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List of publications

This thesis is based on a systematic review and three original studies, described in the following five papers. Moreover, it includes an updated literature search to complement the findings of the systematic review. The papers will be referred to in the text by their respective Roman numerals and/or short title. Permission to reprint the original articles has been obtained from the respective journals.

STUDY I

Paper I **Gade J**, Quick A.A, Beck AM, Vinther A. SARC-F in hospitalized, geriatric medical patients – feasibility, prevalence of risk of sarcopenia, and characteristics of the risk group, including one-year follow-up. Manuscript submitted in September 2019 to *Clinical Nutrition*.

Short title in thesis: SARC-F study

SYSTEMATIC REVIEW

Paper II **Gade J**, Pedersen RJ, Beck AM. Effect of Protein or Essential Amino Acid Supplementation During Prolonged Resistance Exercise Training in Older Adults on Body Composition, Muscle Strength, and Physical Performance Parameters: A Systematic Review. Rehabilitation Process and Outcome. 2018. doi.org/10.1177/1179572718765760

Short title in thesis: Systematic review

STUDY II

Paper III **Gade J**, Beck AM, Bitz C, Christensen B, Klausen TW, Vinther A, Astrup A. Protein-enriched, milk-based supplement to counteract sarcopenia in acutely ill geriatric patients offered resistance exercise training during and after hospitalisation: study protocol for a randomised, double-blind, multicentre trial. BMJ Open. 2018;8: e019210. doi:10.1136/bmjopen-2017-019210

Paper IV **Gade J**, Beck AM, Andersen H.E, Christensen B, Rønholt F, Klausen T.W, Vinther V, Astrup A. Protein supplementation combined with low-intensity resistance training in geriatric medical patients during and after hospitalization: a randomized, double-blind, multicenter trial. British Journal of Nutrition. 2019 [Epub ahead of print]. doi.org/10.1017/S0007114519001831

Short title in thesis: PEPOP study

Acronym for 'Protein and Exercise is Positive for Older People'

STUDY III

Paper V **Gade J**, Astrup A, Vinther A., Zerahn B. Comparison of a dual-frequency bio-impedance analyzer with dual-energy X-ray absorptiometry for assessment of body composition in geriatric medical patients ≥ 70 years. Manuscript submitted in September 2019 to *Clinical Physiology and Functional Imaging*.

Short title in thesis: DF-BIA vs. DXA

Contributions to the papers

Paper I **JG**, AMB, and AV designed the study; AAQ collected data under the supervision of **JG** and AV; **JG** analyzed data; **JG** was responsible for writing the manuscript, for which AAQ assisted with the method section. The manuscript was revised and approved by all authors.

Paper II **JG** designed the study; **JG** and RJP were responsible for data collection under the supervision of AMB; **JG** was responsible for writing the manuscript, which was revised and approved by all authors.

Paper III AMB, **JG**, AV, BC and AA designed the study; **JG** prepared the data-analysis plan supported by TWC; **JG** was responsible for writing the manuscript, which was revised and approved by all authors.

Paper IV AMB, **JG**, AV, BC, and AA designed the study; **JG** was responsible for trial execution and data collection under the supervision of AMB, AV, BC and AA; FR and HEA provided access to potential study participants and facilitated study recruitment; **JG** analyzed data supported by TWC; **JG** was responsible for writing the manuscript, which was revised and approved by all authors.

Paper V **JG**, AV, AA and BZ designed the research; **JG** was responsible for trial execution and data collection under the supervision of BZ; BZ and **JG** analyzed data; **JG** wrote the first draft of the manuscript, which was critically reviewed and improved by all authors. **JG** was responsible for writing the manuscript, which was revised and approved by all authors.

Summary

Sarcopenia is the progressive loss of muscle mass and strength with advancing age, which in severe cases can lead to loss of physical performance. Moreover, sarcopenia is associated with increased morbidity and mortality. The primary causes are age-related physiological decline, while secondary causes are related to inactivity/disuse, inadequate nutrition, and/or disease. Consequently, geriatric medical patients are particularly vulnerable. The proportion of older adults is increasing, and sarcopenia, therefore, has a growing significant public health impact. Yet even though this is a major research area, geriatric medical patients are highly understudied in the context of sarcopenia. Thus, the overall objective of this PhD project was to investigate aspects related to sarcopenia in geriatric medical patients by means of a systematic literature review, as well as original studies.

