The interplay between sarcopenia, physical and cognitive functions and the role of nutrition as a modifiable factor in aging

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« La vieillesse est un compte en banque, tu retires ce que tu as amassé. »
-Berthe Labadie-Tessier

À mes très chers grands-parents que j'ai perdus durant mes études doctorales,
Jacqueline, Berthe et Robert.

ABSTRACT

Background: Aging may lead to sarcopenia and cognitive impairment. These conditions are associated with loss of autonomy, disabilities and mortality. It is imperative to identify elders at risk of cognitive and functional decline and target modifiable risk factors e.g., diet, to attenuate this decline. Dairy product intake, though typically low in older adults, has been positively associated with lean mass and mobility. As well, protein, leucine, vitamin D and omega-3 fatty acids have all individually shown positive effects on physical function, lean mass or cognition. Providing a combined supplement of these nutrients may be beneficial at improving functional and cognitive status with aging. This dissertation aimed to: (**1**) identify sarcopenia diagnostic criteria in community dwelling older adults, (**2**) to determine if sarcopenia is an independent predictor of cognitive decline, (**3**) to investigate associations between dairy product intake and cognitive functions, and (**4**) to test the feasibility (recruitment, eligibility, consent rates) and adherence of a combined nutritional supplement to improve physical and cognitive functions in older adults with a loss of autonomy.

Methods & main findings: Aims 1-3 data are from the large (n=30,097), contemporary (baseline 2011-2015) Canadian Longitudinal Study on Aging comprehensive cohort (11 centers across 7 provinces). Men and women ≥65 years (n=12,646) were included in all analyses. (**Aim 1**) At baseline, appendicular lean mass (ALM), was determined by dual-energy X-ray absorptiometry (DXA), ALM index (ALM/ht^2) was used to identify sarcopenia; handgrip strength was measured through dynamometry; physical function using gait speed, timed up and go, balance and chair rise tests and a weighted composite score using a factor analysis was created. Classification and regression trees (CART) were used to identify sex-specific cut-points for low strength as a determinant of impaired physical performance (<1.5 SD below composite score mean) and for low ALM as a determinant of low strength. The cut-points for low ALM were <7.30 kg/m^2 for men and <5.42 kg/m^2 for women and the cut-points obtained for low grip strength were <33.1 kg and <20.4 kg for men and women, respectively. The results established the first Canadian empirically-derived sarcopenia cut-points. (**Aim 2**) Memory, executive functions and psychomotor speed domains were evaluated with 7 tests at baseline and at 3-year follow-up. Sarcopenia was tested as an independent predictor of the 3-year cognitive change using multiple linear regressions. After adjusting for covariates including physical activity and fat mass, sarcopenia was associated with greater executive function decline over 3 years (std β: -0.032, p=0.028). Sarcopenia was not an independent predictor of memory and psychomotor speed decline. Identifying older adults with sarcopenia may help in the prediction of cognitive impairment. (**Aim 3**) Dairy product consumption frequency was collected

using the validated semi-quantitative short diet questionnaire. Cross-sectional associations were examined using multivariate analysis of covariance adjusted for covariates. Total dairy product, cheese and low-fat dairy product intakes were positively associated with the executive function domain and yogurt intake with the memory domain (all $p<0.05$), independently of covariates including diet quality and socioeconomic status. Intakes of cheese and low-fat dairy were associated with verbal fluency specifically (all $p<0.05$). Dairy product intake ≥2.5 times/d as part of a high-quality diet may represent a preventive strategy to cognitive decline. (**Aim 4**) Older adults referred to two geriatric day hospitals were invited to participate in a 16-week double-blind, randomized controlled feasibility trial. Participants were randomized to either: placebo (CTR; corn oil and maltodextrin) or intervention (EXP; 2 g n-3 fatty acids with 1500 IU vitamin D_3 1x/d + 20-30 g whey protein (according to body weight), with 3 g leucine 2x/d). ALM (DXA) was assessed at weeks 0 and 16, handgrip and knee extension strength (dynamometry), physical performance tests, blood tests, dietary intake (3-day food diary), physical activity (accelerometry) were evaluated at weeks 0, 8 and 16. Adherence was assessed using self-reported consumption of supplements, collection of empty powder and oil containers and objectively using plasma phospholipid n-3 fatty acids measurement (gas chromatography-mass spectrometry). Over 2 years, 244 patients were screened, 46 were eligible (19%; 95% CI: 15, 23), 20 were randomized, 10 completed the study (n=4 in EXP, n=6 in CTR); median age was 87 (range: 77-94) years. Self-reported adherence to powder was 96% (95% CI: 83, 108) and to oil, 85% (95% CI: 63, 107) supporting the acceptability of the supplement. Proportions of plasma eicosapentaenoic acid and docosahexaenoic increased significantly 3- and 1.5-fold respectively at week 8 in EXP, with no change in CTR. The EXP median protein intake alone surpassed the target 1.2–1.5 g/kg/d for older adults, without altering usual diet. Nonetheless, the low eligibility rate demonstrated the non-feasibility of the trial in this population, at least with the present design. More liberal eligibility criteria should be considered in future trials.

Conclusion: The studies of this thesis provide novel understanding of determinants of sarcopenia and cognition, and lay out groundwork for future dietary interventions aimed to delay the loss of autonomy in older adults, including the most vulnerable.

RÉSUMÉ

Contexte: Le vieillissement peut mener à la sarcopénie et à des troubles cognitifs. Ces conditions sont associées à la perte d'autonomie, à l'incapacité et à la mortalité. Il est impératif d'identifier les personnes âgées à risque de déclin fonctionnel et cognitif et de cibler les facteurs de risque modifiables, tels que l'alimentation, pour le ralentir. La consommation de produits laitiers, bien que généralement faible chez les personnes âgées, a été positivement associée à la masse maigre et à la mobilité. De plus, l'apport en protéines, leucine, vitamine D et acides gras oméga-3 a montré, pour chacun des nutriments individuellement, des effets positifs sur la fonction physique, la masse maigre ou la cognition. Offrir un supplément combinant ces nutriments aux personnes âgées pourrait être bénéfique pour améliorer l'état fonctionnel et cognitif. Cette thèse visait donc à : (**1**) identifier des critères diagnostiques de la sarcopénie chez les personnes âgées vivant dans la communauté, (**2**) déterminer si la sarcopénie est un prédicteur indépendant du déclin cognitif, (**3**) étudier les associations entre la fréquence de consommation de produits laitiers et les fonctions cognitives et (**4**) tester la faisabilité (taux de recrutement, d'éligibilité, de consentement) et l'observance d'un supplément nutritionnel combiné pour améliorer les fonctions physiques et cognitives chez les personnes âgées en perte d'autonomie.

Méthodologie et résultats principaux: Les données des objectifs 1 à 3 proviennent de la grande cohorte exhaustive (n=30 097) et contemporaine (recrutement 2011-2015) de l'Étude longitudinale canadienne sur le vieillissement (11 centres à travers 7 provinces). Les hommes et les femmes de ≥65 ans (n=12 646) ont été inclus dans les analyses. (**Objectif 1**) En début d'étude, la masse maigre appendiculaire (ALM) a été déterminée par absorptiométrie à rayons X en double énergie (DXA), l'indice de masse musculaire appendiculaire (ALM/ht^2) a été utilisé pour identifier la sarcopénie; la force de préhension a été mesurée par dynamométrie; la fonction physique par les tests de vitesse de marche, de lever-marcher chronométré, d'équilibre debout, du lever de chaise (5 répétitions), et un score composite pondéré a été créé à partir d'une analyse factorielle. Des modèles d'arbres de classification et de régression (CART) ont été utilisées pour identifier les seuils d'une faible force de préhension comme déterminant d'une performance physique limitée (<1.5 ÉT sous la moyenne du score composite) et d'une faible ALM comme déterminant d'une faible force de préhension, séparément pour les hommes et les femmes. Les seuils d'une faible ALM étaient <7,30 kg/m^2 pour les hommes et <5,42 kg/m^2 pour les femmes, et les seuils d'une faible force de préhension étaient <33,1 kg and <20,4 kg pour les hommes et les femmes, respectivement. Ces résultats ont établi les

premiers seuils de sarcopénie au Canada, obtenus de manière empirique. (**Objectif 2**) Les domaines cognitifs de la mémoire, des fonctions exécutives et de la vitesse psychomotrice ont été évalués avec 7 tests en début d'étude et après 3 ans. La sarcopénie a été testée comme prédicteur indépendant du changement cognitif sur 3 ans à partir de régressions linéaires multiples. Après ajustement pour plusieurs covariables incluant l'activité physique et le % de masse grasse, la sarcopénie était associée à une plus grande diminution des fonctions exécutives après 3 ans (std β: -0,032, p=0,028). Par contre, celle-ci n'était pas un prédicteur indépendant du déclin de la mémoire et de la vitesse psychomotrice. L'identification des personnes âgées sarcopéniques pourrait aider à prédire les troubles cognitifs. (**Objectif 3**) La fréquence de consommation de produits laitiers a été évaluée à l'aide du questionnaire semi-quantitatif court sur l'alimentation (SDQ) validé. Les associations transversales ont été examinées à l'aide d'analyse multivariées de covariance ajustées. La consommation de produits laitiers totaux, de fromage et de produits laitiers faibles en gras étaient positivement associées aux fonctions exécutives, et la consommation de yogourt à la mémoire (tous $p<0,05$), indépendamment de la qualité de l'alimentation et du statut socio-économique, parmi d'autres covariables. L'apport en fromage et en produits laitiers faibles en gras était spécifiquement associé à la fluidité verbale (tous $p<0,05$). La consommation de produits laitiers ≥2,5 fois/j dans une alimentation de haute qualité peut contribuer à une stratégie préventive du déclin cognitif. (**Objectif 4**) Les personnes âgées référées à deux hôpitaux de jour gériatriques ont été invitées à participer à un essai de faisabilité contrôlé randomisé en double aveugle de 16 semaines. Les participants ont été randomisés pour recevoir soit le placebo (CTR; huile de maïs et maltodextrine) ou le traitement actif (EXP; 2 g d'acides gras oméga-3 avec 1500 UI de vitamine D_3 1x/j + 20-30 g de protéines de lactosérum (selon le poids corporel), avec 3 g de leucine 2x/j). La masse maigre (DXA) a été évaluée aux semaines 0 et 16, la force de préhension et d'extension du genou (dynamométrie), les tests de performance physique, les tests sanguins, l'apport alimentaire (journal alimentaire de 3 jours), l'activité physique (accélérométrie) ont été évalués aux semaines 0, 8 et 16. L'observance a été évaluée par la consommation des suppléments auto-rapportée, la collecte des contenants de poudre et d'huile vides et par le niveau plasmatique des acides gras omega-3 des phospholipides (chromatographie en phase gazeuse-spectrométrie de masse, GCMS). Sur 2 ans, l'éligibilité de 244 patients a été vérifiée, 46 étaient éligibles, (19%; IC à 95% : 15, 23), 20 ont été randomisés, 10 ont complété l'étude (EXP : n=4, CTR : n=6); l'âge médian était de 87 (intervalle : 77-94) ans. L'observance auto-rapportée était de 96% (IC à 95% : 83, 108) pour la poudre et 85% (IC à 95% : 63, 107) pour l'huile soutenant l'acceptabilité du supplément. Les proportions d'acides

eicosapentaénoïque (EPA) et docosahexaénoïque (DHA) plasmatiques ont augmenté de 3 et 1,5 fois respectivement à la semaine 8 dans le groupe EXP, sans changement dans le CTR. L'apport protéique médian du groupe EXP a dépassé la cible de 1,2 à 1,5 g/kg/j recommandée pour les personnes âgées, sans modifier leur diète usuelle. Toutefois, le faible taux d'éligibilité a démontré la non-faisabilité de l'essai, tel qu'élaboré présentement, dans cette population. Des critères d'éligibilité plus libéraux devraient être envisagés pour les essais futurs.

Conclusion: Les études de cette thèse fournissent une nouvelle compréhension des déterminants de la sarcopénie et de la cognition, et constituent le fondement de futures interventions nutritionnelles visant à retarder la perte d'autonomie chez les personnes âgées, incluant les plus vulnérables.

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Preface and advancement of scholarly knowledge

This dissertation is based on the Canadian Longitudinal Study on Aging (CLSA) baseline (2011-2015) and follow-up data (3 years) of the comprehensive cohort, a large nationally-representative cohort of community-dwelling adults; and on a pilot study that enrolled older adults experiencing a loss of autonomy from Montreal, Quebec, Canada who took part in a rehabilitation program at the Montreal General Hospital (MGH) and *Institut Universitaire de Gériatrie de Montréal* (IUGM). This dissertation starts by analysing observational data to establish potential independent associations, to provide evidence for generating hypotheses and to support the investigation of the effect of an intervention also included in this thesis. In the context of aging, it aims to examine the interplay between (1 and 2) body composition, strength and physical performance and cognitive functions; (3) nutrition and cognitive functions; and (4) to investigate whether providing a nutritional supplement to improve body composition and physical performance is feasible in older adults losing autonomy. These studies together establish a novel Canadian diagnosis for low lean mass and strength, and support a positive and independent relationship between lean mass, strength, specific food intake and cognitive functions in healthy community dwelling older adults; however, it invalidates the feasibility of a randomized trial of a nutritional supplement to improve these outcomes in older adults losing autonomy.

Originality and contribution to the scientific literature of this dissertation stems from the large sample size and contemporary nature of the CLSA; elaborated statistical models that included adjustment for important confounders, avoiding spurious associations and permitting strong conclusions; valid, reliable and objective assessment of body composition, physical performance and cognitive functions in all studies. More specific to the pilot study, the unique yet growing population studied; the use of sensitive biomarkers reflective of dietary intake to test adherence; and the overall high-quality evidence-based protocol testify to the novelty of this research work. The findings of this thesis offer the first Canadian empirical diagnostic criteria for sarcopenia and shed important light on the newly investigated relationship between sarcopenia and cognitive decline. It provides original leads on dietary strategies in the prevention of sarcopenia and cognitive impairment.

This dissertation is presented in a manuscript-based thesis, with most manuscripts submitted, accepted or published in peer-reviewed journals. The first manuscript published in the Journal of Cachexia Sarcopenia and Muscle, the cornerstone of this dissertation, aimed to provide a new diagnosis for sarcopenia and dynapenia as predictors of impaired physical performance. The data presented in this study are unique as they include body composition assessment by dual-energy X-ray

absorptiometry and numerous strength and physical performance tests from over 9,000 participants of the contemporary (2011-2015) CLSA cohort at baseline. To our knowledge this was the first study to derive cut-points for sarcopenia and dynapenia diagnosis in a Canadian cohort. Building on previous work we have identified cut-points from an empirical approach and demonstrated that the original definition of sarcopenia should be chosen for its diagnosis in the clinical and research field. The second manuscript shows a comprehensive analysis of the association between sarcopenia and cognitive decline over 3 years. To our knowledge, this was the first study to investigate this relationship by accounting for strength in the models and to robustly support an independent link of low appendicular lean mass with executive functions decline. The third manuscript reports the cross-sectional association between dairy products intake and cognitive functions. The data presented are unique as dairy products were also investigated individually with different cognitive domains, expanding the understanding of potential mechanisms. The fourth study's aim was to assess the feasibility of a multi-nutrient supplement (fish oil and whey protein enriched with leucine) provided to older adults to improve appendicular lean mass and physical function in a randomized controlled trial design. Very low recruitment rate though excellent adherence (90%) to the supplement and physical tests were noted for participants who completed the study. This study is also one of the very few to have characterized plasma phospholipid fatty acids profile in this unique population of seniors with a loss of autonomy.

This assemblage of manuscripts will notably contribute to the literature related to fundamental physiological outcomes impacted by aging, i.e., lean mass, strength, physical performance, cognitive functions, and nutrition in elderly adults that has not been examined in a very large Canadian population before. It also provides insight on feasibility of a trial to improve these outcomes in older adults experiencing a loss of autonomy.

Published research articles in peer-reviewed journals

- **Tessier A-J**, Chevalier S. An update on protein, leucine, omega-3 fatty acids, and vitamin D in the prevention and treatment of sarcopenia and functional decline. Nutrients. 2018 Aug 16; 10(8), 1099. (Chapter 3)
- **Tessier A-J,** Wing SS, Rahme E, Morais JA, Chevalier S. Physical function-derived cut-points for the diagnosis of sarcopenia and dynapenia from the Canadian longitudinal study on aging. J Cachexia Sarcopenia Muscle. 2019 Jul 15; 10(5), 985-999. (Chapter 4)
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- **Tessier A-J**, Presse N, Rahme E, Ferland G, Bherer L, Chevalier S. Milk, yogurt and cheese intake is positively associated with cognitive executive functions in older adults of the Canadian Longitudinal Study on Aging. J Gerontol A Biol Sci Med Sci. 2021 Jun 11; glab165 (Chapter 6)
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losing autonomy – a pilot study. Curr Dev Nutr. June 2020; 4(Supplement_2):79.

- **Tessier A-J,** Presse N, Ferland G, Bherer L, Chevalier S. Dairy product intake frequency is associated with cognitive executive functions in older adults of the Canadian Longitudinal Study on Aging. Appl Physiol Nutr Metab. May 2019; 44:S48. Poster competition finalist.
- **Tessier A-J**, Wing SS, Rahme E, Morais JA, Chevalier S. New cut-points for the diagnosis of sarcopenia and dynapenia in Canadian older adults. Appl Physiol Nutr Metab. May 2018; 43:S39. George Beaton Award recipient.
- **Tessier A-J,** Wing SS, Rahme E, Morais JA, Chevalier S. Empirical criteria for the definition of low muscle mass and strength from the Canadian Longitudinal Study on Aging. J Frailty Aging. March 2018; 7(Supplement 1):144.
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- **Tessier A-J,** Farsijani S, Payette H, Morais JA, Gaudreau P, Shatenstein B, Chevalier S. Dairy product intake is associated with cognition but not cognitive decline in the NuAge community-dwelling older adult cohort. FASEB J. 3 Oct 2018; 31(S1):150.5. Winner of American Society of Nutrition Emerging Leader Poster Competition.

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- **Tessier A-J,** Presse N, Rahme E, Ferland G, Bherer L, Chevalier S. Dairy product intake frequency is associated with cognitive executive functions in older adults of the Canadian Longitudinal Study on Aging. Canadian Nutrition Society, Niagara Falls, ON, Canada. May 2019.
- **Tessier A-J**, Wing SS, Rahme E, Morais JA, Chevalier S. New cut-points for the diagnosis of sarcopenia and dynapenia in Canadian older adults. Canadian Nutrition Society, Halifax, NS, Canada. May 2018. 2nd prize winner of the Nestlé Graduate Student Competition.
- **Tessier A-J,** Farsijani S, Payette H, Morais JA, Gaudreau P, Shatenstein B, Chevalier S. Dairy product intake is associated with cognition, but not cognitive decline in the NuAge community-dwelling older adult cohort. Canadian Nutrition Society, Montreal, QC. May 2017.

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- **Tessier A-J,** Farsijani S, Payette H, Morais JA, Gaudreau P, Shatenstein B, Chevalier S. Dairy product intake is associated with cognition, but not cognitive decline. 14ème édition des Journées de la recherche du Réseau québécois de recherche sur le vieillissement du FRQS. Montreal, QC. September 2017.

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- **Tessier, A-J,** Lévy-Ndejuru J, Morais JA, Plourde M, Beauchet O, Chevalier S. Supplément nutritionnel visant à améliorer la fonction physique et cognitive. 14ème édition des Journées de la recherche du Réseau québécois de recherche sur le vieillissement du FRQS. Montreal, QC. September 2017.

Contribution of authors

For chapters 4 and 5: Dr. S Wing (primary applicant) and Dr. S Chevalier (co-applicant) applied for the CLSA data access (application ID #160609). Drs. Wing and Chevalier obtained ethics approval from the MUHC Research Ethics Board (REB) for the use of study data and maintained annual ethics approvals for subsequent use (2017-1787, 16-068-MUHC).

For chapters 4-6: The candidate and Dr. S Chevalier conceptualized the design and statistical approach. The candidate cleaned, managed and transformed the CLSA data, performed all assumption testing and statistical analyses. The candidate was the primary author, produced all data tables and figures, wrote the first draft of the manuscript and edited all subsequent drafts. Before submission to peer-review, the CLSA had the opportunity to review all manuscripts.

Chapter 4: Dr. S Chevalier provided revisions to the manuscript. Dr. S Wing, Dr. E Rahme provided feedback regarding the manuscript prior to submission. The candidate and S Chevalier replied to the reviewers.

Chapter 5: The candidate applied for the CLSA Follow-Up 1 data access under the guidance of Dr. S Chevalier. Dr. Rahme confirmed statistical approaches. Dr. S Wing, Dr. E Rahme provided feedback regarding the manuscript prior to submission.

Chapter 6: Dr. S Chevalier obtained ethics approval from the McGill REB (REB-46-0618) and CLSA data access (agreement ID #2002004). Dr. Rahme confirmed statistical approaches. Drs. N Presse, G Ferland, L Bherer and E Rahme provided feedback regarding the manuscript prior to submission.

Chapter 7: Dr. S Chevalier is the primary investigator of the pilot study. The candidate created the study forms under the guidance of S Chevalier. The candidate screened all patients coming at the MGH Geriatric Day Hospital and recruited participants at both sites. The candidate coordinated the study visits, was trained to perform and performed the physical and cognitive tests measurements, anthropometrics assessments, prepared supplements, conducted participant teaching of the use of supplement, accelerometers and filling of food diaries. The candidate did visits at home for all participants to provide and collect supplements/empty vials in between study visits. Additionally, the candidate was responsible for data entry and auditing. Finally, the candidate conducted plasma fatty acid extraction and separation at the University of Alberta, Edmonton, Canada, under the supervision of Dr. V Mazurak. S Chevalier obtained ethics

approval from MUHC REB for recruitment at MGH and IUGM and maintained annual renewals for subsequent use of the study data (2017-2470, 15-633-MUHC; NCT04454359).

- Ms. J Lévy-Ndejuru, A Moyen, M Lawson participated in recruitment, study visits and food diary analyses.
- Dr. J Morais participated in blood collection, when necessary validated eligibility.
- Dr. F Andria participated in screening of patients at IUGM.
- Ms Marie Lamarche participated in the coordination of study visits, conducted blood sample processing, glucose and insulin analyses and supplements preparation.
- Dr. V Mazurak provided material for plasma phospholipid fatty acid analysis and oversaw gas chromatography mass spectrometry (GCMS) analyses performed by Mr. A Bhullar.
- Dr. S Chevalier aided the candidate with the intervention preparation and assisted in overseeing measurements and intervention, helped with recruitment and at-home visits when needed.

All co-authors provided feedback regarding the manuscript prior to submission.

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$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
3DFD	Three-day food diaries
AD	Alzheimer's disease
AFT	Animal fluency test
AI	Adequate Intake
ALM	Appendicular lean soft tissue mass
aMD	Adjusted mean difference
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
APOE	Apolipoprotein E
ASA24	Automated self-administered 24-hour
AWGS	Asian Working Group for Sarcopenia
BDNF	Brain-derived neurotrophic factor
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CART	Classification and regression tree
CCHS	Canadian Community Health Survey
CES-D 10	Center for Epidemiological Studies Short Depression Scale
CFG	Canada's food guide
C-HEI	Canadian healthy eating index
CLIA	Chemiluminescence immunoassay
CLSA	Canadian Longitudinal Study on Aging
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disease
COWAT	Controlled oral word association test
CRP	C-reactive protein
CRT	Choice reaction time
CT	Computed tomography
CTR	Control
d	Day
DASH	Dietary Approaches to Stop Hypertension
DFE	Dietary folate equivalents
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DXA	Dual-energy X-ray absorptiometry

EAR	Estimated Average Requirements
EPA	Eicosapentaenoic acid
EPIDOS	Epidémiologie de l'Ostéoporose
EWGSOP	European Working Group on Sarcopenia in Older People
EXP	Experimental
FA	Fatty acids
FFM	Fat-free mass
FFQ	Food frequency questionnaire
FNIH	Foundation for the National Institute of Health
GDH	Geriatric day hospital
ICC	Intraclass correlation coefficient
IGF-1	Insulin-like growth factor-1
IL-	Interleukin
IU	International units
IUGM	Institut de Gériatrie de Montréal
IWGS	International Working Group on Sarcopenia
kcal	Kilocalorie
LM	Lean soft tissue mass
MANCOVA	Multivariate analysis of covariance
MAT	Mental alternation test
MCI	Mild cognitive impairment
mg	Milligram
MGH	Montreal General Hospital
min	Minute (s)
MIND	Mediterranean-DASH Intervention for Neurodegenerative Delay
mL	Milliliter (s)
MMSE	Mini-mental state examination
MNA-SF	Mini nutritional assessment – short form
MoCA	Montreal cognitive assessment
MPS	Muscle protein synthesis
MyoPS	Myofibrillar protein synthesis
MRI	Magnetic resonance imaging
MTORC1	Mammalian target of rapamycin complex 1
MUHC	McGill University Health Centre
NPA	Negative percent agreement
NuAge	Quebec Longitudinal Study on Nutrition and Aging
OR	Odds ratio
PASE	Physical activity scale for the elderly

PPA	Positive percent agreement
PUFA	Polyunsaturated fatty acids
Q	Quartile (s)
RAE	Retinol activity equivalents
RAVLT	Rey auditory verbal learning test
RCT	Randomized controlled trial
RDA	Recommended Dietary Allowance
REB	Research ethics board
SCFA	Short chain fatty acids
SCREEN II-AB	Abbreviated seniors in the community risk evaluation for eating and nutrition, version II
SD	Standard deviation
SDQ	Short diet questionnaire
SPPB	Short physical performance battery
std	Standardized
TUG	Timed up and go
VDR	Vitamin D receptor
WB	Whole body
WC	Waist circumference
WHO	World Health Organization

CHAPTER 1: Introduction

1.1 Background and thesis rationale

Canadians are living longer and there are now more older adults, as defined by age at or above 65 years, than children under the age of 14 years (1). There are over 6.6 million older Canadians (18% of the total population) (2) and they are expected to account for 23-25% of the population by 2036 (3). This shift in distribution towards older ages is also observed at the worldwide level with a projected increase from 1 to 1.4 billion older persons (above 60 years) between 2020 and 2030 (4). As the population is aging, serious health problems are becoming more prevalent. The number one fear of older adults is to lose their independence (5). Indeed, aging is associated with the decline in physical and cognitive functions which may lead to deleterious consequences such as the onset of dementia (6), decreased quality of life (7, 8), institutionalization (9), frailty (10), disability (11) and mortality (12, 13). The unprecedented shift in demographics has led the Public Health Agency of Canada to place healthy and independent aging at the forefront of priorities to benefit the health and social care system, societies, families and individuals (14).

While some individuals may never experience disability related to aging, several others will. The MacArthur model illustrates three major components to successful aging (**Figure** 1-1) (15, 16). To "avoid disease and disability" is one of the successful aging components. As early as from the age of 30 years, a loss of muscle mass and strength can be observed; the decline is estimated to follow a rate of 0.5-1% and 2-3% per year, respectively, after the age of 50 years (17). Sarcopenia, originally defined as the age-related loss of muscle mass, may lead to debilitating health outcomes including disability and mortality (18-20). It was officially recognized as a disease state in 2016 (21). Muscle strength was recently added to the definition of sarcopenia by some groups, but a lack of consensus remains among researchers to date (22). Also, no diagnostic cut-points for sarcopenia were ever derived from a Canadian population. Considering differences in ethnicities and BMI in Canada compared to populations used to derive diagnostic cut-points, and the time of recruitment of the latter (up to 20-year-old data), deriving new cut-points using the data from the contemporary, newly released, nationally representative CLSA cohort is timely and needed for a uniform and more precise characterization of sarcopenia.

Another component of successful aging is to maintain "high cognitive and physical function". Some degree of cognitive decline is also inevitable with aging and may transition to cognitive impairment (23). Unfortunately, no cure for cognitive impairment exists. It is thus imperative to find approaches for early detection, before the manifestation of symptoms, for instituting preventive measures. Low BMI during late life was shown to be a predictor of cognitive decline (24). Although

BMI is often available, it does not inform on the contribution of different body compartments. Sarcopenia (low lean mass) was associated with 2.3 times (95% CI: 1.7-3.0) higher chance of having cognitive impairment (25) and may be a more sensitive and stronger predictor of cognitive decline. Yet studies examining the relationship between sarcopenia and cognition are mostly cross-sectional, have used sarcopenia definitions that embedded strength, and measured cognitive function with general tests or binary outcomes (mild cognitive impairment (MCI) or dementia) (25). Identifying independent predictors of different cognitive domains will help to recognize individuals at risk of cognitive dysfunction, will guide the elaboration of preventive strategies and extend understanding of potential mechanisms.

