard H, Khan Y, Hamarsland H, Koll L, Hanestadhaugen M, Lie Eriksen A, Grindaker E, Whist JE, Buck D, Ahmad R, Strand TA, Rønnestad BR, Ellefsen S

In general, two types of analyses are performed per outcome measure. First, main effect (i.e. "general efficacy of intervention") was examined using mixed modelling with absolute values of the dependent variable and the time points as repeated statements/observations (in addition, training modality, i.e. 10RM and 30RM, as a repeated statement for unilateral measures) with random intercepts. For main analyses, i.e. comparison of effect between vitamin D_3 and placebo arm, mixed modelling were used with change scores (% or absolute changes) for the dependent variable with supplementation arms (vitamin D_3 and placebo arm) as the fixed effect, unless otherwise stated in "statistical model" column. Interaction effects were checked between the fixed effect and health status (COPD vs. non-COPD) and training modality for the unilateral measures. For blood variables were the interaction between the fixed effect and sex examined as well. Definitions of 'n': Number of 'pre-to-post-measures' per supplementation arm. Time points: Pre-RCT (week -1); pre-intro resistance training (week 11-13); post-intro resistance training (week 17); post-RCT (week 28-30). See Figure 2 for overview of the study protocol.

				General efficacy of intervention	**Comparison of efficacy, vitamin D_3 vs placebo arm**										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time (p < 0.05)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
Strength variables															
1RM knee extension	Pre-RCT – Pre-intro resistance training	Leg	Unilateral	Yes (↑)	% change	Vitamin D - placebo	4.0 (12.9) vs 10.6 (15.7)	72 vs 90	-8.35	(-14.45, -2.24)	0.008	Mixed model with baseline values as covariate	-	No	
1RM knee extension	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	26.0 (19.5) vs 18.6 (15.8)	64 vs 84	7.16	(-0.71, 15.04)	0.074	"	-	Yes, with training modality (p = 0.001)	Placebo: 10RM, 23.6 (17.9, 29.3); 30RM, 16.5 (10.8, 22.1) Vitamin D: 10RM, 28.4 (22.2, 34.6); 30RM, 26.0 (19.8, 32.1)
1RM knee extension	Post-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	19.0 (14.9) vs 12.0 (10.5)	59 vs 74	6.79	(1.29, 12.30)	0.016	"	Figure 4	Yes, with training modality (p = 0.004)	Placebo: 10RM, 14.8 (10.3, 19.2); 30RM, 10.0 (6.1, 14.0) Vitamin D: 10RM, 22.3 (17.3, 27.2); 30RM 16.1 (11.6, 20.6)
1RM knee extension	Pre-RCT – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	29.9 (20.9) vs 30.5 (23.8)	64 vs 75	-2.37	(-12.09, 7.35)	0.628	"	-	Yes, with training modality (p = 0.024) and health status (p = 0.029)	Placebo: 10RM, 37.6 (30.1, 45.0); 30RM, 31.8 (25.1, 38.5) Vitamin D: 10RM, 34.4 (26.4, 42.5); 30RM, 30.1 (23.0, 37.3) Placebo: Healthy, 26.7 (20.3, 33.2); COPD, 42.6 (30.9, 54.3) Vitamin D: Healthy, 27.2 (19.8, 34.5); COPD, 37.4 (25.3, 49.5)
1RM leg press	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	40.4 (25.2) vs 33.2 (21.0)	63 vs 84	6.75	(-4.66, 18.15)	0.242	"	-	No	
1RM leg press	Post-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	25.6 (20.5) vs 22.9 (14.2)	58 vs 74	1.55	(-7.89, 10.99)	0.744	"	Figure 4	No	

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time ($p < 0.05$)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
1RM chest press	Pre-RCT – Pre-intro resistance training	Arm	Bilateral	Yes (↑)	"	"	4.3 (10.5) vs 5.7 (9.3)	34 vs 43	-0.41	(-5.37, 4.55)	0.871	"	-	No	
1RM chest press	Pre-intro resistance training – Post-RCT	Arm	Bilateral	Yes (↑)	"	"	21.7 (8.4) vs 23.2 (11.8)	32 vs 38	-2.60	(-8.38, 3.18)	0.372	"	-	No	
1RM chest press	Post-intro resistance training – Post-RCT	Arm	Bilateral	Yes (↑)	"	"	14.8 (7.6) vs 14.9 (8.4)	29 vs 35	-2.25	(-6.76, 2.29)	0.325	"	Figure 4	No	
1RM chest press	Pre-RCT – Post-RCT	Arm	Bilateral	Yes (↑)	"	"	27.7 (12.8) vs 30.1 (15.1)	32 vs 38	-2.54	(-10.11, 5.03)	0.505	"	-	No	
Handgrip strength	Pre-RCT – Pre-intro resistance training	Arm	Unilateral	No	"	"	0.6 (7.9) vs 3.9 (17.3)	36 vs 45	-3.29	(-10.53, 3.95)	0.369	"	-	No	
Handgrip strength	Pre-intro resistance training – Post-RCT	Arm	Unilateral	No	"	"	0.6 (13.9) vs -0.2 (21.1)	33 vs 43	1.00	(-8.5, 10.5)	0.835	"	-	No	
Handgrip strength	Post-intro resistance training – Post-RCT	Arm	Unilateral	No	"	"	0.2 (13.4) vs. -2.8 (8.7)	30 vs 37	2.22	(-3.93, 8.38)	0.473	"	Figure 4	No	
Handgrip strength	Pre-RCT – Post-RCT	Arm	Unilateral	No	"	"	0.4 (10.3) vs 2.1 (20.0)	33 vs 43	-2.08	(-10.95, 6.80)	0.642	"	-	No	
Peak torque 60°/s	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	6.2 (11.9) vs 6.7 (14.7)	64 vs 85	-1.18	(-6.65, 4,29)	0.668	"	-	Yes, with health status ($p = 0.008$)	Placebo: Healthy, 4.5 (0.9, 8.1); COPD, 14.1 (7.5, 20.6) Vitamin D: Healthy, 4.0 (-0.1, 8.2); COPD, 12.1 (5.2, 19.0)
Peak torque 60°/s	Post-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	7.4 (13.0) vs 4.9 (10.3)	59 vs 77	2.482	(-2.76, 7.72)	0.346	"	Figure 4	No	
Peak torque 180°/s	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	8.4 (13.9) vs 10.6 (14.7)	64 vs 84	-3.51	(-10.04, 3.02)	0.287	"	-	No	
Peak torque 180°/s	Post-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	7.1 (12.3) vs 7.9 (11.3)	59 vs 76	-1.67	(-6.61, 3.27)	0.501	"	Figure 4	No	
Peak torque 240°/s	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	8.5 (21.2) vs 9.8 (15.9)	64 vs 84	-0.45	(-7.26, 6.36)	0.895	"	-	Yes, with training modality ($p = 0.038$)	Placebo: 10RM, 9.3 (3.0, 15.6); 30RM, 12.8 (7.9, 17.8) Vitamin D: 10RM, 15.2 (8.1, 22.3); 30RM, 6.0 (0.5, 11.4)
Peak torque 240°/s	Post-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	7.7 (14.0) vs 9.5 (13.4)	59 vs 77	-0.92	(-7.01, 5.17)	0.764	"	Figure 4	No	
Leg strength factor	Post-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	14.5 (8.1) vs 11.6 (7.3)	56 vs 69	2.51	(-1.00, 6.02)	0.158	"	Figure 4	No	

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time ($p < 0.05$)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
Lower-limb muscle mass variables															
Type 1 fibre-CSA, dominant leg	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	No	% change	Vitamin D - placebo	7.6 (32.0) vs 1.0 (23.3)	35 vs 38	0.09	(-15.78, 15.96)	0.991	Mixed model with baseline values as covariate, weighted for lowest number of included muscle fibres	-	No	
Type 1 fibre-CSA	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	15.5 (34.8) vs 15.8 (26.6)	58 vs 69	4.07	(-8.97, 17.12)	0.537	"	Figure 5	No	
Type 1 fibre-CSA	Post-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	27.6 (46.2) vs 17.7 (31.1)	47 vs 62	8.31	(-10.41, 27.03)	0.379	"	-	No	
Type 2 fibre-CSA, dominant leg	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	No	"	"	12.3 (36.2) vs 6.0 (30.5)	35 vs 38	-3.31	(-17.87, 11.25)	0.651	"	-	No	
Type 2 fibre-CSA	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	24.4 (35.8) vs 23.1 (36.8)	58 vs 69	3.15	(-11.8, 18.1)	0.675	"	Figure 5	No	
Type 2 fibre-CSA	Post-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	24.7 (49.6) vs 26. 1 (44.0)	47 vs 63	-5.06	(-28.91, 18.78)	0.672	"	-	Yes, with health status ($p = 0.042$)	Placebo: Healthy, 20.3 (4.6, 36.0); COPD, 57.4 (31.1, 83.8) Vitamin D: Healthy, 25.8 (8.6, 43.0); COPD, 41.8 (10.2, 73.4)
Rectus femoris thickness	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	15.4 (3.8) vs 12.2 (10.9)	65 vs 82	7.49	(1.80, 13.18)	0.011	Mixed model with baseline values as covariate	Figure 5	Yes, with health status ($p = 0.046$)	Placebo: Healthy, 12.0 (8.3, 15.7); COPD, 10.7 (3.8, 17.7) Vitamin D: Healthy, 13.7 (9.4, 18.0); COPD, 24.1 (17.0, 31.2)
Vastus lateralis thickness	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	7.0 (7.6) vs 7.6 (7.1)	64 vs 81	-0.30	(-3.20, 2.60)	0.838	"	Figure 5	Yes, with training modality ($p = 0.009$)	Placebo: 10RM, 7.3 (5.0, 9.7); 30RM, 6.7 (4.3, 9.1) Vitamin D: 10RM, 4.2 (1.5, 6.9); 30RM, 9.2 (6.5, 11.9)
Leg lean mass	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	1.0 (4.8) vs 2.5 (3.9)	58 vs 74	-1.82	(-3.93, 0.29)	0.090	"	Figure 5	No	
Muscle mass factor	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	8.4 (7.9) vs 9.0 (8.3)	51 vs 58	0.39	(-3.55, 4.33)	0.843	"	Figure 5	No	
Muscle quality															
Muscle strength factor / muscle mass factor	Baseline – post intervention	Leg	Unilateral	Yes (↑)	% change	"	5.6 (9.1) vs 2.4 (10.0)	44 vs 47	1.92	(-3.00, 6.84)	0.436	Mixed model with baseline values as covariate	Figure 5	No	
Muscle fibre characteristics															

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time ($p < 0.05$)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
Total RNA per tissue weight, dominant leg	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	No	% change	Vitamin D - placebo	3.8 (31.5) vs 1.1 (19.7)	37 vs 42	-5.11	(-17.63, 7.41)	0.419	Mixed model with baseline values as covariate	-	No	
Total RNA per tissue weight	Pre-intro resistance training – Post-intro resistance training	Leg, muscle	Unilateral	Yes (↑)	"	"	15.1 (36.0) vs 18.5 (33.2)	61 vs 73	-10.69	(-24.00, 2.62)	0.114	"	-	No	
Total RNA per tissue weight	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	21.6 (41.7) vs 14.2 (28.2)	62 vs 76	-2.32	(-13.22, 8.59)	0.673	"	Figure 8	No	
Myonuclei per fibre, type 1 fibres, dominant leg	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	No	% change	Vitamin D - placebo	-16 (39) vs -9 (35)	16 vs 22	8.39	(-17.48, 34.27)	0.514	Mixed model with baseline values as covariate, weighted for lowest number of included muscle fibres	-	No	
Myonuclei per fibre, type 2 fibres, dominant leg	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	Yes (↓)	"	"	-13.3 (31.8) vs -7.9 (26.3)	16 vs 22	-6.40	(-28.73, 15.93)	0.564	"	-	No	
Myonuclei per fibre, type 1 fibres	Pre-intro resistance training – Post-intro resistance training	Leg, muscle	Unilateral	Yes (↑)	"	"	23.2 (29.4) vs 24.5 (61.4)	25 vs 33	-2.13	(-26.57, 22.30)	0.861	"	-	No	
Myonuclei per fibre, type 2 fibres	Pre-intro resistance training – Post-intro resistance training	Leg, muscle	Unilateral	Yes (↑)	"	"	21.6 (59.4) vs 32.2 (90.6)	25 vs 33	-19.97	(-52.45, 12.50)	0.222	"	-	No	
Myonuclei per fibre, type 1 fibres	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	25.7 (69.1) vs 48.1 (98.1)	29 vs 32	-0.40	(-40.13, 39.33)	0.984	"	Figure 7	No	
Myonuclei per fibre, type 2 fibres	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	"	"	24.9 (48.3) vs 34.3 (103.4)	29 vs 32	3.91	(-27.65, 35.46)	0.804	"	Figure 7	No	
Myonuclear domain (mean fibre CSA per myonucleus), type 1 fibres, dominant leg	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	Yes (↑)	"	"	22.9 (32.9) vs 27.1 (49.2)	16 vs 22	-2.77	(-33.78, 28.24)	0.857	"	-	No	
Myonuclear domain (mean fibre CSA per myonucleus), type 2 fibres, dominant leg	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	No	"	"	18.1 (39.3) vs 29.1 (67.6)	16 vs 22	-6.77	(-46.87, 33.32)	0.733	"	-	No	
Myonuclear domain (mean	Pre-intro resistance	Leg, muscle	Unilateral	Yes (↓)	"	"	-14.4 (18.1) vs -9.0 (45.9)	25 vs 33	-10.61	(-23.24, 2.03)	0.098	"	-	No	

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	Time points	Body part	Uni- or bilateral limb	Main effect of time ($p < 0.05$)	Units	Compa-rison	Raw mean (SD)	n	Esti-mate	95% CI	P-value	Statistical model	Figure /Table	Interactions	Estimates (95% CI)
fibre CSA per myonucleus), type 1 fibres	training – Post-intro resistance training														
Myonuclear domain (mean fibre CSA per myonucleus), type 2 fibres	Pre-intro resistance training – Post-intro resistance training	Leg, muscle	Unilateral	Yes (↓)	"	"	-3.1 (21.6) vs -13.8 (24.6)	25 vs 33	8.71	(-0.31, 17.74)	0.058	"	-	No	
Myonuclear domain (mean fibre CSA per myonucleus), type 1 fibres	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↓)	"	"	-11.0 (28.0) vs -9.5 (31.0)	29 vs 32	-12.35	(-25.05, 0.36)	0.057	"	-	No	
Myonuclear domain (mean fibre CSA per myonucleus), type 2 fibres	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	No	"	"	-1.7 (24.7) vs 2.8 (29.1)	29 vs 32	-14.53	(-26.94, -2.12)	0.023	"	-	No	
Proportion of fibre type I, dominant leg (IHC)	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	No	Fraction (n fibres type I/total n fibres)	"	Vitamin D: Pre-interv., 0.62 (0.13); Pre-intro ST, 0.63 (0.14). Placebo: Pre-interv., 0.57 (0.16); Pre-intro ST, 0.63 (0.17)	-	-	-	0.546	Generalized mixed model with binomial error distribution and logit-link, weighted for number of included fibres. Interaction of time and supplementation arm	-	-	
Proportion of fibre type IIA, dominant leg (IHC)	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	No	Fraction (n fibres type 2X/total n fibres)	"	Vitamin D: Pre-interv., 0.27 (0.11); Pre-intro ST, 0.24 (0.13). Placebo: Pre-interv., 0.30 (0.11); Pre-intro ST, 0.25 (0.11)	-	-	-	0.841	"	-	-	
Proportion of fibre type IIX, dominant leg (IHC)	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	No	Fraction (n fibres type 2X/total n fibres)	"	Vitamin D: Pre-interv., 0.09 (0.06); Pre-intro ST, 0.10 (0.06). Placebo: Pre-interv., 0.10 (0.09); Pre-intro ST, 0.10 (0.07)	-	-	-	0.347	"	-	-	
Proportion of hybrid fibres IIA/IIX, dominant leg (IHC)	Pre-RCT – Pre-intro resistance training	Leg, muscle	Unilateral	No	Fraction (n fibres type 2AX/total n fibres)	"	Vitamin D: Pre-interv., 0.02 (0.02); Pre-intro ST, 0.02 (0.03). Placebo:	-	-	-	0.200	"	-	-	

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time (p < 0.05)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
							Pre-interv., 0.03 (0.03); Pre-intro ST, 0.03 (0.03)								
Proportion of fibre type I (IHC)	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	No	Fraction (n fibres type I/total n fibres)	"	Vitamin D: Pre-intro ST, 0.62 (0.15); Post-interv., 0.58 (0.13). Placebo: Pre-intro ST, 0.61 (0.16); Post-interv., 0.61 (0.16).	-	-	-	0.888	"	Figure 7	-	
Proportion of fibre type IIA (IHC)	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	Fraction (n fibres type 2A/total n fibres)	"	Vitamin D: Pre-intro ST, 0.25 (0.11); Post-interv., 0.30 (0.12). Placebo: Pre-intro ST, 0.26 (0.12); Post-interv., 0.29 (0.12).	-	-	-	0.710	"	Figure 7	-	
Proportion of fibre type IIX (IHC)	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↓)	Fraction (n fibres type 2X/total n fibres)	"	Vitamin D: Pre-intro ST, 0.10 (0.07); Post-interv., 0.07 (0.06). Placebo: Pre-intro ST, 0.10 (0.07); Post-interv., 0.07 (0.06).	-	-	-	0.925	"	Figure 7	-	
Proportion of hybrid fibres IIA/IIX (IHC)	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	Yes (↑)	Fraction (n fibres type 2AX/total n fibres)	"	Vitamin D: Pre-intro ST, 0.03 (0.03); Post-interv., 0.03 (0.03). Placebo: Pre-intro ST, 0.03 (0.02); Post-interv., 0.03 (0.03).	-	-	-	0.595	"	-	-	

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time (p < 0.05)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
Endurance performance (one-legged and whole-body)															
Muscular performance, knee extension	Pre-RCT – Pre-intro resistance training	Leg	Unilateral	Yes (↑)	% change	Vitamin D - placebo	9.6 (23.8) vs. 15.3 (29.2)	72 vs 88	-2.76	(-13.87, 8.28)	0.621	Mixed model with baseline values as covariate	-	No	
Muscular performance, knee extension	Pre-RCT – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	97.5 (70.5) vs 84.1 (46.7)	65 vs 82	8.63	(-18.90, 36.16)	0.534	"	-	No	
Muscular performance, knee extension	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	80.2 (55.7) vs 65.8 (40.7)	65 vs 84	6.16	(-16.16, 28.47)	0.584	"	-	No	
Muscular performance, knee extension	Post-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	53.5 (33.3) vs 42.3 (29.8)	59 vs 74	4.32	(-9.98, 18.63)	0.548	"	Figure 6	Yes, with health status (p = 0.014)	Placebo: Healthy, 42.6 (32.8, 52.5); COPD, 41.0 (24.9, 57.0) Vitamin D: Healthy, 61.4 (50.5, 72.3); COPD, 30.8 (13.1, 48.5)
Muscular performance, chest press	Pre-RCT – Pre-intro resistance training	Arm	Bilateral	Yes (↑)	"	"	73.6 (112.1) vs 68.5 (80.9)	34 vs 42	-8.67	(-50.66, 33.31)	0.682	"	-	No	
Muscular performance, chest press	Pre-RCT – Post-RCT	Arm	Bilateral	Yes (↑)	"	"	188.3 (172.2) vs 227.1 (305.4)	32 vs 37	-90.04	(-210.12, 30.05)	0.138	"	-	No	
Muscular performance, chest press	Pre-intro resistance training – Post-RCT	Arm	Bilateral	Yes (↑)	"	"	66.6 (32.0) vs 95.3 (173.5)	32 vs 38	-45.13	(-113.97, 23.70)	0.195	"	-	No	
Muscular performance, chest press	Post-intro resistance training – Post-RCT	Arm	Bilateral	Yes (↑)	"	"	41.7 (30.3) vs 56.8 (109.2)	29 vs 35	-15.07	(-62.43, 32.29)	0.527	"	Figure 6	No	
Maximal workload, one-legged cycling	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	8.6 (13.4) vs 9.0 (7.3)	62 vs 74	1.84	(-3.04, 6.71)	0.455	"	Figure 6	No	
Maximal workload, bicycling	Pre-intro resistance training – Post-RCT	Whole-body	Bilateral	Yes (↑)	"	"	7.8 (8.5) vs 7.4 (11.4)	32 vs 41	-0.25	(-5.79, 5.30)	0.929	"	Figure 6	No	
6-min step-test	Pre-intro resistance training – Post-RCT	Whole-body	Bilateral	Yes (↑)	"	"	9.6 (10.6) vs 6.0 (7.5)	31 vs 44	3.68	(-1.25, 8.61)	0.141	"	Figure 6	No	
1-min sit-to-stand test	Pre-intro resistance training – Post-RCT	Whole-body	Bilateral	Yes (↑)	"	"	9.3 (13.1) vs 7.8 (11.6)	32 vs 43	3.37	(-2.43, 9.17)	0.251	"	Figure 6	No	
One-legged endurance performance factor	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	"	"	24.7 (18.9) vs 21.5 (11.1)	61 vs 73	2.44	(-4.84, 9.72)	0.505	"	Figure 6	No	
Whole-body performance factor	Pre-intro resistance training – Post-RCT	Whole-body	Bilateral	Yes (↑)	"	"	8.7 (8.0) vs 7.0 (6.5)	30 vs 40	1.78	(-2.09, 5.65)	0.362	"	Figure 6	No	

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time ($p < 0.05$)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
Endurance variables (one-legged and whole-body)															
VO_2max (mL min^{-1}), one-legged cycling	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↑)	% change	Vitamin D - placebo	0.3 (9.4) vs 2.9 (9.1)	62 vs 72	-1.19	(-5.91, 3.54)	0.618	Mixed model with baseline values as covariate	-	No	
Work economy, one-legged cycling	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↓)	"	"	-3.4 (7.0) vs -4.9 (6.4)	120 vs 146	1.60	(-1.72, 4.92)	0.539	Mixed model with power output as covariate; training modality and the two 5-min steps as repeated measures	-	No	
VO_2max (mL min^{-1}), bicycling	Pre-intro resistance training – Post-RCT	Whole-body	Bilateral	No	"	"	0.6 (12.8) vs 3.7 (11.6)	31 vs 38	-5.90	(-12.89, 1.08)	0.096	Mixed model with baseline values as covariate	-	No	
VO_2max (mL kg^{-1} min^{-1}), bicycling	Pre-intro resistance training – Post-RCT	Whole-body	Bilateral	No	"	"	0.9 (13.1) vs 4.1 (11.8)	31 vs 38	-5.81	(-13.08, 1.47)	0.116	"	-	No	
Dual-energy x-ray absorptiometry variables															
Whole-body lean mass	Pre-intro resistance training – Post-RCT	Whole-body	-	Yes (↑)	% change	Vitamin D - placebo	1.2 (2.7) vs 1.5 (2.0)	30 vs 37	-0.44	(-1.72, 0.85)	0.498	Mixed model with baseline values as covariate	Table S8	No	
Upper-body lean mass	Pre-intro resistance training – Post-RCT	Upper-body	-	Yes (↑)	"	"	1.4 (3.7) vs 1.3 (2.8)	30 vs 37	0.04	(-1.74, 1.82)	0.964	"	-	No	
Whole-body bone mineral density	Pre-intro resistance training – Post-RCT	Whole-body	-	No	"	"	-0.2 (2.6) vs -0.4 (1.9)	30 vs 37	0.43	(-0.77, 1.64)	0.476	"	Table S8	No	
Leg bone mineral density	Pre-intro resistance training – Post-RCT	Leg	Unilateral	No	"	"	-0.5 (2.5) vs 0.0 (2.1)	58 vs 74	-0.30	(-1.27, 0.68)	0.547	"	-	Yes, with health status ($p = 0.046$)	Placebo: Healthy, 0.3 (-0.4, 1.0); COPD, -1.1 (-2.2, 0.1) Vitamin D: Healthy, -0.2 (-1.0, 0.6); COPD, -1.1 (-2.3, 0.0)
Whole-body fat mass	Pre-intro resistance training – Post-RCT	Whole-body	-	Yes (↓)	"	"	-2.1 (6.7) vs -3.3 (8.6)	30 vs 37	0.96	(-3.29, 5.21)	0.654	"	Table S8	No	
Leg fat mass	Pre-intro resistance training – Post-RCT	Leg	Unilateral	Yes (↓)	"	"	-2.1 (6.6) vs -2.2 (8.8)	58 vs 74	-0.57	(-4.61, 3.47)	0.778	"	-	No	
Whole-body fat percentage	Pre-intro resistance training – Post-RCT	Whole-body	-	Yes (↓)	Change	"	-0.8 (1.7) vs -0.9 (1.8)	30 vs 37	0.17	(-0.80, 1.14)	0.722	"	-	No	