Specifically, the screening tool SARC-F, developed to identify individuals at risk of sarcopenia, was evaluated in an observational cohort study with consecutive screening of all patients admitted to a Danish medical ward (Paper I). Feasibility and prevalence of risk were assessed, and the risk group was characterized according to selected outcomes at baseline and after one year. Results showed that the screening tool was feasible for patients without severe cognitive impairment, corresponding to 85% in the clinical setting. The prevalence of risk was high, at around 65%, and several sarcopenia-related adverse outcomes characterized the group at risk, such as lower muscle strength and physical performance, but also increased length of hospitalization (men only) and one-year mortality (women only). These results emphasize the importance and relevance of the identification of sarcopenic patients in clinical practice and the initiation of appropriate treatment.

Regarding sarcopenia prevention and treatment strategies, RT is known to induce hypertrophy and strength, although a combination of RT and PrS potentially be more effective than RT alone. This theory was investigated in a systematic literature review of RCTs, including older adults > 60 years, to examine the combined effect of prolonged RT and PrS/amino acids, compared to RT alone, with or without a placebo supplement (Paper II and an updated literature search). The studies included were heterogeneous, of varying quality, and many had small sample sizes. The evidence of an independent effect of PrS/amino acids during RT was inconsistent and considered to be low to moderate. Results indicated that the weakest patients might derive greatest benefit from the intervention, likely related to low habitual protein intake. However, protein quality and training status also seem to play an important role. Further research is needed to support an interpretation of the importance of the study population and design.

In the systematic review, no studies of geriatric medical patients were retrieved. This verifies the novelty of the double-blinded, multicenter RCT that was performed to investigate the same theory in geriatric medical patients, of whom many were expected to have an inadequate protein intake. A

study protocol was published (Paper III) to verify adherence to original intentions, eliminate publication bias, and inform other researchers about the ongoing study. Specifically, the RCT investigated whether PrS could increase the adaptive response to RT in 165 acutely ill, geriatric medical patients. Eligible patients were enrolled during admission to one of three medical departments and continued the intervention for 12 weeks after discharge. Also, there was a six-month follow-up period without intervention. The intervention consisted of either 27.5 g of whey protein/day or iso-caloric placebo supplements, while all participants had daily supervised, standardized RT during admission, continuing as self-training at home after discharge (target of four times/week). On conducting the study, compliance after discharge from hospital proved difficult, as the resources to engage and appetite were low. The results revealed improvements for most endpoints of muscle strength, mass, physical performance, and QoL in both groups, with no increased benefit from PrS (Paper IV). The lack of effect may be due to the small difference in total protein intake between the groups for most of the intervention period. During hospitalization, the difference in protein intake was 0.4 g/kg/d. For the 12-week period after discharge, however, the difference was reduced to 0.2 g/kg/d, due to low compliance in both groups. Although, it is also possible that confounding factors, such as medical treatment of the acute condition and total RT/physical activity, influenced the results and/or masked possible effects.

Finally, the agreement of body composition measurements between DF-BIA and DXA was investigated in a subpopulation of 31 participants from the RCT (Paper V). Both tools are widely used in research and clinical settings, and LBM measurement is a prerequisite for the diagnosis of sarcopenia and important for evaluating treatment effects. Measurements were conducted during hospitalization and repeated 12 weeks after discharge, with replicate measurements being made on one of the two occasions. The methods proved to have good replicability, indicating that both can be used for monitoring. The great variation in the individual agreement should be taken into consideration, however. Furthermore, Bland Altman plots for direct comparison of the methods, and for monitoring changes, revealed both significant fixed and negative proportional bias. On measuring total LBM, for instance, DF-BIA had higher values than DXA, and vice versa for total fat mass. Moreover, gender differences were discovered, and the division of body segments differed between the methods. Collectively, the results illustrate that body size affects the agreement between the methods, and a cautious approach should be taken to comparing studies that do not use the same measurement tool or include populations with very different body sizes. Moreover, only DXA, using clear anatomical references, can be recommended for segmental analysis. Regarding Papers III+IV, the monitoring of LBM using DF-BIA seems to be acceptable at population level, yet the measurements of segmental LBM should be evaluated critically.

Resumé (Danish summary)

Sarkopeni er det progressive og aldersrelaterede tab af muskelmasse og styrke, som i svære tilfælde kan føre til fysisk funktionsnedsættelse. Desuden er sarkopeni forbundet med øget sygelighed og dødelighed. Den primære årsag er et aldersbetinget fysiologisk tab, mens sekundære årsager er relateret til inaktivitet/sengeleje, utilstrækkelig ernæring og/eller sygdom. Derfor er geriatriske medicinske patienter særligt udsatte. Andelen af ældre voksne er stigende, og sarkopeni har derfor en betydelig og voksende folkesundhedsmæssig indvirkning. Selv om der er tale om et større forskningsområde, er geriatriske medicinske patienter kun sparsomt undersøgt i relation til sarkopeni. Det overordnede formål med denne ph.d.-afhandling var derfor at undersøge aspekter relateret til sarkopeni hos geriatriske medicinske patienter vha. en systematisk litteraturgennemgang samt originale studier.