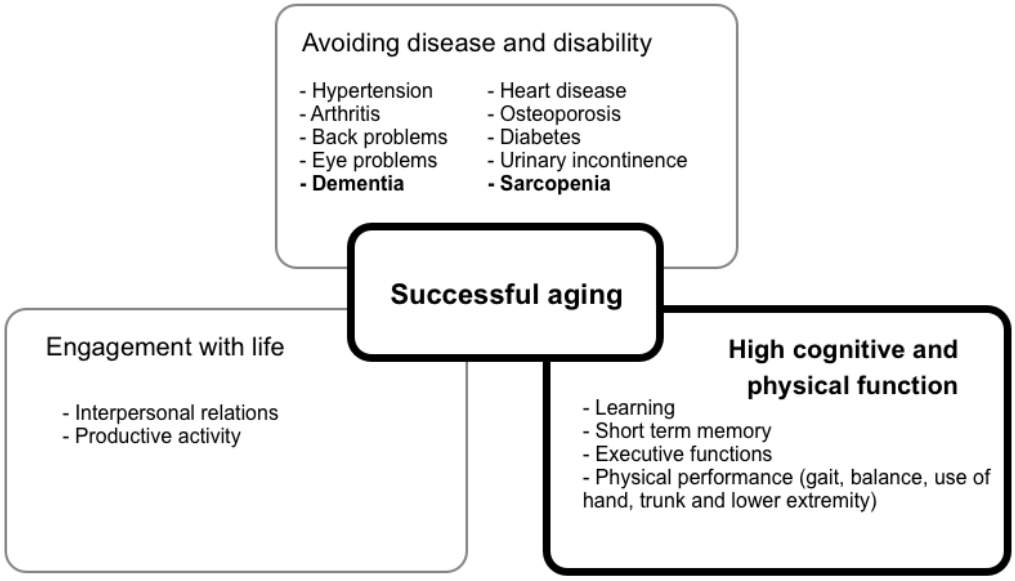
Factors other than sarcopenia may lead to cognitive dysfunction and physical disability, including suboptimal nutritional status, sedentary behavior, smoking, insulin resistance, declining sex hormone levels, and others (**Figure** 1-2). Among these are modifiable behavioral factors which can be targeted in interventions, namely nutritional status. The prevalence of malnutrition in the elderly population is ~20% across living arrangements and as high as ~50% in rehabilitation settings (multinational pooled analysis) (26). Poor nutritional status is associated with impaired cognition (27), physical function capacity and frailty (28).

Whole foods from dairy products are nutrient dense which addresses many barriers encountered with increasing nutritional intake in older adults due to lack of appetite, early satiety and chewing difficulties. There has been a gain in interest for dairy products and cognitive functions as dairy may exert a neurocognitive role possibly through their content of bioactive peptides, vitamin B_{12}, vitamin D, and calcium, and by-products of fermentation (29, 30). To date, few studies have investigated the association between dairy product intake and cognitive function. In fact, a systematic review published in 2010 concluded that inconsistencies remain with regard to this association (31). Importantly, the review included studies with numerous design limitations, namely the lack of serving size definition, specification of dairy fat content and analysis of dairy product types (i.e., yogurt, cheese, milk) (31). This suggests that further work is needed to clarify the link between specific dairy products and cognitive function domains, giving insights as to which matrix may play a role in defined functions and potential areas of the brain.

Supplementation with nutrients that have beneficial effects on physical function, e.g., whey protein, leucine, vitamin D and n-3 polyunsaturated fatty acids (PUFA), also represent a conceivable approach to preserving autonomy and quality of life in older adults. The current Recommended Dietary Allowance (RDA) for protein in the adult population (≥19 years), including older individuals,

is set at 0.8 g/kg/d and is based on nitrogen balance studies to meet basal biological needs to avoid protein deficiency (32). While current protein intake in older men and women in Quebec is 1 g/kg/d (33), several authors support ≥1.2 g/kg/d should be recommended to healthy older adults for skeletal muscle health (34, 35). The effect of protein supplementation on the skeletal muscle may largely depend on protein quality, dose and timing of ingestion (36), and presence of anabolic resistance (37, 38). A 15 g supplementation of protein from milk (80% casein and whey protein) 2x/d resulted in an increased Short Physical Performance Battery (SPPB) score (physical function) after 24 weeks in prefrail and frail older adults (n=65, ≥65 years) (39). In contrast, another study providing 2.5 g leucine with each main meal to community-dwelling older adults (n=30, 71 ± 4 year) did not find any significant improvement in muscle parameters after 12 weeks potentially due to insufficient total amino acid availability for muscle protein synthesis (MPS) stimulation at each meal (40). Regarding vitamin D, high-dose supplements (800-1,000 IU) were shown to have a favorable effect on balance, strength and physical performance in elderly populations (41). Improvements in physical performance and lean mass were also reported following supplementation with 3 g of n-3 fatty acids in healthy older adults (42, 43). Very few studies have tested the effect of combining some of these nutrients in a supplement and found improvement in the number of chair stands (44), appendicular muscle mass (44-46), and strength (46, 47). However, populations studied were relatively healthy and durations were short (6 and 13 weeks), and only one included n-3 PUFA (46). The provision of a rigorously designed nutritional supplement that combines the important nutrients related to muscle health (soluble milk protein, leucine, vitamin D and n-3 PUFA) may prove more efficient than single components to improve muscle mass, muscle function and cognition in frail older individuals.

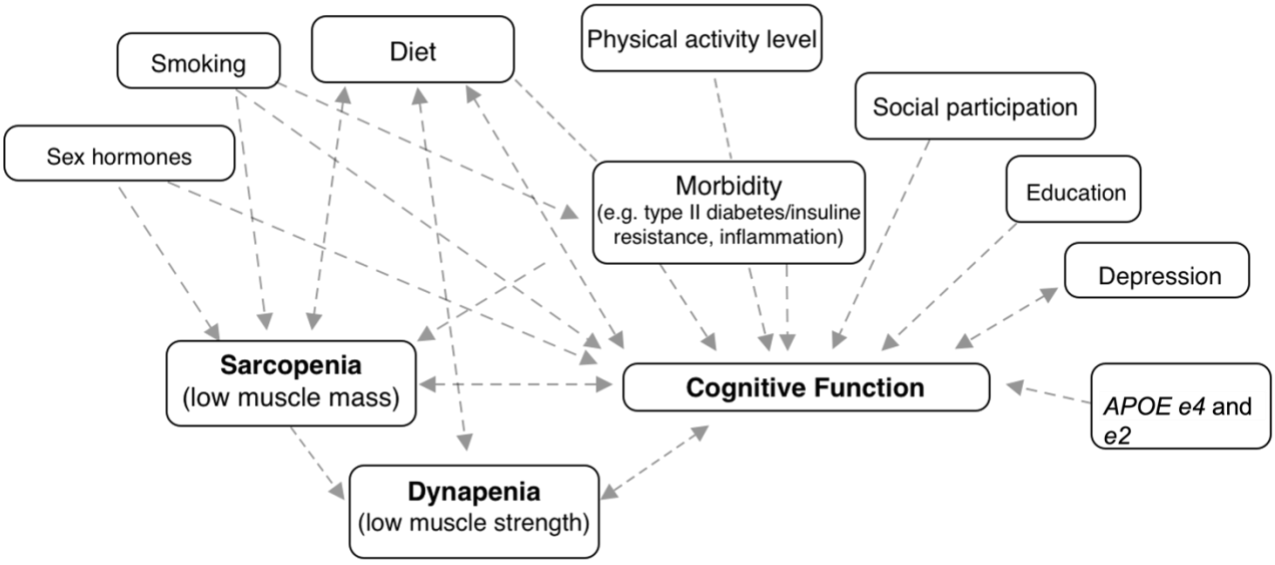
Figure 1-1 Determinants of successful aging



Adapted from (15, 16, 48).

Bold sections represent the focus of this dissertation.

Figure 1-2 Potential causes and risk factors of sarcopenia and cognitive impairment



1.2 Study objectives and hypotheses

This thesis includes three main objectives addressing some of the identified knowledge gaps from previous studies (illustrated in **Figure** 1-3**)**. Each objective is presented in separate chapters.

Observational studies (Aims 1, 2 & 3)

Aim 1: Definition and diagnosis of sarcopenia: (Chapter 4, Manuscript 1)

To identify cut-points for dynapenia, i.e., low muscle strength as a determinant of impaired physical performance, and for the diagnosis of sarcopenia, i.e., low appendicular lean mass (ALM) as a determinant of low muscle strength in the large CLSA cohort.

Hypothesis: ALM index is associated with handgrip strength, and handgrip strength is associated with physical performance in Canadian older men and women.

Aim 2: Sarcopenia and cognitive decline: (Chapter 5, Manuscript 2)

To determine if sarcopenia is a determinant of cognitive decline over 3 years in older adults of the CLSA.

Hypothesis: Sarcopenia (low ALM), as identified using the diagnostic criteria determined in Aim 1, is an independent predictor of cognitive decline in 3 domains over a 3-years follow-up in men and women community-dwelling older Canadian adults.

Aim 3: Dairy product intake and cognitive functions: (Chapter 6, Manuscript 3)

To investigate the relationship between total dairy product intake and three cognitive domains in the large comprehensive cohort of the CLSA; and to examine associations with specific dairy types; milk, yogurt, cheese, regular-fat and low-fat, and fermented products.

Hypothesis: Older adults with a higher consumption of dairy products will perform better in all three cognitive domains, independently of their diet quality and other confounding factors and covariates.

Interventional study (Aim 4)

Aim 4: Pilot RCT: (Chapter 7, Manuscript 4)

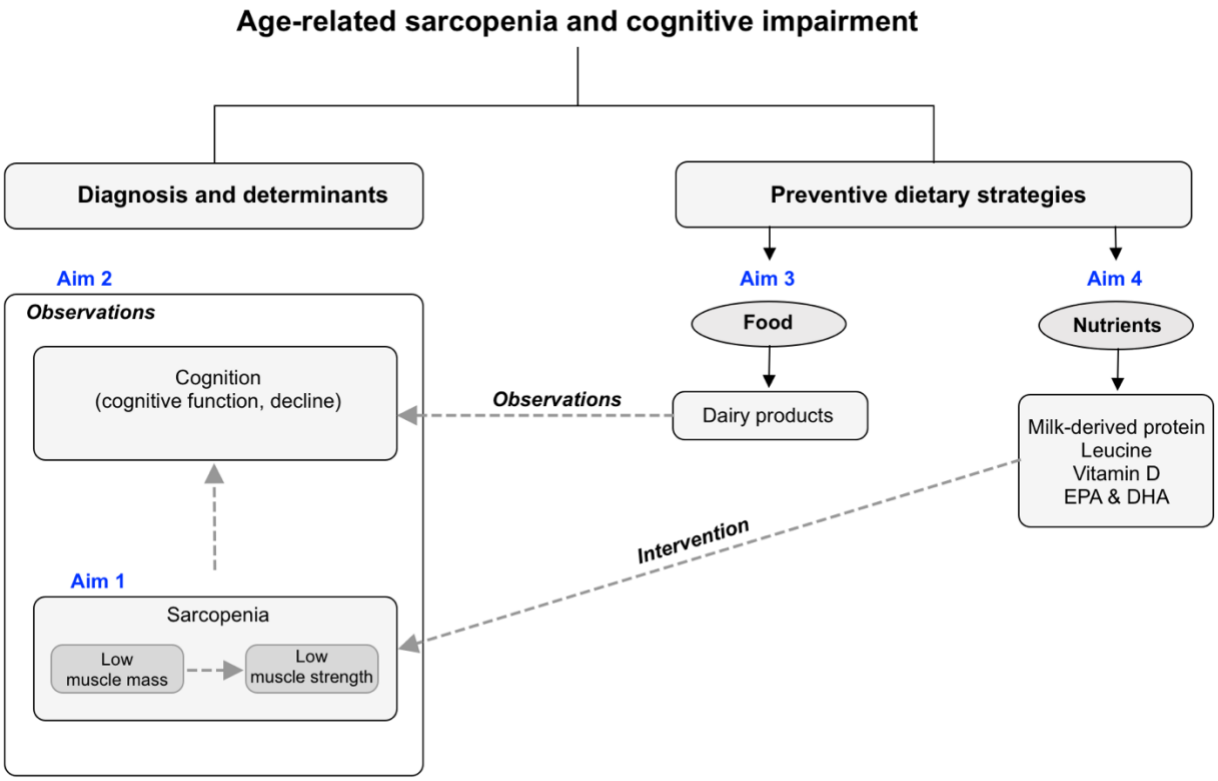
To determine if a 16-week randomized controlled trial of a combined nutritional supplement aimed to increase lean soft tissue mass and improve physical performance in older adults taking part in a geriatric day hospital (GDH) rehabilitation program is feasible in terms of recruitment (40 participants within 1 year), adherence, ability to complete the study assessment, and potential of adverse events.

The secondary outcome was to characterize the nutritional and functional status of this specific population of seniors with impaired autonomy.

Hypothesis: At least 50% of expected recruitment will be reached, ≥30% of patients admitted at the GDH will be eligible, ≥50% of them will consent to participate in the study, and the adherence to the supplement will be ≥80%.

Prior to testing these hypotheses to meet objectives, the following comprehensive literature review (**Chapters 2 & 3**) aims to briefly present the physiological consequences of aging, discuss the current definition of arising age-related well-recognized conditions including sarcopenia and cognitive impairment, and explore the link between these consequences (observational studies; **Chapter 2**). Additionally, the review will examine the role of nutrition as a modifiable risk factor for these age-related consequences with a focus on dairy products and fish intake, and highlighting specific nutrients including whey protein, leucine, vitamin D, n-3 PUFA (from observational and interventional studies; **Chapter 3**). This review will further justify the rationale of the observational study objectives leading to the elaboration of a pilot RCT involving these nutrients.

Figure 1-3 Thesis framework and objectives



CHAPTER 2: Literature review

2.1 Aging

Over the last 60 years, the proportion of older adults has been continually growing in Canada; the year of 2016 marked the first time the older adult population outnumbered that of children under the age 14 years (1). According to Statistics Canada, 6.6 million older adults ≥65 years could be counted as of July 1st 2019, representing 18% of the total population (2), and this proportion is expected to reach 23-25%, or 1 in 4 individuals by 2036 (3). This demographic change comes with health and support system challenges to older adults, caregivers and society. Therefore, successful aging of Canadian older adults is considered of high priority in this country. Maintaining "high physical and cognitive functions" is an important component of successful aging (**Figure** 1-1). The next two sections will cover the impact of aging on these functions as they are the focus of this dissertation.

2.2 Physical decline related to aging

All humans experience a loss in muscle mass and strength with aging. The peak in strength and mass is typically reached at the age of 30 years and the trajectory follows a declining rate of 0.5-1% and 2-3% per year, respectively after the age of 50 years (17). This loss in skeletal muscle and function may eventually lead to the recognized geriatric condition, sarcopenia, i.e., low muscle mass and strength, the consequences of which are debilitating (**Figure** 2-1).

The observed decline in skeletal muscle mass characterized by a decrease in muscle fiber count and protein content of remaining cells is thought to contribute to decreased strength (49, 50) and fitness (lower metabolic rate (51, 52) and maximal oxygen consumption (53)). Sarcopenia is also associated with poor health outcomes namely decreased mobility, increased disability, impaired autonomy, frailty and mortality (18-20).

The causes of sarcopenia are complex and remain elusive. Schematically, aging causes both a decrease in, and resistance to anabolic stimuli and an increase in catabolic factors, all of which negatively impact skeletal muscles (50). More specific to muscle mass, sex steroids, growth hormone, insulin and insulin-like growth factor (IGF-1) were all shown to have an anabolic effect, yet the production of these hormones declines with advancing age (50) which may contribute to sarcopenia. Modifiable behavioural factors including physical inactivity and suboptimal nutritional status were also identified as causes of low muscle mass (22).

Interestingly, low muscle strength appears to originate from age-related defects pertaining to the muscular system, and also to the nervous system (19). Specific alterations include decreases in motor-unit recruitment rate, excitation-contraction uncoupling, increase in adipocyte and fat infiltration into muscles or muscle fiber type transition (fast to slow) (54). Mitochondrial dysfunction and content reduction were also reported with aging and likely contributes to the loss of muscle mass and strength (55).

Although low muscle mass and strength were pooled in recent definitions of sarcopenia, their respective etiological factors suggest they differ and may be related to different health implications (54). Therefore, a brief overview of the history, existing definitions and current diagnosis of sarcopenia is necessary.

2.2.1 Defining sarcopenia

Sarcopenia - or "poverty of flesh", in Greek - was originally identified as the term to signify age-related loss of lean body mass by Irwin Rosenberg in 1988 (56). Since then, the definition has evolved to include the loss of muscle strength, the condition was recognized as an official disease in 2016 (21) and yet, no global consensus on the operational definition of sarcopenia exists. Importantly, these limitations prevent standard diagnosis in clinical settings and comparison between research studies (57). Also, the different proposed diagnostic criteria were derived from cohorts of limited sample sizes, pooled cohorts (diverse instrument models, software and quality control methods) with various representations of ethnic or racial groups or from noncontemporary data. It is recognized that obesity prevalence, which has increased over the past years, and race/ethnicity (e.g., African American, Asian, Hispanic, White) influence body composition, and therefore cut-points for low muscle mass (58). To date, no cut-points for age-related sarcopenia have been derived from Canadian representative populations.

Prevailing criteria for sarcopenia along with their respective specifics are listed by working groups in **Table** 2-1. The European Working Group on Sarcopenia in Older People (EWGSOP), the International Working Group on Sarcopenia (IWGS), The Foundation for the National Institute of Health (FNIH) and the Asian Working Group for Sarcopenia (AWGS) have been investigating approaches to determine cut-off points of muscle mass, strength and physical performance in populations representative of the Europe (22), United-States (59), and East and Southeast Asia (60), respectively.

These groups proposed different cut-points for low muscle mass as measured by dual-energy X-ray absorptiometry (DXA) and only the AWGS consensus also endorses cut-points measured by bioelectrical impedance analysis (BIA). The EWGSOP, IWGS and FNIH all selected sex-specific criteria for low muscle mass derived from divergent approaches including T-scores (based on younger adults' peak in muscle mass), 20% below an older adult population mean and classification trees to predict an outcome (weakness). Though similar between groups, sex-specific cut-points proposed for low strength slightly differ from one another and were derived from populations independent of those used to establish muscle mass criteria, as shown in **Table** 2-1. All except the IWGS include an assessment of strength in the identification of sarcopenia and measured it by handgrip dynamometry, a valid marker of overall body strength (61). Physical performance has been added in recent sarcopenia definitions to better identify individuals at risk of adverse outcomes (62). For example, the EWGSOP2 uses low physical performance (combined to low muscle mass and strength) to further characterize sarcopenia as severe (62). The assessment of physical performance is heterogenous among proposed operational definitions and includes the Short Physical Performance Battery (SPPB), gait speed, timed up and go (TUG), 400 m walk, and the chair-rise test (62, 63) which evaluate a large range of performances namely balance, gait, leg strength and endurance. From criteria determined by the EWGSOP and the IWGS, the prevalence of sarcopenia was estimated to be substantial in several older population settings (age ≥50 years); free-living (1-29%), long-term care (14-33%) and acute hospital-care (10%) settings (64). Moreover, 56% (95% CI: 46, 65) of patients in post-acute inpatient rehabilitation settings were shown to live with sarcopenia (meta-analysis of 6 studies, n=1,507, high heterogeneity) (65).

Different technological approaches may be used to measure muscle mass (66, 67). In sarcopenia research, DXA, a 3-compartment model, is the most commonly used and reference method for measuring appendicular lean soft tissue mass. DXA works with two X-ray beams, one of high energy that measures bone tissue and the other of low energy that measures soft tissue. The degree of X-ray absorption allows to differentiate the tissue type using an algorithm (68). Lean soft tissue mass excludes bone (10% of total body mass) and fat masses (19% of total body mass) (67); appendicular lean soft tissue mass further excludes other organs (18% of total body mass) and mainly consists of skeletal muscle except for a small proportion of water, skin, other connective tissues and fat-free adipose tissues (67, 69).

The gold standards for measuring body composition are multicompartment models that combine multiple measurements as it minimizes assumptions, e.g., body volume measurement by air or water displacement, total body water by doubly-labeled water and bone mass by DXA (66). Other than multicompartment models, computed tomography (CT) scan and magnetic resonance imaging (MRI) provide the most accurate measure of body composition as each provides a direct measure of organ tissue volume (66). However, these techniques are not easily accessible, they are costly and are typically used in highly specialized settings. BIA is another approach to estimating muscle mass, it provides a measure of fat mass and fat-free mass (FFM) (2 compartments) via electrodes that send a weak electric current throughout the body and receive the voltage to calculate the body's resistance (66). Sarcopenia cut-points derived from this method were reported in the literature and are endorsed by the AWGS (70). Details regarding BIA are not covered in this review as this tool was not used in any of this thesis research projects. Changes observed with aging are not limited to physical features; cognitive function is also affected and will be discussed in the next section as it is relevant to this dissertation.

Table 2-1 Latest updated cut-points for sarcopenia diagnosis from prevailing working groups

	European Working Group on Sarcopenia 2 (EWGSOP 2; 2019 update (62))	**International Working Group on Sarcopenia (IWGS 2011)(63)**	**The Foundation for the National Institute of Health (FNIH 2015) (71)**	**Asian Working Group for Sarcopenia (AWGS; 2019 update (72))**
Reference population	**Low ALM (73):** Australian, Geelong osteoporosis study (1993-1997, 2001-2006 and 2004-2008) n=682 (45% women) 20-39 y 99% Caucasian **Low grip strength (74):** 12 pooled British cohorts (1990-2012) n= 49,964 4-90 y (n≈2,407; 30-35 y; 40% W) Race/ethnicity not reported	**Low ALM (75):** 2 US communities (Health Aging and Body Composition Study, 1997-98) n=2,984 (52% women) 70-79 y 41% Black	9 pooled American cohorts (1967-2007) n=26,625 (57% women) 65+ y 89.9-90.5% Caucasian **Low ALM (76):** n=11,270 (33% women) **Low grip strength (77):** n=20,847 (53% women)	**Low ALM** : Pooled cohorts (Hong Kong, Japan, Taiwan) (unpublished data; cut-points reported in (60, 72)) **Low grip strength (78)**: 8 pooled East and Southeast Asian cohorts n=21,984 (53% women) 65+ y
Specifics of cut-points	**Low ALM:** Sex-specific, T-score ≤ – 2.0 SD (reference: 20-39 y) **Low grip strength:** Sex-specific, T-score ≤ – 2.5 SD (reference: peak at 32 y)	**Low ALM:** Sex-specific, lowest 20% of the distribution of the index	**Low ALM:** Sex-specific, determinant of low strength, using CART analysis **Low grip strength:** Sex-specific, determinant of gait speed <0.8 m/s, using CART analysis	**Low ALM:** Sex-specific, ≤ – 2.0 SD (reference: 20-40 y) **Low grip strength:** Sex-specific, lowest quintile

Criteria				
Low lean mass (DXA)	**ALM normalized for height squared** Women: <5.5 kg/m^2 Men: <7.0 kg/m^2	**ALM normalized for height squared** Women: ≤5.67 kg/m^2 Men: ≤7.23 kg/m^2	**ALM normalized for body mass index** Women: <0.512 Men: <0.789	**ALM normalized for height squared** Women: <5.4 kg/m^2 Men: <7.0 kg/m^2
Low strength	**Grip strength** Women: <16 kg Men: <27 kg **Chair stand** >15 s for five rises	Not included	**Grip strength** Women: <16 kg Men: <26 kg	**Grip strength:** Women: <18 kg Men: <28 kg
Low physical performance	**Gait speed** ≤0.8 m/s **SPPB** ≤8 point score **TUG** ≥20 s **400 m walk test** non-completion or ≥6 min for completion	**Gait speed** <1.0 m/s	Gait speed <1.0 m/s (used as the outcome in CART analysis)	**Gait speed** <1.0 m/s **SPPB** ≤9 point score **Chair stand** ≥12 s for five rises

EWGSOP, European Working Group on Sarcopenia; IWGS, International Working Group on Sarcopenia; FNIH, Foundation for the National Institute of Health; AWGS, Asian Working Group for Sarcopenia; SD, standard deviation; ALM, appendicular lean mass; DXA, dual-energy X-ray absorptiometry; SPPB, short physical performance battery; TUG; timed up and go.
Presented ALM cut-points are those measured by DXA as this instrument is recommended by all expert groups. Cut-points by BIA are also endorsed by the AWGS.
EWGSOP2 2019: low muscle strength=sarcopenia probable; low muscle strength + low muscle quantity=sarcopenia confirmed; the previous criteria + low physical performance=sarcopenia severe.
AWGS 2019: low muscle mass + strength or low physical performance=sarcopenia; low muscle mass + low strength + low physical performance=severe sarcopenia.
IWGS: low muscle mass + low gait speed=sarcopenia.
FNIH: no endorsement of sarcopenia definition, use of term low lean mass and weakness.

2.3 Cognitive decline

Cognitive decline is inevitable with aging. Crystallized cognitive ability (or intelligence), i.e., well-entrenched skills and knowledge, increases until 60-70 years and starts to slowly decline then. Distinctively, fluid intelligence, i.e. psychomotor abilities and processing speed, peaks at the age of 30 years and declines at a rate of -0.02 SD/year from then (79). While these trajectories are non-pathological, different degrees of cognitive decline exist and may progress to cognitive dysfunctions such as MCI and dementia (23) (**Figure** 2-1). MCI is recognized from a deficit in ≥1 of the following domains: ability to acquire and remember new information, reasoning and handling of complex tasks, visuo-spatial abilities, language functions, or from a change in personality or behavior, that does not interfere with independence (80). Dementia is distinct from MCI by its severity, and is typically described by the presence of a deficit in ≥2 of the aforementioned domains that impairs social and professional life (80). Dementia is a highly diverse condition that may be further delineated by the deposition of amyloid ß-peptide (senile plaque), the manifestation of tangle from tau hyperphosphorylation, along with dysfunction in synapse and neurons; these features are specifically observed in Alzheimer's disease (AD), a condition that falls under the umbrella term, dementia (81, 82). Notably, it is suspected that AD starts to develop one to two decades before the condition is recognised (83). Individuals with MCI experience a progress of their condition to AD at a rate of 10-15% per year compared to 1-2% in individuals with normal cognitive status (23). In Canada, 17% of adults ≥65 years live with MCI, and 7% ≥65 years and 25% ≥85 years live with dementia (84, 85).

Risk factors and consequences

Risk factors for accelerated cognitive decline and cognitive impairment include metabolic, behavioral, environmental and genetic risk factors. Among metabolic risk factors, chronic inflammation, hypertension, diabetes, and mid-life obesity were associated with increased risk of cognitive decline (86, 87). Lifestyle habits identified as behavioral risk factors include poor diet quality, physical inactivity, excessive alcohol consumption, social isolation and smoking (87). Being a carrier of the *APOE e4* allele is the biggest genetic risk factor that was related to late-onset AD and conversely, *APOE e2* appears to be protective of AD (88). Low level of education, depression and hearing loss were also linked to increased risk of cognitive impairment (87). Importantly, this progressive degeneration disorder eventually impairs

autonomy of affected individuals, decreases quality of life and may lead to frailty, hospitalisation and mortality (8, 10, 13, 87, 89, 90). Unfortunately, no drug treatment exists to slow down the progression of the disease, to stop it or reverse brain damages to date (91). The existing treatments may solely temporarily alleviate the symptoms.

2.4 Body composition, sarcopenia related to cognitive functions

Body weight and body mass index (BMI; kg/m^2) are biometrics mainly reflective of weight in muscle, adipose and bone tissues. Accelerated involuntary weight loss after mid-life was identified as a pertinent biomarker that indicates the imminent onset of MCI (24% higher risk of MCI with 5 kg weight loss/decade) (92) and AD (>10 years before diagnosis) (83, 93, 94). This association is not fully understood; however, particular preclinical behavioral changes such as decreased caloric intake or diet quality due to gustatory changes, decreased interest in food, and lower physical activity level are possible explanations (83, 93, 95).

It is well-known that overweight (i.e., BMI 25-29.9) and obesity (i.e., BMI ≥ 30 kg/m^2) are associated with other chronic diseases (96) and mortality (97, 98) in adults. However, the associations seem to differ in older adults ($\geq$65 years). Although overweight and obesity were associated with less successful patterns of aging (i.e., presence of disability and disease), overweight was protective of early mortality compared to "normal" and underweight BMI (99). Higher BMI was shown to promote survival; low BMI (<23 kg/m^2) was associated with all-cause mortality (U shaped association with reduced risk between 23-29 kg/m^2, increased risk >33 kg/m^2) (98, 100). Comparably, higher BMI (BMI $\geq$25-29.9 and $\geq$30 kg/m^2) during mid-adulthood and lower BMI (<20 kg/m^2) in later life were both associated with greater incidence of dementia (95, 101) and cognitive decline (24, 102). The protective effect of normal or high BMI on cognitive functions in late-life may be explained by greater adipose tissue-secreted hormones circulating levels such as estradiol in women (103) and possibly in men (104). Low BMI may also be the result of a weight loss in certain cases. Lastly, it is possible that specific body compartments further explain this relationship and are more sensitive biomarkers of cognitive decline and dementia risk, but few groups have investigated the associations of muscle or lean soft tissue and adipose tissue masses separately.

Lean soft tissue mass was shown to be reduced in individuals with early AD compared to normal individuals and was associated with brain atrophy (105). Few cross-sectional studies

investigated the association between sarcopenia (combination of muscle mass, strength and/or physical performance) and cognitive impairment; while the majority found an association (106-108), another did not (109). These studies were reviewed by Chang et al. in 2016 (110) and by Peng et al. in 2019 (25). Despite heterogenous study designs, the authors of the two meta-analyses concluded that persons with sarcopenia were 2.3 times more likely to have concomitant cognitive impairment compared to those without (adjusted models, 95% CI: 1.2, 4.2 and 1.7, 3.0; 6-11 studies, n=5,994 and n=10,710) (25, 110). Nonetheless, included studies were limited by small sample sizes, the use of global cognitive tests which prevents the assessment of different cognitive domains, heterogenous definitions of sarcopenia (with or without strength or physical performance) and importantly, by the lack of model adjustment for muscle function and physical activity level which may have mediated the observed associations.