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time (p < 0.05)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
Visceral fat	Pre-intro resistance training – Post-RCT	Abdomen	-	Yes (↓)	% change	"	-3.2 (15.1) vs -8.0 (24.2)	28 vs 35	3.843	(-7.78, 15.47)	0.511	"	Table S8	No	
Lung function															
Forced vital capacity	Pre-RCT – Post-RCT	Lungs	-	Yes (↓)	% change	Vitamin D - placebo	-0.9 (9.8) vs -2.8 (7.9)	34 vs 44	2.33	(-2.21, 6.87)	0.310	Mixed model with baseline values as covariate	-	No	
FEV_1/FVC	Pre-RCT – Post-RCT	Lungs	-	No	Change	"	0.38 (4.54) vs 1.05 (4.34)	34 vs 44	-2.89	(-5.11, -0.67)	0.012	"	-	Yes, with health status (p = 0.001)	Placebo: Healthy, 1.7 (-0.3, 3.6); COPD, 0.2 (-3.5, 4.0) Vitamin D: Healthy, 2.3 (0.5, 4.1); COPD, -6.1 (-10.5, -1.8)
FEV_1	Pre-RCT – Post-RCT	Lungs	-	No	% change	"	-1.1 (10.1) vs -1.1 (6.8)	34 vs 44	-2.65	(-6.15, 0.85)	0.221	"	-	No	
Predicted FEV_1	Pre-RCT – Post-RCT	Lungs	-	No	Change	"	-0.6 (7.6) vs -0.4 (6.8)	34 vs 44	-2.98	(-6.71, 0.74)	0.114	"	-	Yes, with health status (p = 0.048)	Placebo: Healthy, -0.8 (-3.8, 2.2); COPD, 2.1 (-3.4, 7.5) Vitamin D: Healthy, 0.7 (-2.2, 3.6); COPD, -5.4 (-11.7, 0.9)
Peak expiratory flow	Pre-RCT – Post-RCT	Lungs	-	No	% change	"	-3.6 (18.0) vs 1.1 (12.3)	34 vs 44	-2.07	(-8.23, 4.01)	0.506	"	-	No	
Blood variables															
25-hydroxyvitamin D	Pre-RCT – Pre-intro resistance training	Blood	-	Yes (↑)	Change in nmol L^{-1}	Vitamin D - placebo	35.3 (19.9) vs -7.4 (10.7)	36 vs 45	44.75	(37.77, 51.72)	<0.001	Mixed model with baseline values as covariate	-	Yes, with health status (p = 0.035)	Placebo: Healthy, -9.2 (14.0, -4.3); COPD, -2.9 (-10.9, 5.0) Vitamin D: Healthy, 32.3 (26.8, 37.8); COPD, 45.1 (35.8, 54.4)
Testosterone	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in nmol L^{-1}	"	-0.05 (2.24) vs 0.29 (1.58)	16 vs 21	-0.64	(-3.48, 2.21)	0.652	"	-	No	
Growth hormone	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in ug L^{-1}	"	0.05 (1.81) vs -0.14 (1.87)	36 vs 45	0.41	(-0.50, 1.32)	0.374	"	-	No	
Androstenedione	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in nmol L^{-1}	"	0.15 (2.39) vs -0.08 (1.09)	36 vs 45	0.11	(-0.85, 1.07)	0.814	"	-	No	
Parathyroid hormone	Pre-RCT – Pre-intro resistance training	Blood	-	Yes (↓)	Change in pmol L^{-1}	"	-0.6 (1.2) vs -0.5 (1.6)	36 vs 45	-0.40	(-1.13, 0.32)	0.272	"	-	No	
IGF-1	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in nmol L^{-1}	"	0.6 (1.6) vs 0.0 (2.3)	36 vs 45	0.17	(-0.84, 1.18)	0.737	"	-	No	
Sex-hormone binding globulin	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in nmol L^{-1}	"	0.7 (10.6) vs 0.9 (9.1)	36 vs 45	0.99	(-3.92, 5.90)	0.688	"	-	No	
Cortisol	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in nmol L^{-1}	"	4.0 (102.8) vs -18.1 (92.4)	36 vs 45	37.79	(-7.23, 82.8)	0.099	"	-	No	
Thyroid-stimulating hormone	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in mIU L^{-1}	"	0.12 (0.48) vs -0.05 (0.72)	36 vs 45	-0.01	(-0.32, 0.30)	0.956	"	-	No	
Free T4	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in pmol L^{-1}	"	0.11 (1.62) vs 0.31 (1.61)	36 vs 45	-0.30	(-1.14, 0.54)	0.477	"	-	No	
Free T3	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in pmol L^{-1}	"	-0.14 (0.47) vs 0.07 (0.47)	36 vs 45	-0.09	(-0.30, 0.11)	0.382	"	-	No	

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time ($p < 0.05$)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
Triglycerides	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in mmol L^{-1}	"	0.08 (0.36) vs -0.03 (0.31)	36 vs 45	0.10	(-0.06, 0.27)	0.206	"	-	No	
HDL	Pre-RCT – Pre-intro resistance training	Blood	-	Yes (↓)	Change in mmol L^{-1}	"	-0.06 (0.18) vs -0.08 (0.29)	36 vs 45	0.06	(-0.07, 0.19)	0.374	"	-	No	
LDL	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in mmol L^{-1}	"	-0.05 (0.48) vs -0.17 (0.57)	36 vs 45	0.15	(-0.11, 0.42)	0.250	"	-	No	
LDL/HDL	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change	"	0.06 (0.32) vs 0.43 (2.87)	36 vs 45	-0.73	(-1.82, 0.35)	0.183	"	-	No	
Iron	Pre-RCT – Pre-intro resistance training	Blood	-	Yes (↓)	Change in µmol L^{-1}	"	-2.6 (7.7) vs -2.2 (5.2)	36 vs 45	0.80	(-2.23, 3.83)	0.599	"	-	No	
Transferrin	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in g L^{-1}	"	0.0 (0.2) vs 0.0 (0.2)	36 vs 45	-0.01	(-0.11, 0.08)	0.805	"	-	No	
Ferritin	Pre-RCT – Pre-intro resistance training	Blood	-	Yes (↓)	Change in µg L^{-1}	"	-10.3 (38.0) vs -17.2 (28.0)	36 vs 44	8.72	(-7.75, 25.18)	0.295	"	-	No	
TIBC	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in µmol L^{-1}	"	0.5 (4.9) vs 0.6 (4.9)	36 vs 45	-0.02	(-2.45, 2.40)	0.984	"	-	Yes, with health status ($p = 0.045$)	Placebo: Healthy, -0.6 (-2.4, 1.1); COPD, 3.3 (0.5, 6.1) Vitamin D: Healthy, 0.2 (-1.7, 2.2); COPD, 2.4 (-0.8, 5.6)
Iron/TIBC	Pre-RCT – Pre-intro resistance training	Blood	-	Yes (↓)	Change	"	-0.04 (0.13) vs -0.04 (0.08)	36 vs 45	0.00	(-0.05, 0.05)	0.905	"	-	No	
Calcium	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in mmol L^{-1}	"	0.01 (0.07) vs 0.02 (0.09)	36 vs 45	-0.01	(-0.05, 0.03)	0.529	"	-	No	
Albumin-corrected calcium	Pre-RCT – Pre-intro resistance training	Blood	-	Yes (↑)	Change in mmol L^{-1}	"	0.01 (0.07) vs 0.02 (0.07)	36 vs 45	-0.02	(-0.05, 0.02)	0.335	"	-	No	
Albumin	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in g L^{-1}	"	0.06 (2.23) vs -0.02 (2.21)	36 vs 45	0.32	(-0.77, 1.42)	0.558	"	-	No	
Creatinine	Pre-RCT – Pre-intro resistance training	Blood	-	Yes (↑)	Change in µmol L^{-1}	"	1.6 (6.4) vs 4.2 (7.8)	36 vs 45	-4.80	(-8.40, -1.20)	0.010	"	-	Yes, with health status ($p = 0.012$)	Placebo: Healthy, 2.3 (-0.2, 4.8); COPD, 9.1 (5.0, 13.2) Vitamin D: Healthy, 2.6 (-0.2, 5.4); COPD, -0.8 (-5.5, 3.9)
Aspartate transaminase	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in units L^{-1}	"	-0.1 (26.0) vs -3.1 (8.7)	36 vs 45	0.31	(-8.95, 9.57)	0.947	"	-	No	
Creatine kinase	Pre-RCT – Pre-intro resistance training	Blood	-	Yes (↓)	Change in units L^{-1}	"	-78 (136) vs -64 (188)	36 vs 45	-14.00	(-39.05, 11.05)	0.269	"	-	Yes, with sex ($p = 0.005$)	Placebo: Female, -85 (-107, -63); Male, -37 (-60, -14) Vitamin D: Female, -87 (-113, -61); Male, -63 (-89, -38)
C-reactive protein	Pre-RCT – Pre-intro resistance training	Blood	-	No	Change in mg L^{-1}	"	-0.4 (3.5) vs -0.2 (5.7)	36 vs 45	-1.23	(-2.90, 0.45)	0.148	"	-	No	
25-hydroxyvitamin D	Pre-RCT – Post-RCT	Blood	-	Yes (↑)	Change in nmol L^{-1}	"	40.7 (21.6) vs -3.9 (15.4)	34 vs 44	46.75	(38.30, 55.21)	<0.001	Mixed model with baseline values as covariate	Figure 2	No	
Testosterone	Pre-RCT – Post-RCT	Blood	-	No	Change in nmol L^{-1}	"	1.34 (2.98) vs -0.02 (2.41)	15 vs 20	0.42	(-3.57, 4.40)	0.832	"	Table S8	No	
Growth hormone	Pre-RCT – Post-RCT	Blood	-	No	Change in ug L^{-1}	"	0.34 (1.56) vs -0.12 (2.39)	29 vs 41	-0.01	(-1.04, 1.02)	0.985	"	Table S8	No	

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time ($p < 0.05$)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
Androstenedione	Pre-RCT – Post-RCT	Blood	-	No	Change in nmol L^{-1}	"	0.27 (1.82) vs -0.10 (1.40)	31 vs 37	0.30	(-0.61, 1.22)	0.507	"	Table S8	No	
Parathyroid hormone	Pre-RCT – Post-RCT	Blood	-	Yes (↓)	Change in pmol L^{-1}	"	-0.69 (1.74) vs -0.20 (1.53)	34 vs 44	-0.61	(-1.44, 0.22)	0.145	"	Table S8	No	
IGF-1	Pre-RCT – Post-RCT	Blood	-	No	Change in nmol L^{-1}	"	-0.49 (2.35) vs -0.52 (2.57)	34 vs 44	0.02	(-1.25, 1.29)	0.971	"	Table S8	No	
Sex-hormone binding globulin	Pre-RCT – Post-RCT	Blood	-	No	Change in nmol L^{-1}	"	1.41 (8.81) vs 0.02 (9.06)	34 vs 44	1.80	(-2.96, 6.56)	0.453	"	Table S8	No	
Cortisol	Pre-RCT – Post-RCT	Blood	-	No	Change in nmol L^{-1}	"	18.0 (93.6) vs -18.0 (95.7)	34 vs 44	48.23	(2.85, 93.60)	0.038	"	Table S8	No	
Thyroid-stimulating hormone	Pre-RCT – Post-RCT	Blood	-	No	Change in mIU L^{-1}	"	0.13 (0.71) vs -0.07 (0.78)	34 vs 44	0.13	(-0.24, 0.50)	0.488	"	-	Yes, with sex (p = 0.033)	Placebo: Female, 0.27 (-0.05, 0.59); Male, -0.29 (-0.62, 0.05) Vitamin D: Female, 0.22 (-0.16, 0.60); Male, 0.02 (-0.36, 0.40)
Free T4	Pre-RCT – Post-RCT	Blood	-	No	Change in pmol L^{-1}	"	-0.03 (1.80) vs -0.14 (1.71)	34 vs 44	0.17	(-0.74, 1.09)	0.707	"	-	No	
Free T3	Pre-RCT – Post-RCT	Blood	-	No	Change in pmol L^{-1}	"	-0.12 (0.37) vs 0.07 (0.60)	34 vs 44	-0.01	(-0.27, 0.25)	0.922	"	-	No	
Triglycerides	Pre-RCT – Post-RCT	Blood	-	Yes (↓)	Change in mmol L^{-1}	"	-0.06 (0.44) vs -0.13 (0.40)	34 vs 44	0.05	(-0.17, 0.27)	0.659	"	Table S8	No	
HDL	Pre-RCT – Post-RCT	Blood	-	No	Change in mmol L^{-1}	"	-0.06 (0.25) vs -0.02 (0.22)	34 vs 44	-0.06	(-0.15, 0.09)	0.570	"	Table S8	No	
LDL	Pre-RCT – Post-RCT	Blood	-	Yes (↓)	Change in mmol L^{-1}	"	-0.21 (0.50) vs -0.14 (0.42)	34 vs 44	-0.04	(-0.26, 0.19)	0.752	"	Table S8	No	
LDL/HDL	Pre-RCT – Post-RCT	Blood	-	No	Change	"	-0.08 (0.36) vs -0.05 (0.32)	34 vs 44	0.02	(-0.15, 0.19)	0.799	"	-	No	
Iron	Pre-RCT – Post-RCT	Blood	-	Yes (↓)	Change in µmol L^{-1}	"	-4.0 (7.2) vs -2.4 (5.5)	34 vs 44	-0.50	(-3.26, 2.26)	0.718	"	Table S8	No	
Transferrin	Pre-RCT – Post-RCT	Blood	-	No	Change in g L^{-1}	"	-0.02 (0.19) vs -0.00 (0.16)	34 vs 44	-0.05	(-0.10, 0.08)	0.782	"	Table S8	No	
Ferritin	Pre-RCT – Post-RCT	Blood	-	Yes (↓)	Change in µg L^{-1}	"	-19.6 (30.8) vs -26.4 (29.8)	34 vs 43	-0.70	(-13.32, 11.92)	0.912	"	Table S8	Yes, with health status (p = 0.005)	Placebo: Healthy, -21.1 (-30.0, -12.2); COPD, 34.3 (-49.1, -19.4) Vitamin D: Healthy, -12.9 (-23.9, -2.7); COPD, -43.9 (-60.3, -27.5)
TIBC	Pre-RCT – Post-RCT	Blood	-	No	Change in µmol L^{-1}	"	-0.38 (4.94) vs 0.50 (3.90)	34 vs 44	-0.62	(-2.89, 1.64)	0.586	"	-	No	
Iron/TIBC	Pre-RCT – Post-RCT	Blood	-	Yes (↓)	Change	"	-0.06 (0.11) vs -0.04 (0.87)	34 vs 44	-0.02	(-0.06, 0.03)	0.496	"	-	No	
Calcium	Pre-RCT – Post-RCT	Blood	-	No	Change in mmol L^{-1}	"	0.00 (0.07) vs 0.01 (0.08)	34 vs 44	-0.02	(-0.05, 0.02)	0.410	"	Table S8	No	
Albumin-corrected calcium	Pre-RCT – Post-RCT	Blood	-	No	Change in mmol L^{-1}	"	0.01 (0.07) vs 0.02 (0.07	34 vs 44	-0.02	(-0.06, 0.01)	0.149	"	Table S8	No	
Albumin	Pre-RCT – Post-RCT	Blood	-	No	Change in g L^{-1}	"	-0.06 (1.76) vs -0.36 (2.40)	34 vs 44	0.70	(-0.23, 1.63)	0.139	"	-	No	
Creatinine	Pre-RCT – Post-RCT	Blood	-	Yes (↑)	Change in µmol L^{-1}	"	4.3 (7.8) vs 4.8 (5.9)	34 vs 44	-1.11	(-4.70, 2.49)	0.542	"	-	No	
Aspartate aminotransferase	Pre-RCT – Post-RCT	Blood	-	Yes (↓)	Change in units L^{-1}	"	-2.7 (10.4) vs -3.0 (7.7)	34 vs 44	-0.38	(-3.29, 2.52)	0.794	"	Table S8	Yes, with health status (p = 0.034)	Placebo: Healthy, -2.2 (-4.2, -0.3); COPD, -5.0 (-8.4, -1.7)) Vitamin D: Healthy, -1.4 (-3.7, 1.0); COPD, -6.6 (-10.6, -2.7)
Creatine kinase	Pre-RCT – Post-RCT	Blood	-	Yes (↓)	Change in units L^{-1}	"	-48.0 (161.3) vs -44.8 (191.8)	34 vs 44	13.37	(-22.09, 48.83)	0.455	"	Table S8	Yes, with sex (p = 0.013)	Placebo: Female, -69.9 (-100.8, -38.9); Male, -36.2 (-69.1, -3.2)) Vitamin D: Female, -71.7 (-108.2, -35.3); Male, -7.6 (-44.0, 28.9)

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time ($p < 0.05$)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
C-reactive protein	Pre-RCT – Post-RCT	Blood	-	No	Change in mg L^{-1}	"	0.29 (5.82) vs -0.50 (5.01)	34 vs 44	-0.55	(-2.45, 1.35)	0.565	"	-	No	
Quartile analysis of [25(OH)D]-status at Pre-RCT															
Leg strength factor	Post-intro resistance training – Post-RCT	Leg	Unilateral	-	% change	Quartiles of [25(OH)D] at Pre-RCT in vitamin D vs placebo arm	Vitamin D: Q1, 12 (5); Q2, 14 (9); Q3, 19 (5); Q4, 13 (8). Placebo: Q1, 12 (6); Q2, 12 (6); Q3, 9 (5); Q4, 13 (7).	56 vs 62	-	-	0.237	Interaction effect quartiles [25(OH)D] Pre-RCT and supplementation arm, baseline values as covariate	Figure S3	-	-
Muscle mass factor	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	-	"	"	Vitamin D: Q1, 7 (7); Q2, 12 (8); Q3, 7 (7); Q4, 4 (10). Placebo: Q1, 12 (8); Q2, 11 (8); Q3, 10 (8); Q4, 3 (7).	51 vs 58	-	-	0.159	"	Figure S3	-	-
One-legged endurance performance factor	Pre-intro resistance training – Post-RCT	Leg	Unilateral	-	"	"	Vitamin D: Q1, 24 (15); Q2, 19 (13); Q3, 36 (29); Q4, 22 (10). Placebo: Q1, 26 (9); Q2, 21 (11); Q3, 20 (10); Q4, 19 (13).	61 vs 73	-	-	0.950	"	Figure S3	-	-
Whole-body performance factor	Pre-intro resistance training – Post-RCT		Whole-body	-	"	"	Vitamin D: Q1, 10 (8); Q2, 9 (4); Q3, 12 (12); Q4, 4 (6). Placebo: Q1, 4 (5); Q2, 11 (8); Q3, 6 (7); Q4, 7 (6).	30 vs 39	-	-	0.266	"	Figure S3	-	-
Quartile analysis of baseline fat percentage															
Leg strength factor	Post-intro resistance training – Post-RCT	Leg	Unilateral	-		Quartiles of fat percentage at Pre-RCT in vitamin D vs placebo arm	Vitamin D: Q1, 13 (8); Q2, 13 (10); Q3, 18 (7); Q4, 14 (6). Placebo: Q1, 9 (6); Q2, 10 (8); Q3, 15 (8); Q4, 9 (5).	48 vs 58	-	-	0.068	Interaction effect quartiles fat percentage at Pre-RCT and supplementation arm, baseline values as covariate	Figure S4	-	-
Muscle mass factor	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	-		"	Vitamin D: Q1, 10 (9); Q2, 5 (9); Q3, 10 (5); Q4, 7 (8). Placebo: Q1, 8 (11); Q2, 11 (8); Q3, 1 (6); Q4, 6 (3).	51 vs 58	-	-	0.428	"	Figure S4	-	-
One-legged endurance performance factor	Pre-intro resistance training – Post-RCT	Leg	Unilateral	-		"	Vitamin D: Q1, 17 (9); Q2, 20 (9); Q3, 32 (26); Q4, 32 (24).	53 vs 62	-	-	0.100	"	Figure S4	-	-

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time (p < 0.05)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
							Placebo: Q1, 14 (8); Q2, 25 (8); Q3, 23 (11); Q4, 29 (11).								
Whole-body performance factor	Pre-intro resistance training – Post-RCT	-	Whole-body	-		"	Vitamin D: Q1, 7 (3); Q2, 6 (6); Q3, 15 (10); Q4, 9 (10). Placebo: Q1, 5 (4); Q2, 8 (9); Q3, 7 (7); Q4, 8 (7).	26 vs 35	-	-	0.192	"	Figure S4	-	-
Quartile analysis of baseline body mass index															
Leg strength factor	Post-intro resistance training – Post-RCT	Leg	Unilateral	-		Quartiles of BMI at Pre-RCT in vitamin D vs placebo arm	Vitamin D: Q1, 16 (8); Q2, 13 (7); Q3, 15 (8); Q4, 15 (9). Placebo: Q1, 12 (7); Q2, 12 (6); Q3, 12 (8); Q4, 10 (8).	56 vs 69	-	-	0.030	Interaction effect quartiles BMI at Pre-RCT and supplementation arms, baseline values as covariate	Figure S4	-	-
Muscle mass factor	Pre-intro resistance training – Post-RCT	Leg, muscle	Unilateral	-		"	Vitamin D: Q1, 9 (8); Q2, 12 (8); Q3, 6 (7); Q4, 7 (8). Placebo: Q1, 10 (6); Q2, 9 (9); Q3, 9 (10); Q4, 8 (7).	51 vs 58	-	-	0.590	"	Figure S4	-	-
One-legged endurance performance factor	Pre-intro resistance training – Post-RCT	Leg	Unilateral	-		"	Vitamin D: Q1, 30 (23); Q2, 30 (24); Q3, 18 (10); Q4, 20 (8). Placebo: Q1, 21 (9); Q2, 17 (11); Q3, 24 (10); Q4, 25 (12).	61 vs 73	-	-	0.127	"	Figure S4	-	-
Whole-body performance factor	Pre-intro resistance training – Post-RCT	-	Whole-body	-		"	Vitamin D: Q1, 9 (10); Q2, 7 (10); Q3, 9 (5); Q4, 10 (9). Placebo: Q1, 4 (5); Q2, 7 (6); Q3, 9 (6); Q4, 9 (8).	30 vs 40	-	-	0.675	"	Figure S4	-	-
Weekly health diary															
Self-reported health$^{\text{-week}}$ (0-10)	Entire intervention period	-	-	Yes (↑)	Avg score	Vitamin D - placebo	6.3 (1.2) vs 6.6 (1.5)	33 vs 42	-0.25	(-0.87, 0.37)	0.433	Independent t-test	Table S8	-	-
Nausea		-	-		Raw, % incidence (events/total number of weeks*100);	"	3.70 (16.69) vs 3.47 (5.07)	33 vs 42	-	-	0.204	Generalized mixed model with binomial error distribution and logit-link	-	-	-
Stomach ache$^{\text{-week}}$	"	-	-	-	"	"	6.87 (19.5) vs 3.54 (7.93)	33 vs 42	-	-	0.916	"	-	-	-

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	**Time points**	**Body part**	**Uni- or bilateral limb**	**Main effect of time (p < 0.05)**	**Units**	**Compa-rison**	**Raw mean (SD)**	**n**	**Esti-mate**	**95% CI**	**P-value**	**Statistical model**	**Figure /Table**	**Interactions**	**Estimates (95% CI)**
Loose stools or diarrhoea^{-week}	"	-	-	-	"	"	7.57 (16.13) vs 10.35 (19.15)	33 vs 42	-	-	0.338	"	-	-	-
Bloated stomach^{-week}	"	-	-	-	"	"	9.04 (18.80) vs 7.64 (17.76)	33 vs 42	-	-	0.762	"	-	-	-
Dizziness^{-week}	"	-	-	-	"	"	5.87 (17.65) vs 7.15 (10.75)	33 vs 42	-	-	0.285	"	-	-	-
Sleep problems^{-week}	"	-	-	-	"	"	11.35 (23.19) vs 17.19 (27.52)	33 vs 42	-	-	0.428	"	-	-	-
Balance problems^{-week}	"	-	-	-	"	"	4.47 (17.71) vs 6.16 (17.70)	33 vs 42	-	-	0.432	"	-	-	-
Rash^{-week}	"	-	-	-	"	"	0.82 (2.07) vs 3.60 (12.53)	33 vs 42	-	-	0.398	"	-	-	-
Itchiness^{-week}	"	-	-	-	"	"	6.57 (21.25) vs 6.92 (17.92)	33 vs 42	-	-	0.696	"	-	-	-
Blood in urine^{-week}	"	-	-	-	"	"	0.30 (1.74) vs 0.00 (0.00)	33 vs 42	-	-	1.000	"	--	-	-
Pain when urinating^{-week}	"	-	-	-	"	"	0.52 (1.91) vs 0.00 (0.00	33 vs 42	-	-	0.997	"	-	-	-
Vitamin D exposure															
Taken the supplement as prescribed^{-week}	Entire intervention period	-	-	-	Fraction (events/total number of weeks)	Vitamin D - placebo	0.993 (0.020) vs 0.993 (0.017)	33 vs 42	Vitamin D: 0.993 (0.983, 0.997) Placebo: 0.993 (0.981, 0.997)		0.998	Generalized mixed model with binomial error distribution and logit-link	-	-	-
Number of hours outdoors^{-week}	"	-	-	-	Avg events/week	"	8.8 (6.0) vs 8.9 (6.4)	33 vs 42	-0.11	(-2.99, 2.78)	0.941	Independent t-test	-	-	-
Fish for dinner^{-week}	"	-	-	-	"	"	1.9 (0.8) vs 1.8 (0.7)	33 vs 42	0.09	(-0.26, 0.43)	0.612	"	-	-	-
Fish to other meals^{-week}	"	-	-	-	"	"	2.0 (1.7) vs 1.6 (1.1)	33 vs 42	-0.40	(-0.27, 1.07)	0.233	"	-	-	-
Cod liver oil (teaspoons^{-week})	"	-	-	-	Raw, incidence (events/total number of weeks); estimate, MLE	"	1.2 (3.8) vs 1.6 (3.4)	33 vs 42	-0.29	(-0.68, 0.10)	0.154	Generalized model with Poisson distribution	-	-	-
Cod liver oil (capsules^{-week})	"	-	-	-	"	"	1.5 (3.8) vs 2.0 (3.8)	33 vs 42	-0.27	(-0.62, 0.08)	0.131	"	-	-	-
Number of eggs eaten^{-week}	"	-	-	-	Events/week	"	3.2 (1.8) vs 2.9 (2.2)	33 vs 42	0.35	(-0.57, 1.26)	0.452	Independent t-test	-	-	-
Habitual dietary data															
Kcal	Week 23	-	-	-	Day^{-1}	Vitamin D - placebo	1777 (529) vs 1985 (611)	32 vs 43	-876	(-1980, 228)	0.118	Independent t-test	-	-	-
Protein	"	-	-	-	Gram kg^{-1} day^{-1}	"	1.26 (0.40) vs 1.27 (0.36)	32 vs 43	-0.02	(-0.20, 0.16)	0.845	"	-	-	-
Fat	"	-	-	-	Gram kg^{-1} day^{-1}	"	0.99 (0.47) vs 1.05 (0.38)	32 vs 43	-0.06	(-0.26, 0.14)	0.580	"	-	-	-
CHO	"	-	-	-	Gram kg^{-1} day^{-1}	"	2.46 (1.05) vs 2.88 (1.03)	32 vs 43	-0.41	(-0.90, 0.07)	0.094	"	-	-	-

				General efficacy of intervention	Comparison of efficacy, vitamin D_3 vs placebo arm										
Variable	Time points	Body part	Uni- or bilateral limb	Main effect of time ($p < 0.05$)	Units	Compa-rison	Raw mean (SD)	n	Esti-mate	95% CI	P-value	Statistical model	Figure /Table	Interactions	Estimates (95% CI)
Alcohol	"	-	-	-	Units day^{-1}	"	0.76 (0.92) vs 0.67 (1.04)	32 vs 43	0.10	(-0.36, 0.55)	0.674	"	-	-	-
Vitamin D	"	-	-	-	IU day^{-1}	"	281 (235) vs 331 (260)	32 vs 43	-49.3	(-163.6, 65.1)	0.393	"	-	-	-

Table S3

Statistical summary table for qPCR data

Variable	Time point	Body part	Units	Comparison	Mean (95% CI)	Estimate (SE)	P-value	Statistical model
Proportion of fibre type I (qPCR, GeneFam)	Pre-RCT	Leg, muscle	Mean, arbitrary units; estimate, fold difference (SE)	Vitamin D - placebo	Vitamin D: 39845 (34003, 46690) Placebo: 55533 (38561, 51199)	0.82 (0.16)	0.181	Generalized mixed model with negative binomial distribution corrected for offset (Pre-RCT) differences
"	Pre-intro resistance training	"	"	"	Vitamin D: 38726 (33377, 44932) Placebo: 41340 (36081, 47365)	1.10 (0.09)	0.289	"
"	Post-intro resistance training	"	"	"	Vitamin D: 42105 (36223, 48943) Placebo: 42888 (37386, 49201)	1.17 (0.09)	0.087	"
"	Post-RCT	"	"	"	Vitamin D: 44931 (38685, 52185) Placebo: 45577 (39773, 52228)	1.15 (0.09)	0.118	"
Proportion of fibre type IIA (qPCR, GeneFam)	Pre-RCT	"	"	"	Vitamin D: 45712 (40764, 51260) Placebo: 42522 (38373, 47119)	1.14 (0.12)	0.274	"
"	Pre-intro resistance training	"	"	"	Vitamin D: 44470 (40197, 49196) Placebo: 41618 (37934, 45660)	1.00 (0.09)	0.993	"
"	Post-intro resistance training	"	"	"	Vitamin D: 54646 (49298, 60573) Placebo: 48031 (43704, 52786)	1.14 (0.09)	0.129	"
"	Post-RCT	"	"	"	Vitamin D: 49781 (44937, 55146) Placebo: 47535 (43320, 52159)	0.94 (0.09)	0.508	"
Proportion of fibre type IIX (qPCR, GeneFam)	Pre-RCT	"	"	"	Vitamin D: 27349 (21559, 34692) Placebo: 27143 (21987, 33509)	1.01 (0.22)	0.944	"
"	Pre-intro resistance training	"	"	"	Vitamin D: 32173 (25926, 39926) Placebo: 34221 (28103, 41672)	0.88 (0.18)	0.444	"
"	Post-intro resistance training	"	"	"	Vitamin D: 20808 (16702, 25924) Placebo: 21913 (17933, 26776)	0.90 (0.19)	0.550	"
"	Post-RCT	"	"	"	Vitamin D: 19737 (15865, 24555) Placebo: 18202 (14932, 22188)	0.95 (0.19)	0.773	"
rRNA species expression per muscle weight								
18s rRNA expression per muscle weight	Pre-intro resistance training	Leg, muscle	Estimate, fold difference (SE)	Vitamin D - placebo	-	1.08 (0.08)	0.279	Mixed model on log-transformed data corrected for Pre-RCT differences between supplementation arms
"	Post-intro resistance training	"	"	"	-	1.02 (0.08)	0.788	"
"	Post-RCT	"	"	"	-	1.03 (0.08)	0.708	"
5.8s rRNA expression per muscle weight	Pre-intro resistance training	"	"	"	-	1.07 (0.10)	0.459	"
"	Post-intro resistance training	"	"	"	-	1.06 (0.10)	0.525	"
"	Post-RCT	"	"	"	-	0.93 (0.10)	0.454	"
28s rRNA expression per muscle weight	Pre-intro resistance training	"	"	"	-	1.03 (0.08)	0.690	"
"	Post-intro resistance training	"	"	"	-	1.00 (0.08)	0.966	"
"	Post-RCT	"	"	"	-	1.00 (0.08)	0.980	"
5s rRNA expression per muscle weight	Pre-intro resistance training	"	"	"	-	1.00 (0.11)	0.965	"
"	Post-intro resistance training	"	"	"	-	0.91 (0.12)	0.365	"
"	Post-RCT	"	"	"	-	0.93 (0.12)	0.482	"
45s pre-rRNA expression per muscle weight	Pre-intro resistance training	"	"	"	-	1.15 (0.10)	0.172	"
"	Post-intro resistance training	"	"	"	-	1.12 (0.11)	0.439	"
"	Post-RCT	"	"	"	-	1.08 (0.10)	0.439	"

Table S4 Computed factors for main outcome domains

Calculation: For each of the singular variables included in a factor, each subject's value (pre and post) was normalized to the highest recorded value during the study conduct as a fraction, thus showing values < 1. Afterwards was the ultimate factor for each subject calculated as the mean of the normalized values for each variable included.