Mere konkret blev Screenings-værktøjet SARC-F, der er udviklet med henblik på at identificere personer med risiko for sarkopeni, evalueret i et observationelt kohortestudie med fortløbende screening af alle patienter, der blev indskrevet på en dansk medicinsk afdeling (Artikel I). Screenings-gennemførligheden samt prævalensen af risiko blev vurderet, og risikogruppen blev karakteriseret ift. udvalgte endepunkter ved baseline og efter et år. Resultaterne viste, at screening var muligt hos patienter uden svær kognitiv svækkelse, svarende til 85% i klinisk praksis. Prævalensen af risiko var høj, ca. 65%, og flere sarkopeni-relaterede følgevirkninger karakteriserede risikogruppen, fx lavere muskelstyrke og fysisk funktionsniveau, men også øget hospitalsindlæggelse (kun mænd) og etårs dødelighed (kun kvinder). Resultaterne understreger betydningen og relevansen af identifikation af patienter med sarkopeni i klinisk praksis samt igangsættelse af passende behandling.

Med hensyn til forebyggelses-og behandlingsstrategier for sarkopeni, er RT kendt for at inducere hypertrofi og styrke, selvom en kombination af RT og PrS potentielt kan være mere effektiv end RT alene. Denne teori blev undersøgt i en systematisk litteraturgennemgang af RCTs, der inkluderede ældre voksne > 60 år, og undersøgte den kombinerede effekt af længerevarende RT og PrS/aminosyrer, sammenlignet med RT alene, med eller uden et placebo-supplement (Artikel II og en opdateret litteratursøgning). De inkluderede studier var heterogene, af varierende kvalitet og mange havde små stikprøvestørrelser. Beviset for en selvstændig effekt af PrS/aminosyrer, når der samtidig bliver styrketrænet, var inkonsekvent og ansås for at være lavt til moderat. Resultaterne indikerede, at de svageste patienter måske har størst gavn af interventionen, sandsynligvis relateret til lavt habituelt proteinindtag. Imidlertid synes proteinkvalitet og træningsstatus også at spille en vigtig rolle. Yderligere forskning er nødvendig for at vurdere betydningen af studiepopulation og design.

I den systematiske litteraturgennemgang blev der ikke fundet studier med geriatriske medicinske patienter. Dette verificerer nyhedsværdien af det dobbeltblindede, multicenter RCT, der blev udført for at undersøge den samme teori i netop denne population, hvor mange forventes at have et utilstrækkeligt proteinindtag. En studieprotokol blev publiceret (Artikel III) for at verificere, at de oprindelige intentioner blev overholdt, eliminere publikationsbias og orientere andre forskere om den igangværende undersøgelse. Konkret undersøgte RCT'et, om PrS kunne øge det adaptive respons til RT hos 165 akut syge, geriatriske medicinske patienter. Egnede patienter blev rekrutteret under deres indlæggelse på én af tre medicinske afdelinger og fortsatte interventionen i 12 uger efter udskrivelse. Derudover var der en seks-måneders opfølgningsperiode uden intervention. Interventionen bestod af enten 27,5 g valleprotein/dag eller iso-kalorisk placebotilskud, mens alle deltagere dagligt havde superviseret, standardiseret RT under hospitalsindlæggelsen, der fortsatte hjemme som selvtræning efter udskrivelse (målet var fire gange/uge). Det viste sig, at komplians efter udskrivelse var vanskelig, da deltagernes ressourcer til at engagere sig samt appetitten var lav. Resultaterne viste forbedringer for de fleste endepunkter relateret til muskelstyrke, masse, fysisk funktionsniveau og QoL i begge grupper, uden nogen øget effekt af PrS (Artikel IV). Manglen på effekt kan skyldes den lille forskel i det samlede proteinindtag mellem grupperne i det meste af interventionsperioden. Under indlæggelse var forskellen i proteinindtag på 0,4 g/kg/d. I den 12-ugers periode efter udskrivelse blev forskellen imidlertid reduceret til 0,2 g/kg/d pga. lav komplians i begge grupper. Det er dog også muligt, at konfunderende faktorer, såsom behandlingseffekten af den akutte sygdomstilstand og total RT/fysisk aktivitet, har påvirket resultaterne og/eller maskeret en mulig effekt.