Several groups observed an association between higher handgrip strength and lesser cognitive decline or decreased risk of dementia (reviewed in (111)). However, little attention has been brought to the association with muscle mass; few longitudinal studies are available to date and results are far from conclusive. Also, none examined the relationship of grip strength and muscle mass independently of one another. In a subsample of the Epidémiologie de l'Ostéoporose (EPIDOS) cohort, van Kan et al. examined the change in percent body fat and muscle mass in relation to cognitive impairment onset after 7 years and did not find any associations, possibly due to low power (total n=181 women, 75+ years, n=15 with incident dementia, n=6 with incident MCI) (112). Another study of small sample size did not report a significant link between ALM, strength and MCI or dementia, but authors did find a protective association with physical performance as assessed by the SPPB score (total n=297 men and women, 65+ years, n=50 with incident MCI, n=5 with incident dementia) (113). Only one prospective study of 131 community-dwelling Japanese older adults found a significant relationship between sarcopenia (as defined by the AWGS) and cognitive deterioration (evaluated with the Mini-Mental State Examination (MMSE)) after 1 year (114). Again, this study had important statistical limitations related to inappropriate choice of test (ANOVA performed on highly unequal groups), limited sample size and associated minimal adjustment of the model. No study investigated the link between muscle mass or sarcopenia and cognitive decline over time.

Mechanisms relating sarcopenia to cognitive functions remain unknown. Chronic inflammation (115, 116), insulin resistance (117, 118) and suboptimal vascular function (108) have been highlighted previously as potential explanations connecting sarcopenia and cognitive functions (110). Chronic low-grade inflammation with advanced age, also called "inflammaging", accelerates the process of aging and has deleterious effects on multiple organs including skeletal muscles and the brain. This state is characterised by increased pro-inflammatory cytokines and natural killer cells (innate immune system). In muscles, these cytokines promote catabolism and impair muscle protein synthesis (MPS) and glucose uptake in response to insulin (119). Inflammation was also shown to be linked to inter- and intra-muscular fat deposition (120), thereby reducing muscle quality and function (121). In parallel, pro-inflammatory cytokines along with oxidative-nitrosative stress leads to increased amyloid- ß peptide deposition and tau hyperphosphorylation of the brain which persists decades before related cognitive decline occurs (122).

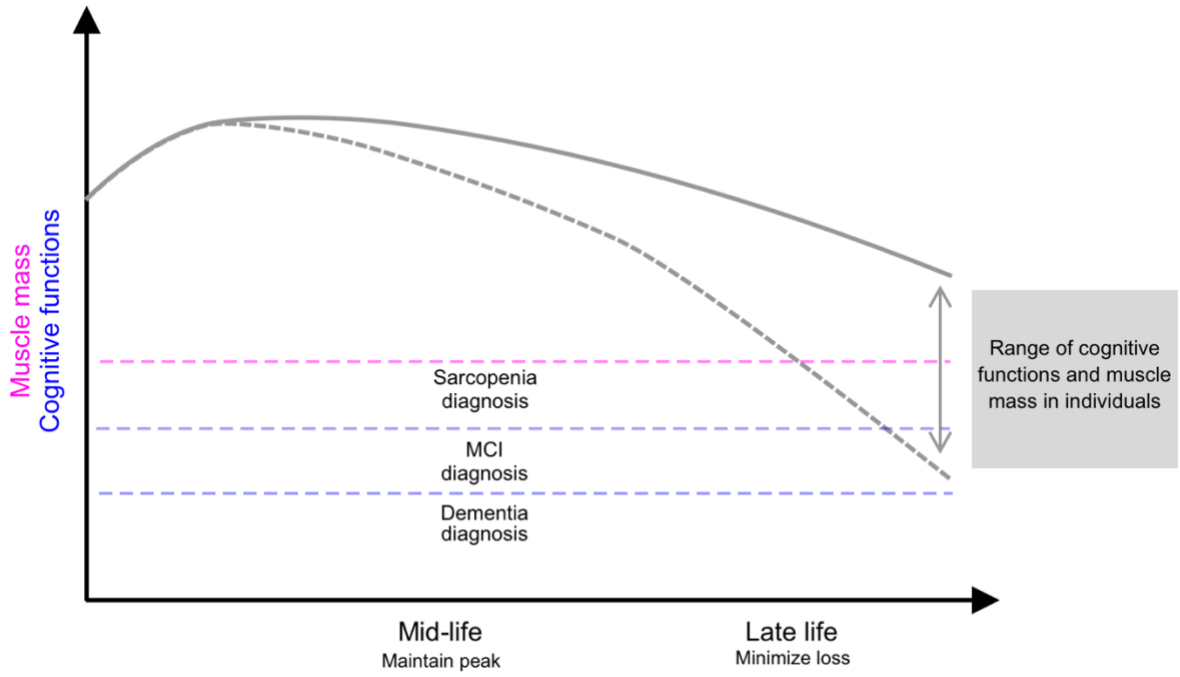
In addition to chronic inflammation, insulin resistance often manifests with aging. Insulin is considered the main anabolic hormone of human adults. With aging, the anabolic effect of insulin on muscle protein synthesis becomes blunted and this defect was associated with lower FFM (123). Insulin is also able to cross the blood brain barrier and has potent neurotrophic actions. Interestingly, both lower levels of insulin in individuals with AD (105, 124), and type 2 diabetes (hyperinsulinemia) and elevated plasma glucose levels in younger dementia-free individuals (125-127) were associated with decreased white matter volume, hippocampal volume and decreased cognitive performance. This suggests that adequate insulin signaling and glucose uptake and utilisation are necessary for proper cognitive functioning.

Arterial stiffness and impaired blood flow were identified in persons living with sarcopenia (low muscle mass) (128, 129) and were also associated with white matter hyperintensities in the brain, a proxy of cerebral small vessel disease and marker of cognitive decline, MCI and incident dementia (130). Results from a large cross-sectional study suggested that the association between sarcopenia and low cognitive function may be partly explained by white matter hyperintensities and arterial stiffness, although the strength of associations were modest (108). Nevertheless, strong data support that physical activity can significantly improve arterial stiffness in older individuals and may therefore represent an important mediator of the relationship (131). The deleterious effects of the above shared processes may be detectable

earlier on muscle mass compared to cognitive functions. Therefore, sarcopenia may be an early preclinical, yet non-specific sign of pathological cognitive decline.

Potential strategies to maintain cognitive functions over the adult life course target modifiable risk factors, possibly muscle mass if shown to be independently related to cognitive decline, and among others, smoking cessation, social participation, physical activity and adequate nutrition. A specific review of the literature on the latter is provided in the following sections.

Figure 2-1 Muscle mass, strength and cognitive declines and related potential diagnosis of sarcopenia, MCI and dementia over the life course



MCI, mild cognitive impairment.

Adapted from (62) and (79).

In this illustration, it is hypothesized that sarcopenia is more likely to be detected before MCI and dementia.

2.5 Dietary intakes of older Canadians

Among the modifiable behavioral factors, diet has been related to both muscle and cognitive health. In Canada, diet occupies the second rank among risk factors with highest disease burden from mortality and morbidity (measured in disability-adjusted life year) in older adults (50-69 years and 70+ years) (132), after tobacco use. Diseases included chronic cardiovascular, diabetes and kidney diseases, which are also related to muscle and cognitive health as discussed in **sections 2.3** and **2.4**.

From national data (2008/2009 Canadian Community Health Survey (CCHS) – Healthy Aging), as high as 34% of Canadian older adults ≥65 years are at risk of poor nutritional status mainly due to gaining/losing weight, frequent meal skipping and negative attitude to cooking (133). The prevalence of nutritional risk is also greater among women compared to men (38% vs. 29%). A decline in energy intake is observed with age (134, 135) as expected from decreasing energy expenditure, loss of appetite and increased sensation of fullness. The majority of older adults are either consuming within or above the Acceptable Macronutrient Distribution Range for macronutrients (136). However, adequacy of nutrient intakes expressed as a percentage of total energy intake should be interpreted with caution. A well-recognized paradox in aging is that diminished energy intake may represent a risk factor for not meeting nutrient requirements which for most, remain or increase with old age (137). Special attention is brought to the increased needs in protein and current intake of older adults in **Chapter 3** of this dissertation.

A new version of the Canada's Food Guide (CFG) was released in January 2019 (138). Recommendations are based on a healthy plate illustrating proportions to be consumed for three different food categories: protein foods, fruits and vegetables and whole grain foods. A unique section on healthy eating tips for seniors is available, but unlike the previous 2007 version no daily number of servings are recommended by food and age group (139). Further, the CCHS were conducted in 2008/2009 and 2015 when CFG 2007 was in use, and most of the literature in Canada and internationally is based on servings from food groups. Hence, recommendations from the CFG 2007 will be used for examination of dietary intake of older adults in this review. The majority of adults meet the daily recommendations for meat and alternatives; however more than half of older adults consume less than 5 servings of vegetables and fruits (recommended 7

servings/d) (134). As for grain products, 66% and 43% of older women and men, respectively, (71+ years) do not meet the recommended 5 servings/d; whereas ~80% of older adults consume <2 servings/d of dairy products and alternatives that is well below the CFG 2007 recommendations of 3 servings/d (134). Improving dietary intake of older adults may specifically help in preserving cognitive and physical function in aging.

2.6 Nutrition and cognition in aging

Evidence supporting a role for nutrients, food groups and dietary patterns in cognition is accumulating. The overall literature is reviewed here from dietary patterns to specific nutrients with an emphasis on dairy products that are specifically investigated in this dissertation.

High-quality dietary patterns

The Mediterranean diet, rich in olive oil, fish, fruits and vegetable, breads, legumes, nuts and seeds, has been extensively investigated and is the most studied among dietary patterns in relation to cognitive outcomes (140). Results from observational and intervention studies are in favor of a (~30%) reduced risk of AD or MCI among individuals with a higher adherence to the diet (141), a beneficial association with episodic memory and global cognition, and improved delayed recall, working memory and global cognition (142). Though there are less data pertaining to the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) and Dietary Approaches to Stop Hypertension (DASH) diets, evidence suggests a protective effect from these on cognition. In fact, the MIND diet shows the greatest and most consistent associations to date (reviewed in (143)). Similar to the Mediterranean diet, the MIND diet is rich in olive oil, fish, vegetables, nuts and more specifically in whole grains, berries and green leafy vegetables (144). The Mediterranean diet along with the DASH diet and high Healthy Eating Index were also shown to be protective of cardiovascular health (145) which may explain their potential indirect favorable effect on cognitive health.

Nutrients and non-nutrient food components

Vitamin D, B vitamins (specifically folate, B_{12}, B_1 and B_6), and antioxidants (mainly polyphenols and selenium) may play a protective role in cognitive functions and appear to be the most studied nutrients in this context (140). Their respective implications are in various neurobiological pathways (apoptosis, neurodegeneration and inflammation), homocysteine metabolism and antioxidative activity (146-148).

Evidence from longitudinal studies suggests that higher vitamin D status is protective of cognitive decline or dementia and AD (140). Interestingly, a meta-analysis of (7 prospective and 1 retrospective studies; n=28,354; 5.6-21 years follow-up duration) that examined the dose-response relationship between vitamin D status and risk of incident dementia or AD found an increase in protection up to serum 25(OH)D levels of ~35 ng/mL (87.4 nmol/L) (149).

An international Consensus Statement published recently reported that elevated and even moderately elevated levels of homocysteine (towards higher end of normal range) in the elderly increases the risk of dementia, as supported by strong data (150). Authors concluded that B vitamins supplementation should be considered in older adults with cognitive impairment and high homocysteine level in the prevention of progression to dementia or cognitive decline given their effectiveness at lowering homocysteine level (when high at baseline) and slowing brain atrophy (150). Yet, a meta-analysis published recently reported no clear benefits of lowering homocysteine levels using B vitamins (151).

Among macronutrients, dietary fats have been the most studied in relation to cognitive functions and decline. Observational studies mostly support beneficial associations from monounsaturated fatty acids and total polyunsaturated fatty acids (PUFA) intakes in cognitive health (140). N-3 PUFA have a role in the modulation of neurological functions from the early developmental stages of life. In older adults, n-3 PUFA may have a protective action on neurological function possibly by improving cerebral blood flow, integrity of neuronal membrane, and by reducing amyloid-β deposition, as well as inflammation (152). Dietary and circulating n-3 PUFA were both associated with better cognition in older adults and reduced risk of AD, suggesting a beneficial effect on slowing the progression of age-related cognitive decline (153). A recent meta-analysis of 10 RCTs on the effect of n-3 PUFA supplementation on cognitive function in older participants with normal cognitive status, MCI or AD (n=2,507, aged ≥50 years) showed a slight, but significant favorable effect from n-3 PUFA (different fatty acids and forms, 240-3,200 mg, 12.8-24 weeks duration) on immediate recall and attention, and processing speed domains among individuals with MCI only (154). It is not clear whether n-3 PUFA supplementation has an effect on cognitive function or decline in normo-cognitive older adults. It is also possible that persons with low DHA and EPA status benefit the most from n-3 PUFA supplementation (155). Mixed results, mostly neutral or negative associations, have been

observed between saturated fat intake and cognition; these will be discussed in the dairy products section of this dissertation.

2.6.1 Food groups and beverages: special focus on dairy products

Fish, particularly fatty fish which are important sources of n-3 PUFA and vitamin D, vegetables, fruits, nuts, legumes, meat and dairy have been studied in relation to cognitive functions. While mostly beneficial associations were found for fish and vegetables, evidence remains very limited and mixed for meat, fruits, legumes and dairy (140).

Compelling evidence from observational studies supports a beneficial role for moderate alcohol intake (1-3 glasses/d, analyses not stratified by sex) on cognitive outcomes, compared to non-consumption or to high alcohol intake (140). These associations would pertain to wine consumption, red wine particularly, possibly because of its content in polyphenols. Coffee has also been well studied although no evidence exists from RCTs. The literature reports mixed results (beneficial or neutral associations) (140).

Dairy products

Dietary intake in older adults

While the associations between certain nutrients and food groups with cognitive function has been well explored, little attention has been brought to dairy products. Based on the 2004 CCHS, adults ≥71 years consumption of dairy products is low (current intake of 1.4 and 1.2 servings/d in men and women (134) vs. 3 servings/d, CFG 2007 recommendation).

When examining high-quality dietary patterns, dairy products are interestingly consumed in moderation as part of the Mediterranean diet and full-fat dairy are actually restricted (143). Dairy products are consumed in higher amounts as part of the DASH diet (low-fat, 2-3 servings/d) and are absent in the MIND diet with cheese being restricted (143). Increased dairy product consumption may contribute to optimize older adults' overall nutritional status, and are of special interest in cognitive health as they may exert a neuroprotective role through their content in whey proteins including alpha-lactalbumin (rich in cysteine and tryptophan), bioactive peptides (intrinsic and derived from digestion of casein and whey proteins), vitamin B_{12}, vitamin D, and fermented products (reviewed in (29)).

Dairy products including milk, cheese and yogurt are nutrient-rich foods and have a unique matrix (156) (**Table** 2-2). In occurrence, 3 servings of milk provide an older adult with

37.5% of the daily 800 IU recommendation in vitamin D, 150% of the 2.4 µg recommendation for vitamin B_{12} and 73% of the 1,200 mg RDA for calcium. Dairy products are also sources of high quality soluble whey (20% or 6.3 g/L; 19% or 1.2 g/L of which is alpha-lactalbumin), insoluble casein (79%) and other proteins (2%) (157).

Table 2-2 Comparison of nutritional values found in a serving of milk, cheese and yogurt.

	White milk (partly skimmed, 2% M.F.) 250 mL/258 g	Cheese (cheddar, regular, 34% M.F.) 50 g	Yogurt (fruit flavored, low-fat, 0.5-1.9% M.F.) 175 g
Energy (kcal)	129.0	203.0	131.3
Water (g)	230.0	18.5	142.8
Protein (g)	8.5	12.0	6.5
Leucine (g)	0.8	1.0	0.7
Tryptophan (g)	0.1	0.4	0.1
Fat (g)	5.1	17.0	2.0
SFA (g)	3.2	9.7	1.3
16:0	1.4	4.3	0.7
18:0	0.6	1.8	0.2
14:0	0.5	1.5	0.2
SCFA			
4:0	0.2	0.3	0.0
6:0	0.1	0.3	0.0
Unsaturated FA (g)	1.6	4.9	0.6
Carbohydrate (g)	12.4	0.7	22.2
Vitamin A (µg, RAE)	163.0	131.5	19.3
Vitamin D (IU)	103.0	3.5	0.0
Folate (µg, DFE)	13.0	13.0	5.3
Riboflavin (mg)	0.5	0.2	0.4
Thiamin (mg)	0.1	0.0	0.1
Vitamin B_6 (mg)	0.1	0.0	0.1
Vitamin B_{12} (µg)	1.4	0.4	0.5
Vitamin K (µg)	0.5	1.5	0.2
Calcium (mg)	309.0	337.5	208.3
Phosphorus (mg)	237.0	236.5	171.5
Magnesium (mg)	28.0	13.5	19.3
Potassium (mg)	361.0	38.0	309.8
Zinc (mg)	1.2	1.7	0.6

SFA, saturated fatty acids; SCFA, short chain fatty acids; RAE, retinol activity equivalents; IU, international units; DFE, dietary folate equivalents. Data from (158). Nutrients reported are the most abundant ones in these products and are nutrients of interest in cognition. Partly skimmed milk (2%) (159), regular cheddar cheese* (160) and low-fat flavored yogurt (161) were selected as examples as they are the most consumed, in their respective category, among Canadians. Servings represented in this table are those recommended in CFG 2007 (139). *Most consumed and produced after specialty cheese.

Dairy components and their potential mechanisms of action

Numerous mechanisms of action have been proposed, with some more investigated than others. For instance, dairy product consumption was positively associated with cerebral glutathione concentration in older adults possibly due to their substantial levels of alpha-lactalbumin-derived cysteine and B vitamins, which are required in glutathione synthesis (30). The antioxidant effect of glutathione may reduce oxidative stress and subsequent neurodegeneration of the aging brain. Similarly, bioactive peptides could also have an antioxidant effect and reduce inflammation (162, 163). Additionally, evidence suggests potential cognitive performance enhancement from a particular whey protein, alpha-lactalbumin, possibly due to increased availability of tryptophan, a key amino acid in the synthesis of serotonin. Short-term diets rich in alpha-lactalbumin were associated with reduced depressive symptoms under stress (164), improved abstract visual memory and simple motor performance in recovered depressed patients (165) and brain-sustained attention processes in subjects with sleep complaints (166).

Dairy products are available as regular-fat and low-fat options at Canadian grocery stores. The main dietary fat fraction of dairy products are saturated fatty acids (60-70%), predominantly palmitic (C16:0; 30-45%), stearic (C18:0; 12-19%) and myristic (C14:0; 11-17%) (157, 158) (**Table** 2-2). As a group of dietary fats, saturated ones have long had a negative reputation because of linked to elevated plasma LDL cholesterol concentrations and increasing risk of cardiovascular disease (CVD) (167), although the risk was demonstrated to be non-significant from a meta-analysis of prospective studies (n=347,747 adults) (168). Consequently, due to their high saturated fatty acid content, dairy products have been identified as products that may contribute to the development of CVD (169), an independent factor for MCI development as discussed in **section 2.6.1**. Importantly, saturated fatty acids are not created equal. They vary in structure and cardiovascular health is impacted differently namely depending on the fatty acid chain length. Concerning dairy product composition, lauric, myristic and palmitic are recognized to raise LDL-cholesterol, while stearic is not; yet, lauric acid generally plays a protective role by increasing HDL-cholesterol (170). Overall, recent evidence is not supportive of a negative effect of regular-fat dairy products (milk, cheese, yogurt) on cardiovascular disease outcomes, and protective associations were shown for total dairy, low-fat dairy, yogurt, cheese, milk or fermented dairy intakes (171, 172). The consumption of dairy products to decrease blood

pressure, risk of type 2 diabetes and stroke as demonstrated from a recent systematic review of meta-analyses (172) may indirectly contribute to cognitive health.

Studies examining the relationship between saturated fats and cognitive scores or risk of MCI, AD or dementia have mostly shown unfavourable associations (reviewed in (140)). Nonetheless, these studies did not account for food sources, type of saturated fatty acids or diet quality which may in part explain the findings. Short chain fatty acids (SCFA) characterized as fatty acids with fewer than 6 carbon atoms are typically produced from fermentation of indigestible polysaccharides by bacteria in the large intestine. As a result of microbial activity in the bovine rumen, these are also found in dairy products in the form of butyric (C4:0; ~0-4% of saturated fatty acids) and caproic (C6:0; ~0-4%) fatty acids (173). Novel evidence is pointing to a potential beneficial role for SCFA in neuropathology and brain functions. SCFA appear to contribute in the preservation of the blood brain barrier integrity; modulation of neurotrophic factors and neurotransmitters levels; and may participate in recovering cognitive functions, namely memory (174).

Fermented dairy products may have specific properties on cognition as a source of lactic acid bacteria, fatty acids, peptides and menaquinones (vitamin K_2) (175). Yogurts, hard/aged cheeses, some soft cheeses (e.g., feta, swiss, emmental), sour cream, buttermilk, kefir are fermented dairy products. Novel evidence from animal studies shows brain dopamine levels and cognitive performance may be enhanced by fungal fermented cheese and yogurt (175). Also, several forms of menaquinones were measured mostly in fermented cheeses and fermented milk, some with substantial amounts, depending on their main microorganism species (176). Given the recently demonstrated link between vitamin K status and cognition (177), there may be a particular potential role for fermented dairy.

Interventional studies of the effect of dairy products intake on cognition

To my knowledge, only one study tested the effect of dairy products intake on cognition in older adults (178). In this randomized crossover trial, 33.4 g/d of camembert cheese was shown to slightly increase serum BDNF of Japanese women with MCI (n=71; ≥ 70 years) after 3 months compared to an isocaloric soft non-fermented processed cheese (group x time, F=5.37, p-value=0.024), but not MMSE. The absence of change in cognitive performance may be attributed to the use of a global cognitive test and limited intervention duration. Another study tested the effect of whole dairy product intake on cognition in middle-aged adults (179). This crossover

study investigated whether a high (4 servings/d) vs. low intake (1 serving/d) of reduced-fat dairy diet for 6 months improves cardiometabolic health among 38 overweight and obese adults (52 ± 2 y), and cognitive function as a secondary outcome. Results revealed a modest, but significant improvement in spatial span backward subdomain of working memory score, but not in other cognitive domains investigated, after following the high dairy diet (p=0.046). Since the study only tested low-fat products, the potential effects of regular fat dairy intake remain to be tested.

Observational epidemiological studies of dairy products intake and cognition

While there is a large body of evidence of the association of dairy product intake and CVD, their direct association with cognitive functions has been marginally explored. A systematic review (2010; 3 cross-sectional and 5 prospective studies; middle-aged and older adults) concluded that inconsistencies remain regarding to the relationship between dairy product consumption and cognitive performance (31). This review included studies with numerous design limitations, namely the lack of specification of dairy fat content, analysis of dairy food (e.g., yogurt, cheese, milk) and serving size definition. All studies used global cognitive assessments, clinical diagnosis of dementia or depression-oriented questionnaires, therefore precluding the findings of associations with cognitive domains. Importantly, dairy was one among other dietary factors observed and none of the studies addressed the quality of the overall diet.

More recent cross-sectional studies conducted in older adult populations have shown positive associations between dairy product intake and cognitive performance (180-182). A new cross-sectional study (2020; n=619; age 72 ± 5 years) examined associations between different dairy product categories (milk, cheese, yogurt, buttermilk, fermented products, full-fat vs. skimmed) assessed by a semi-quantitative food frequency questionnaire and cognitive functions evaluated using multiple tests belonging to 4 different domains (181). Authors only found cheese intake to be associated with 33% lower probability of poor processing speed (below 10% of the study population distribution) (prevalence ratio=0.67, 95% CI: 0.47-0.97; fully adjusted logistic regression model). None of the dairy categories were associated with any of the cognitive domains in the fully adjusted linear regression models (covariates: BMI, education, smoking, alcohol consumption, habitual physical activity and total energy intake). One study prospectively investigated associations over 13 years and revealed the absence of a link between dairy intake

and cognitive function in the SU.VI.MAX 2 study cohort (n=3,076, ≥45 years) (183). However, diet was only evaluated at baseline and thus results do not account for potential changes in dairy consumption over time. Considering the scarce evidence available, as well as the heterogeneity in cognitive outcome measures and dietary assessments, conclusions regarding these associations remain outstanding. Further work should be reported to strengthen the current state of literature.

Connecting Statement 1

The next chapter is the continuation of this thesis literature review. The section covers the evidence pertaining to the relationships and effects of protein, vitamin D and n-3 PUFA on muscle mass, strength and physical performance in healthy and vulnerable older adults. The number and sample size of intervention studies combining these nutrients in a supplement to improve the above outcomes were still limited and designs highly heterogenous. Hence, a narrative format was chosen instead of a systematic review and meta-analysis design. This review is of particular relevance to **Chapter 7**, the feasibility RCT of a combined nutritional supplement of whey protein, leucine, vitamin D and n-3 PUFA to improve muscle mass and function in older adults taking part in a rehabilitation program.

CHAPTER 3: Literature review – nutrition, sarcopenia and physical function in aging

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An update on protein, leucine, omega-3 fatty acids, and vitamin D in the prevention and treatment of sarcopenia and functional decline

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3.1 Abstract

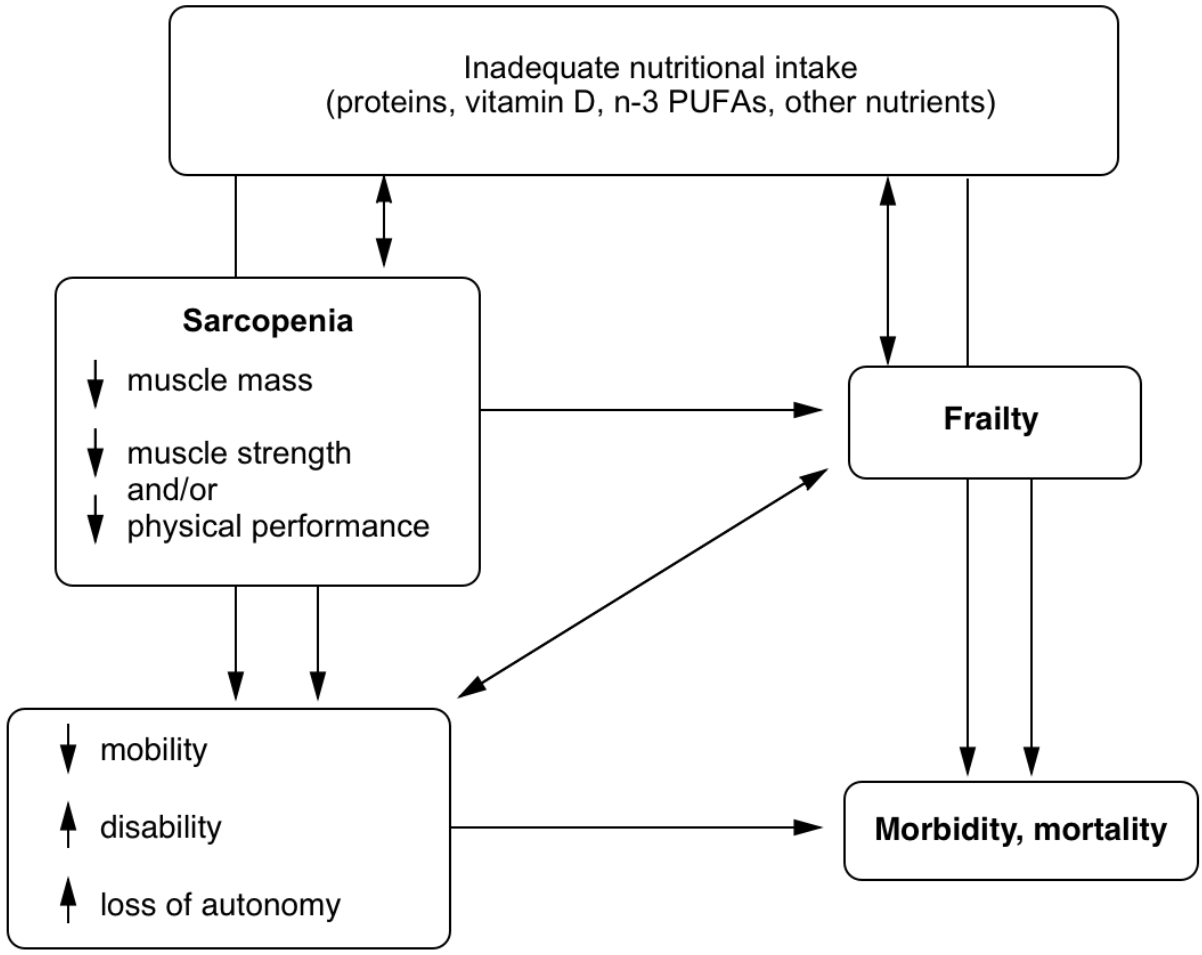
Aging is associated with sarcopenia and functional decline, leading to frailty and disability. As a modifiable risk factor, nutrition may represent a target for preventing or postponing the onset of these geriatric conditions. Among nutrients, high-quality protein, leucine, vitamin D and omega-3 polyunsaturated fatty acids (n-3 PUFA) are of particular interest for their demonstrated effects on skeletal muscle health. This narrative review aims to examine the recent observational and interventional evidence on the associations and the role of these nutrients in muscle mass, strength, mobility and physical function of free-living older adults, healthy or at risk of frailty. Recent evidence supports a higher protein intake recommendation of 1.0-1.2 g/kg/d in healthy older adults; an evenly distributed mealtime protein intake or minimal protein per meal may be beneficial. In addition, vitamin D supplementation of 800-1,000 IU, particularly when vitamin D status is low, and doses of ~3 g/d of n-3 PUFA may be favorable for physical function, muscle mass and strength. Reviewed studies are highly heterogenous, yet the quantity, quality and timing of intakes should be considered when designing intervention studies. Combined protein, leucine, vitamin D and n-3 PUFA supplements may convey added benefits and may represent an intervention strategy in the prevention of sarcopenia and functional decline.