Muscle mass factor										
	Included variables	***Explanation***	***Baseline (avg ± SD)***	***Post intervention (avg ± SD)***	***Estimate, change (95% CI)***	***Main effect of time (p value)***	***Correlation with factor at baseline, r value (p value)***	***Correlation for change score with change score for factor, r value (p value)***	***Eigenvalue***	***% variance explained***
	1. Muscle fibre CSA	The combined measure of fibre type 1 and 2 CSA	0.43 (0.13)	0.49 (0.15)	0.064 (0.042, 0.085)	<0.001	0.737 (<0.001)	0.939 (<0.001)	-	-
	2. Muscle thickness	The combined measure of muscle thickness of vastus lateralis and rectus femoris	0.60 (0.10)	0.66 (0.11)	0.058 (0.049, 0.067)	<0.001	0.644 (<0.001)	0.406 (<0.001)	-	-
	3. Leg lean mass	Lean mass in the legs	0.64 (0.15)	0.65 (0.15)	0.011 (0.006, 0.016)	<0.001	0.914 (<0.001)	0.331 (<0.001)	-	-
	Muscle mass factor	-	**0.56 (0.10)**	**0.61 (0.11)**	**0.048 (0.038, 0.057)**	**<0.001**	-	-	**1.283**	**43**
Muscle strength factor										
	Included variables	***Explanation***	***Baseline (avg ± SD)***	***Post intervention (avg ± SD)***	***Estimate, change (95% CI)***	***Main effect of time (p value)***	***Correlation with factor at baseline, r value (p value)***	***Correlation for change score with change score for factor, r value (p value)***	***Eigenvalue***	***% variance explained***
	1. Leg muscle strength	The combined measure of 1RM knee extension and leg press	0.44 (0.14)	0.52 (0.15)	0.082 (0.072, 0.092)	<0.001	0.953 (<0.001)	0.775 (<0.001)	-	-
	2. Leg muscle torque	The combined measure of torque (Nm) achieved during knee extension at 60°, 180° and 240°/sec	0.48 (0.16)	0.51 (0.17)	0.033 (0.022, 0.044)	<0.001	0.968 (<0.001)	0.728 (<0.001)	-	-
	Muscle strength factor	-	**0.46 (0.14)**	**0.52 (0.15)**	**0.056 (0.049, 0.064)**	**<0.001**	-	-	**1.132**	**57**
One-legged muscle endurance factor										
	Included variables	***Explanation***	***Baseline (avg ± SD)***	***Post intervention (avg ± SD)***	***Estimate, change (95% CI)***	***Main effect of time (p value)***	***Correlation with factor at baseline, r value (p value)***	***Correlation for change score with change score for factor, r value (p value)***	***Eigenvalue***	***% variance explained***
	1. Muscle performance	Number of repetitions at 50% of 1RM knee extension	0.13 (0.03)	0.22 (0.09)	0.088 (0.077, 0.099)	<0.001	0.602 (<0.001)	0.917 (<0.001)		
	2. Maximal power output	Maximal power output achieved during one-legged cycling	0.49 (0.17)	0.52 (0.17)	0.035 (0.029, 0.040)	<0.001	0.988 (<0.001)	0.292 (<0.001)		
	One-legged endurance performance factor	-	**0.31 (0.09)**	**0.37 (0.11)**	**0.063 (0.057, 0.070)**	**<0.001**			**1.114**	**56**

Whole-body endurance performance factor										
	Included variables	***Explanation***	***Baseline (avg ± SD)***	***Post intervention (avg ± SD)***	***Estimate, change (95% CI)***	***Main effect of time (p value)***	***Correlation with factor at baseline, r value (p value)***	***Correlation for change score with change score for factor, r value (p value)***	***Eigenvalue***	***% variance explained***
	1. Maximal power output	Maximal power output achieved during bicycling	0.44 (0.17)	0.47 (0.18)	0.029 (0.020, 0.039)	<0.001	0.872 (<0.001)	0.544 (<0.001)	-	-
	2. 6-min step test	Number of steps achieved during a 6-min test	0.59 (0.17)	0.63 (0.19)	0.044 (0.033, 0.056)	<0.001	0.959 (<0.001)	0.638 (<0.001)	-	-
	3. 1-min sit-to-stand test	Number of sit-to-stands achieved during a 1-min test	0.58 (0.13)	0.62 (0.14)	0.042 (0.027, 0.057)	<0.001	0.858 (<0.001)	0.763 (<0.001)	-	-
	Whole-body endurance performance factor	-	0.54 (0.14)	0.58 (0.15)	0.039 (0.031, 0.047)	**<0.001**	-	-	1.290	43

Each factor was calculated to accommodate methodological limitation inherent to the singular measures. Corroborating with this, each measure separately correlated with the computed muscle mass factor at baseline (r = 0.602 - 0.988) and change scores for each of these measures at post-RCT correlated with the change score for the corresponding factor (r = 0.292 – 0.939), suggesting the feasibility of our approach.

Table S5 Functional annotation analysis of placebo compared to Vitamin D_3 supplementation

Comparison	Gene ontology category	Gene ontology	Significance category[a]	Set size[b]	Rank *P*-value[c]	% MSD > 0[d]	GSEA *P*-value[e]	NES	LE[f]	Log_2 Fold-change in LE [min, max]
Pre-intro RT - Pre-RCT: ΔVitamin D_3 vs. Δplacebo	Cellular component	Cell cortex	Consensus	246 (318)	0.041	24.8%	0.034	-1.38	67 (64.2%)	-0.61 [-1.94, -0.2]
		Cell substrate junction	Consensus	386 (414)	0.041	23.1%	0.010	-1.37	107 (57%)	-0.59 [-1.5, -0.24]
		Inner mitochondrial membrane protein complex	Consensus	119 (135)	0.018	29.4%	3.33e-05	-1.72	48 (66.7%)	-0.61 [-1.28, -0.25]
		Mitochondrial protein complex	Consensus	244 (260)	0.041	24.6%	5.99e-07	-1.69	83 (66.3%)	-0.57 [-1.28, -0.2]
		Organelle inner membrane	Consensus	471 (534)	0.005	24.6%	4.59e-07	-1.55	142 (69.7%)	-0.58 [-1.34, -0.24]
		Respirasome	Consensus	82 (100)	0.018	35.4%	1.47e-04	-1.73	38 (68.4%)	-0.63 [-1.28, -0.25]
		Respiratory chain complex	Consensus	69 (85)	0.005	40.6%	5.09e-04	-1.70	35 (71.4%)	-0.64 [-1.28, -0.25]
	Biological process	Extracellular structure organization	GSEA	267 (373)	0.516	17.6%	0.002	-1.56	92 (41.3%)	-0.64 [-1.54, -0.22]
		Mitochondrial gene expression	GSEA	166 (165)	0.524	23.5%	0.008	-1.59	56 (64.3%)	-0.46 [-1.07, -0.15]
		Mitochondrial respiratory chain complex assembly	GSEA	88 (96)	0.339	30.7%	0.008	-1.69	34 (76.5%)	-0.55 [-1.23, -0.2]
		Mitochondrial translation	GSEA	137 (137)	0.504	24.8%	0.008	-1.63	51 (62.7%)	-0.45 [-1.07, -0.15]
		Mitochondrion organization	GSEA	479 (528)	0.129	24%	0.002	-1.46	120 (70%)	-0.59 [-1.49, -0.25]
		Oxidative phosphorylation	GSEA	126 (144)	0.129	27.8%	0.008	-1.66	49 (65.3%)	-0.6 [-1.24, -0.2]
	Cellular component	Collagen containing extracellular matrix	GSEA	287 (408)	0.292	19.5%	2.26e-06	-1.64	103 (49.5%)	-0.67 [-1.54, -0.21]
		Extracellular matrix	GSEA	346 (531)	0.474	17.9%	4.39e-06	-1.59	116 (46.6%)	-0.66 [-1.54, -0.21]
		Mitochondrial matrix	GSEA	444 (471)	0.406	22.3%	6.63e-06	-1.53	141 (62.4%)	-0.5 [-1.49, -0.15]
	Molecular function	Extracellular matrix structural constituent	GSEA	126 (165)	0.215	26.2%	0.004	-1.63	46 (67.4%)	-0.72 [-1.54, -0.25]
		Oxidoreductase activity acting on nad p h	GSEA	85 (107)	0.678	27.1%	0.007	-1.65	31 (67.7%)	-0.6 [-1.23, -0.2]
		Structural molecule activity	GSEA	482 (670)	0.399	19.5%	1.16e-06	-1.55	145 (50.3%)	-0.59 [-1.54, -0.24]
	Cellular component	Golgi lumen	Rank	48 (100)	0.018	22.9%	0.243	-1.45	9 (66.7%)	-1.32 [-4.22, -0.7]
		Oxidoreductase complex	Rank	95 (115)	0.028	33.7%	0.100	-1.45	37 (73%)	-0.61 [-2.21, -0.22]

[a] Consensus significance indicates agreement between directional (GSEA) and non-directional (Rank) hypothesis test of overrepresentation (see methods for details). [b] Indicates number identified genes in gene set and total number of gene in gene set in parentheses. [c] Rank-based enrichment test based on minimum significant difference identifies gene-sets that are over-represented among top-ranked genes without a directional hypothesis. [d] Fraction of genes in gene set with unadjusted 95% CI not spanning zero i.e. minimum significant difference (MSD) > 0. [e] Gene-set enrichment analysis (GSEA) tests for over-representation among top and bottom genes based on Log_2 fold-changes × $-log_{10}$(P-values) in comparing changes from pre-RCT to pre-intro RT (Δ) in Δ vitamin D_3 (n = 11) to Δ placebo arm (n = 13). Positive normalized enrichment scores (NES) indicates gene sets with higher expression in post-intro resistance training (RT) or Post-RCT compared to pre-intro RT, negative NES indicates gene sets with lower expression at respective time-points. [f] Number of genes in leading edge (LE, genes that contributes to the enrichment score) with the fraction of leading edge genes with unadjusted 95% CI not spanning zero (MSD > 0). *P*-values are adjusted for FDR.

Table S6 Functional annotation analysis of placebo compared to Vitamin D_3 supplementation using KEGG and Hallmark gene set collections

Database	Gene set	Significance category[a]	GSEA *P*-value[b]	NES	Rank *P*-value[c]
Hallmark	Glycolysis	Consensus	0.0222660	-1.416601	0.0035221
	Oxidative phosphorylation	Consensus	0.0000008	-1.705525	0.0164380
	Apical junction	Consensus	0.0002589	-1.581541	0.0243787
	Myogenesis	GSEA	0.0000597	-1.621807	0.0801965
	Spermatogenesis	GSEA	0.0222660	1.608497	0.8502940
	Adipogenesis	Rank	0.2330750	-1.225707	0.0035221
	Hypoxia	Rank	0.5569932	-1.052236	0.0243832
KEGG	Focal adhesion	Consensus	0.0408189	-1.455807	0.0108574
	Oxidative phosphorylation	Consensus	0.0005303	-1.718086	0.0306703
	Parkinsons disease	Consensus	0.0129755	-1.610688	0.0306703
	Alzheimers disease	Consensus	0.0274953	-1.533430	0.0328614
	Ecm receptor interaction	GSEA	0.0129755	-1.679383	0.1129705
	Pathogenic escherichia coli infection	GSEA	0.0361533	-1.623002	0.2248120

[a] Consensus significance indicates agreement between directional (GSEA) and non-directional (Rank) hypothesis test of overrepresentation (see methods for details). [b] Gene-set enrichment analysis (GSEA) tests for over-representation among top and bottom genes based on Log_2 fold-changes × $-log_{10}$(P-values) in comparing changes from pre-RCT to pre-intro RT (Δ) in Δ vitamin D_3 (n = 11) to Δ placebo arm (n = 13). [c] Rank-based enrichment test based on minimum significant difference identifies gene-sets that are over-represented among top-ranked genes without a directional hypothesis. *P*-values are adjusted for FDR.

Table S7 Genes identified as differentially expressed between Vitamin D_3 after the supplementation period (Time × Treatment)

Ensembl gene ID	Gene Symbol	Log fold-change	SE	*Z*-value	*P*-value	Adjusted *P*-value[a]	Uniformity (*P*-value)[b]
ENSG00000145819	ARHGAP26	0.45	0.08	5.405	6.49e-08	9.49e-04	0.993
ENSG00000184898	RBM43	0.50	0.10	5.285	1.26e-07	9.49e-04	0.826
ENSG00000170619	COMMD5	-0.74	0.15	-5.021	5.14e-07	0.002	0.939
ENSG00000012211	PRICKLE3	-0.70	0.14	-4.874	1.10e-06	0.003	0.517
ENSG00000276023	DUSP14	-0.63	0.13	-4.806	1.54e-06	0.003	0.381
ENSG00000113916	BCL6	0.57	0.12	4.694	2.68e-06	0.004	0.916
ENSG00000241399	CD302	0.54	0.12	4.625	3.74e-06	0.005	0.947
ENSG00000122884	P4HA1	0.40	0.09	4.464	8.04e-06	0.010	0.807
ENSG00000117410	ATP6V0B	-0.40	0.09	-4.308	1.65e-05	0.016	0.967
ENSG00000167772	ANGPTL4	-1.57	0.37	-4.285	1.83e-05	0.016	0.878
ENSG00000072657	TRHDE	0.87	0.21	4.254	2.10e-05	0.018	0.781
ENSG00000112394	SLC16A10	0.42	0.10	4.177	2.96e-05	0.020	0.509
ENSG00000184307	ZDHHC23	-0.85	0.20	-4.195	2.73e-05	0.020	0.924
ENSG00000248713	C4orf54	0.47	0.11	4.186	2.84e-05	0.020	0.976
ENSG00000211899	Not mapped[d]	-1.52	0.37	-4.141	3.47e-05	0.023	0.387
ENSG00000130402	ACTN4	-0.57	0.14	-4.116	3.85e-05	0.023	0.992
ENSG00000146278	PNRC1	0.44	0.11	4.125	3.70e-05	0.023	0.904
ENSG00000279668	Not mapped[d]	0.73	0.18	4.108	4.00e-05	0.023	0.797
ENSG00000145358	DDIT4L	0.63	0.16	4.081	4.49e-05	0.024	0.583
ENSG00000156804	FBXO32	0.56	0.14	4.070	4.71e-05	0.024	0.993
ENSG00000138379	MSTN	0.60	0.15	4.036	5.44e-05	0.026	0.965
ENSG00000091136	LAMB1	-0.39	0.10	-4.004	6.23e-05	0.027	0.441
ENSG00000232079	LINC01697	0.53	0.13	4.016	5.91e-05	0.027	0.157
ENSG00000250978	Not mapped[d]	0.68	0.17	3.988	6.67e-05	0.027	0.283
ENSG00000013306	SLC25A39	-0.79	0.20	-3.958	7.56e-05	0.028	0.854
ENSG00000138376	BARD1	0.38	0.10	3.940	8.16e-05	0.029	0.821
ENSG00000167553	TUBA1C	-0.54	0.14	-3.942	8.08e-05	0.029	0.683
ENSG00000164823	OSGIN2	0.35	0.09	3.922	8.77e-05	0.029	0.489
ENSG00000149923	PPP4C	-0.65	0.17	-3.896	9.80e-05	0.030	0.629
ENSG00000172890	NADSYN1	-0.46	0.12	-3.885	1.03e-04	0.031	0.642
ENSG00000143554	SLC27A3	-0.72	0.19	-3.866	1.10e-04	0.033	0.463
ENSG00000172493	AFF1	0.36	0.09	3.853	1.17e-04	0.033	0.995
ENSG00000182492	BGN	-0.85	0.22	-3.821	1.33e-04	0.036	0.901
ENSG00000138600	SPPL2A	0.38	0.10	3.793	1.49e-04	0.038	0.843
ENSG00000266524	GDF10	-0.90	0.24	-3.796	1.47e-04	0.038	0.454
ENSG00000274180	NATD1	-0.59	0.16	-3.800	1.45e-04	0.038	0.823
ENSG00000099991	CABIN1	-0.39	0.10	-3.760	1.70e-04	0.041	0.569
ENSG00000156219	ART3	0.36	0.10	3.736	1.87e-04	0.042	0.919
ENSG00000160783	PMF1	-0.65	0.17	-3.736	1.87e-04	0.042	0.870
ENSG00000113272	THG1L	0.38	0.10	3.730	1.92e-04	0.042	0.885
ENSG00000007312	CD79B	-1.20	0.32	-3.713	2.05e-04	0.042	0.689
ENSG00000115461	IGFBP5	0.43	0.12	3.714	2.04e-04	0.042	0.552
ENSG00000125845	BMP2	0.64	0.17	3.709	2.08e-04	0.042	0.691
ENSG00000197070	ARRDC1	-0.90	0.24	-3.720	1.99e-04	0.042	0.941
ENSG00000137727	ARHGAP20	0.85	0.23	3.695	2.20e-04	0.043	0.509
ENSG00000159228	CBR1	-0.54	0.15	-3.701	2.15e-04	0.043	0.723
ENSG00000165915	SLC39A13	-0.93	0.25	-3.685	2.29e-04	0.044	0.738
ENSG00000176108	CHMP6	-0.70	0.19	-3.676	2.37e-04	0.045	0.926
ENSG00000224051	CPTP	-0.78	0.21	-3.677	2.36e-04	0.045	0.748
ENSG00000069667	RORA	0.47	0.13	3.671	2.42e-04	0.045	0.988
ENSG00000214970	Not mapped[d]	0.59	0.16	3.664	2.49e-04	0.046	0.766
ENSG00000182809	CRIP2	-1.35	0.37	-3.647	2.66e-04	0.048	0.706
ENSG00000162989	KCNJ3	0.66	0.18	3.635	2.77e-04	0.049	0.271
ENSG00000121068	TBX2	-0.70	0.19	-3.622	2.92e-04	0.050	0.967

[a] *P*-values are adjusted for FDR. [b] Raw *P*-values from simulation based tests of uniformity of residuals where low values indicates problematic models (see methods). [d] No official gene symbol available, not included in enrichment analyses.

Table S8 Functional annotation analysis of resistance training effects averaged over treatment arms.

Comparison	Gene ontology category	Gene ontology	Significance category[a]	Set size[b]	Rank *P*-value[c]	% MSD > 0[d]	GSEA *P*-value[e]	Normalized enrichment score	LE[f]	Log_2 Fold-change in LE [min, max]
Post-intro RT vs. pre-intro RT	Biological process	Blood vessel morphogenesis	Consensus	455 (686)	6.93e-18	51.2%	4.94e-04	1.38	126 (100%)	0.52 [0.16, 2.1]
		Extracellular structure organization	Consensus	251 (373)	7.24e-36	59.8%	7.78e-08	1.54	94 (100%)	0.66 [0.2, 2.1]
		Inflammatory response	Consensus	436 (765)	4.23e-23	53.9%	0.017	1.32	152 (100%)	0.51 [0.17, 1.89]
		Leukocyte migration	Consensus	272 (502)	1.26e-21	62.1%	0.046	1.35	101 (100%)	0.5 [0.15, 1.8]
	Cellular component	Collagen containing extracellular matrix	Consensus	262 (408)	1.45e-37	62.2%	6.59e-07	1.50	94 (100%)	0.67 [0.2, 2.08]
		Collagen trimer	Consensus	54 (87)	1.31e-15	72.2%	0.019	1.53	21 (100%)	0.89 [0.39, 2.08]
		External side of plasma membrane	Consensus	184 (388)	1.31e-19	65.2%	0.008	1.44	79 (100%)	0.53 [0.17, 3.09]
		Extracellular matrix	Consensus	321 (531)	8.26e-42	59.8%	6.59e-07	1.48	107 (100%)	0.7 [0.2, 2.08]
		Side of membrane	Consensus	324 (582)	2.20e-16	56.2%	0.007	1.36	103 (100%)	0.49 [0.15, 3.09]
	Molecular function	Extracellular matrix structural constituent	Consensus	111 (165)	2.29e-27	67.6%	8.67e-04	1.58	45 (100%)	0.8 [0.2, 2.08]
	Biological process	Nuclear transcribed mRNA catabolic process	GSEA	174 (208)	0.678	48.3%	1.13e-06	-2.04	85 (84.7%)	-0.17 [-0.49, -0.09]
		Ribosome biogenesis	GSEA	268 (290)	1.000	38.4%	2.19e-10	-2.24	137 (73%)	-0.14 [-0.54, -0.07]
		RNA splicing via transesterification reactions	GSEA	311 (345)	0.941	49.2%	1.33e-08	-2.08	138 (93.5%)	-0.13 [-0.3, -0.05]
		rRNA metabolic process	GSEA	204 (221)	1.000	38.2%	5.82e-06	-2.01	108 (70.4%)	-0.13 [-0.31, -0.07]
		Translational initiation	GSEA	158 (192)	0.360	51.3%	1.13e-06	-2.12	83 (86.7%)	-0.17 [-0.4, -0.08]
		Viral gene expression	GSEA	167 (194)	0.807	47.9%	4.97e-05	-1.91	85 (83.5%)	-0.17 [-0.31, -0.08]
	Cellular component	Ribosomal subunit	GSEA	158 (186)	1.000	44.3%	1.26e-06	-1.98	77 (81.8%)	-0.18 [-0.87, -0.08]
		Ribosome	GSEA	196 (228)	1.000	42.3%	6.59e-07	-2.03	84 (83.3%)	-0.18 [-0.87, -0.08]
		Spliceosomal complex	GSEA	169 (186)	0.950	52.1%	1.13e-04	-1.78	83 (90.4%)	-0.13 [-0.25, -0.05]
	Molecular function	Structural constituent of ribosome	GSEA	132 (162)	0.992	47%	4.03e-05	-2.05	70 (81.4%)	-0.17 [-0.31, -0.08]
	Biological process	Cell chemotaxis	Rank	178 (306)	1.13e-14	59.6%	0.066	1.39	66 (100%)	0.56 [0.15, 1.8]
		Collagen fibril organization	Rank	36 (55)	2.14e-14	72.2%	0.089	1.53	18 (100%)	0.93 [0.32, 2.08]
		Leukocyte cell cell adhesion	Rank	210 (343)	2.84e-13	53.3%	0.112	1.34	77 (100%)	0.49 [0.17, 1.78]
		Lymphocyte activation	Rank	430 (736)	5.72e-14	49.5%	0.080	1.28	117 (100%)	0.5 [0.19, 1.78]
		Positive regulation of cell adhesion	Rank	269 (401)	4.09e-13	52%	0.139	1.29	72 (100%)	0.53 [0.17, 1.78]
		Regulation of cell adhesion	Rank	466 (695)	1.20e-14	48.1%	0.057	1.27	120 (100%)	0.5 [0.17, 1.78]
		Response to wounding	Rank	434 (687)	4.09e-13	50.7%	0.051	1.29	108 (100%)	0.47 [0.15, 2.08]
		T cell activation	Rank	291 (468)	5.86e-13	50.5%	0.068	1.31	87 (100%)	0.52 [0.2, 1.78]
		Taxis	Rank	407 (652)	1.54e-14	51.8%	0.135	1.23	136 (99.3%)	0.46 [0.15, 1.8]
	Molecular function	Integrin binding	Rank	108 (135)	8.32e-13	59.3%	0.052	1.48	35 (100%)	0.67 [0.2, 2.08]
	Biological process	Blood vessel morphogenesis	Consensus	455 (686)	1.56e-25	52.1%	5.70e-08	1.65	127 (100%)	0.45 [0.16, 1.64]