Til sidst blev overensstemmelsen af målinger for kropssammensætning mellem DF-BIA og DXA undersøgt i en delpopulation på 31 deltagere fra RCT'et (Artikel V). Begge apparater anvendes i udstrakt grad indenfor forskning og i klinisk praksis, og LBM-måling er en forudsætning for at diagnosticere sarkopeni samt vurdere behandlingseffekter. Målingerne blev gennemført under indlæggelse og gentaget 12 uger efter udskrivelsen, og der blev foretaget på hinanden gentagne målinger ved én af de to lejligheder. Metoderne viste sig at have god replikerbarhed, hvilket indikerer, at de begge kan bruges til monitorering af ændringer. Den store variation i de individuelle målinger skal dog tages i betragtning. Desuden viste Bland Altman plots, for direkte sammenligning af metoderne og monitorering af forandringer, både signifikant systematisk forskel og negativ proportional bias. Ved målingen af den totale LBM havde DF-BIA fx højere værdier end DXA, og omvendt for total fedtmasse. Desuden blev der fundet kønsforskelle, og inddelingen af kropssegmenter adskiller sig for metoderne. Samlet set viser resultaterne, at kropsstørrelse påvirker overensstemmelsen mellem metoderne, og der bør derfor tages forbehold ved sammenligning af studier, der ikke anvender samme apparater, eller inkluderer populationer med meget forskellige kropsstørrelser. Desuden kan kun DXA, der benytter tydelige anatomiske referencer, anbefales til segmental analyse. Med hensyn til Artikel III+IV synes monitoreringen af LBM med DF-BIA at være acceptabel på populationsniveau, men målingerne af segmental LBM bør evalueres kritisk.

Thesis at a glance

	Objectives	Methods	Results	Conclusions
PAPER I	What is the feasibility of the SARC-F screening tool to identify the risk of sarcopenia in geriatric medical patients? What is the prevalence of risk? And what are the characteristics of the risk group?	Observational cohort study with consecutive screening of all admitted, non-isolated patients ≥ 65 years for 6 months. Baseline testing and 1-year follow-up.	Feasibility in the clinical setting was 85% and prevalence was 64.5% (n=301). Risk was significantly associated with reduced muscle strength, physical function, and health, as well as longer admission (M) and 1-year mortality (F).	SARC-F is feasible for geriatric medical patients without severe cognitive impairment. The risk of sarcopenia is high and negatively associated with clinically important outcomes. Identification and initiation of treatment are thus important and relevant.
PAPER II & updated search	What do we already know about the effect of prolonged PrS and RT, versus RT alone, from RCTs of older adults? How many studies have been performed in a geriatric setting?	Systematic literature search in four databases. Two independent reviewers. Only PrS in addition to RT, versus RT alone, in older adults with mean age ≥ 60 years. Moreover, an updated literature search in a single database.	Identification of 16 studies for Paper II and four from the updated search. All studies with heterogenous design. Inconsistent evidence of PrS to increase the adaptive response to RT and consequently counteract sarcopenia.	Inconsistent evidence. The frailest may improve more from a combined intervention. Protein quality and training status may also play an important role. Studies of geriatric medical patients are lacking.
PAPERS III & IV	Can PrS during admission and 12 weeks after discharge increase the adaptive response to RT in geriatric medical patients, and subsequently counteract sarcopenia?	Double-blind, multicenter RCT (n=165). Recruitment from three medical departments. 27.5 g PrS/d or iso-caloric placebo. Both groups had RT supervised daily during admission and 4x/week for 12 weeks after discharge. Measurement of muscle strength, mass and function, QoL, and 6-month follow-up on hospital admission(s) and mortality.	Both groups improved for most endpoints, with no increased benefit from PrS. Total protein intake was as follows: protein group: 1.0 g/kg/d during admission and 1.1 after discharge; and placebo group: 0.6 g/kg/d during admission and 0.9 g/kg/d after discharge. About 50% in both groups were compliant with the RT program.	PrS combined with RT was not superior to RT alone in geriatric medical patients (n=141, ITT analysis). An effect of RT (and extra energy) in both groups was indicated.
PAPER V	How does DF-BIA compare with DXA in geriatric medical patients for measurement of body composition (replicability, direct comparisons as well as monitoring)? In relation to Papers III+IV, how valid are the measurements of LBM?	Subpopulation of 31 participants from Papers III+IV. DF-BIA and DXA measurements during admission and 12 weeks after discharge. Replication of measurements either during or after discharge.	Both DF-BIA and DXA have good replicability. However, significant fixed and proportional bias was present for both the direct comparison and for the monitoring of changes. Moreover, gender-specific differences were indicated.	DF-BIA and DXA are not interchangeable in geriatric medical patients. Both are subject to confounders and bias. DF-BIA cannot be recommended for segmental analysis but may be used for monitoring of total LBM.