3.2 Introduction

Sarcopenia is defined as the generalized and progressive loss of muscle mass and strength (184, 185) leading to declines in physical function and mobility. These are integral components of frailty defined by a decrease in function of several physiological and psychological systems, increasing vulnerability to stressors (185). In addition to reducing the quality of life (7), these age-related conditions increase risks of morbidity (11, 186), hospitalization (187) and its associated costs (188) and mortality (12). Sarcopenia has a multifactorial etiology, namely neuromotor dysfunction, chronic low-grade inflammation, physical inactivity, decreased endocrine function, and poor nutritional status (184). The latter, resulting from a reduction in dietary intake among other causes, was previously associated with lower physical function and frailty among older adults from various settings (hospitals, community, rehabilitation) (189-191). Approximately two third of older adults are estimated malnourished or at risk of becoming malnourished (26). Adding to a poor nutritional status in a context of advanced age is the presence of anabolic resistance. It is characterized by a reduced response to anabolic stimuli including dietary protein, leading to higher protein needs compared to those of younger adults (192).

As a modifiable risk factor, nutrition is a potential target to improve or prevent the loss of physical function in older adults (**Figure** 3-1). Specific nutrients are of particular interest for their demonstrated role on the muscular system and have been the object of earlier and more recent studies, either as single supplements or in combination with other supplements. These include proteins, especially those rich in leucine which is the most potent branched-chain amino acid at stimulating muscle protein synthesis (MPS) (193), vitamin D and n-3 polyunsaturated fatty acids (n-3 PUFA). This narrative review aims to examine the latest observational and interventional (supplementation alone, without physical exercise) evidence on the associations and the role of these nutrients in muscle mass, strength, mobility and physical function of free-living older adults, healthy, frail or at risk of functional decline.

Figure 3-1 Potential role of nutrition on the physical health of older adults



Short arrows within boxes: increase or decrease. Long arrows between boxes: may lead to. Double-sided arrows: the relationship may be bidirectional. Arrows passing through boxes: factors in box could be mediators.

3.3 Proteins and amino acids

3.3.1 Total dietary protein intake

Inadequate dietary protein intake is generally recognized as an etiologic factor contributing to sarcopenia (184). In an effort to prevent sarcopenia, maintain physical function and long-term optimal health, in 2013 and 2014, large international groups of experts issued a consensus to increase protein recommendations at 1.0-1.2 g/kg/d for healthy individuals, 1.2 g/kg/d for active individuals and 1.2-1.5 g/kg/d for those with chronic or acute diseases (except renal) (34, 35) from the current RDA of 0.83 g/kg/d (194). This consensus was based on substantial evidence emanating from metabolic, interventional and observational studies then available and continues to be supported by more recent evidence, comprehensively reviewed elsewhere (195). In brief, metabolic studies concur to show greater requirements than previously estimated by nitrogen balance, and the majority of observation studies to show higher lean or muscle mass and strength and lesser risk of losses and functional decline in subgroups of the older population consuming more dietary protein.

Interventional studies

On the other hand, randomized clinical trials of protein or amino acid supplements have reported opposing results on muscle mass or strength. From 9 trials included in a recent meta-analysis, no significant effect of supplements was found on lean body mass, leg and handgrip strength (196). Heterogeneity was evident for trials differed in studied population (healthy, frail, diabetic or sarcopenic individuals), duration and supplement forms and doses. Importantly, usual dietary protein intake was not measured in all studies therefore limiting data interpretation as to additional protein effect. The issue of compliance, difficult to measure with precision, is also to be considered since these supplements are not always palatable and become monotonous over long periods of time. In that respect, Mitchell et al. (2017) conducted a well-controlled feeding study of 35 healthy older men (>70 years), providing all prepared foods to test a diet at the current protein RDA against twice the RDA, i.e., 0.8 g/kg/d or 1.6 g/kg /d, for 10 weeks (197). While the appendicular lean mass (ALM) remained unchanged in individuals following the 1.6 g/kg/d diet, it decreased in the RDA group. This strengthens the higher protein needs for maintenance of muscle mass with aging. With regards to functional outcomes, benefits in favor of supplements versus placebo were reported as increased grip strength (198), improvement in functional limitations (199), absence of deterioration (200) or improvement (39) in physical

performance (Short Physical Performance Battery score, SPPB) whereas no improvement was observed in others (197, 201).

Again, this general lack of effect of protein supplements as opposed to positive associations between protein intake and muscle mass and function observed in cohort studies could be due to the type, dose and timing of supplements, compliance, as well as the population studied. Indeed, from the above studies, beneficial effects of protein supplements on physical function appear to be seen especially in frail, malnourished individuals or those at risk of malnutrition.

3.4 Meal distribution of dietary protein.

Beyond total daily protein intake, the effect of its mealtime distribution is a topic of emerging interest in the field of sarcopenia research. To equally distribute protein intake across the three daily meals is based on the concept of reaching a per-meal anabolic threshold (38, 202). A combination of factors such as insulin resistance (123, 203, 204), sedentary lifestyle or short-term immobilization (205, 206), inflammation, lipotoxicity and oxidative stress (207) are thought to cause anabolic resistance (38, 207). An elevated anabolic threshold translates into greater needs to achieve maximal stimulation of MPS in older compared to younger individuals (208-210). Earlier studies agreed upon a desirable dose of 25-30 g of high-quality protein per meal, providing ≥ 15 g of essential amino acids, recognized as the main amino acids responsible for stimulating MPS (211).

Interventional studies

The even distribution hypothesis was first confirmed in a crossover study in young adults (n=8) comparing a 7-day diet of evenly (30/30/30 g) versus unevenly (11/16/63 g) distributed protein intake. MPS measured by incorporation of $^{13}C_6$-phenylalanine over 24 hours was significantly higher following the diet with an even distribution (212). Contrastingly, a randomized trial testing the distribution, at two levels of intake (0.8 and 1.5 g/kg/d) during 4 days in older adults (52-75 years), concluded that quantity of dietary protein intake, but not distribution, resulted in an increased MPS and net protein balance (213). These studies were of too short duration to demonstrate effects on muscle mass or strength. To date, the only longer-term intervention study (42 days) testing distribution has been conducted in malnourished hospitalized elderly patients. They consumed on average 1.3 g/kg/d from a diet that provided

either >70% of proteins at lunch (bolus) or divided into 4 meals (spread; n=30-36/group). Lean body mass significantly increased in the group fed the protein bolus diet. No change in grip strength was reported in neither of the groups (214). It is possible that none of the four meals in the spread diet provided enough protein to reach the anabolic threshold, which would theoretically be higher in these malnourished and inactive patients. Clearly, the response to protein intake may differ according to health status, which justifies further research in vulnerable persons at risk of malnutrition.

Observational studies

We examined the loss of lean mass related to protein intake distribution using data from 351 and 361 free-living men and women aged 67-84 years of the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge) (215). We found that a more evenly distributed protein intake, regardless of quantity, was associated with higher lean mass at baseline and 2-years follow-up, after adjustment for relevant covariates (216). However, neither total intake nor protein distribution were related to the rate of loss over the 2-years follow-up, a period perhaps not long enough to detect association with a marginal decline (2% in 2 years). Muscle strength and mobility were also studied in this cohort (n=1,741; 3-years follow-up). A composite score was created for each of these parameters, embedding handgrip, arm and leg strength, and timed up and go (TUG), chair-stand and walking speed, respectively. In both sexes, a more evenly distributed protein intake was positively associated with muscle strength throughout the study, but not with mobility, and did not predict strength or mobility rate of decline (33). A recent and smaller cross-sectional study (n=140) reported a positive association between a more even protein distribution and gait speed, but not with total SPPB and its other single components. Muscle mass and strength were not measured (217). Finally, no such associations were found in a small cross-sectional study (n=99) of successful agers, though not representing a broad range of functional capacity, thus pointing to the fact that dietary protein intake and distribution may have more impact in those with impaired muscle health (218). Since both total and mealtime distribution of protein may influence muscle mass and function, a per-meal minimal intake has been advocated (219). Testing this rationale in 4,123 adults >50 years of the 2011-2014 National Health and Nutrition Examination Survey (NHANES), grip strength was positively associated with consumption of ≥25 g protein/meal on 2 or more eating occasions compared to only one eating occasion of the same amount. This relationship disappeared when adjusted for multiple

covariates including total daily protein intake which was related with grip strength (220). In contrast, in 1081 older adults (50-85 years) of the 1999-2002 NHANES, Loenneke et al. (2016) found that participants consuming at least 2 meals containing 30-45 g protein/meal had the greatest leg lean mass and strength, and those consuming at least one meal/d of ≥30 g protein/meal had greater responses, than those with no meal reaching the 30 g threshold (221). Adjusted models included age, sex, ethnicity, smoking, physical activity, total carbohydrate and fat intakes and relative protein intake (g/kg). It appears that a threshold of 30 g vs. 25 g may explain discrepancies between studies but more likely, that lower limb muscles respond more to protein intake due to solicitation for mobility than handgrip strength.

In summary, in healthy older adults, mealtime protein distribution may have more impact when other anabolic stimuli are minimal, i.e., at low total protein intakes and physical activity levels, which remains to be investigated in longer-term intervention studies. To this date, per-meal protein doses sufficient to generate anabolism in persons at risk of malnutrition and sarcopenia are still unknown.

3.5 Leucine

Leucine is the most potent amino acid to stimulate MPS from activation of the nutrient and growth factor-sensing mammalian target of rapamycin complex 1 (mTORC1) and in turn, its downstream targets ribosomal protein S6 kinase 1, translation initiation factor 4EBP-1 and elongation factor 2 (222, 223). Though not fully elucidated, the mTORC1 activation by leucine is thought to occur at the lysosome through a cascade involving Ragulator Rag GTPases and vacuolar H+-ATPase (224, 225). Three to four fold increments in plasma leucine appear to be required to elevate intracellular concentrations and augment MPS (226) provided that other essential amino acids are also available to sustain greater protein synthesis (227).

Interventional studies

Very few cohort studies have associated dietary leucine intake to muscle mass. Because leucine being ubiquitously found in all proteins, though in animal more than plant proteins, its intake is practically impossible to dissociate from total protein intake from foods. Thus, most of the evidence on leucine's effect has been accrued from supplement studies, most of short-term duration. Two meta-analyses published in 2015 concluded differently (228, 229). The one from Xu et al. (2015) included 9 randomized controlled trials (RCTs), 4 testing acute post-challenge

responses (three in healthy, one in cancer participants) and 5 longer-term, ranging from 10 days to six months (in participants either healthy, with type 2 diabetes, or had polymyalgia, and were on bed rest, or exercising). A pooled effect of 1.08 (95% CI: 0.50-1.67) was found on acutely increasing MPS but no effect was observed on lean body mass or leg lean mass from longer-term interventions. The meta-analysis by Komar et al. (2015) included 16 studies testing leucine-rich supplements in a wider variety of participants, also including frail, sarcopenic and institutionalized. Subgroup analysis revealed a mean effect of 1.14 kg (95% CI:0.55-1.74) increase in lean body mass in favor of leucine-rich supplements in sarcopenic, but not in healthy participants. In both meta-analyses, leucine was either given as pure crystalline powder, or as part of essential amino acid drinks, complete medical formula or whey protein, at doses ranging from 2 g/d to 17.6 g/d. This considerable heterogeneity in population, study design and type of supplements studied precludes firm conclusions but point to plausible effects in persons having or at risk of sarcopenia. The question as to why the acute stimulation of MPS by leucine supplements does not seem to translate into measurable changes in lean body mass in healthy older adults remains open. Insufficient usual dietary protein intake, poor long-term compliance and perhaps habituation to a sustained stimulus may explain negative findings.

More recent studies from the group of Phillips et al. have revived a promising anabolic role for leucine (230-232). Using an integrative measure of myofibrillar protein synthesis (MyoPS) over 3 d in well-controlled crossover feeding studies, providing all foods to older men, 5 g of leucine added to each of the 3 daily meals resulted in augmented MyoPS in both the rested leg and the one submitted to unilateral resistance exercise (232). Interestingly, this effect was seen at both low (0.8 g/kg/d) and higher (1.2 g/kg/d) daily protein intakes. In healthy older women, 10 g of whey protein added with 3 g of leucine were compared to 25 g of whey protein intrinsically containing 3 g leucine, taken twice daily for six day. Results showed that the lower protein, leucine-matched supplement was as effective as the 25 g protein dose at increasing acute and integrated MyoPS which could represent a practical alternative for older women with typical low appetite (230). Lastly, Devries et al (2018) tested the twice daily consumption of 15 g milk protein drink containing 4.2 g leucine against 15 g mixed protein drink containing 1.3 g leucine, as part of a diet providing 1 g protein/kg/d, under the same protocol in older women (231). Greater acute postprandial and integrated MyoPS responses over six days were found with the higher leucine containing drink. Altogether, these positive results obtained in rigorous conditions

are promising and warrant corroboration in longer-term interventions to demonstrate potential benefits of leucine for preserving muscle mass and function.

3.6 Vitamin D

3.6.1 Vitamin D, physical function, and muscle mass and strength

Vitamin D is a key nutrient in musculoskeletal health. In adults, vitamin D deficiency is associated with bone diseases including osteomalacia, osteopenia and osteoporosis, and increases the risk of fractures (233). However, bone health is not the only physiological dimension to be impacted by vitamin D since its involvement has been evidenced in cardiovascular disease, autoimmune diseases, and cancer prevention (234), among others. There has been growing interest in the implications of vitamin D status in physical function of older adults given the high prevalence of vitamin D deficiency in this population (235). The ubiquity of vitamin D receptors (VDR) in various tissues, including muscles, is well recognized (236). From its binding to VDRs, vitamin D mediates genomic and non-genomic effects in muscle cells; it namely promotes muscle contractility through calcium influx, myoblast differentiation and insulin sensitivity of muscles (237). The current RDA for vitamin D intake is 600 IU/d for persons aged 1-70 years and 800 IU/d for older adults (≥71 years) which translates into serum 25-hydroxyvitamin D (25(OH)D) level ≥50 nmol/L for skeletal health (238).

Observational studies

While large cross-sectional studies corroborate a relationship between insufficient level of serum 25(OH)D (<50 nmol/L) and low physical performance (239-244), mobility (239, 241-243), muscle strength (239, 240, 242, 243, 245, 246) and greater disability (239, 246) in free-living older adults, the association with muscle strength was not found in a cohort of older women (>90% with vitamin D insufficiency) (247). In 2017, a meta-analysis of 17 cross-sectional and five longitudinal studies (n=54-4,100) provided fair evidence that seniors with low vitamin D status, regardless of the cut-point used for its definition, had slower usual gait speed compared to those with normal status (-0.18 m/s in vitamin D deficient; ≤25 nmol/L) (248). Physical performance (by TUG) was also associated with vitamin D deficiency. More recently, Vaes et al. (2018) confirmed cross-sectional associations between vitamin D insufficiency, gait speed (n=745) and TUG (n=488) in older men and women aged ≥65 years; interestingly, frail individuals were at higher risk of being vitamin D insufficient compared to non-frail (249), an

association that has also been ascertained in a meta-analysis (250). No link with muscle strength was found in this cohort.

Vitamin D insufficiency has also been longitudinally associated with greater risks of disability (242, 251, 252), decline in physical performance (244, 253) and handgrip strength (254) in healthy older adults. However, few groups did not find such associations (n=988-2,099; 2.5 to six years follow-up) (242, 255). Since then, Granic et al (2017) studied very old adults (n=845, age ≥85 years) and found a greater handgrip strength decline in men of the lowest 25(OH)D season-specific quartile over 5 years, but this was not seen in women (256). Though causal effect cannot be concluded from these observational studies, altogether the evidence suggests that interventions should aim at targeting at risk populations, namely individuals with vitamin D insufficiency or deficiency, and frail seniors to favour better mobility or delay the onset of disabilities.

Interventional studies

Systematic reviews and meta-analyses examined the benefits of vitamin D supplementation on physical performance, muscle mass and strength in community- and/or institution-dwelling seniors (41, 257-259). The importance of considering baseline serum 25(OH)D concentrations has been emphasized, since individuals with vitamin D deficiency appear to be more responsive to supplementation (257). Modest mean differences in TUG (3 studies; n=551) and postural sway (3 studies; n=413) independent of dose were reported following vitamin D supplementation in a first meta-analysis; yet authors observed that all studies providing high daily vitamin D doses (i.e., 800 IU to 1,000 IU) supported beneficial effects on balance and lower extremity muscle strength (41). A second meta-analysis's age subgroup analysis showed a favorable effect of vitamin D supplementation, with or without calcium, on muscle strength in older adults ≥65 years and especially, greater improvement in institutionalized compared to free-living individuals (259). In line with these previous studies, the most recent meta-analysis of RCTs (2017) conducted in community-dwelling older adults confirmed a slight improvement of -0.3 seconds (95% CI: -0.1 to -0.5; 5 studies; n=1,260) in the TUG test following supplementation, but no overall increase in handgrip strength was detected (7 studies; n=1,452) (258). The included RCTs assessing TUG performance provided doses ranging from 800 IU to 2000 IU/d during 10 weeks to 20 months, and one study 150,000 IU every 3 month for a 9-month period. The latter revealed an effect in favor of the placebo (260),

and no effect was found from 2000 IU/d for 10 weeks (261). Further, one additional study by Bischoff-Ferrari et al. (2016), not included in the abovementioned meta-analysis, established a null effect of monthly doses of 24,000 IU + calcifediol and 60,000 IU on lower extremity function (by SPPB) after 1 year and led to higher fall incidence compared to 24,000 IU/month (262). This finding strengthens that benefits are observed with vitamin D doses within the range of 800-1000 IU/d, but not necessarily at higher doses.

Heterogeneity between RCTs (doses and type of supplement, duration of the intervention, participants baseline vitamin D status) and few discrepancies among meta-analyses with regards to selection criteria makes comparison between studies difficult. Nonetheless, in light of the pooled evidence, vitamin D supplementation should be considered to improve physical performance in older adults and perhaps for muscle strength in most likely frail seniors.

3.6.2 Vitamin D and fall prevention

Unintentional falls are the leading cause of injury death in seniors aged ≥65 years in the US (263). Beyond musculoskeletal-related functions, risk factors for falls belong to multiple intrinsic (biological) and extrinsic (socio-economic, environmental, and behavioural) dimensions; balance, gait abnormalities, chronic conditions such as neurological disorders, cognitive impairment, vision, fear of falling, use of medications, environmental hazards all contribute to the occurrence of falls (264).

Interventional studies

Vitamin D and falls is an area that has been extensively studied; trials have tested the impact of vitamin D supplementation on the prevention of falls and numerous meta-analyses examined the overall reported effects. However, due to substantial differences in selection criteria between meta-analyses (265), conflicting results remain. In their high-quality meta-analysis of 14 trials and 27,522 participants, Bolland et al. reported a non-meaningful effect of vitamin D, with or without calcium, on the relative risk (0.96; 95% CI: 0.91-1.01), and highlighted the fact that it would unlikely change with additional future studies (266, 267).

In 2018, an updated evidence report and systematic review of fall prevention interventions in community-dwelling older adults was published for the US Preventive Service Task Force. From the 7 studies of vitamin D supplementation included in the review, authors found inconsistent results possibly due to high heterogeneity (268). Also, one trial in older

women showed deleterious effects of annual 500,000 IU vitamin D_3 dose on falls and fractures (269). Following this report, the new recommendation advises against vitamin D supplementation for fall prevention in community-dwelling non-osteoporotic and non-vitamin D deficient older adults ≥65 years (270).

3.7 N-3 polyunsaturated fatty acids

N-3 PUFA are consumed as eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) or as alpha-linolenic acid (ALA; 18:3 n-3) of which a very limited fraction is converted to EPA (8 and 21% conversion rates) and DHA (~0 and 9%) in men and women, respectively (271, 272). N-3 PUFA are well-known for their anti-inflammatory properties and their role in the development and maintenance of neuro-cerebral functions (273-275). In a large prospective cohort study of older adults, plasma n-3 PUFA levels were associated with a 27% reduction in total mortality risk across quintiles, increasing the life expectancy of individuals in the highest quintile by ~2 years (276). This association with mortality was essentially ascribed to docosapentaenoic acid (DPA; 22:5 n-3), DHA and EPA status, and was more pronounced for cardiovascular disease mortality, including coronary heart disease and ischemic stroke. No RDA recommendations exist for n-3 PUFA, only Adequate Intake (AI) were established for ALA (1.6 g/d for men and 1.1 g/d for women, aged ≥14 years) (194); however, the majority of expert groups endorse intakes of 250-500 mg/d EPA and DHA for cardiovascular health, which translates into ~2 servings (140 g, 5 oz) of fatty fish per week (277). Though older adults tend to consume more fish that are high in n-3 PUFA compared to younger adults, their intake remains suboptimal with a mean of 0.19 ± 0.02 oz/d (278). Although still elusive, the anabolic role of n-3 PUFA on skeletal muscle is thought to be owed to a reduction in pro-inflammatory cytokines, myosteatosis, improvement of insulin sensitivity (279), stimulation of muscle protein synthesis via the mTOR-p70S6k signalling pathway (280) and a diminution of mitochondrial reactive oxygen species emission (281).

Observational studies

Inconsistent associations were found between n-3 PUFA dietary intakes and muscle function from scarce cross-sectional evidence (282, 283) and to our knowledge, no study examined this relationship since then. Previous evidence showed higher plasma n-3 PUFA levels, an objective biomarker of dietary intake (284), to be associated with better physical

performance and gait speed in healthy older adults at baseline and with a lower risk of poor physical performance (odds ratios, OR=0.21, 95% CI: 0.08-0.53; n=884) over three years (relationship owing to EPA and DHA) (285). This cross-sectional association with gait speed was also confirmed recently (286); however one study did not find red blood cell membrane EPA and DHA levels to be associated with physical performance in participants at risk of cognitive decline after controlling for covariates (287). As for muscle strength, the relationship is unclear as evidenced by one study reporting no association with plasma n-3 PUFA once adjusted for covariates (288).

Longitudinal analyses with follow-up periods ranging from three to five years were performed in the cohorts cited-above. One study found baseline total plasma n-3 PUFA and DHA to be associated with the incidence of self-reported mobility disability after five years in healthy older women only (OR=0.48, 95% CI: 0.25-0.93) suggesting that there may be a sex-specific biological role for DHA in mobility (289). However, other studies found no relationship with the relative change in muscular parameters (288), decline in gait speed (289, 290) or physical performance (290) after three and five years. As frequently encountered with longitudinal studies, the decline in the main outcome or the incidence of the condition being looked at may not have been sufficient to detect significant associations. Also, considering the limited evidence available, it can only be concluded that cross-sectional and longitudinal associations of plasma or erythrocyte n-3 PUFA concentrations and muscle mass or function remain uncertain. In future studies, analysis with EPA and DHA levels should consistently be reported, as detected relationships with total n-3 PUFA were driven by these specific fatty acids.

Interventional studies

One study previously reported a modest effect of a six-month 1.2 g/d n-3 PUFA (720 mg EPA and 480 mg DHA) supplementation on gait speed in 126 seniors aged ≥65 years, but not on body composition and strength (291). Three additional recent studies have tested daily 1.1-3.36 g EPA and DHA supplementation during three to six months. Following a 6-month 4 g/d n-3 PUFA supplementation (1.86 g EPA, 1.5 g DHA) compared to corn oil placebo, Smith et al. (2015) detected gains of 2.3 kg in handgrip strength (95% CI: 0.8, 3.7 kg) and of 3.6 % (95% CI: 0.2, 7.0 %) in thigh muscle volume in community-dwelling older adults (n = 60; aged 60 to 85 years) (43). The authors estimated that these beneficial changes would result in a 2 to 3 year-prevention of muscle mass and function decline normally associated with aging. Similarly,

Logan et al. (2015) reported a 4% increase (1.6 kg ± 0.7 kg, p=0.01) in muscle mass as measured by bioelectrical impedance analysis (BIA) and a 7% improvement in the TUG in the intervention group (5 g/d fish oil; 2 g EPA, 1 g DHA) compared to an olive oil placebo, after three months (n=24 healthy women; aged 60-76 years; those who consumed >1 meal of fish per week or took n-3 supplements were excluded) (42). Yet, another three-month trial showed no effect of a 1.3 g/d n-3 PUFA supplementation (660 mg EPA, 440 mg DHA) on body composition (by BIA), TUG, handgrip strength and gait speed in 53 older adults (aged ≥65 years) at risk (1 SD below the mean ALM index of a reference population) or having low lean mass at baseline (292). This absence of effect is possibly due to the low EPA and DHA doses provided and the relatively short duration of the intervention (291, 292). Though biomarker measure of n-3 PUFA was not reported in the latter study, those that provided doses of 3.0 and 3.36 g EPA and DHA effectively increased serum and red blood cells n-3 PUFA concentrations after three (42) and six (43) months respectively, versus no change in the control groups. The effect of doses between 1.1 and 3.0 g was not tested in untrained older adults.

It is of note that the methodologies of these trials used different approaches to body composition assessment, duration of interventions, measures of n-3 PUFA status and doses of supplement which prevents direct comparison of studies. Future trials should report at least one measure of n-3 PUFA circulating levels as well as its changes to allow proper interpretation of the efficacy of the supplement (284). Yet, a three-month intervention appears long enough to see improvements, but only doses ≥3.0 g/d showed compelling increment in functional measures and muscle mass and volume (42, 43). While n-3 PUFA may prevent sarcopenia in healthy older adults, its effect in sarcopenic seniors and those losing autonomy remains to be investigated.

3.8 Combined supplements of protein, leucine, vitamin D and n-3 PUFA

While increased protein intakes and supplements in leucine, vitamin D and n-3 PUFA support potential gains in muscle mass and function when consumed individually, the combination of these nutrients may provide further benefits. Four recent, randomized, double blind placebo-controlled studies tested a combined supplement of high-quality protein and vitamin D, without exercise intervention, on lean mass, strength and physical performance (44-47); two of them included added leucine to the mix (44, 45), and one included n-3 PUFA and creatine (46). A 13-week multicenter study conducted in 380 sarcopenic older individuals aged

≥65 years with high disability risk found a beneficial effect of a 800 IU vitamin D, 3 g leucine and 20 g whey protein supplement, given twice daily, on the chair-stand time (-1.01 s, 95% CI: -1.77, -0.19), but not on physical performance, mobility and strength compared to the control group receiving an isocaloric placebo (44). A very slight increase in ALM (0.17 kg, 95% CI: 0.004, 0.338) was reported, though within the 1.2% lean body mass measurement error by dual-energy X-ray absorptiometry (DXA) (293). The same supplement was provided, before breakfast only, to 24 healthy older men and similarly showed a modest gain in ALM (estimate difference of 0.37 kg) and leg lean mass after 6 weeks (45). Interestingly, dietary protein intake was displaced by the supplement in the intervention group as shown by the same total protein intake between groups when accounting for the supplement. The modest improvement in outcomes may be attributable to the change in meal protein intake distribution, as breakfast protein consumption was higher in the intervention group at the end of the trial with an average of >25 g at each meal compared to ~10 g at breakfast, at baseline and in the control group. Again, the supplement did not show any improvement on handgrip strength and physical performance. Bell et al. examined the effect of a similar multi-nutrient supplement of 30 g whey protein, 2.5 g creatine, 500 IU vitamin D, 400 mg calcium and 1.5 g n-3 PUFA; 700 mg EPA, 445 mg DHA consumed 2x/d (1 h after breakfast and 1 h before going to bed) against a placebo of 22 g maltodextrin on strength and lean mass in 49 healthy older men (aged 73 ± 1 years) during six weeks alone and in combination with high intensity interval trainings during the 12 following weeks (46). After six weeks of supplementation alone, both lean mass and strength increased in the intervention group; the authors reported gains of 0.4 kg for ALM and 3% for the sum of isotonic strength, but the intervention was only superior to the control for the latter. Similarly, Bo et al. found an improvement in handgrip strength (1.91 ± 4.24 kg, p=0.020) from a long, six-month intervention of 22 g whey protein, 702 IU vitamin D and 109 mg vitamin E, consumed before breakfast and dinner, in 60 sarcopenic men and women aged 65-80 years (vs. isocaloric placebo) (47). Though participants receiving the treatment did not gain muscle mass, a trend in decline was seen in the control group, suggesting that the supplementation was protective of muscle mass loss over six months. The authors did not find any effect from the intervention on mobility and physical performance.

Importantly, none of these studies reported serious adverse effects from supplements. Combined supplements showed favorable effects on muscle mass but may be more effective at

stimulating MPS in healthy and promoting muscle mass maintenance in sarcopenic older adults. Their impact on muscle strength is inconsistent and no improvement in physical performance was observed. Though isolating the effect of each nutrients from a combined supplement is impossible, providing high-quality proteins, leucine, vitamin D and n-3 PUFA all together appears to be promising in the prevention of sarcopenia, while being safe. Indeed, more longer-term (i.e., >six weeks) research of multi-nutrient supplementation involving populations at risk of greater muscle mass decline, such as frail individuals and those losing autonomy is needed.

3.9 Conclusion

Proteins, leucine, vitamin D and n-3 PUFA may individually play a protective role in skeletal muscle health. Inadequate intakes of these nutrients could therefore lead to several prejudicial conditions such as sarcopenia, frailty, loss of mobility and physical function, morbidity and mortality. Groups of authors recommend protein intakes of 1.0-1.2 g/kg/d for older adults, which is higher than the currently established RDA but may not always be achievable through dietary sources for some individuals with reduced appetite. The manifestation of anabolic resistance associated with aging explains, among other factors, the increase in protein requirements. In addition, an even distribution of proteins throughout the day may favor the reach of the anabolic threshold to maximally stimulate MPS. However, this effect remains to be confirmed by long-term intervention studies and may differ between healthy and malnourished individuals. The effect of vitamin D on physical function seems to be essentially beneficial in individuals with prior vitamin D deficiency. Also, doses of 800-1,000 IU/d appear more effective compared to lower doses. With respect to n-3 PUFA, recent evidence suggests that EPA and DHA doses of ~3 g/d may have positive impact on physical performance, muscle strength and muscle mass in older adults and this minimal amount may be required for beneficial effects when provided alone, i.e., not combined to other nutrients. Heterogeneity makes comparison of studies difficult such that future studies should have standardized methods.