Post-RCT vs. pre-intro RT		Extracellular structure organization	Consensus	251 (373)	5.53e-32	59.8%	3.48e-08	1.77	90 (100%)	0.54 [0.16, 1.64]
		Leukocyte migration	Consensus	272 (502)	1.36e-10	47.8%	0.014	1.53	69 (100%)	0.45 [0.16, 1.35]
		Regulation of vasculature development	Consensus	246 (425)	5.30e-13	52.8%	5.51e-04	1.64	70 (100%)	0.44 [0.17, 1.64]
	Cellular component	Basement membrane	Consensus	72 (95)	9.21e-13	61.1%	0.006	1.72	23 (100%)	0.62 [0.18, 1.15]
		Collagen containing extracellular matrix	Consensus	262 (408)	3.91e-23	52.7%	3.48e-09	1.77	77 (100%)	0.59 [0.17, 1.64]
		Collagen trimer	Consensus	54 (87)	1.86e-10	57.4%	0.003	1.69	21 (100%)	0.69 [0.28, 1.53]
		Extracellular matrix	Consensus	321 (531)	1.09e-26	52.6%	4.67e-09	1.74	90 (100%)	0.6 [0.17, 1.64]
	Molecular function	Extracellular matrix structural constituent	Consensus	111 (165)	1.65e-22	64%	5.84e-05	1.78	40 (100%)	0.67 [0.18, 1.64]
		Extracellular matrix structural constituent conferring tensile strength	Consensus	28 (41)	2.79e-10	67.9%	0.042	1.62	14 (100%)	0.79 [0.42, 1.53]
	Biological process	mRNA processing	GSEA	452 (503)	1.000	43.4%	1.13e-07	-1.80	167 (95.8%)	-0.13 [-0.33, -0.06]
		NcRNA metabolic process	GSEA	424 (471)	1.000	34.4%	6.67e-06	-1.72	118 (93.2%)	-0.15 [-0.37, -0.08]
		NcRNA processing	GSEA	346 (378)	1.000	35.8%	6.41e-07	-1.81	108 (93.5%)	-0.15 [-0.37, -0.08]
		Ribonucleoprotein complex biogenesis	GSEA	383 (419)	0.983	41.3%	2.16e-15	-2.11	141 (96.5%)	-0.16 [-0.38, -0.07]
		Ribosome biogenesis	GSEA	268 (290)	1.000	38.8%	2.34e-09	-2.07	90 (97.8%)	-0.17 [-0.38, -0.08]
		RNA catabolic process	GSEA	339 (404)	0.064	47.2%	1.24e-07	-1.86	107 (100%)	-0.21 [-0.44, -0.09]
		RNA splicing	GSEA	390 (433)	0.971	45.6%	5.29e-10	-1.91	156 (95.5%)	-0.13 [-0.33, -0.06]
		RNA splicing via transesterification reactions	GSEA	311 (345)	0.865	47.6%	3.15e-09	-1.94	132 (97%)	-0.13 [-0.33, -0.06]
		rRNA metabolic process	GSEA	204 (221)	1.000	38.2%	6.67e-06	-1.93	79 (91.1%)	-0.15 [-0.37, -0.07]
	Cellular component	Spliceosomal complex	GSEA	169 (186)	0.675	51.5%	1.68e-05	-1.98	68 (100%)	-0.14 [-0.33, -0.08]
	Biological process	Cell substrate adhesion	Rank	262 (348)	1.19e-09	48.9%	0.144	1.40	54 (100%)	0.45 [0.17, 1.53]
		Collagen fibril organization	Rank	36 (55)	7.77e-12	72.2%	0.076	1.60	17 (100%)	0.75 [0.25, 1.53]
		Epithelial cell proliferation	Rank	289 (441)	1.37e-10	46.4%	0.079	1.46	62 (100%)	0.45 [0.16, 1.33]
		Positive regulation of cell adhesion	Rank	269 (401)	1.38e-08	46.8%	0.256	1.29	72 (100%)	0.39 [0.17, 1.35]
		Positive regulation of locomotion	Rank	411 (604)	5.47e-11	46.7%	0.074	1.38	91 (100%)	0.41 [0.17, 1.35]
		Regulation of cell adhesion	Rank	466 (695)	5.55e-11	45.5%	0.122	1.32	114 (100%)	0.39 [0.16, 1.35]
		Taxis	Rank	407 (652)	4.06e-10	46.4%	0.075	1.37	108 (99.1%)	0.41 [0.13, 1.25]
	Molecular function	Cell adhesion molecule binding	Rank	403 (501)	4.33e-08	45.4%	0.078	1.38	68 (100%)	0.47 [0.17, 1.53]
		Integrin binding	Rank	108 (135)	6.13e-11	52.8%	0.096	1.50	31 (100%)	0.56 [0.17, 1.53]
		Structural molecule activity	Rank	433 (670)	2.48e-19	51.7%	0.078	1.37	56 (100%)	0.62 [0.18, 1.64]

[a] Consensus significance indicates agreement between directional (GSEA) and non-directional (Rank) hypothesis test of overrepresentation (see methods for details). [b] Indicates number identified genes in gene set and total number of gene in gene set in parentheses. [c], rank-based enrichment test based on minimum significant difference identifies gene-sets that are over-represented among top-ranked genes without a directional hypothesis. [d] Fraction of genes in gene set with unadjusted 95% CI not spanning zero i.e. minimum significant difference (MSD) > 0. [e], Gene-set enrichment analysis (GSEA) tests for over-representation among top and bottom genes based on Log_2 fold-changes × $-log_{10}$(P-values) in time-point with effects averaged over treatment arms (n = 53). Positive normalized enrichment scores (NES) indicates gene sets with higher expression in post-intro resistance training (RT) or post-RCT compared to pre-intro RT, negative NES indicates gene sets

with lower expression at respective time-points. [f] Number of genes in leading edge (LE, genes that contributes to the enrichment score) with the fraction of leading edge genes with unadjusted 95% CI not spanning zero (MSD > 0). *P*-values are adjusted for FDR.

Table S9 Functional annotation analysis time-effect between vitamin D_3 and placebo treatment

Comparison	Gene ontology category	Gene ontology	Significance category[a]	Set size[b]	Rank *P*-value[c]	% MSD > 0[d]	GSEA *P*-value[e]	Normalized enrichment score	LE[f]	Log_2 Fold-change in LE [min, max]
Post-intro RT: Vitamin D_3 vs. placebo	Biological process	Acetyl coa metabolic process	GSEA	33 (38)	0.859	6.1%	0.038	-1.94	3 (66.7%)	-1.05 [-2.62, -0.2]
		Blood vessel morphogenesis	GSEA	452 (686)	0.612	5.1%	0.013	1.63	75 (24%)	0.34 [0.12, 0.94]
		Cell cell junction organization	GSEA	136 (188)	0.750	6.6%	0.030	1.76	30 (30%)	0.33 [0.1, 1.35]
		Cell junction organization	GSEA	231 (293)	0.907	5.2%	0.030	1.69	43 (25.6%)	0.32 [0.1, 1.35]
		Muscle cell differentiation	GSEA	280 (383)	0.753	5.7%	0.037	1.63	35 (34.3%)	0.43 [0.12, 1.35]
		Muscle system process	GSEA	342 (470)	0.909	4.7%	0.030	1.60	34 (32.4%)	0.46 [0.18, 1.39]
		Striated muscle cell differentiation	GSEA	213 (291)	0.760	6.1%	0.030	1.70	32 (31.2%)	0.42 [0.11, 1.35]
		Thioester metabolic process	GSEA	85 (105)	1.000	2.4%	0.030	-1.92	3 (66.7%)	-1.05 [-2.62, -0.2]
		Tissue migration	GSEA	233 (363)	0.938	3.4%	0.037	1.64	33 (21.2%)	0.34 [0.12, 1.39]
		Heterophilic cell cell adhesion via plasma membrane cell adhesion molecules	Rank	27 (46)	0.018	18.5%	0.432	1.48	6 (66.7%)	0.69 [0.26, 1.35]
		Negative regulation of cell differentiation	Rank	465 (750)	0.018	5.4%	0.075	1.48	68 (26.5%)	0.37 [0.13, 1.2]
		Negative regulation of notch signaling pathway	Rank	36 (44)	0.014	16.7%	0.825	1.08	16 (25%)	0.3 [0.1, 0.62]
		Regulation of notch signaling pathway	Rank	76 (107)	0.018	13.2%	0.840	1.05	14 (35.7%)	0.38 [0.22, 0.62]
Post-RCT: Vitamin D_3 vs. placebo		Blood vessel morphogenesis	Consensus	452 (686)	0.002	9.5%	9.78e-08	1.79	135 (28.1%)	0.31 [0.12, 1.27]
		Endothelial cell proliferation	Consensus	115 (191)	0.046	10.4%	0.032	1.60	33 (30.3%)	0.31 [0.14, 0.68]
		Establishment of endothelial barrier	Consensus	31 (43)	0.022	22.6%	0.031	1.66	12 (58.3%)	0.28 [0.15, 0.44]
		Actin filament organization	GSEA	299 (393)	0.481	7.7%	1.73e-04	1.73	72 (25%)	0.3 [0.11, 0.95]
		Cell junction organization	GSEA	231 (293)	0.614	7.4%	5.48e-05	1.80	62 (24.2%)	0.3 [0.12, 0.78]
		Endothelial cell migration	GSEA	170 (275)	0.691	7.1%	2.64e-04	1.79	56 (21.4%)	0.26 [0.13, 0.7]
		Extracellular structure organization	GSEA	251 (373)	0.730	6.4%	2.64e-04	1.73	79 (17.7%)	0.3 [0.11, 1.27]
		Leukocyte migration	GSEA	275 (502)	0.155	8.7%	2.06e-04	1.72	63 (33.3%)	0.35 [0.13, 0.95]
		Lymphocyte activation	GSEA	429 (736)	0.813	5.6%	5.48e-05	1.68	89 (20.2%)	0.35 [0.15, 0.77]
		Regulation of cell activation	GSEA	354 (619)	0.735	6.2%	1.91e-04	1.68	79 (21.5%)	0.34 [0.15, 0.76]
		Regulation of supramolecular fiber organization	GSEA	273 (351)	0.640	6.2%	2.64e-04	1.71	63 (23.8%)	0.3 [0.11, 0.95]
		Regulation of vasculature development	GSEA	244 (425)	0.083	10.2%	3.63e-04	1.71	77 (29.9%)	0.29 [0.14, 0.88]
		T cell activation	GSEA	292 (468)	0.876	6.5%	2.64e-04	1.71	60 (25%)	0.36 [0.15, 0.77]

		Negative regulation of notch signaling pathway	Rank	36 (44)	0.041	16.7%	0.268	1.44	16 (31.2%)	0.36 [0.17, 0.75]
		Regulation of notch signaling pathway	Rank	76 (107)	0.037	10.5%	0.236	1.41	16 (43.8%)	0.41 [0.19, 0.75]

[a] Consensus significance indicates agreement between directional (GSEA) and non-directional (Rank) hypothesis test of overrepresentation (see methods for details). [b] Indicates number identified genes in gene set and total number of gene in gene set in parentheses. [c], rank-based enrichment test based on minimum significant difference identifies gene-sets that are over-represented among top-ranked genes without a directional hypothesis. [d] Fraction of genes in gene set with unadjusted 95% CI not spanning zero i.e. minimum significant difference (MSD) > 0. [e], Gene-set enrichment analysis (GSEA) tests for over-representation among top and bottom genes based on Log_2 fold-changes × $-log_{10}$(P-values) in comparing changes over time (Δ) in Δ vitamin D_3 (*n* = 24) to Δ placebo arm (*n* = 29). Positive normalized enrichment scores (NES) indicates gene sets with higher expression in post-intro resistance training (RT) or Post-RCT compared to pre-intro RT, negative NES indicates gene sets with lower expression at respective time-points. [f] Number of genes in leading edge (LE, genes that contributes to the enrichment score) with the fraction of leading edge genes with unadjusted 95% CI not spanning zero (MSD > 0). *P*-values are adjusted for FDR.

Table S10 Functional annotation analysis of placebo compared to Vitamin D_3 supplementation combined with training using KEGG and Hallmark gene set collections

Comparison	Database	Gene set	Significance category[a]	GSEA *P*-value[b]	NES	Rank *P*-value[c]
Post-intro RT: Vitamin D_3 vs. placebo	Hallmark	Apical junction	GSEA	0.008	1.65	0.274
Post-RCT: Vitamin D_3 vs. placebo		Coagulation	GSEA	0.007	1.70	1.000
		Epithelial mesenchymal transition	GSEA	0.007	1.60	1.000
	KEGG	Cytokine cytokine receptor interaction	GSEA	0.010	1.65	0.201
		Leukocyte transendothelial migration	GSEA	0.008	1.73	0.201
		Chemokine signaling pathway	GSEA	0.010	1.66	1.000
		Ecm receptor interaction	GSEA	0.019	1.67	1.000
		Fc gamma r mediated phagocytosis	GSEA	0.019	1.69	1.000
		Focal adhesion	GSEA	0.008	1.65	1.000
		Natural killer cell mediated cytotoxicity	GSEA	0.026	1.60	1.000
		Regulation of actin cytoskeleton	GSEA	0.019	1.57	1.000

[a] Consensus significance indicates agreement between directional (GSEA) and non-directional (Rank) hypothesis test of overrepresentation (see methods for details). [b] Gene-set enrichment analysis (GSEA) tests for over-representation among top and bottom genes based on Log_2 fold-changes × $-log_{10}$(P-values) in comparing changes from pre-intro RT to Post-RCT (Δ) in Δ vitamin D_3 (n = 24) to Δ placebo arm (n = 29). [c] Rank-based enrichment test based on minimum significant difference identifies gene-sets that are over-represented among top-ranked genes without a directional hypothesis. *P*-values are adjusted for FDR.

Table S11 Blood and health variables

	Vitamin D_3 arm		Placebo arm			
	Baseline	**Post-RCT**	**Baseline**	**Post-RCT**	**Time effect ($p < 0.05$)**	**Vitamin D_3 vs placebo arm (p-value)**
Dual-energy x-ray absorptiometry						
Whole-body bone mineral density ($g \cdot cm^2$)	1.15 (0.16)	1.15 (0.16)	1.14 (0.18)	1.13 (0.18)	No	0.476
Total lean mass (kg)	47.7 (11.1)	48.2 (11.1)	47.7 (9.0)	48.4 (9.2)	Yes (↑)	0.498
Total fat mass	25.6 (8.5)	25.0 (7.9)	25.6 (11.2)	24.9 (11.3)	Yes (↓)	0.654
Visceral fat (gram)	1411 (1004)	1296 (906)	1124 (988)	1060 (1011)	Yes (↓)	0.865
Hormones						
Cortisol ($nmol \cdot L^{-1}$)	367 (89)	378 (79)	356 (95)	339 (119)	No	***0.038***
Growth hormone ($\mu g \cdot L^{-1}$)	1.04 (1.51)	1.40 (1.59)	1.38 (1.73)	1.22 (2.36)	No	0.985
IGF-1 ($nmol \cdot L^{-1}$)	14.1 (3.6)	13.5 (3.3)	14.8 (2.9)	14.3 (3.7)	No	0.971
Testosterone ($nmol \cdot L^{-1}$) *	10.8 (2.4)	12.1 (3.1)	12.1 (4.7)	12.0 (4.0)	No	0.832
Sex-hormone binding globulin ($nmol \cdot L^{-1}$)	57 (22)	59 (23)	61 (27)	61 (27)	No	0.453
Androstenedione ($nmol \cdot L^{-1}$)	4.0 (2.5)	4.3 (2.9)	3.4 (1.5)	3.3 (1.8)	No	0.507
Parathyroid hormone ($pmol \cdot L^{-1}$)	5.5 (2.2)	4.8 (1.5)	5.9 (2.3)	5.8 (2.8)	Yes (↓)	0.145
Lipid profile variables						
Triglycerides ($mmol \cdot L^{-1}$)	1.26 (0.42)	1.20 (0.59)	1.15 (0.57)	1.02 (0.54)	Yes (↓)	0.659
HDL ($mmol \cdot L^{-1}$)	1.69 (0.54)	1.65 (0.50	1.76 (0.47)	1.75 (0.49)	No	0.570
LDL ($mmol \cdot L^{-1}$)	3.3 (1.0)	3.0 (0.8)	3.4 (1.0)	3.3 (1.0)	Yes (↓)	0.752
Iron biology variables						
Fe^{2+} ($\mu mol\ L^{-1}$)	21.3 (4.7)	17.5 (5.9)	20.1 (5.2)	17.7 (4.9)	Yes (↓)	0.718
Transferrin ($g \cdot L^{-1}$)	2.50 (0.25)	2.48 (0.30)	2.42 (0.36)	2.43 (0.40)	No	0.782
Ferritin ($\mu g \cdot L^{-1}$)	135 (78)	116 (71)	155 (91)	126 (75)	Yes (↓)	0.912
Calcium status						
Calcium ($mmol \cdot L^{-1}$)	2.38 (0.11)	2.39 (0.11)	2.36 (0.08)	2.37 (0.08)	No	0.410
Albumin-corrected calcium ($mmol \cdot L^{-1}$)	2.28 (0.12)	2.28 (0.11)	2.27 (0.07)	2.30 (0.09)	No	0.149
Renal function						
Creatinine ($\mu mol \cdot L^{-1}$)	77.8 (17.8)	82.0 (19.6)	80.3 (22.2)	85.0 (24.7)	Yes (↑)	0.542
Tissue damage variables						
Aspartate transaminase ($units \cdot L^{-1}$)	28.1 (11.5)	24.9 (6.7)	28.4 (9.8)	25.2 (6.4)	Yes (↓)	0.794
Creatine kinase ($units \cdot L^{-1}$)	168 (148)	126 (85)	172 (192)	124 (60)	Yes (↓)	0.455
Self-reported health						
Avg. score per week (0-10)	6.3 (1.2)		6.6 (1.5)		Yes (↑)	0.433

For assessing the efficacy of vitamin D_3 supplementation, mixed models with change scores as the dependent variable and baseline values as a covariate was performed. For self-reported health, an independent t-test was performed for the same purpose. *, men-only were included in the testosterone analysis. Alpha level at $p < 0.05$. Values are means with standard deviation.

For the general health benefits of the intervention, the -6 % reductions in triglyceride levels and the -4 % reductions of LDL in serum are of particular interest (with no changes being observed for HDL). This lowered the number of participants with diagnostically elevated LDL levels (≥ 4.1 $mmol \cdot L^{-1}$)[1] from 17 to 13, emphasizing the potential benefits of resistance training for lipid profiles, as has previously been shown to be equivocal.[2,3] This was accompanied by -2.7 % reductions in whole-body fat mass and -5.9 % reductions in visceral fat mass. The observed reduction in visceral fat mass is noteworthy, as its relative change was 2.1-fold greater than the change in overall fat mass, though they were largely correlated (Pearson's $r = 0.70$; $p < 0.001$). This is not an uncommon observation[4–6] and suggests that resistance training leads to targeted metabolism of visceral fat. Overall, these data support the notion that resistance training is an effective strategy for improving long-term cardiovascular health.[2,7]

The intervention was associated with alterations in serum levels of markers of iron biology. Specifically, serum levels of Fe^{2+} and ferritin decreased (-12 % and -16 %, respectively), while levels of transferrin remained unchanged (-0.4 %). Speculatively, this may have affected biological processes such as hemoglobin production and the oxygen-carrying capacity of blood, which was not measured. However, no traces of such adverse effects were found in maximal oxygen uptake (Table S2), which did not change over the course of the training intervention and is known to be closely correlated with total hemoglobin mass.[8] The observed alteration in iron biology may have been due to the daily intake of 500 mg calcium in both supplementation arms, which is known to exert negative effects on iron absorption in humans.[9] The rationale behind including calcium supplementation as part of the study protocol was to ensure sufficient levels of calcium in both supplementation arms, facilitating potential accretion of bone in response to resistance training, particularly so in the vitamin D_3 arm.

Serum levels of markers of muscle tissue damage (creatine kinase and aspartate aminotransferase) decreased during the intervention. This may have been affected by pre-RCT testing, as these were performed during the week preceding blood sampling, and may have contributed to increased levels of creatine kinase and aspartate aminotransferase.[10] Such responses are typically upon frequent conduction of exercise.[11]

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Appendix IV

Supplementary material for Paper III

Chronic Obstructive Lung Disease Does Not Impair Responses to Resistance Training

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Supplementary Tables

Supplementary Table 1. Computed factors for the core outcome domains lower-body muscle mass, lower-body muscle strength, one-legged endurance performance and whole-body endurance performance. Each factor consists of multiple singular outcome measures. First, for each outcome measure, each subject's value (pre and post) was normalized to the highest recorded value during the study conduct, thus providing values <1. Then, for each subject, an ultimate factor was computed for lower-body muscle mass, lower-body muscle strength, one-legged endurance performance and whole-body endurance performance, respectively, calculated as the mean of normalized values for the various variables included.

Lower-body muscle mass factor										
	Included variables	***Explanation***	***Baseline (avg ± SD)***	***Post intervention (avg ± SD)***	***Estimate, change (95% CI)***	***Main effect of time (p value)***	***Correlation with factor at baseline, r value (p value)***	***Correlation for change score with change score for factor, r value (p value)***	***Eigenvalue***	***% variance explained***
	1. Muscle thickness	The combined measure of muscle thickness of *vastus lateralis* and *rectus femoris*	0.60 (0.10)	0.66 (0.11)	0.06 (0.05, 0.07)	<0.001	0.83 (<0.001)	0.84 (<0.001)	-	-
	2. Leg lean mass	Lean mass in the legs	0.64 (0.15)	0.65 (0.15)	0.01 (0.01, 0.02)	<0.001	0.92 (<0.001)	0.63 (<0.001)	-	-
	Lower-body muscle mass factor	-	**0.63 (0.11)**	**0.66 (0.12)**	**0.04 (0.03, 0.04)**	**<0.001**	-	-	**1.10**	**55**
Lower-body muscle strength factor										
	Included variables	***Explanation***	***Baseline (avg ± SD)***	***Post intervention (avg ± SD)***	***Estimate, change (95% CI)***	***Main effect of time (p value)***	***Correlation with factor at baseline, r value (p value)***	***Correlation for change score with change score for factor, r value (p value)***	***Eigenvalue***	***% variance explained***
	1. Leg muscle strength	The combined measure of 1RM knee extension and leg press	0.44 (0.14)	0.52 (0.15)	0.08 (0.07, 0.09)	<0.001	0.95 (<0.001)	0.78 (<0.001)	-	-
	2. Leg muscle torque	The combined measure of torque (Nm) achieved during knee extension at 60°, 180° and 240°/sec	0.48 (0.16)	0.51 (0.17)	0.03 (0.02, 0.04)	<0.001	0.97 (<0.001)	0.73 (<0.001)	-	-
	Lower-body muscle strength factor	-	**0.46 (0.14)**	**0.52 (0.15)**	**0.06 (0.05, 0.06)**	**<0.001**	-	-	**1.13**	**57**
One-legged endurance performance factor										
	Included variables	***Explanation***	***Baseline (avg ± SD)***	***Post intervention (avg ± SD)***	***Estimate, change (95% CI)***	***Main effect of time (p value)***	***Correlation with factor at baseline, r value (p value)***	***Correlation for change score with change score for factor, r value (p value)***	***Eigenvalue***	***% variance explained***
	1. Muscle performance	Number of repetitions at 50% of 1RM knee extension	0.13 (0.03)	0.22 (0.09)	0.09 (0.08, 0.10)	<0.001	0.60 (<0.001)	0.92 (<0.001)		
	2. Maximal power output	Maximal power output achieved during one-legged cycling	0.49 (0.17)	0.52 (0.17)	0.04 (0.03, 0.04)	<0.001	0.99 (<0.001)	0.29 (<0.001)		
	One-legged endurance performance factor	-	**0.31 (0.09)**	**0.37 (0.11)**	**0.06 (0.06, 0.07)**	**<0.001**			**1.11**	**56**

Whole-body endurance performance factor										
	Included variables	***Explanation***	***Baseline (avg ± SD)***	***Post intervention (avg ± SD)***	***Estimate, change (95% CI)***	***Main effect of time (p value)***	***Correlation with factor at baseline, r value (p value)***	***Correlation for change score with change score for factor, r value (p value)***	***Eigenvalue***	***% variance explained***
	Maximal power output	Maximal power output achieved during bicycling	0.44 (0.17)	0.47 (0.18)	0.03 (0.02, 0.04)	<0.001	0.87 (<0.001)	0.54 (<0.001)	-	-
	6-min step test	Number of steps achieved during a 6-min test	0.59 (0.17)	0.63 (0.19)	0.04 (0.03, 0.06)	<0.001	0.96 (<0.001)	0.64 (<0.001)	-	-
	1-min sit-to-stand test	Number of sit-to-stands achieved during a 1-min test	0.58 (0.13)	0.62 (0.14)	0.04 (0.03, 0.06)	<0.001	0.86 (<0.001)	0.76 (<0.001)	-	-
	Whole-body endurance performance factor	-	0.54 (0.14)	0.58 (0.15)	0.04 (0.03, 0.05)	**<0.001**	-	-	**1.29**	**43**

Supplementary Table 2. Genes identified as differentially expressed at baseline between COPD and Healthy in genome-wide transcriptome analyses (RNA-seq). RNA-seq analyses were performed as previously described.[E1,4]