Key nutrient supplementation in older adults is of interest in the prevention of sarcopenia and frailty since it is a simple treatment approach without major side effects and of low costs. Intervention studies testing a combined nutritional supplement are to be explored given the potentially additive effects of proteins, vitamin D and n-3 PUFA in the prevention of muscle mass and function loss.

Connecting Statement 2

The literature review summarized the evidence on aging and related conditions, namely sarcopenia and cognitive impairment. An overview of observational and intervention studies on the associations and roles of nutrition, particularly dairy products, in muscle and cognitive health was provided.

The currently available criteria and definitions for sarcopenia diverge in the literature. Expert groups used various approaches to determine cut-points; some are recommending normalization for height, others for BMI or adjustment for fat mass; and some encourage the combination of muscle mass and strength or physical performance, while others do not. The lack of a consensus to define sarcopenia and the distancing from its original definition in recent research motivated Bulow et al. to publish a call for viewpoints to which I felt important to respond:

Start of commentary

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Uprooting sarcopenia from its origins by introducing body fat to its definition

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TO THE EDITOR: We are in complete agreement with the Viewpoint of Bulow et al. (294) and wish to raise another argument in favor of rejuvenating the term sarcopenia. Not only is the consensus definition confusing enough by combining two distinct—although related—entities, lean mass and physical function, but new cut points for low lean mass involving measures of body fat further confuses defining the condition. Indeed, the FNIH Sarcopenia Project suggested to normalize ALM to BMI as an indicator of low lean mass (295). Because excess body fat negatively impacts physical function, especially gait speed, balance, and any

weight-bearing physical test, lower ratio of lean mass relative to body weight or BMI best predicts poor physical function, logically. Largely influenced by high BMI, a low ratio masks the absolute estimation of lean mass, which may be normal in many. This could lead to misidentification of true sarcopenia, i.e., low ALM relative to height, which in itself carries significant morbidity and mortality risks in several clinical conditions. In contrast, having some degree of excess fat with normal muscle mass may be impeding physical function but may be protective for survival in older adults (296) and in clinical conditions, such as in cancer (297). We strongly support reserving the term sarcopenia for defining low lean mass relative to height, which would resonate in geriatrics but other clinical fields as well, and using dynapenia, as suggested by others (54), to define low strength. The term sarco-dynapenia could then be self-explanatory to all.

End of commentary

Before deriving empirical cut-points for low strength (dynapenia), to identify persons at risk of limited physical performance and for low ALM (sarcopenia), as a determinant of low strength, I interrogated the CLSA database as the choice database to objectively understand the associations between muscle mass, strength, fat mass and physical performance. In addition, I compared the prevalence and characteristics of individuals identified using different cut-points, i.e., ALM/ht^2 vs. ALM/BMI. This study performed in the large CLSA cohort was designed to provide standard population- and sex-specific criteria for sarcopenia and dynapenia to be used by clinicians and researchers.

CHAPTER 4: *Manuscript 1:* Definition and diagnosis of sarcopenia

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Physical function-derived cut-points for the diagnosis of sarcopenia and dynapenia from the Canadian Longitudinal Study on Aging

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Because of a mislabelling of appendicular lean mass coming from the CLSA database, a Corrigendum was published on October 7, 2021 (doi: 10.1002/jcsm.12811). This version of the manuscript includes corrected results.

4.1 Abstract

Background: Aging is associated with sarcopenia (low muscle mass) and dynapenia (weakness) leading to disability and mortality. Widely used previous cut-points for sarcopenia were established from dated, small or pooled cohorts. We aimed to identify cut-points of low strength as a determinant of impaired physical performance and cut-points of low appendicular lean mass (ALM) as a predictor of low strength in a single, large and contemporary cohort of community-dwelling older adults and compare these criteria with others.

Methods: Cross-sectional analyses were conducted on baseline data from 4,725 and 4,363 community-dwelling men and women (65-86 years, 96.8% White) of the Canadian Longitudinal Study on Aging (CLSA) comprehensive cohort. Physical performance was evaluated from gait speed, timed up and go, chair rise, and balance tests; a weighted-sum score was computed using factor analysis. Strength was measured by handgrip dynamometry; ALM, by dual-energy X-ray absorptiometry and ALM index (kg/m^2) was calculated. Classification and regression tree (CART) analyses determined optimal sex-specific cut-points of ALM index predicting low strength and of strength predicting impaired physical performance (score <1.5 SD below the sex-specific mean).

Results: Modest associations were found between ALM index and strength, and between strength and physical performance score in both sexes. ALM index was not an independent predictor of physical performance score. Cut-points of <33.1 kg and <20.4 kg were found to define dynapenia in men and women respectively, corresponding to 21.5% and 24.0% prevalence rates. Sarcopenia cut-points were <7.31 kg/m^2 in men and <5.43 kg/m^2 in women; prevalence rates of 22.9% and 15.6%. Overall, 8.6% of men and 6.1% of women had sarco-dynapenia. Sarcopenic were older and had lower fat mass and BMI than non-sarcopenic participants. While the agreement between current criteria and the updated European Working Group for Sarcopenia in Older Persons recommendations (EWGSOP2) was fair, we found only slight agreement with the FNIH Sarcopenia Project. Older persons identified with sarcopenia as per the FNIH criteria (using ALM/BMI as the index) have higher BMI and fat mass compared to non-sarcopenic and have normal ALM index as per our criteria.

Conclusions: The proposed function-derived cut-points established from this single, large and contemporary Canadian cohort should be used for the identification of sarcopenia and dynapenia in Caucasian older adults. We advise on using criteria based on ALM index in the diagnosis of

sarcopenia. The modest agreement between sarcopenia and dynapenia denotes potential distinct health implications justifying to study both components separately.

4.2 Introduction

The population is aging therefore increasing the prevalence of poor health outcomes. Muscle mass and strength decline at a rate of 0.5-1% and 2-3% per year, respectively, after the age of 50 years (17) leading to increased disability, loss of autonomy, morbidity, decreased quality of life and mortality (184). Sarcopenia, originally defined as the age-related loss of muscle mass by Rosenberg (298), was attributed an international classification of disease-10th revision (ICD-10) code in 2016 recognizing the condition as a disease (21). Though the term sarcopenia is now consensually used to define the combined entities , i.e. the loss of muscle mass and strength (184), some authors argue that sarcopenia (low muscle mass) should be considered separately from dynapenia (low muscle strength) (54). Recently, Bulow et al. have called for rejuvenating the term sarcopenia arguing that adding muscle strength and physical function, i.e., gait speed, to the definition of sarcopenia for the condition to be clinically relevant resulted in a tautology (294, 299). Four major groups have been working toward a consensus for defining sarcopenia: the European Working Group on Sarcopenia in Older People (EWGSOP), the International Working Group on Sarcopenia (IWGS), the Asian Working Group on Sarcopenia and the Foundation for the National Institute of Health (FNIH) Sarcopenia Project. While the former three endorsed cut-points determined arbitrarily for low lean mass, strength and gait speed from previously published work (60, 62, 63, 184), the FNIH determined empirical cut-points of low lean mass relative to body mass index (BMI), and strength from data of pooled cohorts (59). Despite these collective efforts, an ongoing debate subsists. The lack of consensus to define sarcopenia and the use of different cut-points prevent standard diagnosis in clinical settings and hinder comparison of research studies. Presently suggested cut-points carry important limitations, namely that they were derived from cohorts of limited sample sizes (73, 300, 301), from up to 20-year-old data (59, 73-75, 295, 300, 301) when evaluation methods and the population have evolved over the last decades e.g. with increased prevalence of obesity, or from the aggregation of cohorts introducing important heterogeneity with regards to assessment methods (59, 74).

To address these limitations from present diagnostic cut-points and based on the hypothesis that low strength is a predictor of low gait speed and that lean mass is a predictor of low strength as demonstrated by the FNIH project (76, 77), our aim was twofold: 1) to define cut-points for dynapenia, i.e., low strength, to identify persons at risk of limited overall physical

performance and for sarcopenia, i.e., low lean mass, as a predictor of dynapenia, and 2) determine the prevalence of each condition separately and combined, i.e. sarco-dynapenia, from the largest and contemporary Canadian Longitudinal Study on Aging (CLSA). These cut-points were to be clinically relevant and literal to their original definitions.

4.3 Methods

Subjects

Baseline data were from the large, nationally representative CLSA of 51,338 community-dwelling men and women aged 45-86 years, recruited from 2011 to 2015. Participants from 11 cities across Canada (Victoria, BC; Vancouver, BC; Surrey, BC; Calgary, AB; Winnipeg, MB; Hamilton, ON; Ottawa, ON; Montreal, QC; Sherbrooke, QC; Halifax, NS; and St-John's, NFLD) were randomly selected based on age and sex strata in each province through the Canadian Community Health Survey (CCHS), provincial healthcare registries and from random digit dialling, and will be followed every 3 years for ≥20 years (302). Subjects were excluded from the sample frame if they were residents of one of the three territories, if they lived on a First Nation reserve, in institutions, or were full-time members of the Canadian Armed Forces. Individuals were not eligible if they were unable to communicate in English or French or had cognitive impairment that precluded the ability to provide informed consent, at baseline. The study was approved by the CLSA research site ethics boards and all participants of the CLSA study provided informed consent to use their data in research (303). This present study was approved by the McGill University Health Centre Ethics Board (REB 16-068-MUHC). Cross-sectional analyses were performed on data from the comprehensive cohort participants (n=30,097, living within 25-50 km from CLSA collection data sites). This specific cohort provided core information by phone interview, and additional information from face-to-face interview questionnaires through a computer-assisted personal interview software and from site-based visits of neuropsychological, physical function, body composition and clinical assessments performed by trained individuals (302). For the current study, participants who were aged <65 years, who had multiple sclerosis, Alzheimer's disease, effects from stroke or transient ischemic attack, Parkinson's disease, surgery within the last 3 months, polio, unstable heart condition within the last 3 months, pulmonary embolism within the last 6 weeks, chemotherapy within the last 4 weeks, dialysis and missing or improper dual-energy X-ray absorptiometry (DXA)

measurement, grip strength or physical tests results were excluded. The total analytic sample was 9,088 participants (4,725 men and 4,363 women) (Online Resource, **Supplementary Figure** 4-1).

Body composition

Lean soft tissue mass (lean mass) and fat mass were measured by DXA (Hologic Discovery A^{TM} densitometer) of the whole body according to standard procedures. Appendicular lean mass (ALM; kg) was calculated as the sum of the upper and lower limbs lean soft mass. ALM index was obtained by dividing ALM over height squared (kg/m^2) as previously suggested by Baumgartner et al. (300). Weight over 204 kg, height over 1.88 m, exposition to an X-ray with contrast material or participation in a nuclear medicine study within the last 7 days before the DXA were contraindications to receiving the scan. There are risks for erroneous ALM assessment for some individuals who unproperly fit within the scanning area of the DXA bed. For this reason, only those with a valid DXA weight measure were retained in the analyses. Using a Bland and Altman plot, a participant's DXA weight was considered to be valid when the value of the difference between their DXA and scale weight was within the 95% confidence interval of the population mean difference (304).

Anthropometry

Anthropometric evaluation included body weight (140-10 Healthweight Digital Physician Scale) and standing height (Seca 213 stadiometer) measured to the nearest 0.1 kg and 0.1 cm, following standard procedures; for each, the average of two measurements was used. Body mass index (BMI) was calculated by dividing the weight (kg) by the height (m) squared.

Muscle strength

Maximum muscle strength was measured by hand-held dynamometry (kg; Tracker Freedom® Wireless Grip). Participants were tested on their dominant hand and were assessed while sitting on a chair without arm rests, feet flat on the floor, arms close to the body with the elbow flexed at 90 degrees and the non-dominant hand supporting the device. Participants were instructed to squeeze the dynamometer as hard as they could, 3 times with a 15-second rest between trials. The highest value was used for the current analyses. This assessment has shown excellent test-retest reliability (intraclass correlation coefficient (ICC)=0.99) (305).

Physical performance tests

Four-meter Walk test

Gait speed was used to assess mobility. Studies have found gait speed to be strongly associated with adverse outcomes including disability, falls and mortality (306, 307). Subjects were asked to walk a 4-meter distance at their regular pace. Participants were allowed one trial before the actual test. Gait speed was calculated as 4 meters divided by the time to walk this distance in seconds (m/s).

Timed up and go (TUG)

TUG test is a valid measure of mobility, balance and the ability to perform activities of daily living (308) and is also associated with mortality (309). Participants were seated in an arm chair and were timed on their ability to stand up from the chair, walk a 3-meter course, turn around, walk back to the chair and sit down again (in seconds). The use of daily living assistive devices was permitted for both the four-meter walk and TUG tests. Excellent test-retest reliability, with an ICC of 0.99, was reported for this test (308).

Chair rise test

This test is used to measure performance of lower extremity and balance (310). Participants were asked to rise from a chair and sit back down, 5 times, as quickly as possible, with their arms crossed on their chest. The time was recorded from the initial sitting position, with their back rested on the back of the chair and their knees bent at a 90-degree angle, to the fifth and final standing position (in seconds). The average time per sit-to-stand was used for analyses. In community-dwelling older adults test-retest reliability ranges from good to high (ICC: 0.890 and 0.957) (311, 312).

Standing Balance test

Standing balance is a valid and reliable test (313), and is used as a predictor of falls (314). Positioned at a one-meter distance from the wall, participants were instructed to stand in balance on one foot, for as long as possible with a maximum time of 60 seconds. The test was repeated on the other leg and the shortest time recorded was used for analyses.

Physical performance score adjusted for BMI

A physical performance score including the four tests, i.e., TUG, gait speed, chair rise and balance tests, was created using the weighted-sum score method. Physical performance tests measured as time were natural-log-transformed for normalisation of the distributions (all except

gait speed). All test results were then scaled to Z scores and entered in a factor analysis to obtain the loading value of each test. For every subject, a physical performance score was calculated; the Z score test result of all four physical tests was multiplied by its associated loading value before summation. This method accounts for the weight each test has in measuring the physical performance factor (315). Given the well-known relationship between BMI and mobility disability, the residual-based method was used to adjust participants' physical performance score for BMI (316, 317). A binary variable was created to classify participants as having limited physical performance using <1.5 SD below the sex-specific mean of the physical performance score adjusted for BMI.

Potential covariates

Covariates included sex, age, ethnicity, smoking, number of medications, four self-reported and ascertained chronic diseases (318), i.e., heart disease, kidney disease, chronic obstructive pulmonary disease (COPD) and type 2 diabetes which were associated with low lean mass, strength or physical performance. Physical activity level was evaluated by the Physical Activity Scale for Elderly (PASE) (319). The abbreviated version of the Seniors in the Community Risk Evaluation for Eating and Nutrition (SCREEN II-AB), an 11-item tool to evaluate weight change, meal intake and its risk factors, was used to evaluate the risk of poor nutritional state (320).

Statistical analysis

Analyses were conducted separately by sex due to recognized differences in lean mass and strength between men and women. Baseline characteristics are presented as means ± standard deviations (SD). Relationships between physical performance score, handgrip strength and ALM index were examined using path analyses. Functionally-derived cut-points were identified using classification and regression tree (CART) analysis which model is represented by a binary tree. It uses recursive partitioning to optimally classify individuals with a condition (binary dependent variable) from one or several factors (independent variables) by computing all possible factors splits while optimizing purity. It is a non-parametric method that can be performed for prediction purposes and establishing diagnostic tests (321). In this study, CART analysis was carried out in two steps: (i) the first analysis was performed to identify cut-points for dynapenia (low handgrip strength) as a predictor of physical performance impairment, (ii) using the cut-points found in (i), a second model was performed to derive cut-points for

sarcopenia (low lean mass) as a predictor of dynapenia. Considering the large sample size, split-sample was used for internal validation of the predictive model (80% random training dataset and remaining 20% test dataset) (322). Analyses were performed using the Gini index as a cost function to maximize homogeneity in groups generated from the split, with regards to the outcome variable. CART analyses were previously used to derive cut-points of low lean mass and strength (76, 77, 323). Agreement between cut-points was examined using positive and negative percent agreement (PPA and NPA) and Cohen's kappa coefficient (κ) as recommended by the Food and Drug Administration US federal agency in the absence of a diagnostic gold standard (324). Sensitivity analysis to evaluate prediction capacity of the cut-points according to subgroup characteristics of the population was conducted using logistic regression models.
To allow comparison with previous sarcopenia definitions, the present cut-points for low strength and lean mass were combined (sarco-dynapenia) and applied to our study population. Characteristics of sarco-dynapenic and non-sarco-dynapenic participants were compared using Mann-Whitney U test for non-normally and independent t-test for normally distributed variables. Differences in prevalence between current cut-points and the ones from other cohorts were compared with the use of chi-square tests. $P<0.05$ was accepted as significant. Data analyses were performed using IBM® SPSS Statistics, Amos and Decision trees (version 24, Chicago, Il, USA).

4.4 Results

Baseline characteristics of participants

Characteristics of the 9,088 participants (48% women) are summarized in **Table** 4-1, by sex. The mean age was 72.7 ± 5.5 in men and 72.5 ± 5.5 years in women and were comparable between sexes. The majority of participants were Caucasian (96.7 %) and 3.3 % were Asian, African-American, Hispanic or other ethnicities. The use of prescribed medications was low in both men and women, but women were taking more medications than men (from Mann-Whitney U test; $p<0.001$). The mean BMI was in the overweight category range (25-29.9 kg/m^2).
As expected, ALM, ALM index and handgrip strength were higher in men than in women. Women also had a lower physical performance score.

Associations between ALM index, handgrip strength and physical performance

To investigate the relationship between ALM index, handgrip strength and physical performance, we used path analyses including all three variables in a first model and adjusting for the covariates fat mass and age in a second model (**Figure** 4-1). Results are reported as standardized β coefficient (std β) and R-squared. We found positive associations between ALM index and handgrip strength (std β=0.29 in men and std β=0.21 in women; all $p<0.001$) and between handgrip strength and physical performance score (std β=0.22 and std β=0.23; all $p<0.001$) that remained after adjustment for covariates. Considered independently of strength, ALM index and physical performance were inversely associated (std β= -0.05 in men, $p<0.001$; and std β=-0.18 in women; $p=0.001$), however this association did not remain after adjusting for age and fat mass, given the opposite effects of fat mass on ALM index and physical performance.

Cut-points for low strength and low ALM, and prevalence rates

The significant association of ALM index with strength, and strength with physical performance along with the absence of an independent relationship between ALM index and physical performance in the adjusted model justified the identification of cut-points of low strength as a predictor of physical performance, and of low ALM index as a predictor of low strength. To avoid obtaining numerous cut-points by age groups and to simplify the clinical use of our criteria, age was not included in the statistical models. From CART analyses performed in random sex-specific training samples (80 % of total sex-specific sample), the optimum splits for handgrip strength to predict limited physical performance were <33.1 kg and <20.4 kg in men and women, respectively (**Figure** 4-2). For low ALM as a predictor of low strength, cut-points identified were <7.31 kg/m^2 in men and <5.43 kg/m^2 in women (**Figure** 4-3). When applied to their respective test datasets, cut-points were shown to have excellent validity as supported by highly similar PPA and NPA measures in the training compared to test datasets (data not shown), given this very large study population.

The prevalence of impaired physical performance in the cohort was 7.4% (**Supplementary Figure** 4-2). **Table** 4-2 presents the prevalence rates and sensitivity analysis for both low strength and low ALM cut-points overall and across different population subgroups. Classifying participants as per the above criteria, 21.5% of men had low strength and were 4.51 (95% CI: 3.58, 5.68) folds more likely of having impaired physical performance compared to

those with normal strength. The prevalence of low strength in women was 24.0% and these persons had 4.66 (95% CI: 3.67, 5.92) folds greater odds for impaired physical performance compared to women with normal strength. The prevalence rates of low ALM were 22.9% and 15.6% in men and women, respectively. Participants with low ALM had 2.99 (95% CI: 2.58, 3.38; in men) and 2.41 (95% CI: 2.02, 2.86 in women) folds greater odds of having low strength than participants with normal ALM. Overall, 8.6% of men and 6.1% of women had both conditions, i.e., low strength and lean mass. Agreement measures including PPA, NPA and Cohen's κ are described in the Online Resource, **Supplementary Table** 4-1 and **Supplementary Table** 4-2.

The prevalence rates of low strength and low ALM varied between age groups and between presence or absence of chronic diseases (**Table** 4-2). Reflective of the path analysis associations (**Figure** 4-1, Model 1), low strength cut-points more strongly predicted impaired physical performance compared to low ALM as a predictor of low strength in all subgroups except in men at risk of poor nutritional state or with a kidney disease. Cut-points for low strength and low ALM both had higher prediction of their respective outcome in younger (65-74 years) than older (≥ 75 years) seniors. In men, the likelihood of impaired physical performance associated with low strength ranged from 2.74 (95% CI: 0.97, 7.76) in individuals with a kidney disease, to 4.79 (95% CI: 3.17, 7.24) in those with a heart disease. In women, low strength better predicted impaired physical performance in younger participants and in those without a risk of poor nutritional state 5.43 (95% CI: 3.39, 8.70). The likelihood of having low strength associated with low ALM ranged from 2.56 (95% CI: 2.09, 3.15) in men without a risk of poor nutritional state, to 4.47 (95% CI: 2.19, 9.10) in men with a kidney disease; and in women, from 0.79 (95% CI: 0.30, 2.04) in persons with a kidney disease to 2.89 (95% CI: 2.28, 3.67) in those without nutritional risk. Interactions between classification (low and normal strength or ALM) and characteristics groups, e.g., presence/absence of a heart disease, were all non-significant with the exception of age groups and low handgrip strength, and between presence/absence of nutritional risk, and of a kidney disease and low ALM index in women. Sensitivity analysis by BMI was not performed as the physical performance score was already adjusted for this variable.

Characteristics of sarco-dynapenic and non-sarco-dynapenic participants applying the CLSA criteria

To allow comparison with previous studies using the broader definition of sarcopenia including both low ALM and strength, individuals with both conditions were identified and defined as having sarco-dynapenia. By definition, sarco-dynapenic participants had lower ALM index, handgrip strength and physical performance score than non-sarco-dynapenic. They were also older, were less physically active, had lower weight, BMI and fat mass (**Table** 4-3).

Comparison with previous sarcopenia criteria

The cut-points for low ALM and strength identified in this study were compared to the EWGSOP2 recommendations and the FNIH criteria. The EWGSOP2 endorses ALM/ht^2 (kg/m^2) ≤7.0 for men and ≤6.0 for women as criteria for low ALM and <27 kg for men and <16 kg for women, for low handgrip strength (62). The FNIH criteria for low ALM, as defined by an index of ALM (kg) divided by BMI (kg/m^2), are <0.789 for men and <0.512 for women (76), and criteria for low handgrip strength are <26 kg for men and <16 kg for women (77). The IWGS defines sarcopenia as the combination of low ALM and low gait speed (<1.0 m/s) (63). Because our cut-points for low strength are derived from limited physical performance, including a measure of gait speed would be redundant and thus, we did not compare our criteria to those suggested by the IWGS. The current population being mainly Caucasian, the Asian criteria (60) were also not studied.

Our cut-points agreement with the EWGSOP2 was fair with κ of 0.14 and 0.43 in men and women respectively. Positive percent agreement of 100% in men and 81.2% in women were found, and 92.0% and 95.6% for negative percent agreement (data not shown). Comparison between our combined cut-points and the FNIH Sarcopenia Project's criteria showed PPA of only 50.8 in men and 28.8% in women, NPA of 92.0 and 94.1%, and κ of 0.12 and 0.08. This observed disagreement was even more pronounced for the comparison of low ALM cut-points alone (PPA=34.8 and 22.9%; NPA=78.4 and 85.0%; κ=0.08 and 0.06). We thus examined the CLSA participants characteristics applying the ALM/BMI cut-points determined by the FNIH.

Characteristics of participants with low ALM applying the CLSA and the FNIH criteria.

Characteristics of participants by presence or absence of low ALM as per the CLSA criteria and as per the FNIH cut-points are displayed in **Table** 4-4 for men and **Table** 4-5 for women. The prevalence rates of low ALM with the CLSA criteria were 22.9% in men and 15.6% in women. Participants characterized as sarcopenic were older, had lower weight and BMI, ALM index, fat mass, strength and physical performance score than non-sarcopenic participants. Applying the FNIH Sarcopenia project criteria to the CLSA cohort, 10% of men and 8% of women were identified with low ALM/BMI (**Supplementary Figure** 4-3). Sarcopenic participants as per the FNIH criteria had lower handgrip strength and physical performance score, they were older and less physically active compared to those with normal ALM/BMI; however, they had only slightly lower ALM and ALM index (in men), but higher weight, BMI and fat mass compared to participants with normal ALM/BMI. In women, ALM index was not different between individuals with low and normal ALM/BMI. Because of the use of the same ALM index and fair agreement between cut-points, we did not examine participants characteristics applying the EWGSOP2 criteria.

4.5 Discussion

Our study showed that greater strength is independently associated with better physical performance and that higher ALM index is independently associated with greater strength, but not with physical performance after adjusting for fat mass and age. From baseline data of the largest Canadian-representative cohort of older adults, we established sex-specific empirical cut-point values to define low strength and low ALM index. Cut-points of handgrip strength <33.1 kg in men and <20.4 kg in women best predicted the risk of limited physical performance and the optimum cut-points of ALM index to identify those at risk of low strength were <7.31 kg/m^2 in men and <5.43 kg/m^2 in women. These cut-points had good predictive capacity across different subgroups of the population. Though the CLSA cut-points agreed with those for sarco-dynapenia endorsed by the EWGSOP2, poor agreement was found with those of the FNIH Sarcopenia Project primarily due to the use of a different ALM index, i.e., ALM/BMI. To our knowledge, our findings represent the most recent cut-points for sarcopenia and dynapenia in a contemporary database (recruitment between 2011-2015) and the first established in a single large national-

representative population addressing generalizability and methodological issues encountered with previously derived cut-points.

Association between ALM index, strength and physical performance

Previous studies have examined the relationship between lean mass or sarcopenia and physical limitations and found that strength (325) and body fat mass (11, 75, 326, 327) were important mediators of the latter. Indeed, in the Health ABC cohort (n=3,075, aged 70-79, African-American and Caucasian ethnicities), Visser et al. showed greater muscle cross-sectional area (by computed tomography imaging) to be associated with lower extremity physical function at baseline (328) and with incident physical limitations over a 2.5-year follow-up (325), both independently of body fat mass. While authors did not adjust cross-sectional results for strength, longitudinal findings were no longer significant after strength was included in the models. Also in the Health ABC cohort, Delmonico et al. reported a protective effect of low ALM/ht^2 on incident lower extremity performance in both sexes before adjustment, and found associations to be attenuated after correcting for body fat mass though, again, not adjusted for strength (n=2976) (11). In sarcopenic men as defined by low ALM/ht^2 (20% below the mean of the distribution) greater odds for mobility limitations were reported by Dufour et al. after adjusting for comorbidities and BMI among other covariates (OR=6.3, 95% CI: 2.5-16.1) (326). Further, these groups investigated the capacity of sarcopenia, relative to fat mass, at predicting physical performance limitations and found the integration of fat mass as part of the index for sarcopenia to be superior compared with ALM index and concluded that sarcopenia should account for fat mass in its definition to allow better identification of individuals at risk of disability. Adding to these findings, we observed the cross-sectional ALM index-physical performance relationship to be completely mediated by fat mass and strength. Accounting for strength in our first model, we found that increased ALM index was associated with lower physical performance. Fat mass being positively associated with ALM index, but negatively linked with physical performance, the association between ALM index and physical performance disappeared after adjustment for fat mass suggesting that individuals carrying more weight perform less in any weight-bearing physical tests. This would explain the higher odds of physical limitations obtained when using ALM relative to fat mass as the predictor. We confirmed that strength and fat mass are drivers of the relationship between ALM index and physical performance in the CLSA cohort. Albeit ALM can be manipulated, e.g., through adjustment for fat mass, to become a clinically relevant

predictor of functional limitations, the absence of a relationship challenges its use for that purpose and emphasizes that ALM and physical performance are two different conditions. We also observed modest, but stronger associations between ALM index and strength, and strength and physical performance which denotes potential distinct underlying mechanisms for low ALM and low strength, but also likely distinct health implications of these conditions. As defended by Clark and Manini, neurologic factors would mostly be responsible for strength along with architectural changes and muscle mass, whereas growth factors, sex-hormones, inflammation status, physical activity and genetic background would be determinants of muscle mass, justifying to consider low strength and low ALM as two separate conditions, i.e., dynapenia and sarcopenia, respectively (54).