Ensembl gene ID	Gene Symbol	Log fold-change	SE	*Z*-value	*P*-value	Adjusted *P*-value[a]
ENSG00000146416	AIG1	-0.48	0.08	-6.025	1.69e-09	2.56e-05
ENSG00000112796	ENPP5	-0.57	0.10	-5.556	2.75e-08	5.81e-05
ENSG00000137942	FNBP1L	-0.37	0.07	-5.537	3.08e-08	5.81e-05
ENSG00000143507	DUSP10	0.44	0.08	5.612	2.00e-08	5.81e-05
ENSG00000146477	SLC22A3	0.88	0.16	5.555	2.78e-08	5.81e-05
ENSG00000152782	PANK1	-0.44	0.08	-5.601	2.14e-08	5.81e-05
ENSG00000189067	LITAF	0.56	0.10	5.585	2.34e-08	5.81e-05
ENSG00000205678	TECRL	-0.67	0.12	-5.620	1.91e-08	5.81e-05
ENSG00000102007	PLP2	0.50	0.09	5.495	3.91e-08	5.90e-05
ENSG00000133816	MICAL2	0.44	0.08	5.478	4.31e-08	5.91e-05
	MICALCL	0.44	0.08	5.478	4.31e-08	5.91e-05
ENSG00000120658	ENOX1	0.80	0.15	5.397	6.78e-08	8.16e-05
ENSG00000150722	PPP1R1C	-0.71	0.13	-5.391	7.02e-08	8.16e-05
ENSG00000113448	PDE4D	0.42	0.08	5.355	8.55e-08	9.22e-05
ENSG00000048052	HDAC9	-0.59	0.11	-5.242	1.59e-07	1.26e-04
ENSG00000105835	NAMPT	-0.38	0.07	-5.253	1.50e-07	1.26e-04
ENSG00000136040	PLXNC1	-0.52	0.10	-5.251	1.51e-07	1.26e-04
ENSG00000073910	FRY	-0.43	0.08	-5.225	1.74e-07	1.31e-04
ENSG00000151746	BICD1	-0.61	0.12	-5.172	2.31e-07	1.65e-04
ENSG00000267296	CEBPA-DT	0.56	0.11	5.165	2.40e-07	1.65e-04
ENSG00000225549	Not mapped[d]	-0.92	0.18	-5.146	2.66e-07	1.73e-04
ENSG00000198729	PPP1R14C	0.44	0.09	5.126	2.96e-07	1.79e-04
ENSG00000237301	Not mapped[d]	0.92	0.18	5.095	3.48e-07	1.95e-04
ENSG00000091879	ANGPT2	0.65	0.13	4.990	6.04e-07	2.95e-04
ENSG00000151276	MAGI1	-0.36	0.07	-4.994	5.90e-07	2.95e-04
ENSG00000196152	ZNF79	0.43	0.09	4.989	6.06e-07	2.95e-04
ENSG00000183625	CCR3	-0.96	0.20	-4.927	8.37e-07	3.83e-04
ENSG00000140416	TPM1	0.46	0.09	4.871	1.11e-06	4.78e-04
ENSG00000130595	TNNT3	0.41	0.08	4.856	1.20e-06	5.02e-04
ENSG00000186352	ANKRD37	0.59	0.12	4.849	1.24e-06	5.07e-04
ENSG00000099194	SCD	1.04	0.22	4.797	1.61e-06	6.40e-04
ENSG00000107282	APBA1	-0.43	0.09	-4.768	1.86e-06	7.20e-04
ENSG00000154814	OXNAD1	-0.40	0.08	-4.762	1.92e-06	7.25e-04
ENSG00000132953	XPO4	-0.54	0.11	-4.727	2.28e-06	7.82e-04
ENSG00000123700	KCNJ2	0.42	0.09	4.668	3.04e-06	9.50e-04
ENSG00000133794	ARNTL	0.54	0.12	4.665	3.09e-06	9.50e-04
ENSG00000164197	RNF180	-0.35	0.08	-4.616	3.91e-06	0.001
ENSG00000144668	ITGA9	0.38	0.08	4.611	4.01e-06	0.001
ENSG00000137804	NUSAP1	0.37	0.08	4.601	4.20e-06	0.001
ENSG00000143549	TPM3	-0.44	0.10	-4.552	5.32e-06	0.001
ENSG00000226306	NPY6R	-0.52	0.11	-4.548	5.41e-06	0.001
ENSG00000116741	RGS2	0.70	0.15	4.544	5.51e-06	0.001
ENSG00000159884	CCDC107	0.43	0.10	4.538	5.68e-06	0.001
ENSG00000184588	PDE4B	0.45	0.10	4.521	6.16e-06	0.002
ENSG00000134986	NREP	-0.48	0.11	-4.513	6.39e-06	0.002
ENSG00000105612	DNASE2	0.51	0.11	4.499	6.84e-06	0.002
ENSG00000066382	MPPED2	-0.48	0.11	-4.489	7.15e-06	0.002
ENSG00000147010	SH3KBP1	-0.36	0.08	-4.469	7.85e-06	0.002
ENSG00000108342	CSF3	-1.16	0.26	-4.407	1.05e-05	0.002
ENSG00000138061	CYP1B1	0.47	0.11	4.404	1.06e-05	0.002
ENSG00000162493	PDPN	0.35	0.08	4.408	1.04e-05	0.002

Ensembl gene ID	Gene Symbol	Log fold-change	SE	*Z*-value	*P*-value	Adjusted *P*-value[a]
ENSG00000196526	AFAP1	0.51	0.12	4.416	1.01e-05	0.002
ENSG00000225613	Not mapped[d]	1.14	0.26	4.407	1.05e-05	0.002
ENSG00000249464	LINC01091	0.67	0.15	4.398	1.09e-05	0.002
ENSG00000139998	RAB15	0.53	0.12	4.385	1.16e-05	0.002
ENSG00000138688	KIAA1109	-0.36	0.08	-4.362	1.29e-05	0.002
ENSG00000174437	ATP2A2	-0.44	0.10	-4.361	1.30e-05	0.002
ENSG00000119771	KLHL29	0.53	0.12	4.351	1.35e-05	0.002
ENSG00000134569	LRP4	0.41	0.09	4.350	1.36e-05	0.002
ENSG00000182985	CADM1	-0.35	0.08	-4.352	1.35e-05	0.002
ENSG00000139209	SLC38A4	0.49	0.11	4.344	1.40e-05	0.002
ENSG00000079156	OSBPL6	0.37	0.08	4.340	1.42e-05	0.002
ENSG00000077150	NFKB2	0.42	0.10	4.333	1.47e-05	0.002
ENSG00000163071	SPATA18	0.52	0.12	4.323	1.54e-05	0.003
ENSG00000180209	MYLPF	0.44	0.10	4.315	1.59e-05	0.003
ENSG00000108960	MMD	0.35	0.08	4.302	1.69e-05	0.003
ENSG00000176909	MAMSTR	0.52	0.12	4.297	1.73e-05	0.003
ENSG00000138759	FRAS1	-0.37	0.09	-4.251	2.13e-05	0.003
ENSG00000186047	DLEU7	0.93	0.22	4.249	2.15e-05	0.003
	DLEU1-AS1	0.93	0.22	4.249	2.15e-05	0.003
ENSG00000164649	CDCA7L	-0.44	0.10	-4.239	2.25e-05	0.003
ENSG00000156265	MAP3K7CL	0.48	0.11	4.221	2.43e-05	0.003
ENSG00000060656	PTPRU	0.52	0.12	4.214	2.51e-05	0.003
ENSG00000162552	WNT4	0.72	0.17	4.193	2.76e-05	0.004
ENSG00000197442	MAP3K5	-0.40	0.09	-4.190	2.78e-05	0.004
ENSG00000223749	Not mapped[d]	1.35	0.32	4.187	2.82e-05	0.004
ENSG00000175567	UCP2	0.44	0.11	4.154	3.27e-05	0.004
ENSG00000087903	RFX2	0.56	0.13	4.135	3.55e-05	0.004
ENSG00000138411	HECW2	-0.50	0.12	-4.134	3.57e-05	0.004
ENSG00000233621	LINC01137	0.67	0.16	4.137	3.52e-05	0.004
ENSG00000260337	Not mapped[d]	0.76	0.18	4.133	3.57e-05	0.004
ENSG00000163823	CCR1	-0.65	0.16	-4.127	3.67e-05	0.004
ENSG00000106070	GRB10	-0.39	0.09	-4.121	3.77e-05	0.004
ENSG00000174791	RIN1	0.96	0.23	4.108	3.99e-05	0.005
ENSG00000196440	ARMCX4	0.40	0.10	4.105	4.05e-05	0.005
ENSG00000111602	TIMELESS	0.39	0.10	4.100	4.14e-05	0.005
ENSG00000144908	ALDH1L1	0.42	0.10	4.095	4.22e-05	0.005
ENSG00000166833	NAV2	-0.40	0.10	-4.093	4.25e-05	0.005
ENSG00000101306	MYLK2	0.35	0.09	4.079	4.51e-05	0.005
ENSG00000285820	Not mapped[d]	1.43	0.35	4.076	4.58e-05	0.005
ENSG00000129910	CDH15	0.35	0.09	3.984	6.76e-05	0.007
ENSG00000254901	BORCS8	0.37	0.09	3.975	7.05e-05	0.007
ENSG00000158486	DNAH3	-0.84	0.22	-3.922	8.79e-05	0.008
ENSG00000260391	Not mapped[d]	1.47	0.37	3.921	8.82e-05	0.008
ENSG00000105327	BBC3	0.72	0.19	3.903	9.50e-05	0.009
ENSG00000183010	PYCR1	0.66	0.17	3.898	9.69e-05	0.009
ENSG00000226833	LOC100505774	-0.51	0.13	-3.892	9.93e-05	0.009
	LOC112267877	-0.51	0.13	-3.892	9.93e-05	0.009
ENSG00000109061	MYH1	0.68	0.18	3.889	1.01e-04	0.009
ENSG00000089101	CFAP61	0.52	0.13	3.878	1.05e-04	0.009
ENSG00000168334	XIRP1	0.42	0.11	3.857	1.15e-04	0.010
ENSG00000178752	ERFE	0.83	0.21	3.851	1.17e-04	0.010
ENSG00000272734	Not mapped[d]	0.43	0.11	3.853	1.17e-04	0.010
ENSG00000105339	DENND3	-0.35	0.09	-3.847	1.20e-04	0.010
ENSG00000115129	TP53I3	0.65	0.17	3.837	1.24e-04	0.010
ENSG00000169710	FASN	0.78	0.20	3.838	1.24e-04	0.010
ENSG00000169515	CCDC8	0.72	0.19	3.827	1.30e-04	0.010

Ensembl gene ID	Gene Symbol	Log fold-change	SE	*Z*-value	*P*-value	Adjusted *P*-value[a]
ENSG00000176749	CDK5R1	0.40	0.11	3.818	1.35e-04	0.010
ENSG00000109771	LRP2BP	0.44	0.12	3.812	1.38e-04	0.011
ENSG00000068724	TTC7A	0.43	0.11	3.809	1.40e-04	0.011
ENSG00000138615	CILP	0.40	0.11	3.806	1.41e-04	0.011
ENSG00000109321	AREG	1.12	0.30	3.799	1.46e-04	0.011
ENSG00000157330	C1orf158	1.58	0.42	3.793	1.49e-04	0.011
ENSG00000196296	ATP2A1	0.43	0.11	3.796	1.47e-04	0.011
ENSG00000228526	MIR34AHG	0.48	0.13	3.792	1.49e-04	0.011
ENSG00000161513	FDXR	0.62	0.16	3.784	1.54e-04	0.011
ENSG00000174032	SLC25A30	-0.39	0.10	-3.775	1.60e-04	0.011
ENSG00000104147	OIP5	0.50	0.13	3.773	1.61e-04	0.011
ENSG00000205106	LINC02716	0.59	0.16	3.772	1.62e-04	0.011
ENSG00000099999	RNF215	0.42	0.11	3.760	1.70e-04	0.012
ENSG00000196482	ESRRG	-0.38	0.10	-3.731	1.91e-04	0.013
ENSG00000267080	ASB16-AS1	0.36	0.10	3.713	2.05e-04	0.014
ENSG00000205959	Not mapped[d]	0.39	0.11	3.684	2.30e-04	0.015
ENSG00000138835	RGS3	-0.53	0.14	-3.674	2.39e-04	0.015
ENSG00000184545	DUSP8	0.46	0.12	3.674	2.39e-04	0.015
ENSG00000137193	PIM1	0.46	0.13	3.669	2.43e-04	0.015
ENSG00000262468	Not mapped[d]	0.51	0.14	3.665	2.47e-04	0.015
ENSG00000023171	GRAMD1B	0.44	0.12	3.661	2.51e-04	0.015
ENSG00000146166	LGSN	-1.09	0.30	-3.658	2.54e-04	0.015
ENSG00000147256	ARHGAP36	0.78	0.21	3.652	2.60e-04	0.016
ENSG00000159259	CHAF1B	0.36	0.10	3.653	2.59e-04	0.016
ENSG00000124587	PEX6	0.44	0.12	3.634	2.79e-04	0.016
ENSG00000215018	COL28A1	0.35	0.10	3.607	3.10e-04	0.017
ENSG00000139292	LGR5	-0.49	0.14	-3.595	3.25e-04	0.018
ENSG00000099308	MAST3	0.66	0.18	3.589	3.32e-04	0.018
ENSG00000102468	HTR2A	-0.81	0.23	-3.589	3.32e-04	0.018
ENSG00000110660	SLC35F2	0.54	0.15	3.586	3.36e-04	0.018
ENSG00000089847	ANKRD24	0.70	0.19	3.583	3.40e-04	0.018
ENSG00000118515	SGK1	0.44	0.12	3.583	3.40e-04	0.018
ENSG00000124935	SCGB1D2	-0.74	0.21	-3.556	3.76e-04	0.020
ENSG00000163492	CCDC141	-0.44	0.12	-3.553	3.81e-04	0.020
ENSG00000184349	EFNA5	0.60	0.17	3.551	3.84e-04	0.020
ENSG00000064655	EYA2	0.60	0.17	3.541	3.99e-04	0.020
ENSG00000091513	TF	0.43	0.12	3.540	4.00e-04	0.020
ENSG00000138379	MSTN	0.47	0.13	3.544	3.94e-04	0.020
ENSG00000184347	SLIT3	0.36	0.10	3.533	4.11e-04	0.020
ENSG00000235070	Not mapped[d]	-0.59	0.17	-3.528	4.19e-04	0.021
ENSG00000163879	DNALI1	0.39	0.11	3.518	4.36e-04	0.021
ENSG00000119969	HELLS	0.53	0.15	3.505	4.57e-04	0.022
ENSG00000175489	LRRC25	-0.57	0.16	-3.495	4.74e-04	0.022
ENSG00000185105	MYADML2	0.36	0.10	3.492	4.79e-04	0.023
ENSG00000104313	EYA1	-0.42	0.12	-3.489	4.85e-04	0.023
ENSG00000258647	Not mapped[d]	0.74	0.21	3.483	4.96e-04	0.023
ENSG00000260604	Not mapped[d]	-0.63	0.18	-3.484	4.94e-04	0.023
ENSG00000278464	Not mapped[d]	0.43	0.12	3.483	4.95e-04	0.023
ENSG00000075240	GRAMD4	0.37	0.11	3.472	5.16e-04	0.023
ENSG00000086967	MYBPC2	0.41	0.12	3.473	5.15e-04	0.023
ENSG00000145626	UGT3A1	0.41	0.12	3.477	5.07e-04	0.023
ENSG00000161036	LRWD1	0.51	0.15	3.471	5.19e-04	0.023
ENSG00000212907	ND4L	-0.35	0.10	-3.464	5.31e-04	0.023
ENSG00000198915	RASGEF1A	-0.62	0.18	-3.459	5.41e-04	0.023
ENSG00000106992	AK1	0.40	0.12	3.454	5.53e-04	0.024
ENSG00000277758	LOC102724488	0.89	0.26	3.432	6.00e-04	0.025

Ensembl gene ID	Gene Symbol	Log fold-change	SE	*Z*-value	*P*-value	Adjusted *P*-value[a]
ENSG00000197361	FBXL22	0.49	0.14	3.404	6.63e-04	0.027
ENSG00000231607	DLEU2	0.35	0.10	3.393	6.90e-04	0.028
ENSG00000158008	EXTL1	-0.58	0.17	-3.392	6.94e-04	0.028
ENSG00000140798	ABCC12	-0.70	0.21	-3.389	7.01e-04	0.028
ENSG00000165887	ANKRD2	0.69	0.21	3.383	7.16e-04	0.028
ENSG00000105877	DNAH11	0.98	0.29	3.383	7.18e-04	0.028
ENSG00000156463	SH3RF2	0.40	0.12	3.373	7.45e-04	0.028
ENSG00000285155	Not mapped[d]	-0.39	0.11	-3.372	7.45e-04	0.028
ENSG00000168528	SERINC2	0.51	0.15	3.366	7.62e-04	0.029
ENSG00000188488	SERPINA5	-0.69	0.21	-3.356	7.90e-04	0.030
ENSG00000125844	RRBP1	0.36	0.11	3.348	8.13e-04	0.030
ENSG00000108932	SLC16A6	0.58	0.18	3.336	8.51e-04	0.031
ENSG00000130600	H19	0.56	0.17	3.330	8.67e-04	0.031
ENSG00000154080	CHST9	-0.56	0.17	-3.336	8.49e-04	0.031
ENSG00000174996	KLC2	0.39	0.12	3.331	8.66e-04	0.031
ENSG00000188582	PAQR9	-0.47	0.14	-3.337	8.48e-04	0.031
ENSG00000284820	Not mapped[d]	0.61	0.18	3.331	8.65e-04	0.031
ENSG00000171617	ENC1	0.41	0.12	3.328	8.74e-04	0.031
ENSG00000047662	FAM184B	0.72	0.22	3.316	9.12e-04	0.032
ENSG00000172932	ANKRD13D	0.41	0.13	3.314	9.21e-04	0.032
ENSG00000158458	NRG2	0.63	0.19	3.313	9.24e-04	0.032
ENSG00000279529	Not mapped[d]	0.43	0.13	3.309	9.37e-04	0.032
ENSG00000284693	LINC02606	-0.48	0.14	-3.311	9.29e-04	0.032
ENSG00000140795	MYLK3	-0.35	0.11	-3.303	9.57e-04	0.033
ENSG00000146005	PSD2	0.79	0.24	3.301	9.63e-04	0.033
ENSG00000148671	ADIRF	0.60	0.18	3.301	9.63e-04	0.033
ENSG00000111245	MYL2	-0.35	0.11	-3.281	0.001	0.034
ENSG00000176134	Not mapped[d]	-0.42	0.13	-3.274	0.001	0.035
ENSG00000071564	TCF3	0.38	0.12	3.272	0.001	0.035
ENSG00000214942	Not mapped[d]	-0.72	0.22	-3.270	0.001	0.035
ENSG00000005206	SPPL2B	0.37	0.11	3.262	0.001	0.036
ENSG00000181418	DDN	0.74	0.23	3.253	0.001	0.037
ENSG00000215187	FAM166B	0.44	0.14	3.254	0.001	0.037
ENSG00000052749	RRP12	0.42	0.13	3.252	0.001	0.037
ENSG00000264343	NOTCH2NLA	0.47	0.14	3.244	0.001	0.037
ENSG00000173546	CSPG4	0.46	0.14	3.231	0.001	0.038
ENSG00000177551	NHLH2	1.15	0.36	3.230	0.001	0.038
ENSG00000117707	PROX1	-0.36	0.11	-3.227	0.001	0.039
ENSG00000225472	Not mapped[d]	-0.51	0.16	-3.227	0.001	0.039
ENSG00000159713	TPPP3	0.55	0.17	3.226	0.001	0.039
ENSG00000205279	CTXN3	-0.72	0.22	-3.221	0.001	0.039
ENSG00000255495	Not mapped[d]	0.40	0.12	3.220	0.001	0.039
ENSG00000149090	PAMR1	0.46	0.14	3.211	0.001	0.040
ENSG00000124374	PAIP2B	-0.35	0.11	-3.193	0.001	0.041
ENSG00000072310	SREBF1	0.48	0.15	3.188	0.001	0.042
ENSG00000104889	RNASEH2A	0.47	0.15	3.184	0.001	0.042
ENSG00000238083	LRRC37A2	0.36	0.11	3.185	0.001	0.042
ENSG00000270021	Not mapped[d]	0.52	0.16	3.184	0.001	0.042
ENSG00000185847	LINC01405	-0.41	0.13	-3.177	0.001	0.043
ENSG00000248587	Not mapped[d]	0.36	0.11	3.173	0.002	0.043
ENSG00000105738	SIPA1L3	0.37	0.12	3.161	0.002	0.044
ENSG00000273301	Not mapped[d]	-0.86	0.27	-3.145	0.002	0.046
ENSG00000077943	ITGA8	-0.41	0.13	-3.136	0.002	0.047
ENSG00000241288	LINC02614	0.43	0.14	3.138	0.002	0.047
ENSG00000127191	TRAF2	0.52	0.17	3.134	0.002	0.047
ENSG00000283563	ZCWPW2	-0.36	0.11	-3.129	0.002	0.047

Ensembl gene ID	Gene Symbol	Log fold-change	SE	*Z*-value	*P*-value	Adjusted *P*-value[a]
ENSG00000140280	LYSMD2	0.37	0.12	3.127	0.002	0.047
ENSG00000070601	FRMPD1	-0.36	0.12	-3.117	0.002	0.048
ENSG00000108231	LGI1	-0.37	0.12	-3.112	0.002	0.049
ENSG00000220563	Not mapped[d]	0.37	0.12	3.110	0.002	0.049
ENSG00000250303	LINC02762	-0.37	0.12	-3.109	0.002	0.049
ENSG00000166123	GPT2	-0.37	0.12	-3.107	0.002	0.049
ENSG00000167037	SGSM1	-0.60	0.19	-3.102	0.002	0.049
ENSG00000153822	KCNJ16	0.59	0.19	3.098	0.002	0.049

[a] *P*-values are adjusted for FDR. [b] Raw *P*-values from simulation based tests of uniformity of residuals where low values indicate problematic models. [d] No official gene symbol available, not included in enrichment analyses.

Supplementary Table 3. Gene ontology (GO) analysis of genome-wide transcriptome data (RNA-seq; COPD vs. Healthy), performed as previously described.[E1,4]

Comparison	Gene set category	Gene set	Significance category[a]	Set size[b]	Rank *P*-value[c]	% MSD > 0[d]	GSEA *P*-value[e]	NES	LE[f]	Log_2 Fold-change in LE [min, max]
Baseline: COPD vs. Healthy	Biological process	Actin filament based movement	Rank	118 (153)	4.47e-05	30.5%	0.760	1.08	20 (85%)	0.6 [0.29, 0.97]
		Actin mediated cell contraction	Rank	92 (123)	4.29e-05	34.8%	0.728	1.12	14 (92.9%)	0.63 [0.41, 0.97]
		Fatty acid metabolic process	Rank	279 (396)	8.48e-06	29.7%	0.602	-1.12	61 (83.6%)	-0.32 [-0.96, -0.15]
		Monocarboxylic acid metabolic process	Rank	469 (672)	8.48e-06	29.4%	0.468	-1.17	72 (97.2%)	-0.36 [-1.17, -0.16]
		Muscle contraction	Rank	252 (362)	4.27e-05	29.4%	0.767	1.05	34 (82.4%)	0.59 [0.25, 1.01]
		Muscle filament sliding	Rank	31 (39)	1.39e-04	54.8%	0.728	1.15	10 (90%)	0.61 [0.29, 0.97]
		Muscle system process	Rank	321 (467)	8.48e-06	29.9%	0.740	1.08	45 (82.2%)	0.56 [0.25, 1.03]
	Cellular component	Inner mitochondrial membrane protein complex	GSEA	114 (138)	0.771	27.2%	0.003	-1.83	39 (76.9%)	-0.22 [-0.37, -0.12]
		Mitochondrial matrix	GSEA	436 (473)	0.122	29.4%	2.19e-04	-1.61	120 (88.3%)	-0.23 [-0.53, -0.12]
		Mitochondrial protein complex	GSEA	234 (265)	0.933	26.1%	3.66e-05	-1.90	70 (80%)	-0.21 [-0.37, -0.1]
		Organelle inner membrane	GSEA	461 (549)	0.826	25.4%	0.005	-1.43	92 (95.7%)	-0.24 [-0.56, -0.14]
		Actin cytoskeleton	Rank	392 (503)	1.87e-04	28.1%	0.304	1.29	93 (74.2%)	0.41 [0.14, 0.98]
		Contractile fiber	Rank	191 (238)	2.24e-05	33%	0.505	1.21	49 (83.7%)	0.41 [0.16, 1]
	Molecular function	G protein coupled receptor activity	GSEA	146 (867)	0.411	22.6%	0.018	-1.75	29 (69%)	-0.52 [-1.39, -0.17]
Post-RT (13 weeks training): ΔCOPD vs ΔHealthy	Biological process	Proteasomal protein catabolic process	Rank	421 (481)	0.019	29.2%	0.591	-1.08	97 (82.5%)	-0.43 [-1.26, -0.19]
		Regulation of cholesterol efflux	Rank	25 (46)	0.019	48%	0.102	-1.50	13 (84.6%)	-0.54 [-1.32, -0.29]
		Regulation of protein catabolic process	Rank	327 (395)	0.019	31.2%	0.293	-1.17	71 (97.2%)	-0.46 [-1.26, -0.23]
	Cellular component	Actin cytoskeleton	Consensus	392 (503)	0.002	29.1%	5.68e-06	-1.38	133 (75.2%)	-0.44 [-1.17, -0.16]
		Actin filament bundle	Consensus	67 (75)	5.45e-04	38.8%	0.016	-1.47	31 (74.2%)	-0.48 [-1.17, -0.2]
		Actomyosin	Consensus	69 (78)	4.62e-04	37.7%	0.011	-1.48	31 (74.2%)	-0.49 [-1.17, -0.2]
		Contractile fiber	Consensus	191 (238)	1.04e-05	33.5%	1.56e-04	-1.44	63 (87.3%)	-0.47 [-1.17, -0.19]
		I band	Consensus	114 (140)	4.71e-04	33.3%	2.28e-04	-1.52	40 (87.5%)	-0.48 [-1.17, -0.2]
		Adherens junction	GSEA	127 (166)	0.244	27.6%	0.005	-1.42	44 (65.9%)	-0.47 [-1.17, -0.16]
		Cell cell junction	GSEA	344 (493)	0.161	26.5%	1.91e-04	-1.34	116 (63.8%)	-0.43 [-1.17, -0.16]
		Cell substrate junction	GSEA	359 (423)	0.305	27.9%	0.003	-1.31	112 (68.8%)	-0.43 [-0.96, -0.16]
		Collagen containing extracellular matrix	GSEA	214 (427)	0.999	20.1%	0.005	-1.34	74 (51.4%)	-0.47 [-1.56, -0.19]
		Extrinsic component of cytoplasmic side of plasma membrane	GSEA	65 (99)	0.305	24.6%	0.003	-1.56	15 (100%)	-0.53 [-0.89, -0.3]
		Extrinsic component of plasma membrane	GSEA	109 (172)	0.458	22%	0.005	-1.45	25 (84%)	-0.51 [-0.89, -0.27]
		Polymeric cytoskeletal fiber	GSEA	437 (756)	0.110	25.9%	0.005	-1.25	135 (71.1%)	-0.43 [-1.17, -0.17]
		Heterochromatin	Rank	63 (78)	0.004	39.7%	0.063	-1.40	19 (94.7%)	-0.48 [-1.05, -0.17]
	Molecular function	Actin binding	Consensus	336 (437)	0.001	30.7%	3.17e-07	-1.42	125 (75.2%)	-0.44 [-1.17, -0.16]
		Actin filament binding	Consensus	162 (206)	0.002	32.7%	0.001	-1.43	65 (70.8%)	-0.46 [-1.13, -0.21]
		Chromatin binding	Consensus	448 (596)	0.001	29.2%	0.025	-1.23	94 (92.6%)	-0.44 [-1.05, -0.17]
		Molecular adaptor activity	Consensus	252 (314)	0.001	30.6%	0.048	-1.25	80 (70%)	-0.44 [-1.14, -0.16]
		Cell adhesion molecule binding	GSEA	407 (544)	0.384	25.3%	5.69e-04	-1.31	120 (75.8%)	-0.45 [-1.56, -0.16]
		Protein kinase activity	GSEA	449 (563)	0.353	25.6%	9.88e-04	-1.29	102 (81.4%)	-0.49 [-1.29, -0.22]
		Protein serine threonine kinase activity	GSEA	361 (434)	0.167	27.1%	0.004	-1.28	83 (84.3%)	-0.48 [-1.26, -0.22]
		Glutamate receptor binding	Rank	31 (46)	0.016	54.8%	0.071	-1.49	16 (100%)	-0.41 [-0.69, -0.16]
		Nuclear receptor binding	Rank	83 (101)	0.016	37.3%	0.812	-1.03	21 (90.5%)	-0.43 [-1.05, -0.16]

Comparison	Gene set category	Gene set	Significance category[a]	Set size[b]	Rank *P*-value[c]	% MSD > 0[d]	GSEA *P*-value[e]	NES	LE[f]	Log_2 Fold-change in LE [min, max]
		Protein macromolecule adaptor activity	Rank	200 (244)	6.58e-04	33%	0.070	-1.28	71 (69%)	-0.44 [-1.14, -0.16]
		Signaling adaptor activity	Rank	54 (68)	0.016	40.7%	0.343	-1.25	22 (77.3%)	-0.42 [-0.77, -0.18]
		Signaling receptor complex adaptor activity	Rank	32 (41)	0.016	43.8%	0.267	-1.32	10 (100%)	-0.49 [-0.77, -0.27]
		Structural constituent of muscle	Rank	33 (43)	0.016	42.4%	0.073	-1.44	13 (92.3%)	-0.49 [-0.97, -0.26]
		Ubiquitin binding	Rank	71 (76)	0.018	38%	0.145	-1.37	24 (91.7%)	-0.42 [-0.88, -0.25]

[a] Consensus significance indicates agreement between directional (GSEA) and non-directional (Rank) hypothesis test of overrepresentation (see methods for details). [b] Indicates number of identified genes in the gene set and total number of genes in the gene set in parentheses. [c] Rank-based enrichment test, based on minimum significant difference (MSD), identifies gene sets that are overrepresented among top-ranked genes without a directional hypothesis. [d] Fraction of genes in gene set with unadjusted 95% CI not spanning zero, i.e. MSD > 0. [e] Gene-set enrichment analysis (GSEA) tests for overrepresentation among top and bottom genes based on Log_2 fold differences or changes × $-\log_{10}$(*P*-values) in comparing differences at baseline or changes from baseline between COPD and Healthy. Positive normalized enrichment score (NES) indicate gene sets with higher expression in COPD than Healthy; negative NES indicate gene sets with lower expression at respective time-points. [f] Number of genes in leading edge (LE, genes that contributes to the enrichment score) with the fraction of leading edge genes with unadjusted 95% CI not spanning zero.