Comparison to existing criteria

Our cut-point values for low strength <33.1 kg in men and <20.4 kg in women are higher than those endorsed by the EWGSOP2, i.e., <27 kg in men and <16 kg in women which increases the sensitivity of finding persons at risk of impaired physical performance. The latter cut-points were determined using a sex-specific T-score of ≤ -2.5 SD based on the mean grip strength of participants aged 32 years (from 4 pooled British cohorts, 1990-2012, n=20,108, 16-90 years) (74). Our values for low strength are rather concordant with those of a T-score ≤ -2.0 SD, 32 kg and 19 kg for men and women, also reported in this cohort. The EWGSOP2 recommended cut-points for low lean mass of <7.0 kg/m^2 for men and <6.0 kg/m^2 for women, were found by Gould et al. from a cohort of young Australian adults (1993-2006, n=682, 20-39 years) using T-scores ≤ -2.0 SD (<6.94 kg/m^2) and ≤ -1.0 SD (<6.07 kg/m^2) in men and women, respectively (73). Though the T-score approach is logical considering that individuals who fall at the extreme left of the ALM distribution have low ALM relative to the rest of the population, it was not derived to identify persons with greater odds of low strength or mobility limitations. Our function-derived empirical cut-points for low ALM, <7.31 kg/m^2 in men and <5.43 kg/m^2 in women showed both fair positive and negative agreement with the EWGSOP2 recommendations which strengthens our argument that our diagnostic values are not only representative of low ALM relative to the mean of younger adults, but also as a predictor of dynapenia. However, greater agreement was found in women than men possibly due to the EWGSOP2 selection of a higher T-score cut-point for women (≤ -1.0 SD vs. -2.0 SD for men).

Using CART, the FNIH recently established cut-points for handgrip strength, <26 kg and <16 kg for men and women, to predict slowness as defined by gait speed <0.8 m/s (77). From these values, cut-points were derived for ALM using an index of ALM (kg) divided by BMI (kg/m^2) of <0.789 for men and <0.512 for women, as predictors of weakness (76). These values were determined from 8 pooled American cohorts and a set of clinical trials including older adults aged ≥65 years recruited between 1992 and 2007 (n=20,847 for low strength criteria and n=11,270 for low ALM criteria; 89.9-90.5 % Caucasian). Of note, though pooling numerous cohorts provides a large sample size, important limitations pertain to this method, e.g., standardization of measurements especially DXA, cross-calibration of instruments, selection bias from clinical trials, etc. Not surprisingly, we observed poor agreement of our combined criteria for sarcopenia and dynapenia with the FNIH Sarcopenia Project's, mainly owed to the use of different ALM indices, i.e., ALM/BMI in contrast to ALM/ht^2. To better understand the discrepancy, we examined the characteristics of CLSA individuals with low versus normal ALM as per the FNIH definition and our definition. Interestingly, the FNIH criteria for low ALM identified seniors with a mean BMI falling within the obese category (men: 31.2 ± 4.6 kg/m^2, women: 32.1 ± 5.4 kg/m^2), normal mean ALM index (7.85 ± 1.07 kg/m^2 and 6.27 ± 1.04 kg/m^2), lower strength, though not low as per our criteria or the FNIH's, and having lower physical performance compared to those with normal ALM/BMI. Many of these persons identified as having low ALM are simply obese, or sarcopenic-obese or "true" sarcopenic. Therefore, though the FNIH criteria predict physical performance well, they are not discriminant to identify body composition characteristics. They would also have a limited use to assess ALM changes in a prospective study design as the ratio would be largely influenced by BMI changes over time. For instance, a loss of ALM and body fat mass (decreased BMI) would be erroneously interpreted as the absence of change in muscle mass.

Prevalence of sarcopenia, dynapenia and sarco-dynapenia

From heterogenous definitions used to identify sarco-dynapenia, the prevalence of the condition was estimated to range from 5-13% in older adults aged 60-70 years and higher prevalence of 11-50% was found in those aged above 80 years (329). The EWGSOP and IWGS also reported substantial prevalence of sarco-dynapenia in several older population settings (≥50 years); free-living (1-29%), long-term care (14-33%) and acute hospital-care (10%) settings (64). In the current CLSA cohort, 7.4% had sarco-dynapenia (both low strength and ALM), a

prevalence that falls within previously reported range for this condition. Importantly, very low prevalence rates for sarco-dynapenia were obtained in the CLSA cohort when applying the EWGSOP2 recommended cut-points (0.7% in men and 2.3% in women) and also applying the FNIH's (1.4% in men and 1.2% in women).

The FNIH reported prevalence rates in free-living conditions for low strength of 5.3% and 17.9% in men and women respectively applying their cut-points (77). In men and women of our study, 21.5% and 24.1% had low strength applying our cut-points. From their CART model, the FNIH obtained an initial split of data for handgrip strength at 31.8 kg in men and 20.0 kg in women, but chose to use the values resulting from a second split of the data (within each lower sex-specific groups) for more conservative cut-points, explaining the apparent lower prevalence reported by the FNIH. When applying the FNIH criteria to the CLSA cohort, we found lower prevalence of low strength, 4.3% in men and 6.2% in women, compared to the prevalence obtained in the cohorts from which criteria were established. Regarding low ALM, i.e., low ALM/BMI, the FNIH reported 20.2% and 16.7 % prevalence rates (76) in men and women and quite similarly, 22.9% and 15.6% had low ALM using the CLSA-derived criteria. Interestingly, the FNIH criteria resulted in roughly two folds lower prevalence rates for low ALM when applied to the CLSA cohort (10% in men and 8% in women). It confirms that applying the same cut-points to different populations leads to divergent prevalence possibly due to disparities in characteristics such as ethnicities, prevalence of obesity which drastically increased over the past decades (330), level of physical activity and presence of other chronic diseases.

Other health implications of low ALM: illnesses and surgery recovery, and survival

Function-derived ALM cut-points identify persons at risk for impaired mobility and daily life activities, but low ALM should also be considered in other contexts. Skeletal muscle mass is the largest reservoir of amino acid of the body (331), it is a highly metabolically active tissue (332) and has important endocrine and immune functions (331). Low muscle mass contributes to frailty and reflects decreased physiological reserves which may promote unfavorable outcomes under physiological stress such as after surgery (333) or during illnesses (334) due to poorer intrinsic adaptive mechanisms. Previous studies showed lean mass and muscle area to be protective of all-cause mortality reducing the incidence by 9-20% independently of indicators of obesity (central obesity and fat tissue mass), chronic diseases and strength (335-337). Low ALM/BMI has also been associated with mortality (338), but it may be possibly explained by

factors that are different than those explaining the association between low ALM index and mortality. For example, a cross-sectional study in Australian (n=1005, mean age 62) and Korean (n=376, mean age 58) cohorts showed greater likelihood of having the metabolic syndrome attributed to higher waist circumference, blood pressure and triglycerides in participants with lower ALM/BMI, while opposite results were observed for sarcopenia as defined by low ALM index (kg/ht^2) (339). The presence of chronic diseases may describe the association between low ALM/BMI and mortality reported by Balogun et al. as results were only adjusted for age (338). Contrastingly, in other clinical conditions such as cancer or hospitalization, higher fat mass and normal ALM as observed with the FNIH criteria may confer a survival benefit (297). Thus, adjusting or normalizing ALM for fat mass, weight or BMI may fail to recognize older adults with "true" low muscle mass, i.e., relative to height and impede prediction of clinical outcomes. This reinforces the importance of clearly defining sarcopenia to inform research directions.

Strengths and limitations

As we aimed to determine clinically relevant and simple to apply sex-specific cut-points, the latter are binary which limits the predictive capacity, as with any cut-point values. This would be enhanced by considering other factors involved but it would complexify its use. Though handgrip strength was shown to be a valid proxy for overall muscle strength (61), a recent study reported moderate to poor correlation between handgrip and knee extension strength (340) questioning the use of handgrip strength as the sole measure of overall muscle strength. Yet, it is an easy to perform measurement that permits to identify older adults at risk of impaired physical performance. Associations being examined in cross-sectional data do not permit causal interpretation. The use of cut-points to predict the incidence of decline in physical performance will be tested with follow-up data. Further, the CLSA population mostly comprising Caucasian older adults may preclude the application of the present cut-points to populations of other ethnicities. On the other hand, the new sarcopenia and dynapenia cut-points are derived from the largest single contemporary cohort in aging, from the use of precise and accurate reference standard for measuring muscle mass, DXA (67, 293) and the use of objective, valid and reliable physical performance measures using standardized procedures, thus ensuring homogeneity in assessments. As well, the creation of a score that encompasses four tests that assess mobility, balance and physical function altogether represents a novel and robust approach to evaluate physical performance. Another strength is the empirical method to determine cut-points for low

strength and ALM, as well as its application as two separate diagnosis which removes complexity to the term sarcopenia and clarifies its definition.

4.6 Conclusion

In conclusion, ALM index is not independently associated with physical performance cross-sectionally; strength and fat mass are drivers of this hypothesized relationship. Therefore, combining lean mass, strength, physical performance within one single sarcopenia definition is not supported and confuses the clinical picture. We reinforce that low ALM should be referred to as sarcopenia and low strength as dynapenia and strongly support the use of ALM/ht^2 as an index of lean mass as opposed to normalizing ALM for body fat mass measures. Our functionally-derived cut-points from a single, large and contemporary cohort are clinically relevant for identifying low strength as a predictor of limited physical performance and low ALM as a predictor of low strength, with a realistic 6-9% prevalence of sarco-dynapenia. These cut-points aim to guide researchers and health professionals in the identification of these two conditions in older Caucasian individuals to help design interventions targeted for muscle mass or strength and of clinical outcomes in addition to mobility and function. Further studies investigating the implications of these newly derived cut-points for low ALM index in hospitalization, length of stay, recovery post-surgery, ability to respond to treatment, and survival rates are therefore warranted.

Acknowledgments

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Conflicts of Interest

Anne-Julie Tessier, Simon S. Wing, Elham Rahme, José A. Morais and Stéphanie Chevalier declare that they have no conflict of interest.

Table 4-1 Baseline characteristics of the CLSA participants by sex, 2011-2015

	Men (n=4,725)	Women (n=4,363)
Age, y	72.7 ± 5.5	72.5 ± 5.5
Caucasian, %	96.0	97.5
Anthropometric measurements		
Height, cm	1.74 ± 0.07	1.60 ± 0.06
Weight, kg	83.9 ± 13.5	70.1 ± 13.5
BMI, kg/m^2	27.8 ± 4.0	27.5 ± 5.1
Current smoker, %	5	5
Nutritional risk (SCREEN II-AB; range 0-48)	39.6 ± 5.5	39.0 ± 5.9
Medication number (range 0-11)	0.8 ± 0.9	1.0 ± 1.0
PASE score (range 0-629)	129 ± 59	111 ± 53
Body composition		
ALM, kg	24.36 ± 3.59	16.23 ± 2.74
ALM index, kg/m^2	8.05 ± 0.99	6.34 ± 0.95
Fat mass, kg	25.02 ± 7.59	29.0 ± 8.89
Strength		
Maximum grip strength, kg	39.8 ± 8.4	23.9 ± 5.1
Physical performance		
BMI-adjusted physical performance, Z score	0.17 ± 2.14	-0.18 ± 2.16
TUG, sec	9.9 ± 1.9	10.0 ± 2.0
Gait speed, m/sec	0.95 ± 0.19	0.92 ± 0.18
Balance (range 0-60 s)	28.6 ± 23.1	25.1 ± 22.3
Chair rise average time, s	2.8 ± 0.8	2.9 ± 0.8

Values are mean ± SD. SCREEN II, seniors in the community risk evaluation for eating and nutrition; PASE, physical activity scale for elderly; ALM, appendicular lean mass; TUG, timed up and go.

Table 4-2 Sensitivity analysis for strength as a predictor of limited physical performance and for low ALM as a predictor of low strength across subgroups in the CLSA cohort, 2011-2015

	N	Prevalence Low HGS (%)	OR (95% CI) for impaired physical performance Low HGS	p for interaction[1]	Prevalence Low ALM (%)	OR (95% CI) for low HGS Low ALM	p for interaction[2]
Men							
Overall	4725	21.5	4.51 (3.58, 5.68)		22.9	2.99 (2.58, 3.38)	
Age							
65-74	2878	14.8	4.50 (2.87, 7.04)	0.162	17.0	2.62 (2.07, 3.31)	0.897
≥75	1847	32.0	3.09 (2.34, 4.07)		32.3	2.56 (2.09, 3.15)	
Nutritional risk (SCREEN II-AB)							
Yes	1332	24.2	3.09 (2.34, 4.07)	0.304	23.8	3.80 (2.95, 4.90)	0.032
No	3393	20.5	4.50 (2.89, 7.04)		21.4	2.66 (2.17, 3.27)	
Heart disease							
Yes	1029	25.4	4.79 (3.17, 7.24)	0.640	23.6	2.72 (1.99, 3.70)	0.474
No	3696	20.4	4.25 (3.21, 5.62)		22.7	3.09 (2.60, 3.67)	
Kidney disease							
Yes	173	28.9	2.74 (0.97, 7.76)	0.339	28.3	4.47 (2.19, 9.11)	0.254
No	4552	21.2	4.61 (3.64, 5.84)		22.7	2.93 (2.51, 3.41)	
COPD							
Yes	273	26.0	3.55 (1.54, 8.20)	0.565	31.9	3.60 (2.04, 6.34)	0.500
No	4452	21.2	4.58 (3.61, 5.82)		22.4	2.94 (2.51, 3.44)	
Type 2 diabetes							
Yes	630	27.6	4.21 (2.57, 6.90)	0.853	21.0	3.77 (2.52, 5.63)	0.253
No	4095	20.6	4.44 (3.41, 5.76)		23.2	2.92 (2.48, 3.44)	

Women							
Overall	4363	24.1	4.66 (3.67, 5.92)		15.6	2.41 (2.02, 2.86)	
Age							
65-74	2706	15.4	5.43 (3.39, 8.70)	0.013	13.3	2.26 (1.74, 2.94)	0.894
≥75	1657	38.2	2.69 (2.02, 3.58)		19.4	2.21 (1.72, 2.83)	
Nutritional risk (SCREEN II-AB)							
Yes	1427	27.4	2.69 (2.02, 3.58)	0.867	16.1	1.87 (1.42, 2.47)	0.019
No	2936	22.4	5.43 (3.39, 8.70)		15.3	2.89 (2.28, 3.67)	
Heart disease							
Yes	519	28.7	3.01 (1.77, 5.11)	0.081	14.5	2.79 (1.64, 4.46)	0.642
No	3844	23.4	5.11 (3.90, 6.70)		15.8	2.38 (1.98, 2.87)	
Kidney disease							
Yes	142	31.0	3.30 (1.25, 8.67)	0.480	18.3	0.79 (0.30, 2.04)	0.019
No	4221	23.8	4.72 (3.69, 6.05)		15.5	2.51 (2.10, 2.99)	
COPD							
Yes	318	27.4	3.31 (1.64, 6.66)	0.314	17.9	1.90 (1.04, 3.36)	0.421
No	4045	23.8	4.85 (3.75, 6.26)		15.4	2.45 (2.05, 2.94)	
Type 2 diabetes							
Yes	376	27.4	4.48 (2.41, 8.34)	0.909	9.3	2.16 (1.06, 4.40)	0.729
No	3987	23.8	4.66 (3.59, 6.05)		16.2	2.46 (2.05, 2.94)	

HGS, handgrip strength; ALM, appendicular lean mass; SCREEN II-AB, abbreviated Seniors in the Community Risk Evaluation for Eating and Nutrition, version II, score < 38 was considered as at risk of poor nutritional state; COPD, chronic obstructive pulmonary diseases.

[1] Interaction for absence/presence of low HGS and subgroup characteristics in the prediction of impaired physical performance.

[2] Interaction for absence/presence of low ALM and subgroup characteristics in the prediction of low HGS.

Table 4-3 Baseline characteristics of men and women by absence or presence of sarco-dynapenia applying CLSA cut-points, 2011-2015

	Men		Women	
	Non-sarco-dynapenic (n=4,335)	Sarco-dynapenic (n=390)	Non-sarco-dynapenic (n=4,123)	Sarco-dynapenic (n=240)
Prevalence, %	91.4	8.6	93.9	6.1
Age, y	72.4 ± 5.4	76.7 ± 5.4**	72.3 ± 5.5	75.8 ± 5.4**
Caucasian, %[2]	96.4	92.9*	97.6	96.3
Weight, kg	85.1 ± 13.3	71.7 ± 9.7**	71.0 ± 13.3	55.8 ± 7.3**
BMI, kg/m^2	28.1 ± 4.0	24.7 ± 2.8**	27.8 ± 5.1	22.7 ± 2.8**
Nutritional risk (SCREEN II-AB; 0-48)	39.8 ± 5.5	38.5 ± 5.9**	39.0 ± 5.9	38.8 ± 6.2
Medication number (0-11)	0.8 ± 0.9	1.0 ± 0.9*	1.0 ± 1.0	1.1 ± 1.0
PASE score (0-629)	131 ± 59	104 ± 57**	112 ± 53	94 ± 48**
Body composition				
ALM, kg	24.8 ± 3.4	19.7 ± 2.1**	16.5 ± 2.6	12.4 ± 1.2**
ALM index, kg/m2	8.18 ± 0.94	6.76 ± 0.45**	6.43 ± 0.92	5.04 ± 0.31**
Total fat mass, kg	25.3 ± 7.6	21.6 ± 6.1**	29.4 ± 8.9	22.3 ± 5.7**
Muscle strength				
Maximal handgrip strength, kg	40.9 ± 7.8	28.3 ± 4.1**	24.4 ± 4.9	17.1 ± 2.7**
Physical performance				
TUG, s	9.8 ± 1.9	10.8 ± 2.2**	9.9 ± 2.0	10.6 ± 2.2**
Gait speed, m/s[1]	0.96 ± 0.18	0.88 ± 0.19**	0.93 ± 0.18	0.87 ± 0.18**
Average chair rise time, s	2.8 ± 0.7	3.0 ± 0.9**	2.9 ± 0.8	3.0 ± 0.9
Balance (0-60 s)	29.5 ± 23.1	18.9 ± 20.2**	25.5 ± 22.3	19.1 ± 20.2**
BMI-adjusted physical performance, Z score	0.31 ± 2.08	-1.32 ± 2.22**	-0.10 ± 2.13	-1.42 ± 2.21**

Values are mean ± SD. SCREEN II-AB, abbreviated Seniors in the community risk evaluation for eating and nutrition, version II; PASE, Physical activity scale for elderly; ALM, appendicular lean mass; TUG, timed up and go. Mann-Whitney U test unless otherwise specified; **p-value <0.001; *p-value <0.05; [1] Independent *t*-test; [2] Chi-square test.

Table 4-4 Descriptive statistics between men with presence or absence of low ALM applying the new Canadian and the FNIH cut-points, in the CLSA cohort

Men	Canadian cut-points			FNIH cut-points		
	Non-sarcopenic (n=3,701)	Sarcopenic (n=1,024)	p[1]	Non-sarcopenic (n=4,254)	Sarcopenic (n=471)	p[1]
Prevalence, %	77.1	22.9		90.0	10.0	
Age, y	72.1 ± 5.3	74.8 ± 5.7	<0.001	72.6 ± 5.5	74.2 ± 5.6	<0.001
Caucasian, %	96.5	94.6	0.004[2]	96.5	92.6	<0.001[2]
Weight, kg	87.1 ± 12.8	73.0 ± 9.5	<0.001	83.7 ± 13.3	86.1 ± 15.4	0.001
BMI, kg/m^2	28.8 ± 3.8	24.4 ± 2.7	<0.001	27.4 ± 4.6	31.2 ± 4.6	<0.001
Nutritional risk (SCREEN II-AB; 0-48)	39.8 ± 5.4	39.1 ± 5.8	0.006	39.8 ± 5.4	38.0 ± 6.1	<0.001
Medication number (0-11)	0.8 ± 0.9	0.8 ± 0.9	0.652	0.8 ± 0.9	1.1 ± 1.0	<0.001
PASE score (0-629)	132 ± 60	118 ± 57	<0.001	131 ± 59	108 ± 56	<0.001
Body composition						
ALM, kg	25.5 ± 3.1	20.5 ± 2.0	<0.001	24.7 ± 3.5	21.6 ± 3.3	<0.001
ALM index, kg/m^2	8.42 ± 0.80	6.83 ± 0.40	<0.001	8.08 ± 0.98	7.85 ± 1.07	<0.001
Total fat mass, kg	26.1 ± 7.7	21.5 ± 6.0	<0.001	24.4 ± 7.2	31.0 ± 8.6	<0.001
Muscle strength						
Maximal handgrip strength, kg	41.1 ± 8.2	35.7 ± 7.4	<0.001	40.5 ± 8.2	33.7 ± 7.6	<0.001[1]
Physical performance						
Gait speed, m/s	0.96 ± 0.19	0.93 ± 0.19	<0.001[1]	0.96 ± 0.19	0.87 ± 0.18	<0.001[1]
TUG, s	9.8 ± 1.9	10.2 ± 2.0	<0.001	9.8 ± 1.9	10.8 ± 2.3	<0.001
Chair time average, s	2.7 ± 0.7	2.9 ± 0.8	<0.001	2.8 ± 0.7	2.9 ± 0.9	<0.001
Balance, (0-60 s)	29.4 ± 23.1	25.9 ± 22.7	<0.001	29.8 ± 23.1	17.4 ± 19.3	<0.001
BMI-adjusted physical performance score, Z score	0.39 ± 2.09	-0.56 ± 2.16	<0.001[1]	0.26 ± 2.12	-0.65 ± 2.19	<0.001[1]

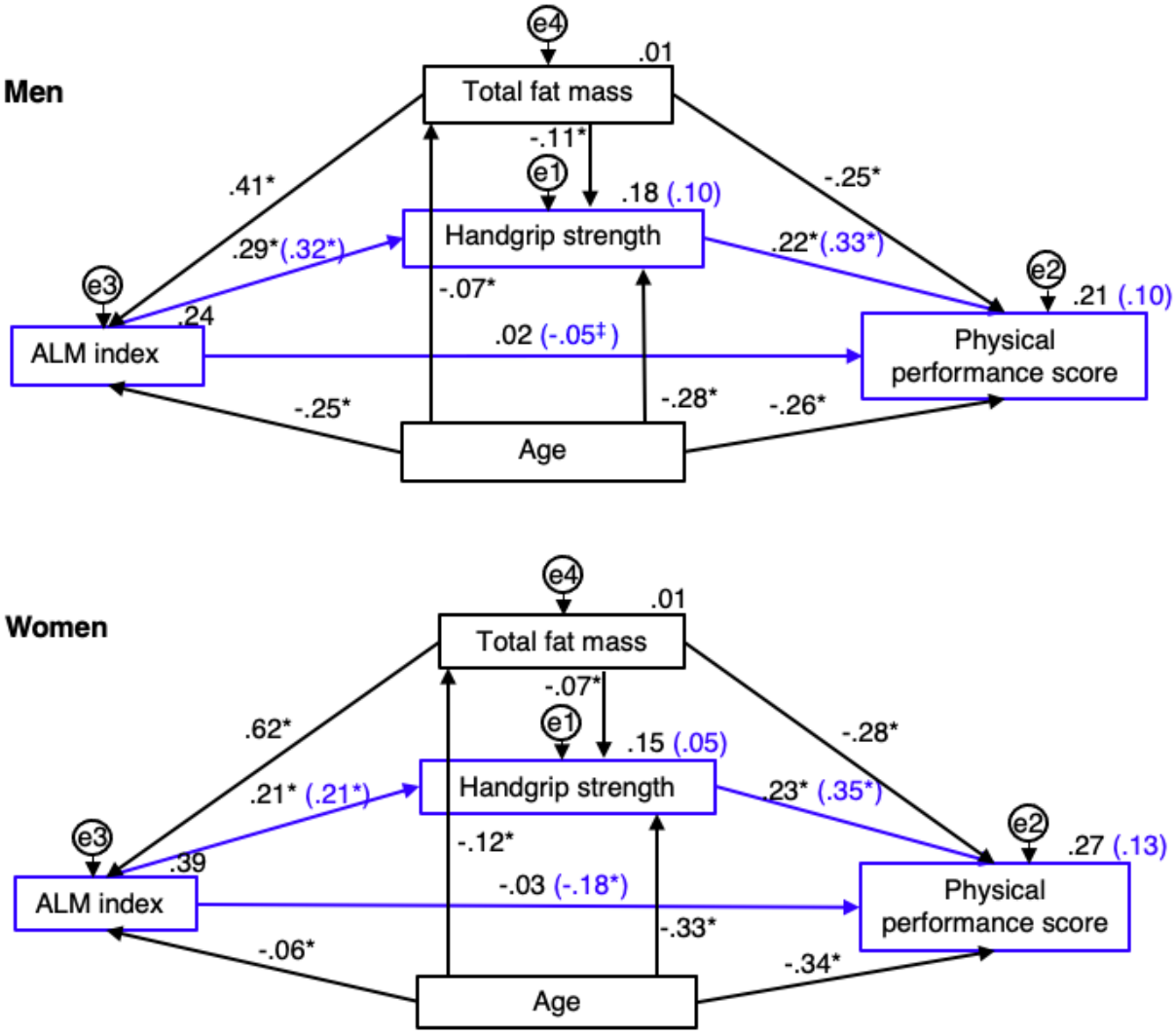
Values are mean ± SD. SCREEN II-AB, abbreviated Seniors in the Community Risk Evaluation for Eating and Nutrition, version II; PASE, Physical activity scale for elderly; ALM, appendicular lean mass; TUG, timed up and go. From Mann-Whitney U test unless otherwise specified; [1] Independent t-test; [2] Chi-square test.

Table 4-5 Descriptive statistics between women with presence or absence of low ALM applying the new Canadian and the FNIH cut-points, in the CLSA cohort

Women	Canadian cut-points			FNIH cut-points		
	Non-sarcopenic (n=3,767)	Sarcopenic (n=596)	p[1]	Non-sarcopenic (n=4,013)	Sarcopenic (n=350)	p[1]
Prevalence, %	84.4	15.6		92.0	8.0	
Age, y	72.3 ± 5.5	73.6 ± 5.8	<0.001	72.5 ± 5.5	72.9 ± 5.7	0.294
Caucasian, %	97.6	97.2	0.595[3]	97.8	94.3	<0.001[2]
Weight, kg	72.6 ± 12.9	56.7 ± 7.4	<0.001	69.8 ± 13.4	74.0 ± 14.0	<0.001
BMI, kg/m^2	28.4 ± 4.9	22.4 ± 2.7	<0.001	27.0 ± 4.9	32.1 ± 5.4	<0.001
Nutritional risk (SCREEN II-AB; 0-48)	39.0 ± 5.8	38.9 ± 6.2	0.891	39.2 ± 5.8	36.7 ± 6.4	<0.001
Medication number (0-11)	1.0 ± 1.0	1.0 ± 1.0	0.419	1.0 ± 1.0	1.5 ± 1.1	<0.001
PASE score (0-629)	112 ± 53	107 ± 51	0.036	112 ± 53	96 ± 51	<0.001
Body composition						
ALM, kg	16.9 ± 2.5	12.8 ± 1.3	<0.001	16.4 ± 2.7	14.4 ± 2.5	<0.001
ALM index, kg/m^2	6.58 ± 0.83	5.05 ± 0.30	<0.001	6.35 ± 0.94	6.27 ± 1.04	0.086
Total fat mass, kg	30.3 ± 8.8	22.2 ± 5.6	<0.001	28.5 ± 8.7	35.4 ± 9.0	<0.001
Muscle strength						
Maximal handgrip strength, kg	24.3 ± 5.1	21.6 ± 4.7	<0.001	24.2 ± 5.1	20.8 ± 4.5	<0.001[1]
Physical performance						
Gait speed, m/s	0.92 ± 0.18	0.92 ± 0.19	0.676[1]	0.93 ± 0.18	0.84 ± 0.17	<0.001[1]
TUG, s	10.0 ± 2.0	10.0 ± 2.0	0.956	9.9 ± 2.0	10.9 ± 2.4	<0.001
Chair time average, s	2.9 ± 0.8	2.9 ± 0.8	0.450	2.9 ± 0.8	2.9 ± 0.9	0.604
Balance, (0-60 s)	24.9 ± 22.2	25.9 ± 22.6	0.257	25.9 ± 22.4	15.4 ± 17.7	<0.001
BMI-adjusted physical performance score, Z score	-0.09 ± 2.14	-0.71 ± 2.21	<0.001[1]	-0.14 ± 2.14	-0.73 ± 2.30	<0.001[1]

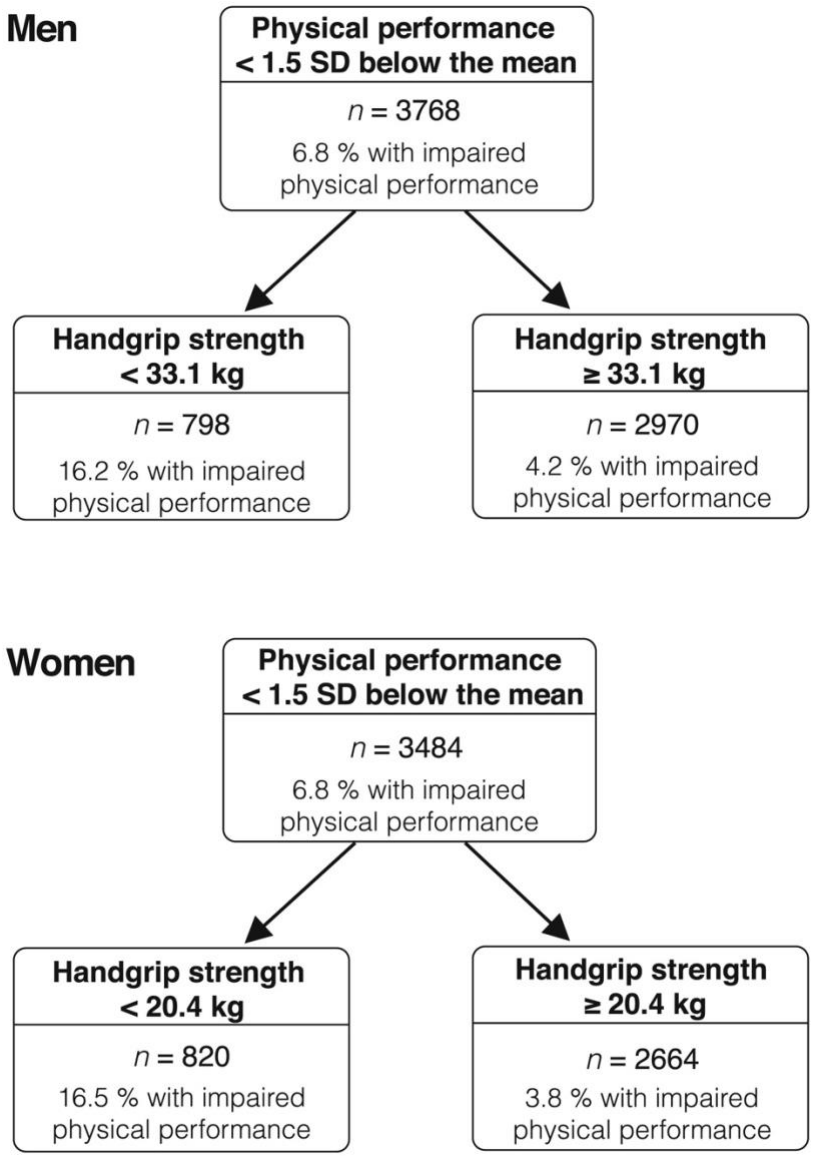
Values are mean ± SD. SCREEN II-AB, abbreviated Seniors in the Community Risk Evaluation for Eating and Nutrition, version II; PASE, Physical activity scale for elderly; ALM, appendicular lean mass; TUG, timed up and go. From Mann-Whitney U test unless otherwise specified; [1] Independent t-test; [2] Chi-square test.

Figure 4-1 Associations between appendicular lean mass index, handgrip strength and physical performance



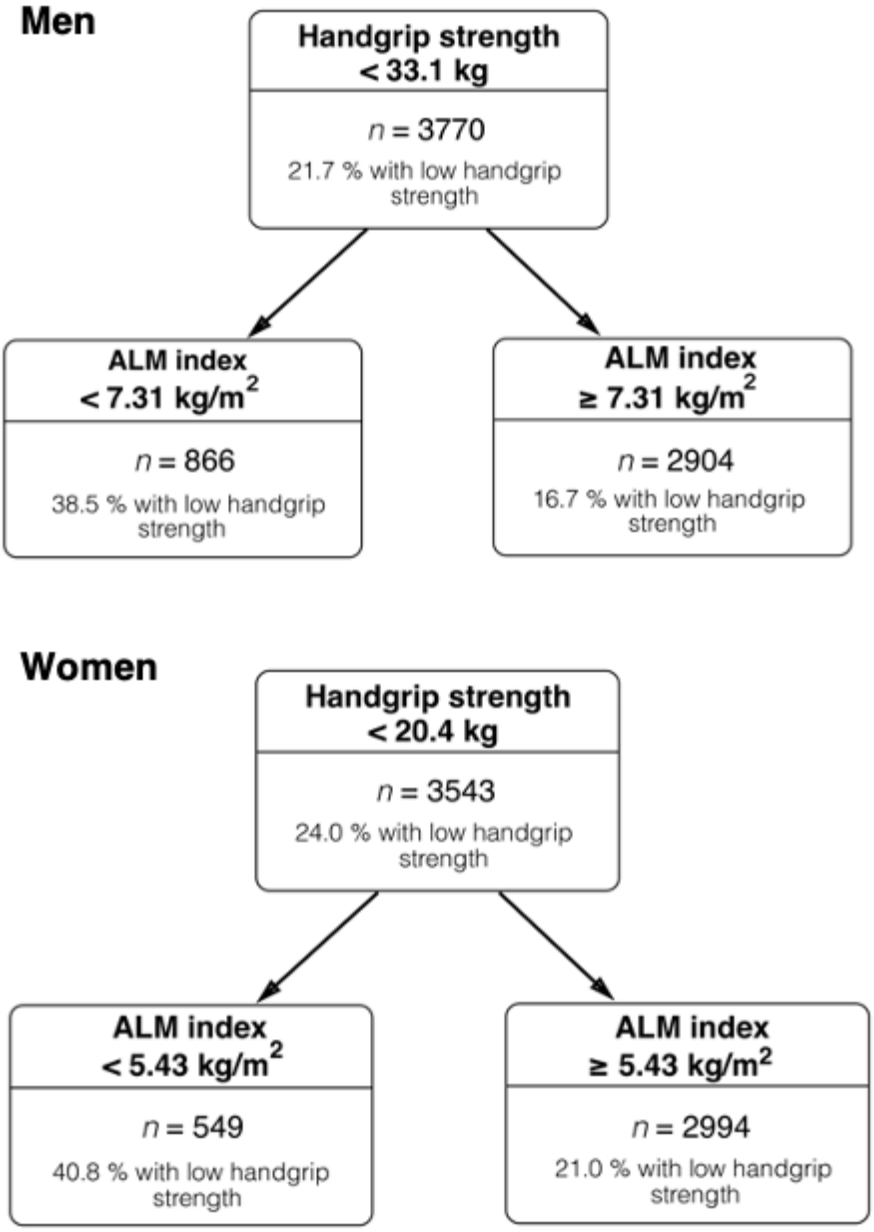
This model was constructed based on the hypothesis that ALM index predicts handgrip strength, and that handgrip strength predicts physical performance. Physical performance score is not adjusted for BMI as fat mass was included in the model. Values along the arrows are expressed as standardized beta coefficient and R^2 (in bold) not adjusted for covariates (Model 1, in blue) and adjusted for fat mass and age (Model 2, in black). *p value < 0.001 ‡ p value <0.05

Figure 4-2 CART results illustrating the handgrip strength cut-points as predictors of impaired physical performance in men and women



Results in training samples representing 80 % of the total study population.

Figure 4-3 CART results illustrating the ALM index cut-points as predictors of low handgrip strength in men and women



Results in training samples representing 80 % of the total study population.

Supplementary Table 4-1 Agreement of low handgrip strength cut-points with impaired physical performance

	Men	Women
Positive percent agreement, %	16.5	16.1
Negative percent agreement, %	95.8	96.1
Cohen's κ	0.164	0.161

Supplementary Table 4-2 Agreement of low lean mass cut-points with low handgrip strength

	Men	Women
Positive percent agreement, %	40.1	25.5
Negative percent agreement, %	81.7	87.5
Cohen's κ	0.213	0.148

Supplementary Table 4-3 Agreement of the CLSA with the FNIH criteria for sarcopenia (low lean mass)

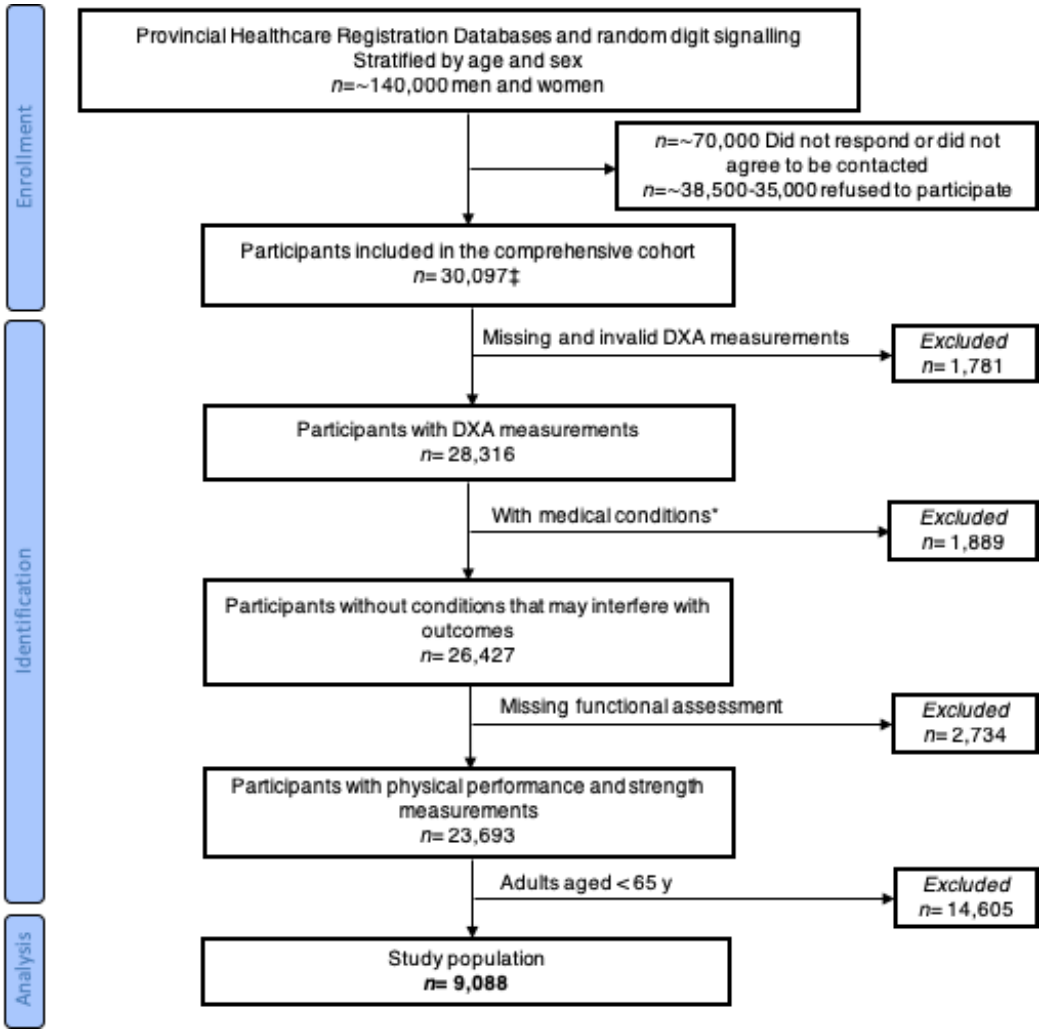
	Men	Women
Positive percent agreement, %	34.8	22.9
Negative percent agreement, %	78.4	85.0
Cohen's κ	0.084	0.055

Supplementary Table 4-4 Agreement of the CLSA with the FNIH criteria for sarco-dynapenia

	Men	Women

Positive percent agreement, %	50.8	28.8
Negative percent agreement, %	92.0	94.1
Cohen’s κ	0.120	0.075

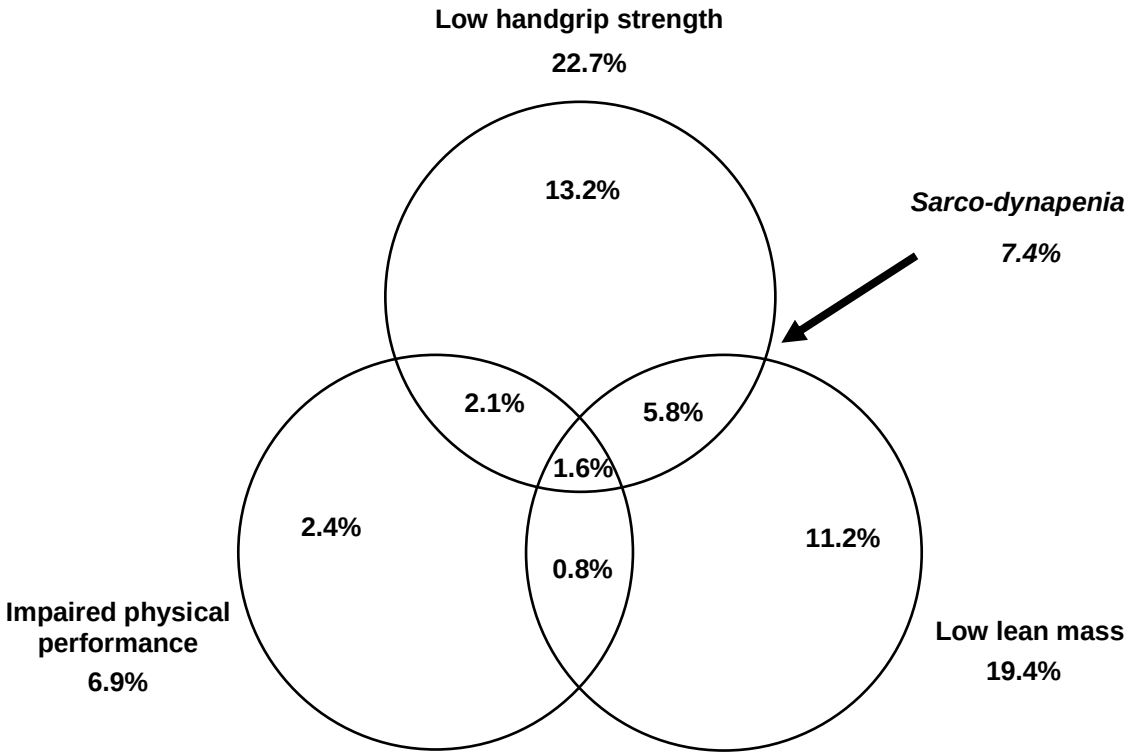
Supplementary Figure 4-1 Flow of participants in the CLSA cohort



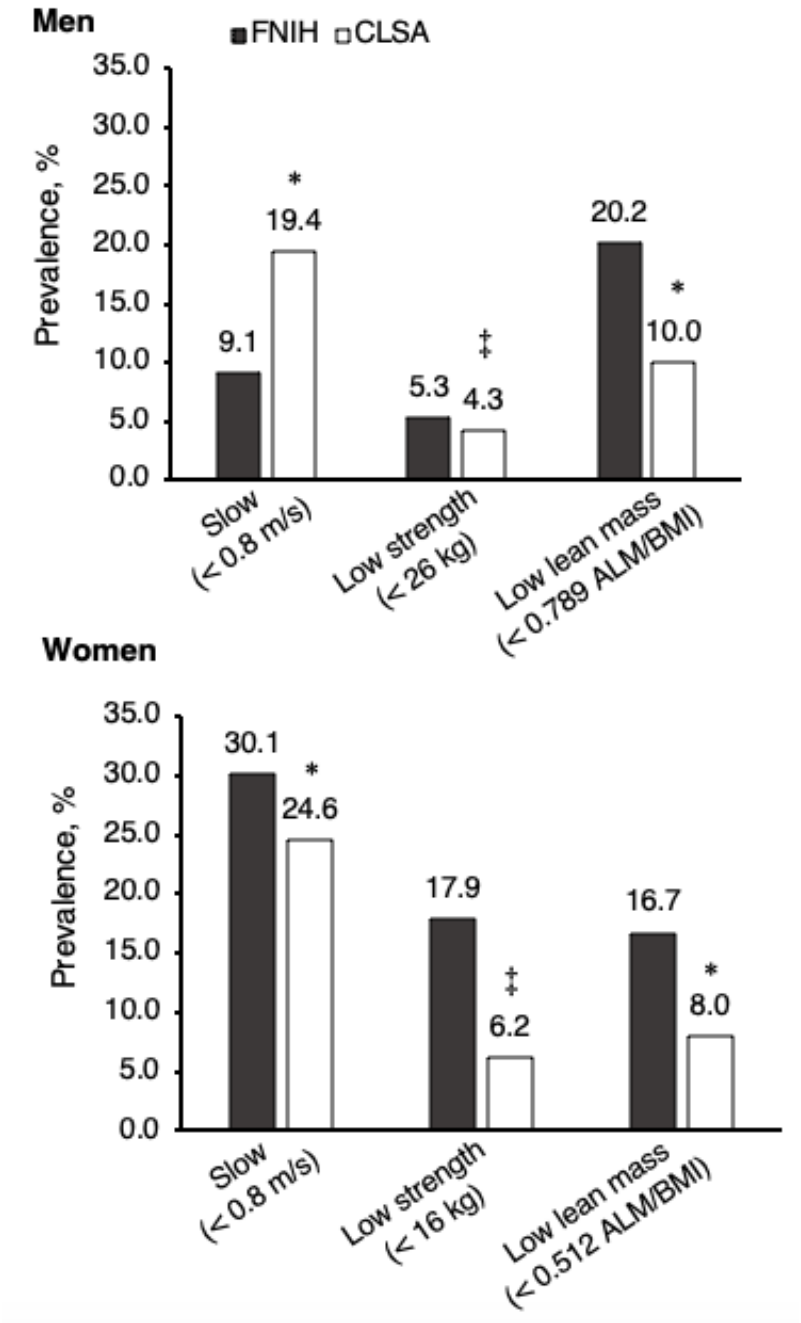
‡ Exclusion criteria are described by Raina et al. (302). *Medical conditions that may have interfered with any of the measured outcomes including multiple sclerosis, Alzheimer's disease, effects from stroke or transient ischemic attack (TIA), Parkinson's

disease, surgery within last 3 months, polio, unstable heart condition within last 3 months, pulmonary embolism within last 6 weeks, chemotherapy within last 4 weeks.

Supplementary Figure 4-2 Prevalence rates of impaired physical performance, low strength and low lean mass



Supplementary Figure 4-3 Prevalence rates of low gait speed, strength and lean mass reported by the FNIH Sarcopenia Project and applying the FNIH criteria to the CLSA


Men
FNIH
CLSA
Prevalence, %
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
9.1
19.4
*
5.3
4.3
‡
20.2
10.0
*
Slow (< 0.8 m/s)
Low strength (< 26 kg)
Low lean mass (< 0.789 ALM/BMI)
Women
Prevalence, %
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
30.1
24.6
*
17.9
6.2
‡
16.7
8.0
*
Slow (< 0.8 m/s)
Low strength (< 16 kg)
Low lean mass (< 0.512 ALM/BMI)

Chi-square test, *p-value < 0.001, ‡ p-value < 0.01 vs. the FNIH prevalence.

Connecting Statement 3

Results of **Chapter 4** confirmed that muscle mass is modestly and independently associated with strength, and that strength is associated with physical performance in both healthy older men and women. These associations informed and permitted to derive the first Canadian cut-points for the diagnosis of sarcopenia (low muscle mass by DXA) and dynapenia (low muscle strength by dynamometry). Low muscle mass and strength are often combined in the definition of sarcopenia (62), yet there may be unique health implications of having low muscle mass (independently of strength). The relationship between strength and cognition has been well investigated (111) and recent evidence points to a unique relationship between low BMI and cognitive decline (24, 102). Further, low muscle mass and impaired cognitive function share underlying mechanisms including chronic inflammation (115, 116), insulin resistance (117, 118) and suboptimal vascular function (108). Therefore, I hypothesized that the presence of low muscle mass is associated with greater cognitive decline, independently of percent body fat mass, strength and physical activity. The prevalence and incidence of MCI and dementia is significant in Canada (84, 85) and there is currently no treatment to reverse these conditions. Hence, it is urgent to find approaches to recognize individuals at risk of the condition. Upon the establishment of diagnostic cut-points for low muscle mass, I aimed to examine whether low muscle mass is an independent predictor of cognitive decline in 3 cognitive domains over 3 years.

CHAPTER 5: *Manuscript 2:* Sarcopenia and cognitive decline

Ready for submission

Sarcopenia (low appendicular lean mass) is independently associated with faster cognitive decline over 3 years in older adults of the Canadian Longitudinal Study on Aging

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5.1 Abstract

Importance: Cross-sectional studies have shown that low muscle mass and strength combined is associated with cognitive impairment. Whether low muscle mass, reflective of physiologic reserve, is independently associated with faster cognitive decline remains unknown.

Objective: To investigate the associations between sarcopenia (low muscle mass) and cognitive decline in three distinct domains.

Design: The contemporary Canadian Longitudinal Study on Aging, a prospective cohort study. Enrolment was between 2011-2015 and follow-up over 3 years.

Setting: Population-based.

Participants: 8,279 community-dwelling older adults (65-86 y; 48% women).

Exposure: Appendicular lean soft tissue mass (ALM) was assessed by dual energy X-ray absorptiometry. Sarcopenia (low ALM) was identified using the sex-specific Canadian cut-points.

Main outcomes and Measures: Memory was assessed using the Rey auditory verbal learning test; executive functions using six tests including the mental alternation, Stroop high interference (words/dot), the animal fluency and the controlled oral word association tests. Psychomotor speed was assessed using computer-administered choice reaction time. Composite scores by domain were created.

Results: A total of 1,605 (19.4%; 15.2% in women and 23.3% in men) participants had sarcopenia at baseline. Participants with sarcopenia were older, had lower BMI and physical activity level. The presence of sarcopenia at baseline was associated with faster 3-year cognitive decline in executive functions and psychomotor speed, from multiple linear regressions. After adjusting for covariates including level of education, % body fat and handgrip strength, sarcopenia remained independently associated with executive function decline (std β: -0.032, p=0.028) only. Sarcopenia was not an independent predictor of memory.

Conclusion and Relevance: This study confirmed longitudinal associations between sarcopenia and cognition in aging. Identification of older adults with low muscle mass, a targetable modifiable factor, may help predict those at risk for accelerated executive function decline. Further longer-term investigation of associations is warranted.

5.2 Introduction

Dementia is increasingly prevalent with age and negatively impacts the quality of life of both patients and families (342). Unfortunately, the pathological changes responsible for dementia appear to be irreversible by the time of diagnosis. Treatments are few, very limited in efficacy and target symptoms (91). Therefore, it is critical to identify early forms of the disorder as well as practical and modifiable biomarkers that can predict subsequent cognitive decline. Such measurable and predictive biomarkers would identify high risk patients appropriate for testing of potential disease modifying therapies.

Depending on its definition, sarcopenia prevalence ranges from 10-40% in community-dwelling older adults (343, 344). Sarcopenia was originally characterised by age-related low skeletal muscle mass. Some groups endorse the inclusion of low muscle strength, but given the added confusion to defining the condition, a consensus is not reached (294, 299). In this article, the term sarcopenia defines low muscle mass only (343). The pathogenetic mechanisms proposed for accelerated cognitive decline – lack of anabolic hormones, vascular diseases, chronic inflammation, insulin resistance, neuronal dysfunction – are similarly implicated in sarcopenia suggesting both may be linked (345). Sarcopenia may be prodromal to the onset of cognitive impairment (92) and may represent a sensitive marker of cognitive decline.

The relationship between muscle strength and cognitive function has been investigated with evidence of lesser cognitive decline or decreased risk of dementia among persons with higher handgrip strength (111). However, little attention has been brought to muscle mass. To date, few cross-sectional studies have explored the relationship between muscle mass, the combination of low muscle mass and strength, and cognitive impairment; conclusions were not uniform with some showing an association between the two conditions (105, 106, 346) and others not (10, 107, 109, 112). To our knowledge, no studies have explored the relationship between sarcopenia, independently of muscle strength, and subsequent cognitive decline. To address this, we examined the associations between sarcopenia (low appendicular lean soft tissue mass, ALM) and 3-year decline in three cognitive domains, memory, executive functions and psychomotor speed, in free-living older adults of the Canadian Longitudinal Study on Aging.

5.3 Methods

Study Population

The nationally representative Canadian Longitudinal Study on Aging (CLSA)'s design and methods have been described elsewhere (302). Briefly, 30,097 community-dwelling men and women 45-86 years across 11 cities in 7 provinces were enrolled in the CLSA comprehensive cohort between 2011-2015. Participants were able to speak French or English, were free of cognitive impairment that precluded the ability to provide informed consent at time of recruitment and underwent in-depth neuropsychological, body composition, physical function and clinical assessments at baseline and at 3-year follow-up. Assessments are and will be repeated every 3 years for 20 years.

The subsample used in the current analyses included participants aged ≥65 years, with complete baseline cognitive, body composition and handgrip strength assessments, who were free of multiple sclerosis, Alzheimer's disease, sequelae of stroke or transient ischemic attack, Parkinson's disease, surgery within the last 3 months, polio, chemotherapy within the past 4 weeks, traumatic brain injury with memory problem, positive screen for post-traumatic stress disorder, or receiving dialysis treatment. Subjects with inaccurate dual-energy X-ray absorptiometry (DXA) measurement at baseline were excluded (method described in (343)). The final analytic sample was 8,279 participants (4,276 men and 4,003 women;

Cognitive test scores No. (%)	All 8279	Without sarcopenia 6674 (80.6)	With sarcopenia 1605 (19.4)	P
Memory				
Rey immediate recall, n words (0-15)	0.4 (1.9)	0.4 (1.9)	0.3 (1.9)	0.03
Rey delayed recall, n words (0-15)	0.3 (1.9)	0.3 (1.9)	0.2 (1.9)	0.24

Executive functions				
Animal naming, n words	-0.4 (4.4)	-0.3 (4.4)	-0.7 (4.3)	0.02
MAT, (0-51)	-1.1 (6.6)	-1.0 (6.6)	-1.6 (6.5)	0.004
F-A-S total, n words	0.4 (8.0)	0.5 (7.9)	-0.1 (8.3)	0.02
Stroop's high interference, s	0.03 (0.73)	0.03 (0.73)	0.04 (0.76)	0.74
Psychomotor speed				
Choice reaction time, ms	-5.3 (180.4)	-7.0 (177.1)	2.0 (194.4)	0.11

Values are Mean (SD). P values are from t-test. An increase in the Stroop's high interference and choice reaction time indicates a decrease in cognitive performance. MAT, Mental Alternation Test.

Figure 5-1). The CLSA study was approved by the research site ethics boards (REB) and all participants provided informed consent (303). The present study was approved by the McGill University REB (REB 46-0618).

Neuropsychological assessment

Thorough neuropsychological testing was performed by a trained staff member at baseline and after 3 years (347, 348). The battery consisted of 10 standard English and French cognitive tests, assessing 3 distinct cognitive domains: memory, executive functions and psychomotor speed, and selected for relevance to diseases of aging and psychometric properties. Memory was assessed using the 15-word Rey auditory-verbal-learning test (RAVLT). Results of the first trial (immediate recall) and second trial (5-min delayed recall) were used. Executive functions were evaluated using four tests: the mental-alternation test (MAT), high interference (color names in incongruent colors/colored dots) of the Stroop test, the animal-fluency test (AFT) and the controlled-oral-word-association for the letters F, A and S (COWAT). For the present analysis, the sum of words across the three letters was calculated to provide a single total COWAT result. Psychomotor speed was assessed using computer-administered choice reaction times. The mean response time was used for analyses. The RAVLT, MAT and AFT were administered in home and the COWAT, CRT and Stroop during the interviews at a CLSA data collection site.

Anthropometry

Anthropometric assessments by trained health assessors included body weight measured wearing light clothing without shoes (140-10 Healthweight Digital Physician Scale; kg), standing height with heels, buttocks and shoulder blades touching the stadiometer (Seca 213; cm). All were measured to the nearest 0.1 unit and the average of two measurements for weight and height was used.

Sarcopenia

Whole body composition, including lean soft tissue mass (lean mass) and fat mass, was estimated at baseline using DXA (Hologic Discovery A^{TM} densitometer) as per standard procedures(349). Appendicular lean mass (ALM; kg) was computed as the sum of the upper and lower limbs lean mass and ALM index (ALMI) calculated by dividing ALM by height squared (m^2). The Canadian cut-points for low ALMI (343) were applied to define sarcopenia: men, <7.30 kg/m^2, and women, <5.42 kg/m^2. Body fat percentage

was calculated as fat mass (kg) divided by total body weight (kg) multiplied by 100. In the CLSA, DXA contraindications were: weight or height exceeding 204 kg or 1.88 m respectively, for accuracy of the measurements, exposition to an X-ray with contrast material or participation in a nuclear medicine study within the last 7 days. Each DXA body weight was ascertained from weight measured by a scale and only participants with weight agreement were included in analyses (343).

Other Covariates

Questionnaires on socio-demographic and lifestyle characteristics were collected at baseline during in-home face-to-face interview. Demographic variables included sex, language (English or French), level of education (categorical), household income (categorical) and ethnicity (White, non-White). Cigarette smoking (current daily, occasional, never) and alcohol use (almost every day, 4-5 times/week, 2-3 times/week, once/week, 2-3 times/month, once/month, less than once/month or never) were self-reported. Social participation was based on the frequency of community-related activities practiced in the last 12 months (none, yearly, monthly, weekly, daily). Symptoms of depression were evaluated with the Center for Epidemiological Studies Short Depression Scale (CES-D10; score 0-30) a higher score denoting more depressive symptoms (350); physical activity level using the Physical Activity Scale for Elderly (PASE), a higher score indicating greater level of physical activity (319); and the risk of poor nutritional state using the abbreviated version of the Seniors in the Community Risk Evaluation for Eating and Nutrition (SCREEN II-AB; score 0-48), a lower score indicating a greater risk (320). Presence of type 2 diabetes was self-reported. Whole blood hemoglobin A1C and serum triglycerides were measured (302). Grip strength was assessed by hand-held dynamometry (kg; Tracker Freedom® Wireless Grip). The highest value of 3 trials was used in analyses.

5.4 Statistical Analysis

Characteristics of participants living with and without sarcopenia (low ALM) at baseline were compared by t-test or Mann-Whitney-U test, for normally and non-normally distributed continuous variables, respectively, and chi-square tests for categorical variables. Cognitive test results measured in time units were converted to negative (Stroop’s high interference and choice reaction

time) for all scores to have the same orientation, i.e., a higher score indicating better cognitive performance. For each cognitive test, the 3-year change was calculated as the difference between year 3 and baseline score, and was standardized to a Z-score to allow comparison between tests within a domain. Composite scores of the change in memory (2 results) and executive functions (4 results; FAS, MAT, animal naming and Stroop's high interference) domains were computed as averages. The change in psychomotor speed domain was represented by a single test (choice reaction time). Baseline composite scores per domain were also computed and used as covariate. Multiple linear regressions were used to examine the relationship between sarcopenia and cognitive change in each domain separately. Three models were applied to the 3 cognitive domains. Model 1 was adjusted for age, sex, education, language, baseline composite score; model 2 for ethnicity, income, smoking, alcohol use, symptoms of depression, type 2 diabetes, A1C, serum triglycerides, physical activity, nutritional risk and body fat percentage in addition to model 1 covariates; model 3, was further adjusted for handgrip strength (continuous). Results were reported in difference between those with versus without sarcopenia in pooled non-standardized mean scores (β) and 95% confidence intervals (CI). $P<0.05$ was accepted as statistically significant.