Appendix V

Supplementary material for Paper IV

Online data supplement

Resistance exercise training increases skeletal muscle mitochondrial respiration in COPD

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Data analyses and statistics

To 1) examine the effects of resistance exercise training (RT) on muscle mass, strength, and endurance performance factors, one-legged maximal oxygen uptake and O_2 cost, and mitochondrial function in controls and COPD separately and to 2) assess the difference in responsiveness to RT between controls and COPD, linear mixed-effects models were used. In these models, both legs' pre- and post-RT measures for each participant were defined as repeated observations. Post-hoc tests, using the Sidak method for correction of multiple comparisons, were conducted to identify within-group differences between 10RM vs. 30RM. As pre biopsies were only sampled from the 30RM leg, the effect of RT modality on mitochondrial function was evaluated by pairwise comparisons between post 10RM and 30RM measurements. For transcriptome analyses, gene counts were modelled using negative binomial generalized linear mixed-effects models with the total library size modelled as the fixed effect [1] together with sex and study conditions (time points and study groups). Genes were regarded as differentially expressed when the absolute $\log_2$ fold-change/difference was greater than 0.5 and the adjusted p-value (false discovery rate adjusted per model coefficient) was <0.05. Enrichment analyses of the Mitocarta pathways v.3.0 [2] were performed using two approaches, the non-parametric rank test (Rank) [3] and the directional gene set enrichment analysis (GSEA) [4] where consensus results of those two analyses were interpreted as having larger biological meaning.

References

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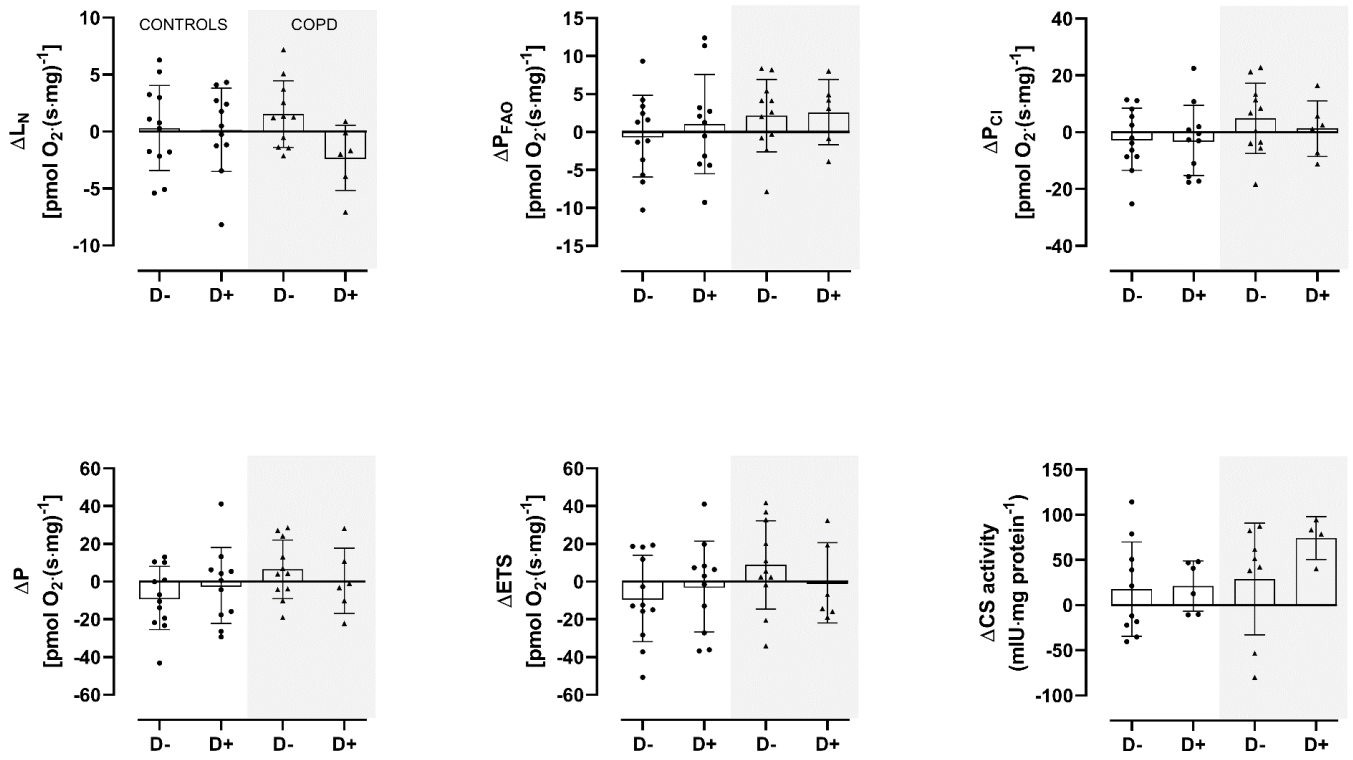
3. Zyla J, Marczyk M, Domaszewska T, Kaufmann SH, Polanska J, Weiner J. Gene set enrichment for reproducible science: comparison of CERNO and eight other algorithms. Bioinformatics. 2019;35:5146-54.
4. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102:15545-50.

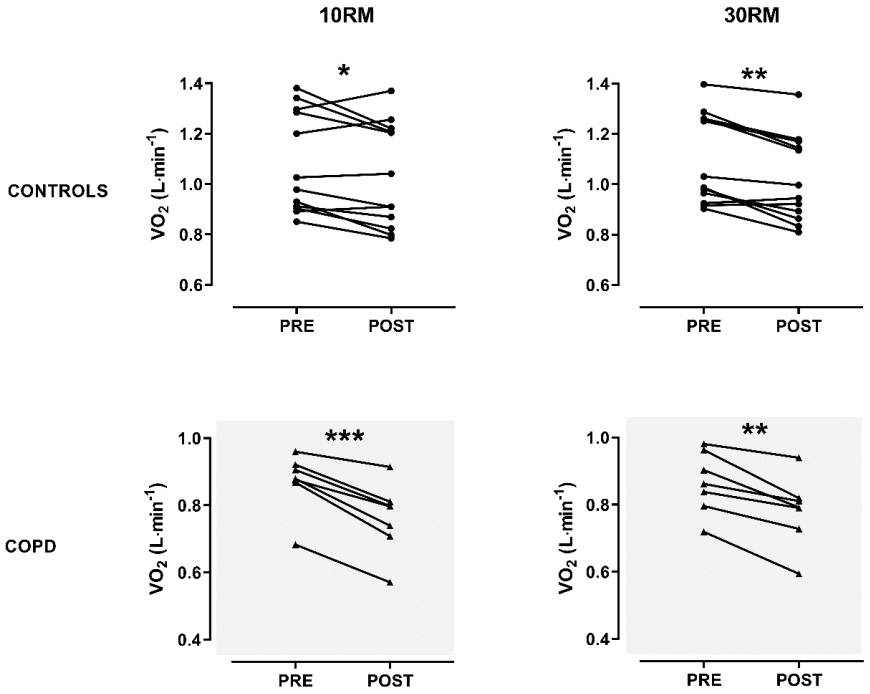
Supplemental figure legends

Supplemental Figure 1. Effect of vitamin D_3 supplementation (D+) and placebo (D-) on resistance exercise training (RT)-induced changes in mitochondrial function.



Circles (CONTROLS) and rectangles (COPD; shaded) represent individual data of changes in mitochondrial respiration and citrate synthase (CS) activity, respectively with 10RM and 30RM RT pooled. Mitochondrial O_2 flux per mg of vastus lateralis muscle tissue with titration of malate and octanoyl carnitine (L_N), ADP (P_{FAO}), glutamate and pyruvate (P_{CI}), succinate (P) and FCCP (ETS). Bars illustrate the mean and lines the SD. To examine the effect of vitamin D_3 on RT-induced changes in mitochondrial function, linear mixed-effects models were used. The 12-week lead-in vitamin D_3 supplementation-only period did not affect baseline mitochondrial function (p_{LN}=0.380, p_{FAO}=0.373, p_{CI}=0.353, p_P=0.599, p_{ETS}=0.847, P_{CS}=0.424). Likewise, there was no modifying effect of vitamin D_3 on RT-induced alterations in mitochondrial function (p_{LN}=0.321, p_{PFAO}=0.358, p_{CI}=0.597, p_P=0.931, p_{ETS}=0.970, P_{CS}=0.471).

Supplemental Figure 2. VO_2 of steady-state submaximal one-legged cycling prior to (PRE) and following 10 RM and 30RM (POST) resistance exercise training (RT).



Differences in PRE vs. POST VO_2 were evaluated with a paired t-test. Note that the workload was different for each individual, but intra-individually constant: CONTROLS: 50 ± 10 W, COPD (shaded): 26 ± 2 W (mean ± SD). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. PRE

Supplemental Table 1. Mitochondrial respiratory capacity prior to (PRE) and following (10RM/30RM) resistance exercise training.

	CONTROLS			P-value		COPD			P-value		P-value	
	PRE	10RM	30RM	Time	Leg	PRE	10RM	30RM	Time	Leg	PRE CONTROLS vs. COPD	Time x condition
Mitochondrial respiration [pmol·(s·mg wet weight)$^{-1}$]												
L_N	8.88 ± 2.07	8.29 ± 2.70	9.62 ± 3.49	0.819	0.528	8.57 ± 2.90	8.83 ± 3.38	9.38 ± 2.46	0.340	0.986	0.794	0.460
P_{FAO}	25.78 ± 5.75	25.16 ± 4.46	27.35 ± 6.31	0.382	0.362	21.56 ± 3.96	23.44 ± 6.56	24.32 ± 2.79	**0.033**	0.926	**0.022**	0.211
P_{CI}	60.89 ± 14.33	57.47 ± 9.48	59.45 ± 13.44	0.696	0.419	48.20 ± 9.00	50.20 ± 10.63	54.30 ± 6.06	0.079	0.694	**0.020**	0.132
P	94.42 ± 21.42	87.78 ± 11.89	90.85 ± 18.93	0.527	0.330	74.38 ± 14.47	74.05 ± 11.07	83.58 ± 6.81	**0.035**	0.175	**0.018**	0.123
ETS	109.61 ± 25.42	102.78 ± 15.33	105.58 ± 21.94	0.566	0.447	89.79 ± 18.77	85.71 ± 12.61	98.87 ± 9.23	0.115	0.136	0.056	0.240
LCR_{FAO} (no unit)	0.36 ± 0.12	0.32 ± 0.08	0.36 ± 0.12	0.933	0.402	0.40 ± 0.11	0.39 ± 0.13	0.39 ± 0.09	0.920	0.906	0.311	0.855
Mitochondrial respiration [pmol·(s·mg wet weight)$^{-1}$]·CS activity^{-1}												
L_N	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.619	0.558	0.06 ± 0.02	0.05 ± 0.03	0.05 ± 0.01	0.681	0.495	0.098	0.857
P_{FAO}	0.14 ± 0.03	0.12 ± 0.02	0.13 ± 0.04	0.699	0.340	0.16 ± 0.04	0.12 ± 0.03	0.12 ± 0.02	0.142	0.223	0.316	0.339
P_{CI}	0.32 ± 0.07	0.27 ± 0.05	0.28 ± 0.08	0.195	0.312	0.34 ± 0.07	0.26 ± 0.07	0.27 ± 0.03	**0.033**	0.152	0.309	0.344
P	0.50 ± 0.11	0.41 ± 0.08	0.43 ± 0.12	0.187	0.256	0.53 ± 0.12	0.40 ± 0.06	0.43 ± 0.04	**0.037**	0.065	0.354	0.337
ETS	0.58 ± 0.14	0.48 ± 0.10	0.49 ± 0.12	0.158	0.328	0.65 ± 0.16	0.46 ± 0.09	0.51 ± 0.07	**0.027**	0.060	0.203	0.229

Mitochondrial O_2 flux in vastus lateralis muscle tissue with titration of malate and octanoyl carnitine (L_N), ADP (P_{FAO}), glutamate and pyruvate (P_{CI}), succinate (P), FCCP (ETS); per mg of muscle tissue and per citrate synthase (CS) activity. Data are presented as means ± SD. P-values represent the effect of time (PRE vs. 10RM/30RM) and of RT mode (pairwise comparisons between POST 10RM vs. 30RM) for CONTROLS and COPD separately, and the interaction of time x condition (CONTROLS vs. COPD).

Supplemental Table 2. Baseline and resistance exercise training-associated differences in gene expression between COPD (n=19) and CONTROLS (n=34)

Comparison	Gene symbol	Log_2 fold-difference	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated MitoPathways[c]
Baseline: COPD vs. CONTROLS	DLD	-0.35	-0.25	0.053	-4.66	9.46e-04	Metabolism, Carbohydrate metabolism, Pyruvate metabolism, TCA cycle, Amino acid metabolism, Branched-chain amino acid metabolism, Branched-chain amino acid dehydrogenase complex, Lysine metabolism, Glycine metabolism, Glycine cleavage system
	TIMM21	-0.32	-0.22	0.051	-4.30	0.002	Mitochondrial central dogma, Translation, Translation factors, Protein import, sorting and homeostasis, Protein import and sorting, TIM23 presequence pathway, OXPHOS, OXPHOS assembly factors, Complex IV, CIV assembly factors
	DECR1	-0.37	-0.26	0.059	-4.33	0.002	Metabolism, Lipid metabolism, Fatty acid oxidation
	AUH	-0.36	-0.25	0.058	-4.32	0.002	Metabolism, Carbohydrate metabolism, Itaconate metabolism, Amino acid metabolism, Branched-chain amino acid metabolism
	SDHD	-0.25	-0.18	0.044	-3.96	0.005	OXPHOS, OXPHOS subunits, Complex II, CII subunits, Metabolism, Carbohydrate metabolism, TCA cycle, Metals and cofactors, Heme-containing proteins
	HADHB	-0.40	-0.27	0.070	-3.90	0.006	Metabolism, Carbohydrate metabolism, Ketone metabolism, Lipid metabolism, Fatty acid oxidation, Amino acid metabolism, Lysine metabolism
	CYCS	-0.36	-0.25	0.064	-3.90	0.006	OXPHOS, OXPHOS subunits, Cytochrome C, Metabolism, Metals and cofactors, Heme-containing proteins, Electron carriers, Cytochromes, Mitochondrial dynamics and surveillance, Apoptosis
	FASN	1.13	0.78	0.205	3.84	0.007	Metabolism, Lipid metabolism
	MCCC1	-0.37	-0.26	0.069	-3.71	0.009	Metabolism, Amino acid metabolism, Branched-chain amino acid metabolism, Vitamin metabolism, Biotin utilizing proteins
	MICU3	-0.37	-0.25	0.069	-3.68	0.010	Small molecule transport, Calcium uniporter, Signaling, Calcium homeostasis, Calcium cycle, EF hand proteins
	PPTC7	-0.41	-0.28	0.078	-3.61	0.012	Signaling
	ACSL1	-0.43	-0.29	0.082	-3.58	0.013	Metabolism, Lipid metabolism, Fatty acid oxidation
	ACADM	-0.38	-0.26	0.074	-3.51	0.016	
	LYRM7	-0.34	-0.23	0.067	-3.49	0.016	OXPHOS, OXPHOS assembly factors, Complex III, CIII assembly factors
	ACADSB	-0.34	-0.24	0.069	-3.43	0.018	Metabolism, Lipid metabolism, Fatty acid oxidation, Amino acid metabolism, Branched-chain amino acid metabolism
	SDHC	-0.30	-0.21	0.062	-3.38	0.020	OXPHOS, OXPHOS subunits, Complex II, CII subunits, Metabolism, Carbohydrate metabolism, TCA cycle, Metals and cofactors, Heme-containing proteins
	NDUFA5	-0.23	-0.16	0.048	-3.35	0.022	OXPHOS, OXPHOS subunits, Complex I, CI subunits
	UQCRC2	-0.23	-0.16	0.048	-3.31	0.024	Protein import, sorting and homeostasis, Protein import and sorting, Preprotein cleavage, OXPHOS, OXPHOS subunits, Complex III, CIII subunits
	NDUFA9	-0.24	-0.16	0.050	-3.29	0.026	OXPHOS, OXPHOS subunits, Complex I, CI subunits
	ETFA	-0.28	-0.19	0.059	-3.24	0.028	Metabolism, Lipid metabolism, Fatty acid oxidation, Amino acid metabolism, Branched-chain amino acid metabolism, Lysine metabolism, Glycine metabolism, Vitamin metabolism, Choline and betaine metabolism, Electron carriers, Q-linked reactions, other
	ATP5PB	-0.21	-0.15	0.046	-3.21	0.029	OXPHOS, OXPHOS subunits, Complex V, CV subunits
	ATP5F1C	-0.26	-0.18	0.056	-3.21	0.029	
	SUCLG2	-0.23	-0.16	0.049	-3.21	0.029	Metabolism, Carbohydrate metabolism, TCA cycle, Itaconate metabolism, Nucleotide metabolism, Nucleotide synthesis and processing
	GUF1	-0.25	-0.18	0.056	-3.17	0.031	Mitochondrial central dogma, Translation
	MRPS35	-0.20	-0.14	0.044	-3.11	0.031	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	GRPEL1	-0.21	-0.14	0.046	-3.12	0.031	Protein import, sorting and homeostasis, Protein import and sorting, Import motor, Metabolism, Metals and cofactors, Fe-S cluster biosynthesis

Comparison	Gene symbol	Log_2 fold-difference	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated MitoPathways[c]
	PDHX	-0.22	-0.15	0.049	-3.14	0.031	Metabolism, Carbohydrate metabolism, Pyruvate metabolism
	COQ10A	-0.35	-0.24	0.078	-3.11	0.031	Metabolism, Metals and cofactors, Coenzyme Q metabolism
	SUCLA2	-0.23	-0.16	0.051	-3.15	0.031	Metabolism, Carbohydrate metabolism, TCA cycle, Itaconate metabolism, Nucleotide metabolism, Nucleotide synthesis and processing
	GPT2	-0.53	-0.37	0.118	-3.11	0.031	Metabolism, Amino acid metabolism, Glutamate metabolism
	COX20	-0.21	-0.14	0.046	-3.13	0.031	OXPHOS, OXPHOS assembly factors, Complex IV, CIV assembly factors
	PDK1	-0.27	-0.19	0.061	-3.10	0.031	Metabolism, Carbohydrate metabolism, Pyruvate metabolism
	MICOS10	-0.32	-0.22	0.071	-3.08	0.033	Mitochondrial dynamics and surveillance, Cristae formation, MICOS complex
	CISD1	-0.20	-0.14	0.046	-3.06	0.034	Metabolism, Metals and cofactors, Fe-S cluster biosynthesis, Fe-S-containing proteins
	TIMM17A	-0.17	-0.12	0.039	-3.05	0.034	Protein import, sorting and homeostasis, Protein import and sorting, TIM23 presequence pathway
	ETFDH	-0.32	-0.22	0.073	-3.05	0.034	Metabolism, Lipid metabolism, Fatty acid oxidation, Amino acid metabolism, Branched-chain amino acid metabolism, Lysine metabolism, Glycine metabolism, Metals and cofactors, Fe-S-containing proteins, Vitamin metabolism, Choline and betaine metabolism, Electron carriers, Q-linked reactions, other
	ECI2	-0.27	-0.19	0.062	-3.06	0.034	Metabolism, Lipid metabolism, Fatty acid oxidation
	MRPL39	-0.20	-0.14	0.046	-3.00	0.038	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	GFM2	-0.20	-0.14	0.046	-2.92	0.045	Mitochondrial central dogma, Translation, Translation factors
	PCCA	-0.24	-0.17	0.057	-2.92	0.045	Metabolism, Carbohydrate metabolism, Propanoate metabolism, Lipid metabolism, Fatty acid oxidation, Vitamin metabolism, Biotin utilizing proteins
	DLAT	-0.25	-0.17	0.058	-2.91	0.045	Metabolism, Carbohydrate metabolism, Pyruvate metabolism
	NDUFS1	-0.25	-0.17	0.061	-2.88	0.047	OXPHOS, OXPHOS subunits, Complex I, CI subunits, Metabolism, Metals and cofactors, Fe-S-containing proteins
	ALDH5A1	-0.30	-0.21	0.072	-2.88	0.047	Metabolism, Amino acid metabolism, GABA metabolism
	SLC25A12	-0.20	-0.14	0.048	-2.88	0.047	Metabolism, Carbohydrate metabolism, Malate-aspartate shuttle, Amino acid metabolism, Glutamate metabolism, Small molecule transport, SLC25A family, Signaling, Calcium homeostasis, EF hand proteins
	FXN	-0.26	-0.18	0.064	-2.86	0.049	Metabolism, Metals and cofactors, Fe-S cluster biosynthesis
	MTIF2	-0.23	-0.16	0.055	-2.86	0.049	Mitochondrial central dogma, Translation, Translation factors
	HSDL2	-0.42	-0.29	0.062	-4.74	9.46e-04	No pathway association
	TPM1	0.66	0.46	0.094	4.87	9.46e-04	
	TPM3	-0.63	-0.44	0.096	-4.55	0.001	
	TMEM65	-0.40	-0.28	0.064	-4.30	0.002	
	ATP2A2	-0.63	-0.44	0.100	-4.36	0.002	
	MYLPF	0.63	0.44	0.102	4.32	0.002	
	RAB10	-0.46	-0.32	0.079	-4.06	0.004	
	RAB3D	0.44	0.30	0.076	4.02	0.004	
	MYH1	0.98	0.68	0.175	3.89	0.006	

Comparison	Gene symbol	Log_2 fold-difference	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated MitoPathways[c]
	CSNK2A2	0.26	0.18	0.048	3.79	0.007	
	ATP2A1	0.62	0.43	0.114	3.80	0.007	
	PFKM	0.29	0.20	0.054	3.71	0.009	
	FAM210A	-0.28	-0.20	0.056	-3.49	0.016	
	MYBPC2	0.59	0.41	0.117	3.47	0.016	
	AK1	0.58	0.40	0.117	3.45	0.017	
	HARS1	0.18	0.12	0.036	3.40	0.019	
	RRP12	0.61	0.42	0.130	3.25	0.028	
	FAM162A	-0.23	-0.16	0.050	-3.25	0.028	
	CTSC	0.35	0.24	0.076	3.20	0.029	
	FRMPD1	-0.52	-0.36	0.117	-3.12	0.031	
	COG4	0.23	0.16	0.052	3.16	0.031	
	ITPR2	-0.28	-0.20	0.062	-3.12	0.031	
	DST	-0.33	-0.23	0.073	-3.11	0.031	
	P4HB	0.27	0.19	0.062	3.03	0.035	
	LRP1B	-0.70	-0.48	0.161	-3.02	0.036	
	RAB4A	-0.20	-0.14	0.045	-2.99	0.038	
	CES1	0.59	0.41	0.136	2.99	0.038	
	PYGB	0.42	0.29	0.098	2.96	0.042	
	ACTN1	0.44	0.30	0.103	2.92	0.045	
	GPD1L	-0.35	-0.24	0.083	-2.92	0.045	
	TNNC2	0.65	0.45	0.156	2.91	0.045	
	RTN4IP1	-0.31	-0.21	0.074	-2.90	0.046	
Post-training: ΔCOPD vs ΔCONTROLS	TXNRD2	-0.49	-0.34	0.083	-4.15	0.030	Metabolism, Detoxification, ROS and glutathione metabolism, Selenoproteins

[a] Estimates provided on the natural log scale together with standard errors (SE) and *Z*-values from generalized mixed linear models. [b] P-values are corrected per coefficient for false discovery rate (FDR) [c] Mitocarta v.3.0 MitoPathways associated with each gene.

Supplemental Table 3. Resistance exercise training-associated changes in gene expression averaged over study groups (COPD, n=19 and CONTROLS, n=34)

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
3th training week vs. baseline	*PDK4*	-0.46	-0.32	0.093	-3.43	0.005	Metabolism, Carbohydrate metabolism, Pyruvate metabolism
	GPT2	-0.28	-0.19	0.050	-3.83	0.002	Metabolism, Amino acid metabolism, Glutamate metabolism
	GPD2	-0.25	-0.18	0.039	-4.45	1.92e-04	Metabolism, Carbohydrate metabolism, Glycerol phosphate shuttle, Electron carriers, Q-linked reactions, other
	ACADL	-0.22	-0.16	0.033	-4.70	7.00e-05	Metabolism, Lipid metabolism, Fatty acid oxidation
	KARS1	-0.22	-0.15	0.020	-7.45	1.49e-11	Mitochondrial central dogma, Translation, mt-tRNA synthetases
	ALDH2	-0.21	-0.14	0.045	-3.15	0.011	Metabolism, Detoxification, Xenobiotic metabolism
	ACSL6	-0.20	-0.14	0.048	-2.92	0.020	Metabolism, Lipid metabolism, Fatty acid oxidation
	SUCLG2	-0.20	-0.14	0.023	-5.94	1.68e-07	Metabolism, Carbohydrate metabolism, TCA cycle, Itaconate metabolism, Nucleotide metabolism, Nucleotide synthesis and processing
	TOMM7	-0.19	-0.13	0.042	-3.23	0.009	Protein import, sorting and homeostasis, Protein import and sorting, TOM, Mitochondrial dynamics and surveillance, Mitophagy, Autophagy
	ALDH6A1	-0.19	-0.13	0.042	-3.10	0.014	Metabolism, Amino acid metabolism, Branched-chain amino acid metabolism
	NDUFB11	-0.19	-0.13	0.046	-2.78	0.028	OXPHOS, OXPHOS subunits, Complex I, CI subunits
	COQ8A	-0.18	-0.12	0.049	-2.56	0.047	Metabolism, Metals and cofactors, Coenzyme Q metabolism
	MICU3	-0.18	-0.12	0.038	-3.20	0.010	Small molecule transport, Calcium uniporter, Signaling, Calcium homeostasis, Calcium cycle, EF hand proteins
	NUDT2	-0.18	-0.12	0.030	-4.02	8.53e-04	Metabolism, Nucleotide metabolism, Nucleotide synthesis and processing
	NFU1	-0.17	-0.12	0.033	-3.57	0.003	Metabolism, Metals and cofactors, Fe-S cluster biosynthesis, Fe-S-containing proteins
	MRPL40	-0.16	-0.11	0.031	-3.51	0.004	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	BNIP3	-0.15	-0.11	0.024	-4.37	2.53e-04	Mitochondrial dynamics and surveillance, Apoptosis
	VPS13D	-0.15	-0.10	0.035	-3.02	0.016	Mitochondrial dynamics and surveillance, Mitophagy, Autophagy
	VDAC1	-0.14	-0.10	0.026	-3.85	0.001	Small molecule transport, Signaling, Calcium homeostasis, Mitochondrial permeability transition pore, Mitochondrial dynamics and surveillance, Organelle contact sites
	MPST	-0.14	-0.10	0.039	-2.55	0.047	Metabolism, Detoxification, Xenobiotic metabolism, Sulfur metabolism
	MAVS	-0.13	-0.09	0.031	-3.01	0.016	Signaling, Immune response
	ACAT1	-0.13	-0.09	0.034	-2.75	0.030	Metabolism, Carbohydrate metabolism, Ketone metabolism, Lipid metabolism, Fatty acid oxidation, Amino acid metabolism, Branched-chain amino acid metabolism, Lysine metabolism
	MRPL51	-0.13	-0.09	0.027	-3.44	0.005	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	TIMM9	-0.13	-0.09	0.027	-3.26	0.008	Protein import, sorting and homeostasis, Protein import and sorting, Protein homeostasis, Chaperones
	HADH	-0.13	-0.09	0.027	-3.30	0.008	Metabolism, Lipid metabolism, Fatty acid oxidation, Amino acid metabolism, Lysine metabolism
	MRPS18C	-0.13	-0.09	0.030	-2.89	0.022	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	MTERF2	-0.13	-0.09	0.029	-3.02	0.016	Mitochondrial central dogma, mtDNA maintenance, mtDNA nucleoid
	LIAS	-0.12	-0.09	0.030	-2.88	0.022	Metabolism, Lipid metabolism, Lipoate insertion, Metals and cofactors, Fe-S-containing proteins
	TOMM20	-0.12	-0.08	0.021	-3.94	0.001	Protein import, sorting and homeostasis, Protein import and sorting, TOM, Mitochondrial dynamics and surveillance

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	NDUFV3	-0.12	-0.08	0.029	-2.88	0.022	OXPHOS, OXPHOS subunits, Complex I, CI subunits
	OMA1	-0.12	-0.08	0.032	-2.55	0.047	Protein import, sorting and homeostasis, Protein homeostasis, Proteases, Mitochondrial dynamics and surveillance, Fusion, Fission
	YME1L1	-0.11	-0.07	0.019	-3.79	0.002	Protein import, sorting and homeostasis, Protein homeostasis, Proteases
	OAT	-0.11	-0.07	0.025	-2.98	0.018	Metabolism, Amino acid metabolism, Proline metabolism
	IDE	-0.11	-0.07	0.028	-2.65	0.038	Protein import, sorting and homeostasis, Protein homeostasis, Proteases
	COQ7	-0.10	-0.07	0.022	-3.28	0.008	Metabolism, Metals and cofactors, Coenzyme Q metabolism
	MRPS25	-0.10	-0.07	0.022	-3.25	0.008	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	MRPL32	-0.10	-0.07	0.024	-2.77	0.029	
	MRPS35	-0.10	-0.07	0.025	-2.68	0.036	
	DMAC2L	-0.09	-0.06	0.019	-3.33	0.007	OXPHOS, OXPHOS subunits, Complex V, CV subunits
	MRPL30	-0.09	-0.06	0.021	-2.86	0.023	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	TSFM	-0.09	-0.06	0.023	-2.54	0.047	Mitochondrial central dogma, Translation, Translation factors
	DAP3	-0.08	-0.05	0.018	-3.07	0.014	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	MTCH2	0.08	0.06	0.019	3.02	0.016	Small molecule transport, SLC25A family, Mitochondrial dynamics and surveillance, Fusion
	ATP5PB	0.09	0.06	0.021	2.86	0.023	OXPHOS, OXPHOS subunits, Complex V, CV subunits
	LYPLA1	0.09	0.07	0.025	2.60	0.042	Metabolism, Lipid metabolism, Signaling
	FH	0.10	0.07	0.026	2.54	0.048	Metabolism, Carbohydrate metabolism, TCA cycle
	LAP3	0.10	0.07	0.017	3.85	0.001	Protein import, sorting and homeostasis, Protein homeostasis, Proteases
	MICU1	0.10	0.07	0.018	3.87	0.001	Small molecule transport, Calcium uniporter, Signaling, Calcium homeostasis, Calcium cycle, EF hand proteins
	SFXN1	0.10	0.07	0.026	2.81	0.026	Metabolism, Amino acid metabolism, Serine metabolism, Vitamin metabolism, Folate and 1-C metabolism, Small molecule transport, Sideroflexins
	IMMT	0.11	0.07	0.025	2.96	0.019	Mitochondrial dynamics and surveillance, Cristae formation, MICOS complex
	SLC25A12	0.11	0.07	0.026	2.87	0.023	Metabolism, Carbohydrate metabolism, Malate-aspartate shuttle, Amino acid metabolism, Glutamate metabolism, Small molecule transport, SLC25A family, Signaling, Calcium homeostasis, EF hand proteins
	GSR	0.11	0.08	0.030	2.52	0.050	Metabolism, Detoxification, ROS and glutathione metabolism
	DECR1	0.11	0.08	0.027	2.81	0.026	Metabolism, Lipid metabolism, Fatty acid oxidation
	NDUFS2	0.12	0.08	0.022	3.78	0.002	OXPHOS, OXPHOS subunits, Complex I, CI subunits, Metabolism, Metals and cofactors, Fe-S-containing proteins
	APOO	0.12	0.09	0.029	3.02	0.016	Mitochondrial dynamics and surveillance, Cristae formation, MICOS complex
	CYCS	0.13	0.09	0.033	2.65	0.038	OXPHOS, OXPHOS subunits, Cytochrome C, Metabolism, Metals and cofactors, Heme-containing proteins, Electron carriers, Cytochromes, Mitochondrial dynamics and surveillance, Apoptosis
	HIBCH	0.13	0.09	0.025	3.54	0.004	Metabolism, Amino acid metabolism, Branched-chain amino acid metabolism
	GLUD1	0.13	0.09	0.021	4.29	3.39e-04	Metabolism, Amino acid metabolism, Glutamate metabolism, GABA metabolism
	ATP5F1A	0.13	0.09	0.027	3.36	0.006	OXPHOS, OXPHOS subunits, Complex V, CV subunits
	ATP5F1B	0.13	0.09	0.026	3.50	0.004	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	HIGD1A	0.13	0.09	0.031	3.00	0.017	OXPHOS, OXPHOS assembly factors, Complex IV, CIV assembly factors, Respirasome assembly
	SLC25A20	0.14	0.10	0.036	2.66	0.037	Metabolism, Lipid metabolism, Fatty acid oxidation, Metals and cofactors, Carnitine synthesis and transport, Carnitine shuttle, Small molecule transport, SLC25A family
	PDHA1	0.14	0.10	0.024	4.04	8.01e-04	Metabolism, Carbohydrate metabolism, Pyruvate metabolism
	CLPB	0.14	0.10	0.036	2.65	0.038	Protein import, sorting and homeostasis, Protein homeostasis, Proteases
	NFS1	0.14	0.10	0.023	4.21	4.46e-04	Metabolism, Metals and cofactors, Fe-S cluster biosynthesis, Fe-S-containing proteins
	TIMM8A	0.14	0.10	0.036	2.74	0.031	Protein import, sorting and homeostasis, Protein import and sorting, Protein homeostasis, Chaperones
	COQ10B	0.14	0.10	0.032	3.06	0.015	Metabolism, Metals and cofactors, Coenzyme Q metabolism
	PRORP	0.15	0.10	0.028	3.67	0.003	Mitochondrial central dogma, mtRNA metabolism, mtRNA granules, Polycistronic mtRNA processing
	NDUFAB1	0.15	0.10	0.038	2.72	0.033	OXPHOS, OXPHOS subunits, Complex I, CI subunits, Metabolism, Lipid metabolism, Type II fatty acid synthesis, Metals and cofactors, Fe-S cluster biosynthesis
	COX7A2	0.16	0.11	0.042	2.62	0.041	OXPHOS, OXPHOS subunits, Complex IV, CIV subunits
	MARS2	0.17	0.12	0.042	2.84	0.024	Mitochondrial central dogma, Translation, mt-tRNA synthetases
	ATP5F1C	0.17	0.12	0.029	4.13	5.97e-04	OXPHOS, OXPHOS subunits, Complex V, CV subunits
	NAXE	0.17	0.12	0.040	3.00	0.017	Metabolism, Metals and cofactors, NAD biosynthesis and metabolism
	CKMT2	0.20	0.14	0.048	2.82	0.026	Metabolism, Nucleotide metabolism, Creatine metabolism
	ACSL1	0.20	0.14	0.042	3.24	0.009	Metabolism, Lipid metabolism, Fatty acid oxidation
	GLS	0.22	0.15	0.027	5.64	8.52e-07	Metabolism, Amino acid metabolism, Glutamate metabolism
	PPTC7	0.26	0.18	0.043	4.18	5.01e-04	Signaling
	CPT1A	0.27	0.19	0.045	4.18	5.01e-04	Metabolism, Lipid metabolism, Fatty acid oxidation, Metals and cofactors, Carnitine synthesis and transport, Carnitine shuttle
	SLC25A5	0.27	0.19	0.043	4.43	2.02e-04	Metabolism, Nucleotide metabolism, Nucleotide import, Small molecule transport, SLC25A family, Signaling, Calcium homeostasis, Mitochondrial permeability transition pore
	MSRA	0.30	0.21	0.051	4.07	7.12e-04	Metabolism, Detoxification, ROS and glutathione metabolism, Sulfur metabolism
	ALDH1B1	0.31	0.22	0.052	4.14	5.73e-04	Metabolism, Detoxification, Xenobiotic metabolism
	NIPSNAP1	0.32	0.23	0.059	3.80	0.002	Mitochondrial dynamics and surveillance, Mitophagy, Autophagy
	ALDH1L2	0.38	0.26	0.057	4.58	1.18e-04	Metabolism, Vitamin metabolism, Folate and 1-C metabolism
	ALDH18A1	0.41	0.28	0.051	5.55	1.26e-06	Metabolism, Amino acid metabolism, Proline metabolism
	SFXN3	0.41	0.29	0.047	6.10	8.38e-08	Metabolism, Amino acid metabolism, Serine metabolism, Vitamin metabolism, Folate and 1-C metabolism, Small molecule transport, Sideroflexins
	SLC25A35	0.57	0.39	0.088	4.48	1.77e-04	Small molecule transport, SLC25A family
	MYH1	-0.83	-0.57	0.091	-6.29	3.03e-08	No pathway association
	CYP4B1	-0.73	-0.51	0.073	-6.96	3.91e-10	
	ATP1A4	-0.40	-0.27	0.069	-3.98	9.58e-04	
	ACTN3	-0.37	-0.26	0.077	-3.35	0.007	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	APOB	-0.37	-0.25	0.097	-2.63	0.039	
	LRP1B	-0.36	-0.25	0.051	-4.91	2.96e-05	
	CKM	-0.31	-0.22	0.037	-5.87	2.41e-07	
	CMYA5	-0.30	-0.21	0.044	-4.76	5.59e-05	
	ARID5B	-0.28	-0.20	0.046	-4.30	3.31e-04	
	RAB10	-0.27	-0.19	0.036	-5.32	4.27e-06	
	ATP2A1	-0.26	-0.18	0.058	-3.16	0.011	
	DMD	-0.25	-0.17	0.043	-3.95	0.001	
	RGS9	-0.24	-0.17	0.055	-3.04	0.015	
	RYR1	-0.24	-0.17	0.046	-3.63	0.003	
	RPL27	-0.23	-0.16	0.050	-3.28	0.008	
	KMT2B	-0.23	-0.16	0.064	-2.56	0.047	
	RPS25	-0.23	-0.16	0.036	-4.43	2.02e-04	
	UBE4A	-0.22	-0.15	0.041	-3.76	0.002	
	RPS13	-0.20	-0.14	0.039	-3.52	0.004	
	HHATL	-0.20	-0.14	0.050	-2.78	0.028	
	ATP2A2	-0.19	-0.13	0.044	-2.92	0.020	
	MBP	-0.18	-0.13	0.033	-3.89	0.001	
	MYBPC1	-0.18	-0.13	0.034	-3.67	0.003	
	LAMP2	-0.18	-0.13	0.030	-4.21	4.46e-04	
	NEDD4L	-0.18	-0.13	0.032	-3.95	0.001	
	UBA52	-0.18	-0.12	0.038	-3.26	0.008	
	RPL23	-0.18	-0.12	0.035	-3.48	0.004	
	MICAL3	-0.18	-0.12	0.043	-2.86	0.023	
	RPS7	-0.17	-0.12	0.045	-2.70	0.034	
	USP24	-0.17	-0.12	0.040	-3.03	0.016	
	PGM1	-0.17	-0.11	0.034	-3.37	0.006	
	PUM1	-0.17	-0.11	0.024	-4.80	4.98e-05	
	PLAAT3	-0.16	-0.11	0.037	-3.06	0.015	
	RAPGEF1	-0.16	-0.11	0.044	-2.59	0.043	
	CCDC58	-0.16	-0.11	0.041	-2.74	0.031	
	ECHDC2	-0.16	-0.11	0.027	-4.10	6.37e-04	
	RPS18	-0.16	-0.11	0.033	-3.34	0.007	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	CACNA1S	-0.16	-0.11	0.036	-3.07	0.014	
	RPL23A	-0.16	-0.11	0.041	-2.66	0.037	
	RTN4	-0.16	-0.11	0.032	-3.47	0.005	
	KDM6A	-0.16	-0.11	0.030	-3.66	0.003	
	ATP8A1	-0.15	-0.11	0.038	-2.79	0.028	
	TMEM38A	-0.15	-0.11	0.035	-3.07	0.014	
	CRYAB	-0.15	-0.10	0.041	-2.53	0.048	
	RPS27A	-0.15	-0.10	0.040	-2.54	0.047	
	CEP350	-0.14	-0.10	0.036	-2.78	0.028	
	ZNF638	-0.14	-0.10	0.028	-3.52	0.004	
	KMT2A	-0.14	-0.10	0.034	-2.84	0.024	
	TMEM109	-0.14	-0.10	0.033	-2.95	0.019	
	HSPA8	-0.14	-0.10	0.029	-3.30	0.008	
	PLGRKT	-0.13	-0.09	0.032	-2.94	0.019	
	SYNE2	-0.13	-0.09	0.022	-4.23	4.35e-04	
	GPD1L	-0.13	-0.09	0.036	-2.56	0.047	
	CLIP1	-0.13	-0.09	0.036	-2.55	0.047	
	PRDX1	-0.13	-0.09	0.029	-3.09	0.014	
	RAB21	-0.13	-0.09	0.023	-3.91	0.001	
	PPID	-0.13	-0.09	0.023	-3.86	0.001	
	ATP1A2	-0.13	-0.09	0.026	-3.37	0.006	
	EIF3B	-0.12	-0.08	0.022	-3.88	0.001	
	TPM3	-0.12	-0.08	0.032	-2.63	0.039	
	VPS13A	-0.12	-0.08	0.031	-2.64	0.039	
	RPL15	-0.12	-0.08	0.033	-2.54	0.047	
	HAX1	-0.11	-0.08	0.025	-3.16	0.011	
	RPSA	-0.11	-0.08	0.027	-2.88	0.022	
	PITHD1	-0.09	-0.07	0.023	-2.89	0.022	
	RAB5B	-0.09	-0.06	0.021	-2.92	0.020	
	RAB2A	-0.08	-0.05	0.020	-2.70	0.034	
	RHOA	0.09	0.06	0.022	2.91	0.021	
	RPN1	0.10	0.07	0.023	2.85	0.023	
	FAM162A	0.10	0.07	0.025	2.77	0.028	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	OPA3	0.12	0.08	0.030	2.78	0.028	
	SPRYD4	0.12	0.09	0.029	2.96	0.018	
	ARF4	0.12	0.09	0.028	3.04	0.015	
	FER	0.13	0.09	0.037	2.55	0.047	
	IQGAP1	0.13	0.09	0.027	3.42	0.005	
	MYL1	0.15	0.10	0.038	2.69	0.035	
	ARF6	0.16	0.11	0.023	4.76	5.59e-05	
	UHRF1BP1L	0.17	0.12	0.038	3.08	0.014	
	RAB6B	0.17	0.12	0.046	2.55	0.047	
	TASOR	0.17	0.12	0.026	4.56	1.25e-04	
	FAM136A	0.17	0.12	0.028	4.21	4.46e-04	
	FLNB	0.17	0.12	0.039	3.06	0.015	
	ACTG1	0.18	0.13	0.048	2.64	0.039	
	PREX2	0.19	0.13	0.035	3.80	0.002	
	HSP90B1	0.19	0.13	0.026	5.11	1.11e-05	
	CA3	0.20	0.14	0.038	3.60	0.003	
	ACSL5	0.20	0.14	0.039	3.63	0.003	
	PGAM1	0.20	0.14	0.040	3.47	0.005	
	RAB8A	0.20	0.14	0.043	3.32	0.007	
	CAMK2D	0.21	0.14	0.041	3.49	0.004	
	PPIA	0.21	0.15	0.039	3.74	0.002	
	FABP4	0.22	0.16	0.047	3.29	0.008	
	RAB8B	0.23	0.16	0.036	4.45	1.92e-04	
	MYH9	0.23	0.16	0.061	2.68	0.036	
	ASAP2	0.24	0.17	0.050	3.35	0.007	
	RAB4B	0.24	0.17	0.065	2.60	0.042	
	CFH	0.25	0.17	0.048	3.61	0.003	
	NME1	0.25	0.18	0.067	2.63	0.039	
	ACTB	0.26	0.18	0.045	4.02	8.56e-04	
	PKM	0.26	0.18	0.048	3.79	0.002	
	CFL1	0.27	0.19	0.045	4.12	6.15e-04	
	MYL6	0.27	0.19	0.048	3.94	0.001	
	ARF3	0.27	0.19	0.032	5.95	1.68e-07	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	FRMPD1	0.28	0.19	0.048	3.99	9.36e-04	
	PDIA3	0.29	0.20	0.035	5.75	4.82e-07	
	ATP1A1	0.29	0.20	0.042	4.79	4.98e-05	
	TUBA1C	0.31	0.22	0.068	3.18	0.011	
	RAB13	0.32	0.22	0.035	6.37	1.98e-08	
	PPP1R9B	0.33	0.23	0.062	3.63	0.003	
	RAB3A	0.33	0.23	0.084	2.69	0.035	
	RHOC	0.34	0.24	0.065	3.64	0.003	
	MYH10	0.35	0.24	0.043	5.57	1.17e-06	
	DMXL2	0.35	0.25	0.047	5.26	5.54e-06	
	EHD3	0.36	0.25	0.067	3.73	0.002	
	EHD2	0.37	0.26	0.058	4.39	2.30e-04	
	TRANK1	0.37	0.26	0.050	5.16	8.79e-06	
	OLFML2A	0.37	0.26	0.048	5.44	2.34e-06	
	HCN3	0.37	0.26	0.089	2.91	0.021	
	MSN	0.38	0.26	0.032	8.31	8.87e-14	
	HMCN1	0.40	0.28	0.052	5.26	5.54e-06	
	S100A6	0.40	0.28	0.091	3.08	0.014	
	CD163	0.40	0.28	0.070	3.96	0.001	
	MYOF	0.41	0.28	0.060	4.69	7.04e-05	
	EHD4	0.41	0.29	0.048	6.01	1.23e-07	
	ANXA2	0.43	0.30	0.049	6.16	6.42e-08	
	S100A4	0.45	0.31	0.070	4.43	2.02e-04	
	PPIB	0.46	0.32	0.071	4.50	1.66e-04	
	CPA3	0.46	0.32	0.086	3.73	0.002	
	CIT	0.48	0.33	0.081	4.10	6.37e-04	
	TPM4	0.49	0.34	0.044	7.56	7.71e-12	
	COL6A1	0.49	0.34	0.097	3.49	0.004	
	THBS1	0.60	0.42	0.084	5.00	1.94e-05	
	COL6A3	0.69	0.48	0.062	7.73	2.48e-12	
	FBN1	0.70	0.48	0.061	7.96	8.06e-13	
	RAC2	0.75	0.52	0.120	4.33	2.91e-04	
	COL1A1	0.98	0.68	0.144	4.70	7.00e-05	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	GAS2L3	1.01	0.70	0.089	7.82	1.67e-12	
	COL1A2	1.04	0.72	0.120	6.03	1.19e-07	
	CENPF	1.07	0.74	0.105	7.07	2.06e-10	
Post-training vs. baseline	*TMEM70*	-0.31	-0.21	0.061	-3.47	0.004	OXPHOS, OXPHOS assembly factors, Complex I, CI assembly factors, Complex V, CV assembly factors
	MGST1	-0.29	-0.20	0.069	-2.95	0.018	Metabolism, Detoxification, Xenobiotic metabolism, ROS and glutathione metabolism
	ACSL6	-0.25	-0.17	0.048	-3.61	0.003	Metabolism, Lipid metabolism, Fatty acid oxidation
	TOMM7	-0.21	-0.15	0.041	-3.63	0.003	Protein import, sorting and homeostasis, Protein import and sorting, TOM, Mitochondrial dynamics and surveillance, Mitophagy, Autophagy
	LIPT2	-0.21	-0.15	0.041	-3.57	0.003	Metabolism, Lipid metabolism, Lipoate insertion
	MRPL40	-0.20	-0.14	0.031	-4.54	1.49e-04	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	MRPL14	-0.20	-0.14	0.046	-3.06	0.014	
	SQOR	-0.20	-0.14	0.029	-4.74	7.29e-05	Metabolism, Electron carriers, Q-linked reactions, other, Sulfur metabolism
	GPD2	-0.20	-0.14	0.039	-3.55	0.003	Metabolism, Carbohydrate metabolism, Glycerol phosphate shuttle, Electron carriers, Q-linked reactions, other
	NUDT2	-0.18	-0.12	0.030	-4.16	5.73e-04	Metabolism, Nucleotide metabolism, Nucleotide synthesis and processing
	NFU1	-0.18	-0.12	0.033	-3.79	0.002	Metabolism, Metals and cofactors, Fe-S cluster biosynthesis, Fe-S-containing proteins
	KARS1	-0.18	-0.12	0.020	-6.21	7.04e-08	Mitochondrial central dogma, Translation, mt-tRNA synthetases
	MRPS18C	-0.17	-0.12	0.030	-3.99	0.001	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	MTERF2	-0.16	-0.11	0.029	-3.90	0.001	Mitochondrial central dogma, mtDNA maintenance, mtDNA nucleoid
	PHB2	-0.16	-0.11	0.042	-2.57	0.043	Protein import, sorting and homeostasis, Protein homeostasis, Chaperones
	NDUFA1	-0.16	-0.11	0.037	-2.89	0.021	OXPHOS, OXPHOS subunits, Complex I, CI subunits
	VDAC1	-0.15	-0.11	0.025	-4.13	6.37e-04	Small molecule transport, Signaling, Calcium homeostasis, Mitochondrial permeability transition pore, Mitochondrial dynamics and surveillance, Organelle contact sites
	NDUFA4	-0.15	-0.11	0.037	-2.82	0.025	OXPHOS, OXPHOS subunits, Complex IV, CIV subunits
	NDUFV3	-0.15	-0.10	0.028	-3.72	0.002	OXPHOS, OXPHOS subunits, Complex I, CI subunits
	MRPS25	-0.15	-0.10	0.021	-4.84	4.62e-05	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	MRPL58	-0.15	-0.10	0.034	-3.06	0.014	Mitochondrial central dogma, Translation, Mitochondrial ribosome, Translation factors
	MRPL51	-0.15	-0.10	0.026	-3.86	0.001	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	NDUFB9	-0.14	-0.10	0.034	-2.93	0.019	OXPHOS, OXPHOS subunits, Complex I, CI subunits
	SLIRP	-0.14	-0.10	0.039	-2.55	0.045	Mitochondrial central dogma, mtRNA metabolism, mtRNA stability and decay, Translation
	NDUFS4	-0.14	-0.10	0.035	-2.80	0.026	OXPHOS, OXPHOS subunits, Complex I, CI subunits
	STOML2	-0.14	-0.10	0.038	-2.58	0.043	Protein import, sorting and homeostasis, Protein homeostasis
	MICU3	-0.14	-0.10	0.038	-2.56	0.045	Small molecule transport, Calcium uniporter, Signaling, Calcium homeostasis, Calcium cycle, EF hand proteins
	SUCLG2	-0.14	-0.10	0.022	-4.27	3.84e-04	Metabolism, Carbohydrate metabolism, TCA cycle, Itaconate metabolism, Nucleotide metabolism, Nucleotide synthesis and processing
	MRPL9	-0.14	-0.09	0.026	-3.58	0.003	Mitochondrial central dogma, Translation, Mitochondrial ribosome

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	IDH3B	-0.13	-0.09	0.032	-2.93	0.019	Metabolism, Carbohydrate metabolism, TCA cycle
	COX6B1	-0.13	-0.09	0.035	-2.58	0.042	OXPHOS, OXPHOS subunits, Complex IV, CIV subunits
	OAT	-0.13	-0.09	0.024	-3.63	0.003	Metabolism, Amino acid metabolism, Proline metabolism
	ATP5PD	-0.13	-0.09	0.031	-2.86	0.023	OXPHOS, OXPHOS subunits, Complex V, CV subunits
	NDUFS5	-0.12	-0.09	0.032	-2.73	0.030	OXPHOS, OXPHOS subunits, Complex I, CI subunits
	MRPS6	-0.12	-0.09	0.031	-2.77	0.028	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	NIT2	-0.12	-0.08	0.018	-4.71	7.97e-05	Metabolism, Detoxification
	TIMM9	-0.12	-0.08	0.027	-3.14	0.011	Protein import, sorting and homeostasis, Protein import and sorting, Protein homeostasis, Chaperones
	HADH	-0.12	-0.08	0.026	-3.15	0.011	Metabolism, Lipid metabolism, Fatty acid oxidation, Amino acid metabolism, Lysine metabolism
	DAP3	-0.12	-0.08	0.018	-4.61	1.20e-04	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	MRPL50	-0.11	-0.08	0.031	-2.52	0.048	
	NIPSNAP2	-0.11	-0.08	0.026	-2.97	0.017	Mitochondrial dynamics and surveillance, Mitophagy, Autophagy
	DIABLO	-0.11	-0.07	0.023	-3.17	0.011	Mitochondrial dynamics and surveillance, Apoptosis
	MRPS35	-0.10	-0.07	0.025	-2.69	0.034	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	DMAC2L	-0.09	-0.07	0.018	-3.60	0.003	OXPHOS, OXPHOS subunits, Complex V, CV subunits
	TOMM20	-0.09	-0.07	0.021	-3.13	0.012	Protein import, sorting and homeostasis, Protein import and sorting, TOM, Mitochondrial dynamics and surveillance
	SAMM50	-0.09	-0.06	0.020	-3.22	0.009	Protein import, sorting and homeostasis, Protein import and sorting, SAM, Mitochondrial dynamics and surveillance, Intramitochondrial membrane interactions
	COX7A2L	-0.09	-0.06	0.023	-2.79	0.027	OXPHOS, OXPHOS subunits, OXPHOS assembly factors, Complex IV, CIV subunits, Respirasome assembly
	MRPS17	-0.08	-0.05	0.020	-2.63	0.038	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	DLST	0.09	0.06	0.023	2.76	0.029	Metabolism, Carbohydrate metabolism, TCA cycle, Amino acid metabolism, Lysine metabolism
	GHITM	0.09	0.07	0.018	3.66	0.003	Mitochondrial dynamics and surveillance, Apoptosis
	ATP5F1B	0.09	0.07	0.026	2.55	0.045	OXPHOS, OXPHOS subunits, Complex V, CV subunits
	LAP3	0.10	0.07	0.017	3.87	0.001	Protein import, sorting and homeostasis, Protein homeostasis, Proteases
	LYPLA1	0.10	0.07	0.025	2.69	0.033	Metabolism, Lipid metabolism, Signaling
	IARS2	0.10	0.07	0.025	2.74	0.030	Mitochondrial central dogma, Translation, mt-tRNA synthetases
	BPHL	0.10	0.07	0.026	2.63	0.038	Metabolism, Detoxification, Xenobiotic metabolism
	PDHX	0.10	0.07	0.020	3.50	0.004	Metabolism, Carbohydrate metabolism, Pyruvate metabolism
	TSFM	0.10	0.07	0.022	3.12	0.012	Mitochondrial central dogma, Translation, Translation factors
	GLRX2	0.10	0.07	0.027	2.65	0.037	Metabolism, Metals and cofactors, Fe-S-containing proteins, Vitamin metabolism, Vitamin C metabolism, Detoxification, ROS and glutathione metabolism
	IDH3A	0.10	0.07	0.027	2.65	0.036	Metabolism, Carbohydrate metabolism, TCA cycle
	DECR1	0.11	0.07	0.027	2.69	0.034	Metabolism, Lipid metabolism, Fatty acid oxidation

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	IMMT	0.11	0.07	0.025	2.99	0.017	Mitochondrial dynamics and surveillance, Cristae formation, MICOS complex
	TIMMDC1	0.11	0.08	0.027	2.79	0.027	OXPHOS, OXPHOS assembly factors, Complex I, CI assembly factors
	ATP5F1A	0.11	0.08	0.026	3.00	0.016	OXPHOS, OXPHOS subunits, Complex V, CV subunits
	GSR	0.12	0.08	0.030	2.67	0.035	Metabolism, Detoxification, ROS and glutathione metabolism
	ETFDH	0.12	0.08	0.030	2.76	0.028	Metabolism, Lipid metabolism, Fatty acid oxidation, Amino acid metabolism, Branched-chain amino acid metabolism, Lysine metabolism, Glycine metabolism, Metals and cofactors, Fe-S-containing proteins, Vitamin metabolism, Choline and betaine metabolism, Electron carriers, Q-linked reactions, other
	LARS2	0.12	0.08	0.030	2.73	0.030	Mitochondrial central dogma, Translation, mt-tRNA synthetases
	NDUFS2	0.12	0.08	0.021	3.86	0.001	OXPHOS, OXPHOS subunits, Complex I, CI subunits, Metabolism, Metals and cofactors, Fe-S-containing proteins
	DBT	0.12	0.08	0.030	2.79	0.027	Metabolism, Amino acid metabolism, Branched-chain amino acid metabolism, Branched-chain amino acid dehydrogenase complex
	MRPS27	0.12	0.08	0.019	4.33	3.02e-04	Mitochondrial central dogma, Translation, Mitochondrial ribosome
	DNAJC11	0.12	0.09	0.025	3.48	0.004	Protein import, sorting and homeostasis, Protein import and sorting, Mitochondrial dynamics and surveillance, Intramitochondrial membrane interactions
	MIPEP	0.12	0.09	0.034	2.55	0.045	Protein import, sorting and homeostasis, Protein import and sorting, Preprotein cleavage, Protein homeostasis, Proteases
	PDHA1	0.13	0.09	0.023	3.73	0.002	Metabolism, Carbohydrate metabolism, Pyruvate metabolism
	CPT2	0.13	0.09	0.028	3.14	0.011	Metabolism, Lipid metabolism, Fatty acid oxidation, Metals and cofactors, Carnitine synthesis and transport, Carnitine shuttle
	MCCC2	0.13	0.09	0.025	3.60	0.003	Metabolism, Amino acid metabolism, Branched-chain amino acid metabolism
	HIBCH	0.13	0.09	0.025	3.63	0.003	
	NLN	0.13	0.09	0.035	2.62	0.039	Protein import, sorting and homeostasis, Protein homeostasis, Proteases
	COQ10B	0.13	0.09	0.032	2.88	0.021	Metabolism, Metals and cofactors, Coenzyme Q metabolism
	GATB	0.13	0.09	0.025	3.66	0.003	Mitochondrial central dogma, Translation, mt-tRNA synthetases
	ACAA2	0.13	0.09	0.027	3.51	0.004	Metabolism, Carbohydrate metabolism, Ketone metabolism, Lipid metabolism, Fatty acid oxidation, Amino acid metabolism, Lysine metabolism
	GLUD1	0.14	0.09	0.020	4.60	1.23e-04	Metabolism, Amino acid metabolism, Glutamate metabolism, GABA metabolism
	PRORP	0.14	0.10	0.027	3.57	0.003	Mitochondrial central dogma, mtRNA metabolism, mtRNA granules, Polycistronic mtRNA processing
	DHODH	0.14	0.10	0.037	2.63	0.038	Metabolism, Nucleotide metabolism, Nucleotide synthesis and processing, Electron carriers, Q-linked reactions, other
	NDUFS1	0.14	0.10	0.031	3.23	0.009	OXPHOS, OXPHOS subunits, Complex I, CI subunits, Metabolism, Metals and cofactors, Fe-S-containing proteins
	IVD	0.15	0.10	0.030	3.39	0.006	Metabolism, Amino acid metabolism, Branched-chain amino acid metabolism
	CYCS	0.15	0.10	0.033	3.17	0.011	OXPHOS, OXPHOS subunits, Cytochrome C, Metabolism, Metals and cofactors, Heme-containing proteins, Electron carriers, Cytochromes, Mitochondrial dynamics and surveillance, Apoptosis
	MLYCD	0.15	0.11	0.042	2.52	0.049	Metabolism, Lipid metabolism
	CLPB	0.16	0.11	0.036	3.06	0.014	Protein import, sorting and homeostasis, Protein homeostasis, Proteases
	PPIF	0.16	0.11	0.038	2.90	0.021	Signaling, Calcium homeostasis, Mitochondrial permeability transition pore
	SLC25A20	0.16	0.11	0.035	3.13	0.012	Metabolism, Lipid metabolism, Fatty acid oxidation, Metals and cofactors, Carnitine synthesis and transport, Carnitine shuttle, Small molecule transport, SLC25A family

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	NFS1	0.16	0.11	0.023	4.88	3.95e-05	Metabolism, Metals and cofactors, Fe-S cluster biosynthesis, Fe-S-containing proteins
	SYNJ2BP	0.16	0.11	0.034	3.32	0.007	Mitochondrial dynamics and surveillance, Organelle contact sites
	TIMM8A	0.18	0.13	0.035	3.58	0.003	Protein import, sorting and homeostasis, Protein import and sorting, Protein homeostasis, Chaperones
	MARS2	0.19	0.13	0.041	3.12	0.012	Mitochondrial central dogma, Translation, mt-tRNA synthetases
	DARS2	0.20	0.14	0.029	4.67	9.36e-05	
	GLS	0.20	0.14	0.027	5.10	1.41e-05	Metabolism, Amino acid metabolism, Glutamate metabolism
	MSRA	0.21	0.15	0.051	2.89	0.021	Metabolism, Detoxification, ROS and glutathione metabolism, Sulfur metabolism
	NIPSNAP1	0.24	0.17	0.059	2.80	0.026	Mitochondrial dynamics and surveillance, Mitophagy, Autophagy
	PPTC7	0.25	0.17	0.042	4.09	7.46e-04	Signaling
	CKMT2	0.26	0.18	0.047	3.89	0.001	Metabolism, Nucleotide metabolism, Creatine metabolism
	ALDH1L2	0.27	0.19	0.057	3.29	0.008	Metabolism, Vitamin metabolism, Folate and 1-C metabolism
	CPT1A	0.28	0.19	0.044	4.36	2.78e-04	Metabolism, Lipid metabolism, Fatty acid oxidation, Metals and cofactors, Carnitine synthesis and transport, Carnitine shuttle
	SFXN3	0.28	0.19	0.047	4.13	6.37e-04	Metabolism, Amino acid metabolism, Serine metabolism, Vitamin metabolism, Folate and 1-C metabolism, Small molecule transport, Sideroflexins
	SLC25A42	0.30	0.21	0.061	3.41	0.005	Metabolism, Metals and cofactors, Coenzyme A metabolism, Small molecule transport, SLC25A family
	ALDH18A1	0.31	0.21	0.051	4.16	5.73e-04	Metabolism, Amino acid metabolism, Proline metabolism
	ACSL1	0.31	0.22	0.041	5.27	6.32e-06	Metabolism, Lipid metabolism, Fatty acid oxidation
	PC	0.40	0.28	0.080	3.52	0.004	Metabolism, Carbohydrate metabolism, Gluconeogenesis, TCA-associated, Vitamin metabolism, Biotin utilizing proteins
	ALDH1B1	0.48	0.33	0.051	6.50	1.53e-08	Metabolism, Detoxification, Xenobiotic metabolism
	SLC25A35	0.51	0.36	0.087	4.07	7.99e-04	Small molecule transport, SLC25A family
	MYH1	-0.87	-0.60	0.089	-6.74	4.89e-09	No pathway association
	ACTN3	-0.72	-0.50	0.080	-6.26	6.19e-08	
	CYP4B1	-0.53	-0.37	0.069	-5.34	5.16e-06	
	ATP1A4	-0.41	-0.29	0.068	-4.24	4.15e-04	
	HCN1	-0.41	-0.28	0.063	-4.46	1.94e-04	
	RPS25	-0.34	-0.23	0.036	-6.51	1.53e-08	
	CES1	-0.33	-0.23	0.059	-3.90	0.001	
	LRP1B	-0.33	-0.23	0.050	-4.58	1.29e-04	
	RGS9	-0.32	-0.22	0.055	-4.00	0.001	
	RPL27	-0.31	-0.22	0.049	-4.39	2.51e-04	
	RPS13	-0.30	-0.21	0.039	-5.32	5.23e-06	
	RPL35	-0.29	-0.20	0.072	-2.75	0.029	
	RPSA	-0.28	-0.19	0.028	-7.00	1.18e-09	
	RPS27A	-0.28	-0.19	0.040	-4.77	6.38e-05	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	Z-value[a]	P-values (FDR)[b]	Associated pathways[c]
	RPL23	-0.27	-0.18	0.035	-5.21	8.06e-06	
	RPL23A	-0.26	-0.18	0.041	-4.35	2.80e-04	
	RPS18	-0.26	-0.18	0.033	-5.37	4.68e-06	
	MYLPF	-0.25	-0.17	0.046	-3.75	0.002	
	UBA52	-0.25	-0.17	0.038	-4.49	1.81e-04	
	RPLP0	-0.24	-0.17	0.048	-3.46	0.005	
	RPL31	-0.23	-0.16	0.041	-3.85	0.001	
	RAB10	-0.22	-0.15	0.035	-4.36	2.78e-04	
	RPS7	-0.22	-0.15	0.044	-3.42	0.005	
	ATP2A1	-0.22	-0.15	0.056	-2.68	0.034	
	RPL11	-0.21	-0.15	0.033	-4.47	1.88e-04	
	CASQ1	-0.20	-0.14	0.025	-5.63	1.28e-06	
	RPL12	-0.20	-0.14	0.055	-2.56	0.045	
	CKM	-0.20	-0.14	0.036	-3.90	0.001	
	MYBPC2	-0.20	-0.14	0.050	-2.73	0.030	
	CMYA5	-0.20	-0.14	0.043	-3.18	0.011	
	RPL26	-0.19	-0.13	0.038	-3.51	0.004	
	RPL18	-0.19	-0.13	0.045	-3.01	0.016	
	RTN4	-0.19	-0.13	0.031	-4.20	5.04e-04	
	PGM1	-0.19	-0.13	0.034	-3.93	0.001	
	HHATL	-0.19	-0.13	0.049	-2.67	0.035	
	TPM2	-0.18	-0.12	0.043	-2.91	0.020	
	CCDC58	-0.18	-0.12	0.041	-3.05	0.015	
	ZNF638	-0.17	-0.12	0.028	-4.27	3.84e-04	
	PTRH2	-0.17	-0.12	0.031	-3.83	0.002	
	ARID5B	-0.17	-0.12	0.044	-2.61	0.040	
	LAMP2	-0.16	-0.11	0.029	-3.85	0.001	
	TPM3	-0.15	-0.11	0.032	-3.40	0.005	
	P4HB	-0.15	-0.11	0.036	-2.95	0.018	
	TMEM38A	-0.15	-0.10	0.034	-3.04	0.015	
	ATP8A1	-0.14	-0.10	0.038	-2.65	0.037	
	CAMK2A	-0.14	-0.10	0.032	-3.14	0.011	
	RAC1	-0.14	-0.10	0.022	-4.52	1.60e-04	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	ECHDC2	-0.14	-0.10	0.027	-3.64	0.003	
	PLGRKT	-0.14	-0.10	0.031	-3.11	0.012	
	HSPA8	-0.13	-0.09	0.029	-3.23	0.009	
	PITHD1	-0.13	-0.09	0.022	-3.94	0.001	
	RAB2A	-0.13	-0.09	0.020	-4.37	2.75e-04	
	KDM6A	-0.13	-0.09	0.029	-2.98	0.017	
	ATP1A2	-0.12	-0.08	0.025	-3.22	0.009	
	PCMT1	-0.11	-0.08	0.020	-4.02	9.45e-04	
	SRL	-0.11	-0.08	0.023	-3.46	0.005	
	PRDX1	-0.11	-0.08	0.028	-2.78	0.027	
	REPS1	-0.11	-0.07	0.025	-3.00	0.016	
	HAX1	-0.10	-0.07	0.025	-2.93	0.019	
	NIF3L1	-0.10	-0.07	0.027	-2.56	0.045	
	RAB21	-0.09	-0.06	0.022	-2.85	0.024	
	EIF3B	-0.09	-0.06	0.021	-2.99	0.016	
	SYPL2	-0.08	-0.06	0.021	-2.80	0.026	
	SPRYD4	0.11	0.08	0.028	2.80	0.026	
	JAK1	0.12	0.08	0.022	3.61	0.003	
	WDR7	0.13	0.09	0.030	3.01	0.016	
	MTOR	0.13	0.09	0.024	3.82	0.002	
	TNS3	0.14	0.09	0.034	2.82	0.025	
	TASOR	0.14	0.10	0.026	3.80	0.002	
	FAM136A	0.15	0.11	0.028	3.80	0.002	
	FER	0.17	0.12	0.036	3.19	0.010	
	THNSL1	0.17	0.12	0.034	3.46	0.005	
	MACF1	0.17	0.12	0.033	3.55	0.003	
	KIAA0754	0.17	0.12	0.033	3.55	0.003	
	ARF3	0.17	0.12	0.032	3.70	0.002	
	CFL1	0.18	0.12	0.046	2.73	0.030	
	ACSL5	0.19	0.13	0.038	3.45	0.005	
	DOP1B	0.19	0.13	0.039	3.37	0.006	
	RYR3	0.19	0.13	0.048	2.75	0.029	
	RAB6B	0.19	0.13	0.046	2.92	0.019	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	CA3	0.21	0.15	0.037	3.90	0.001	
	STAT1	0.21	0.15	0.048	3.06	0.014	
	ASAP2	0.23	0.16	0.050	3.15	0.011	
	MYOF	0.24	0.17	0.061	2.76	0.029	
	TPM4	0.25	0.17	0.045	3.78	0.002	
	EHD3	0.25	0.17	0.067	2.61	0.040	
	FBN1	0.26	0.18	0.062	2.84	0.024	
	GATD3A	0.26	0.18	0.059	3.04	0.015	
	CAMK2D	0.26	0.18	0.040	4.48	1.82e-04	
	EZR	0.26	0.18	0.070	2.59	0.042	
	XIRP2	0.26	0.18	0.058	3.15	0.011	
	RAB13	0.26	0.18	0.034	5.33	5.16e-06	
	CACNA1C	0.27	0.19	0.050	3.72	0.002	
	PPP1R9B	0.27	0.19	0.062	3.03	0.015	
	MSN	0.27	0.19	0.032	5.92	3.30e-07	
	FABP4	0.27	0.19	0.047	4.03	8.96e-04	
	ATP1A1	0.27	0.19	0.042	4.53	1.56e-04	
	RAB4B	0.27	0.19	0.064	2.94	0.019	
	PREX2	0.28	0.19	0.034	5.64	1.28e-06	
	MYH9	0.30	0.21	0.060	3.50	0.004	
	DMXL2	0.31	0.21	0.047	4.59	1.27e-04	
	ARFGEF3	0.32	0.22	0.068	3.26	0.009	
	RHOC	0.32	0.22	0.064	3.43	0.005	
	EHD2	0.32	0.22	0.058	3.80	0.002	
	PITPNM2	0.33	0.23	0.064	3.61	0.003	
	EHD4	0.34	0.24	0.047	5.02	2.06e-05	
	HCN3	0.35	0.25	0.089	2.76	0.028	
	COL6A1	0.36	0.25	0.097	2.58	0.043	
	CIT	0.38	0.26	0.081	3.21	0.010	
	TRANK1	0.39	0.27	0.049	5.40	4.32e-06	
	OLFML2A	0.40	0.27	0.047	5.86	3.94e-07	
	FRMPD1	0.40	0.28	0.047	5.89	3.67e-07	
	HMCN1	0.41	0.28	0.052	5.43	3.83e-06	

Comparison	Gene symbol	Log_2 fold-change	Estimate[a]	SE[a]	*Z*-value[a]	P-values (FDR)[b]	Associated pathways[c]
	RAC2	0.47	0.33	0.122	2.67	0.035	
	COL6A3	0.47	0.33	0.063	5.23	7.85e-06	
	CENPF	0.48	0.33	0.109	3.04	0.015	
	MYH10	0.51	0.35	0.042	8.39	4.75e-14	
	PRUNE2	0.63	0.43	0.115	3.77	0.002	
	COL1A1	0.67	0.46	0.144	3.22	0.009	
	COL1A2	0.67	0.47	0.119	3.94	0.001	
	GAS2L3	0.78	0.54	0.090	6.02	2.03e-07	

[a] Estimate on the natural log scale together with standard errors (SE) and *Z*-values from generalized mixed linear models. [b] P-values are corrected per coefficient for false discovery rate (FDR) [c] Mitocarta v.3.0 pathways associated with each gene.

Appendix VI

Approval letter from The Ethical Committee

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Anette Solli Karlsen	22845522	13.02.2014	2013/1094/REK sør-øst A
			Deres dato:	**Deres referanse:**
			23.10.2013	

Vår referanse må oppgis ved alle henvendelser

Stian Ellefsen
Høgskolen i Lillehammer

2013/1094 KOLS, vitamin D-supplement og styrketrening

Forskningsansvarlig: Høgskolen i Lillehammer
Prosjektleder: Stian Ellefsen

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 20.06.2013. Vurderingen er gjort med hjemmel i helseforskningsloven (hfl.) § 10, jf. forskningsetikklovens § 4.

Prosjektbeskrivelse
Hensikten med studien er å undersøke om tilskudd av D-vitamin kan gi bedre effekt av trening hos KOLS pasienter og å utvikle mer effektive treningsmetoder for denne gruppen.

30-70 % av alle KOLS pasienter lider av tap av muskelmasse, spesielt i benmuskulatur. Fysisk rehabilitering av KOLS pasienter har vanligvis vært utholdenhetstrening. Det er imidlertid vanskelig for pasientene å komme opp i treningsintensiteter som er høye nok til å få stimulert muskulaturen på en hensiktsmessig måte, og det er derfor behov for å utvikle treningsmetoder som er mer effektive.

20-30 % av KOLS pasientene opplever i liten grad fremgang når de trener. En hypotese er at lave nivåer i blodet av vitamin D kan være med på forklare dette. Det er imidlertid gjort mange studier på effekt av vitamin D på muskelstyrke og utholdenhet uten at de har gitt noe entydig svar.

Målsettingen i denne undersøkelsen er å finne ut mer om virkningen av vitamin D-supplement på funksjonell og biologisk tilpasning i forhold til to ulike typer styrketreningsprogram. Prosjektet legges opp som en dobbeltblindet randomisert kontrollert studie.

60 KOLS pasienter over 55 år med lave nivåer av D-vitamin vil rekrutteres fra Sykehuset Innlandet - Granheim lungesykehus eller via fastleger og annonser. De randomiseres til to intervensjonsgrupper, der den ene får vitamin D3 tilskudd og den andre får placebo.

Kontrollgruppen består av vitamin D-sufficiente friske personer og rekrutteres via oppslag i lokale media og hjemmesidene til Høgskolen i Lillehammer.

Pasienter og kontroller skal gjennomføre samme styrketreningsprogram. Det varer i 15 uker der de første 3 er en progressiv introduksjon til treningsprotokollene. Deltakerne vil gjennomgå en rekke fysiske og fysiologiske tester før, under og etter intervensjonen. Det samles også inn informasjon om medisinforbruk sykdomshistorikk og generell helse. Det tas EKG, MR, DXA og blodprøver. Deltakerne skal også svare på skjemaer om livskvalitet (SGRQ og MRC).

Biologisk materiale skal oppbevares i en ny biobank, "COPing with life through exercise and vitamin D" som planlegges å vare til 31.12 2029. Ansvarshavende for biobanken er Stian Ellefsen. Det skal gjøres genetiske undersøkelser av biologisk materiale. Deltakeren samtykker til innsamling, oppbevaring og bruk av materialet.

Saksbehandling
Prosjektet ble behandlet i møte 20.06.2013, og det ble fattet et utsettende vedtak. Komiteen ba om tilbakemelding på følgende merknader:

1) Komiteen ønsker en bedre redegjørelse for hvilke doser med D-vitamin som gis i studien med hensyn på mulige bivirkninger og sikkerhet ved disse dosene.

2) Informasjonsskrivet kan være vanskelig å forstå for målgruppen. Det bør derfor forenkles noe, og man bør unngå å bruke vanskelige medisinske faguttrykk. Deltakerne må også informeres om at materialet kan blir sendt til Danmark for analyse.

3) Sluttdato for prosjektet er angitt til 31.12.2029. Det er ikke klart fra søknaden hvorfor et prosjekt som skal måle effekt av en 12 ukers styrketreningsperiode skal ha en varighet på 16 år.

Prosjektleders tilbakemelding ble mottatt 23.10.2013.
Det fremkommer av tilbakemeldingen at det skal gis daglige doser av 10 000 IU vit D de første to ukene i intervensjonen, deretter 1 000 IU/dag i 25 uker.
Informasjonsskrivet er revidert i forhold til komiteens merknader, og det fremkommer tydelig at biologisk materiale skal utføres for analyse.
Vedrørende prosjektets varighet fremgår det av tilbakemeldingen at prøver skal innsamles til generell biobank "TrainsOME". Biobanken har varighet til 31.12.2038.

Tilbakemeldingen er vurdert av komiteens leder på fullmakt, dette etter at biobanken er godkjent i REK sør-øst C. Tilbakemeldingen ansees som tilfredsstillende i forhold til komiteens merknader.

Vedtak
Komiteen godkjenner at prosjektet gjennomføres i samsvar med det som fremgår av søknaden.

Godkjenningen gjelder til 31.12.2038.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren». Opplysningene skal ikke oppbevares lenger enn det som er nødvendig for å gjennomføre prosjektet, deretter skal opplysningene anonymiseres eller slettes.

Prosjektet skal sende sluttmelding på eget skjema, se helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jfr. helseforskningsloven § 10, 3 ledd og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst A.

Klagefristen er tre uker fra mottak av dette brevet, jfr. forvaltningsloven § 29.

Med vennlig hilsen

Knut Engedal
Professor dr. med.
Leder

Anette Solli Karlsen
Komitesekretær

Kopi til: kari.kjenndalen@hil.no; post@hil.no