The proportion of missing data for baseline covariates ranged from 0.3-9.3% and that of missing follow-up cognitive test scores, 16-24%. To account for missing information and to reduce bias, multiple imputation with 30 replications was applied. The Markov-Chain-Monte-Carlo algorithm was used to impute data; the predictive model included covariates from model 3 and 3-year change in each cognitive test score. Results were reported both as complete case analysis and following multiple imputation, pooled using Rubin's rules. The CLSA sample weights were considered in all analyses for results to be representative of the Canadian population. All data analyses were performed using IBM® SPSS Statistics (version 27, Chicago, Il, USA).

5.5 Results

Baseline characteristics of the 8,279 participants (mean age 72.9 ± 5.6, 48% women) including baseline cognitive test scores are summarized altogether and by sarcopenic and non-sarcopenic in **Table** 5-1 (STROBE diagram,

Cognitive test scores	All	Without sarcopenia	With sarcopenia	P

No. (%)	8279	6674 (80.6)	1605 (19.4)	
Memory				
Rey immediate recall, n words (0-15)	0.4 (1.9)	0.4 (1.9)	0.3 (1.9)	0.03
Rey delayed recall, n words (0-15)	0.3 (1.9)	0.3 (1.9)	0.2 (1.9)	0.24
Executive functions				
Animal naming, n words	-0.4 (4.4)	-0.3 (4.4)	-0.7 (4.3)	0.02
MAT, (0-51)	-1.1 (6.6)	-1.0 (6.6)	-1.6 (6.5)	0.004
F-A-S total, n words	0.4 (8.0)	0.5 (7.9)	-0.1 (8.3)	0.02
Stroop's high interference, s	0.03 (0.73)	0.03 (0.73)	0.04 (0.76)	0.74
Psychomotor speed				
Choice reaction time, ms	-5.3 (180.4)	-7.0 (177.1)	2.0 (194.4)	0.11

Values are Mean (SD). P values are from t-test. An increase in the Stroop's high interference and choice reaction time indicates a decrease in cognitive performance. MAT, Mental Alternation Test.

Figure 5-1). The majority were White (97%), English-speaking (81%), highly educated (72.5% with post-secondary degree or diploma) and the mean BMI was 27.7 kg/m^2. A total of 1,605 participants (19.4%) had sarcopenia at baseline. Participants with sarcopenia were more likely to be men and current daily smokers; they were older, had lower BMI, and lower physical activity level compared to those without sarcopenia (**Table** 5-1). No differences in education and income levels, and prevalence of type 2 diabetes were observed. At baseline, sarcopenic individuals had lower immediate and delayed recall (memory), animal naming score (executive function) and had poorer performance at the choice reaction time (psychomotor speed).

After 3 years, the mean memory performance increased in the immediate recall and delayed recall (both $p<0.001$); within the executive function domain, the animal naming, MAT test and Stroop's high interference results deteriorated, while the FAS total score increased; and psychomotor speed performance did not change significantly. Individuals with sarcopenia experienced a slighter increase in the immediate recall memory test score, a greater decrease in the animal naming, MAT test score and a decrease in the FAS score compared to those without sarcopenia (**Table** 5-2).

Figure 5-2 illustrates the non-adjusted significant 3-year decline in the executive function composite Z-score in individuals with and without sarcopenia, showing significantly greater decline in the former. For all 3 cognitive domains, respective baseline composite score was the strongest predictor of the change over 3 years; participants with a lower cognitive score at baseline experienced a greater decline. Age (std β: -0.208, $p<0.001$, model 3), sex (men associated with greater decline, std β: 0.238, $p<0.001$, model 3) and income (std β: 0.065, $p<0.001$, model 3) predicted most strongly the change in memory performance. Whereas sarcopenia was not a significant predictor of memory change (**Figure** 5-3), grip strength was (std β: 0.049, $p=0.027$, model 3). Age (std β: -0.115, $p<0.001$, model 3) and education (std β: 0.061, $p<0.001$, model 3) were the strongest predictors of executive function changes. The presence of sarcopenia was associated with greater decline in executive functions independently of all covariates including physical activity level, grip strength and body fat percentage (std β: -0.032, $p=0.028$, model 3; **Figure** 5-3). Age (std β: -0.077, $p<0.001$, model 3) and education (std β: 0.048, $p<0.001$, model 3) were also the main predictors of psychomotor speed change. Sarcopenia was significantly associated with a decline in the latter domain in model 1 (std β: -

0.025, p=0.012), but the association did not remain after full adjustment for covariates (std β: 0.007, p=0.52, model 3; **Figure** 5-3).

5.6 Discussion

This large cohort study provided evidence that community-dwelling older adults living with sarcopenia (low ALM) may experience greater cognitive decline, more specifically of the executive function domain, over 3 years compared to persons without sarcopenia. Results revealed that sarcopenia was associated with executive function change independently of important related factors including body fat percentage and grip strength, of note physical activity was no longer a predictor in model 3. However, no associations were found between sarcopenia and changes in the memory and psychomotor speed domains after adjustments for covariates. The present study adds understanding to the complex relationship between body composition, muscle strength and performance change in 3 cognitive domains using data from a longitudinal Canadian-representative population of seniors.

Previous evidence showed mid-life obesity to be associated with worsen cognitive functions in later years of life (95, 101), but low BMI in older age to be associated with greater cognitive decline (24, 102) and incidence of dementia (101). The protective effect of normal or higher BMI on cognitive functions in late-life may be partly explained by higher circulating levels of anabolic hormones such as testosterone and estrogens (103). It is also possible that low BMI commonly results from weight loss, a marker of MCI reflective of preclinical behavioural changes (92). Involuntary weight loss at an older age inevitably includes muscle. Cross-sectional associations between sarcopenia and cognitive impairment are accumulating (reviewed in (25, 110)) and support a positive link between both conditions. These two recent meta-analyses showed that persons with sarcopenia (heterogeneous definitions) were 2.3 times more likely to have concomitant cognitive impairment compared to non-sarcopenic (adjusted models, 95% CI: 1.2, 4.2 and 1.7, 3.0; 6-11 studies, n=5,994 and n=10,710). Very few longitudinal studies are available to date. In a subsample of the EPIDOS cohort, van Kan et al. examined the 7-year change in percent body fat and muscle mass in relation to cognitive impairment onset at 7 years of follow-up and did not find any associations, possibly due to low power (total n=181 women, 75+ years, n=15 with incident dementia, n=6 with incident MCI) (112). Another study of limited sample size did not report a significant link between ALM, strength and MCI or dementia, but

authors did find a protective association with physical function assessed with the Short Physical Performance Battery score (total n=297 men and women, 65+ years, n=50 with incident MCI, n=5 with incident dementia) (113). To our knowledge the present study is the first to explore and identify an association between low ALM and cognitive decline independently of grip strength which is typically combined in most recent definition of sarcopenia and thought to drive the relationship (106, 351).

Although grip strength was also related, the independent association of low ALM suggests that components specific to skeletal muscle as an endocrine organ may play a protective role in maintaining cognitive executive functions. Indeed, the induction of muscle contraction through exercising may stimulate the release of myokines IL-6 (pleiotropic), IL-8, IL-15, and Brain-Derived Neurotrophic Factor (BDNF) with anti-inflammatory effects (352) and explain part of the potential protective effect of preserving muscle mass for brain health, though results in the current study remained significant after adjustment for physical activity. Additional to the hormonal theory, insulin resistance, oxidative stress and low-grade chronic elevation of pro-inflammatory markers including IL-1, IL-6 and tumor necrosis factor alpha were recognized to be involved in both the pathogeneses of sarcopenia and dementia (345, 353). Whether low muscle mass is an early marker or a causal factor of cognitive decline cannot be resolved from our study. Further elucidation of the mechanisms that link muscle mass to cognitive functions is required to orient the elaboration of treatment and preventive strategies to slow the progression of cognitive decline in seniors.

In the current study, associations with sarcopenia (low muscle mass) were only observed for executive functions among the 3 cognitive domains assessed. The explanation to this specific finding remains to be elucidated. Few studies examined these associations with cognitive subdomains and none in a longitudinal design. One study performed in a Korean population (n=1,887, 70-84 years) showed cross-sectional associations between the Asian Working Group on Sarcopenia (AWGS)-defined sarcopenia (combination of low muscle mass and strength and/or physical performance; odds ratio (OR): 2.98; 95% CI: 1.51, 5.89), European Working Group on Sarcopenia in Older Person 2 (EWGSOP-2)-sarcopenia (OR: 2.78; 95% CI: 1.45, 5.31) and impaired executive functions in men only; however, low muscle mass alone (as per AWGS criteria) was not associated with any cognitive subdomains (351). Also, associations with processing speed were significant, but not with memory. Authors concluded that the significant

relationships were driven by low grip strength and low gait speed, among the sarcopenia criteria. Greater physical activity and cardiorespiratory fitness, possibly linked to greater muscle mass and blood flow to the brain (354), were shown to favor executive functioning particularly (reviewed in (355)). Nonetheless, our statistical models accounted for baseline physical activity level as assessed by the validated PASE score. Executive functions are involved in task initiation, problem solving, attention, organization, working memory, inhibition and others. Persons affected by amnestic MCI, a prodromal stage to Alzheimer's disease, were shown to have significantly worse executive function performance compared to normo-cognitive elderly adults (356). Accelerated decline in these functions may interfere with basic and instrumental daily living activities such as financial and shopping skills earlier in life (357).

Surprisingly, mean scores of both immediate and delayed memory tests increased over 3 years in sarcopenic and non-sarcopenic individuals, whereas memory loss is typically expected with aging (358). Although the RAVLT test is sensitive in cognitive impairment detection (359), our findings may be due to a retest effect or time saving modifications brought to the RAVLT test in the CLSA (1 trial vs. 5 in the original version and 5-min vs. 30-min delay) (360) potentially impairing reliability. The increase in the immediate recall was nonetheless significantly blunted in individuals with sarcopenia vs. those without, suggesting a deficit in the memory domain. The overall improvement during follow-up in both memory scores may have obscured identification of such deficits and their association with sarcopenia. Also, the number of tests to assess each domain differed with more executive functions tests available which may have introduced bias. It remains possible that the memory and psychomotor speed domains are impacted upon further repeated measures and this can be addressed as future follow-up data become available in CLSA.

Numerous strengths pertain to this study, lending confidence in the observed results. These include data collected in a large and modern cohort of older adults and sample weights applied in all analyses allowing generalizability to the Canadian population. Also, seven objective, valid and reliable (potentially excluding the modified version of the RAVLT) cognitive tests were used, more than in most studies (348). Lastly, lean soft tissue mass was evaluated using precise and accurate DXA, the reference method for estimating muscle mass (67) and study population-specific empirical sarcopenia cut-points were applied (343). The current study design is a limitation as it prevents causal inference. However, our results were

consistent before and after statistical adjustment for many key confounding factors, rendering alternative explanations for the observed relationships less likely plausible. Finally, models were not adjusted for *APOE e4* as it was not measured at the time of our analyses.

Our study results suggest that sarcopenia is an independent risk factor for executive functions decline in older adults. Importantly, DXA is widely available and measures of sarcopenia could be routinely incorporated into the image analyses. Clinical screening of older adults to identify those with low muscle mass may provide insight regarding their risk of developing cognitive impairment and thereby guide the testing and application of preventative or therapeutic interventions.

Acknowledgements

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Conflict

The authors declare no conflict of interest.

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Table 5-1 Baseline characteristics of participants in the CLSA cohort and by presence and absence of sarcopenia

	All	Without sarcopenia	With sarcopenia	P
No. (%)	8,279	6,674 (80.6)	1,605(19.4)	
Sex, No. (% Women)	4,003 (48)	3,393 (50.8)	610 (38)	<0.001[a]
Age, y	72.9 (5.6)	72.2 (5.4)	74.3 (5.8)	<0.001
White, No. (%)	8,005 (97)	6,476 (97)	1,529 (95)	<0.001[a]
Language, No. (% French)	1,598 (19)	1,232 (18.5)	366 (22.8)	<0.001[a]
BMI, kg/m^2	27.7 (4.7)	28.6 (4.5)	23.6 (2.9)	<0.001
Education, No. (%)				0.67[a]
<secondary school graduation	705 (8.5)	567 (8.5)	138 (8.6)	
Secondary school graduation, no post-secondary	903 (10.9)	741 (11.1)	162 (10.1)	
Some post-secondary education	641 (8.1)	535 (8.0)	136 (8.5)	
Post-secondary degree/diploma	5,979 (72.2)	4,816 (72.2)	1,163 (72.5)	
Income, No. (%)				0.21[a]
<$20,000	452 (5.5)	353 (5.3)	99 (6.2)	
$20,000-$50,000	2,479 (29.9)	1,985 (29.7)	494 (30.8)	
$50,000-$100,000	3,118 (37.7)	2,504 (37.5)	614 (38.3)	
$100,000-$150,000	1,057 (12.8)	874 (13.1)	183 (11.4)	
≥$150,000	529 (6.4)	433 (6.5)	96 (6.0)	
Type 2 diabetes, No. (%)	918 (11)	759 (11)	159 (10)	0.093[a]
A1C, %	5.7 (0.7)	5.7 (0.7)	5.7 (0.6)	<0.001
Triglycerides, mmol/L	1.7 (0.9)	1.8 (0.9)	1.5 (0.9)	<0.001
Physical activity (PASE)	118.8 (57.5)	121.8 (57.9)	113.4 (55.0)	<0.001
Depression scale (CES-D 10; 0-30)	4.7 (4.1)	4.5 (4.0)	4.8 (4.1)	0.14
Nutritional risk (SCREEN II; 0-40)	39.3 (5.8)	39.5 (5.6)	39.0 (6.0)	0.04
Alcohol consumption, almost everyday No. (%)	1,876 (22.7)	1,462 (21.9)	414 (25.8)	<0.001[a]
Current daily smoker, No. (%)	423 (5)	290 (4.3)	133 (8.3)	<0.001[a]

Cognitive test scores				
Memory				
Rey immediate recall, n words (0-15)	5.3 (1.8)	5.4 (1.8)	5.0 (1.7)	<0.001
Rey delayed recall, n words (0-15)	3.4 (2.0)	3.5 (1.9)	3.1 (1.9)	<0.001
Executive functions				
Animal naming, n words	17.9 (5.2)	18.2 (5.2)	17.6 (5.1)	< 0.001
MAT, (0-51)	24.6 (8.5)	25.1 (8.5)	24.7 (8.0)	0.06
F-A-S total, n words	37.4 (12.7)	37.8 (12.6)	37.7 (13.1)	0.25
Stroop's high interference, s	2.32 (0.70)	2.30 (0.67)	2.32 (0.79)	0.68
Psychomotor speed				
Choice reaction time, ms	875.2 (193.4)	866.7 (187.9)	884.2 (215.5)	< 0.001

Values are Mean (SD) unless otherwise indicated. P values are from Mann–Whitney *U* test unless otherwise indicated. PASE, Physical activity scale for elderly; CES-D 10, Center for Epidemiological Studies Short Depression Scale; SCREEN II, seniors in the community risk evaluation for eating and nutrition; MAT, Mental Alternation Test.

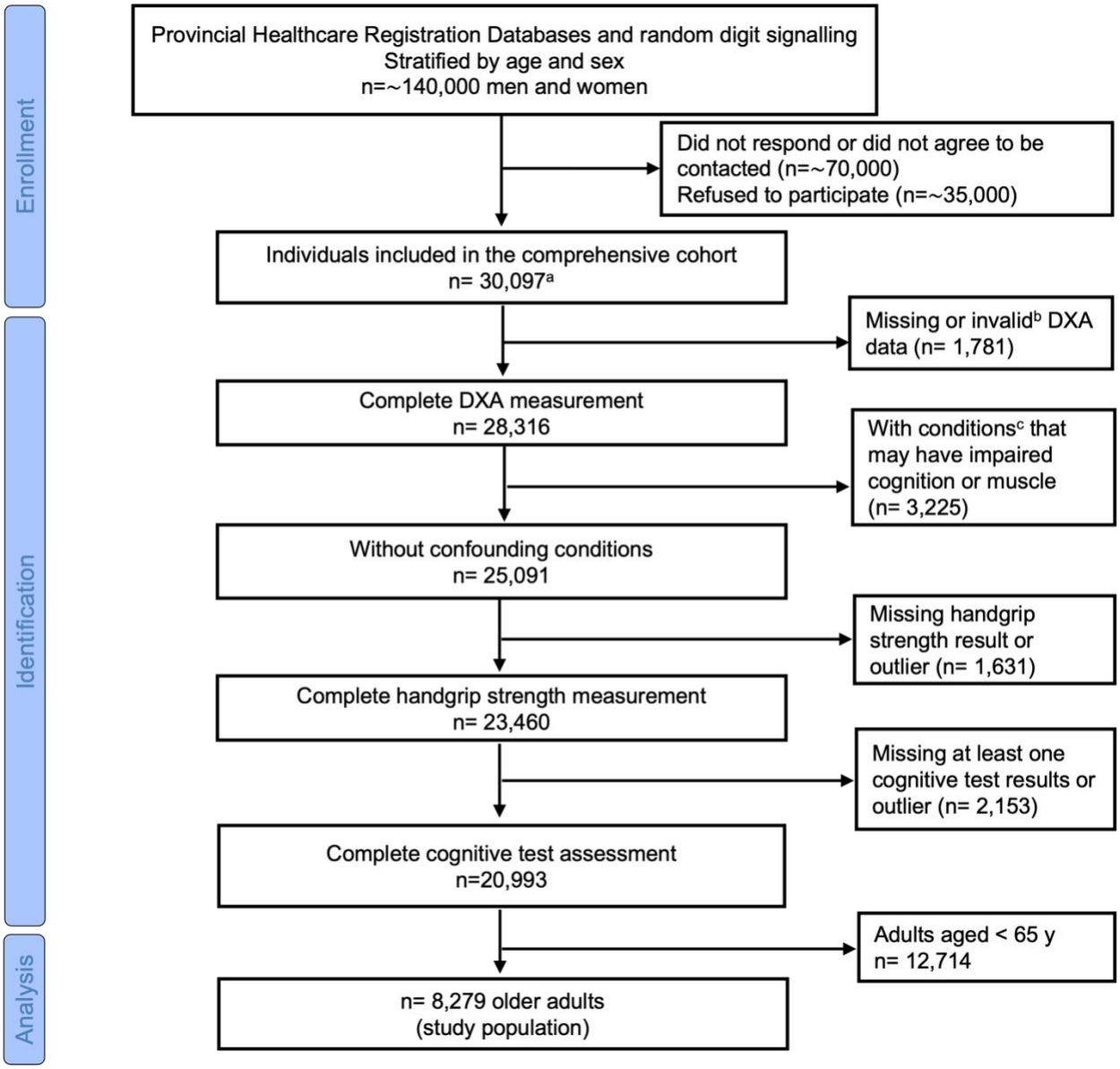
[a] From chi-square test

Table 5-2 . 3-year change in individual cognitive tests of participants in the CLSA cohort and by presence and absence of sarcopenia

Cognitive test scores No. (%)	All 8279	Without sarcopenia 6674 (80.6)	With sarcopenia 1605 (19.4)	P
Memory				
Rey immediate recall, n words (0-15)	0.4 (1.9)	0.4 (1.9)	0.3 (1.9)	0.03
Rey delayed recall, n words (0-15)	0.3 (1.9)	0.3 (1.9)	0.2 (1.9)	0.24
Executive functions				
Animal naming, n words	-0.4 (4.4)	-0.3 (4.4)	-0.7 (4.3)	0.02
MAT, (0-51)	-1.1 (6.6)	-1.0 (6.6)	-1.6 (6.5)	0.004
F-A-S total, n words	0.4 (8.0)	0.5 (7.9)	-0.1 (8.3)	0.02
Stroop's high interference, s	0.03 (0.73)	0.03 (0.73)	0.04 (0.76)	0.74
Psychomotor speed				
Choice reaction time, ms	-5.3 (180.4)	-7.0 (177.1)	2.0 (194.4)	0.11

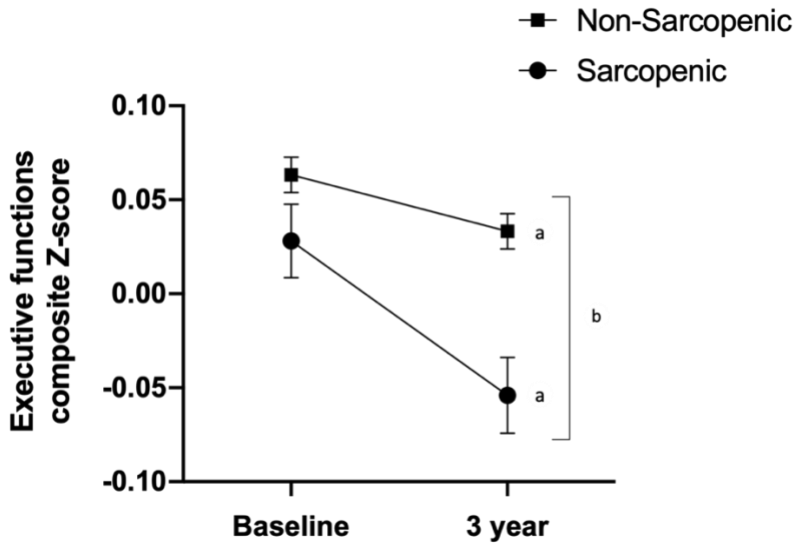
Values are Mean (SD). P values are from t-test. An increase in the Stroop's high interference and choice reaction time indicates a decrease in cognitive performance. MAT, Mental Alternation Test.

Figure 5-1 STROBE diagram of participants in the CLSA cohort



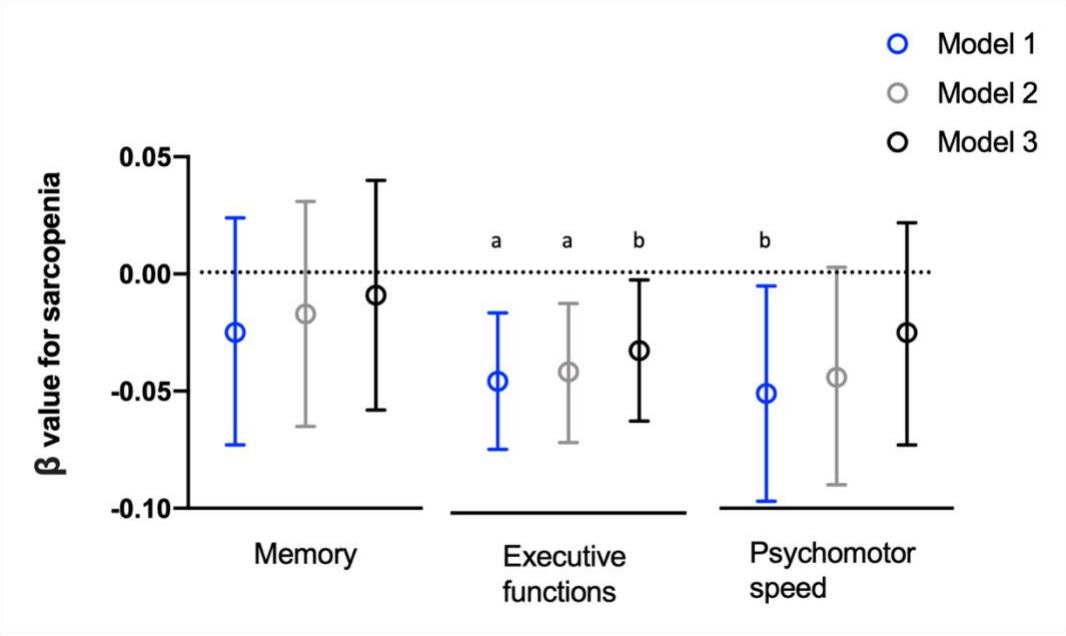
[a]Exclusion criteria are described by Raina et al. (302).
[b]Determined using Bland-Altman agreement plot that compared participants' weight measured by DXA and by scale as described in (343).
[c]Multiple sclerosis, Alzheimer's disease, effects from stroke or transient ischemic attack (TIA), Parkinson's disease, surgery within last 3 months, polio, chemotherapy within last 4 weeks, traumatic brain injury with memory problem, positive screen for post-traumatic stress disorder, receiving dialysis treatment.

Figure 5-2 3-year executive function decline in persons living with and without sarcopenia



Non-adjusted for covariates means ± standard errors. Complete case analysis.
[a] p< 0.001, from paired t-tests.
[b] p< 0.001, from independent t-test.

Figure 5-3 Linear regressions of the association between sarcopenia and cognitive decline over 3 years by cognitive domain



Non-standardized, adjusted β values and 95% confidence intervals. [a]p< 0.01, [b]p< 0.05
Model 1: adjusted for age, sex, education, language, and baseline cognitive composite score (memory, executive functions or psychomotor speed respectively). R^2: memory, 0.18; executive function, 0.14; psychomotor speed, 0.37.
Model 2: adjusted for model 1 covariates and ethnicity, social participation, physical activity, income, alcohol consumption, smoking, blood A1C, triglycerides, type 2 diabetes, percent fat mass. R^2: memory, 0.18; executive function, 0.14; psychomotor speed, 0.39.
Model 3: adjusted for model 2 covariates and grip strength. R^2: memory, 0.18; executive function, 0.15; psychomotor speed, 0.40.
Missing data are from multiple imputation. Analytic weights were considered in all analyses.
A negative β is indicative of a greater cognitive score decline in sarcopenic individuals.

Connecting Statement 4

In **Chapter 5**, the results of the observational longitudinal analyses showed that low muscle mass at baseline, as characterized using the diagnostic criteria determined in **Chapter 4**, was a significant predictor of the executive function cognitive domain decline, but not of memory and psychomotor speed, in healthy older adults. These results were independent of important confounders thought to affect muscle mass and cognitive decline, namely physical activity, % body fat mass and interestingly, handgrip strength. The findings contribute to support that muscle mass is a distinct construct from muscle strength and strengthens the potential implications of having low muscle mass in cognitive decline with aging.

Upon the early identification of individuals at-risk of greater cognitive decline and potentially MCI and dementia, nutritional advice may be tailored to preserve autonomy or to delay the onset of severe cognitive dysfunction. As described in **Chapter 2, Section 2.6.1**, dairy product intake including milk, cheese and yogurt, is generally low in older Canadian adults; yet these foods have a unique matrix and are valuable sources of several nutrients (156) possibly related to cognition in adults (29). European and Australian cross-sectional (180-182) and prospective (183) data showed positive associations between dairy product intake (total dairy products (180, 182), cheese only (181)), or neutral associations (183), with cognitive functions. However, these studies were few and some did not report analyses by dairy fat content. Since there were no such studies conducted in a Canadian population with a possibly different dairy product consumption and diet, I questioned if there would be an association between greater dairy product intake and function in 3 cognitive domains in healthy Canadian older adults. I postulated that greater total dairy product intake and also individual dairy types (milk, cheese, yogurt, fermented dairy, low-fat and regular-fat dairy) are associated with higher cognitive performance in executive functions, memory and psychomotor speed, independently of diet quality and other confounding factors. This large cross-sectional study with robust assessment of cognitive function will provide evidence for public health, longitudinal studies and future trials.

CHAPTER 6: *Manuscript 3*: Dairy products and cognitive functions

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Milk, yogurt and cheese intake is positively associated with cognitive executive functions in older adults of the Canadian Longitudinal Study on Aging

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6.1 Abstract

Background

Dairy products provide essential nutrients such as calcium, vitamins B12 and D, and include bioactive peptides and fermented products, which may be beneficial for cognition, especially in older adults. Yet, few studies of large contemporary cohorts have investigated this relationship using sensitive domain-specific cognitive tests.

Methods

In community-dwelling older adults of the Canadian Longitudinal Study on Aging (2011-2015), we examined cross-sectional associations between total and specific dairy product intake and performance in three cognitive domains (executive functions, memory, psychomotor speed). Cheese, milk, yogurt, regular-fat, low-fat and fermented dairy product intake frequencies were estimated using a food frequency questionnaire; participants were classified into quartiles. MANCOVA models were applied to estimate differences.

Results

In 7,945 participants (65-86 y, 49% women, 97% Caucasian), the mean dairy product intake was 1.9 (1.1) times/d. Total dairy product, cheese and low-fat dairy product intake were positively associated with the executive function domain and yogurt intake with the memory domain (all $p<0.05$), independently of important covariates including age, gender, education and diet quality. Intakes of total dairy product, cheese and low-fat dairy were associated with verbal fluency specifically (all $p<0.05$). Participants with a dairy product intake > 2.5 times/d had a higher score compared to those consuming less. No associations were found with psychomotor speed.

Conclusions

This large cohort study suggests a specific role for dairy components in executive function phonemic verbal fluency and memory. Dairy product intake, a modifiable factor, may be targeted in cognitive health-promoting interventions.

6.2 Background

Cognitive functions such as memory and executive functions often decline with age, and factors including inflammation, vascular diseases, diabetes and obesity may accelerate this decline (86). The global prevalence of dementia among adults aged over 60 years was estimated at 35.6 million in 2010, projected at 115.4 million individuals in 2050 (89). Cognitive impairment leads to the loss of autonomy, decreased quality of life, frailty and mortality, and imposes a heavy burden on the family and health care systems (8, 10, 13, 89). Considering that current pharmacological therapy cannot reverse or treat cognitive impairment, there is an urgent need to identify modifiable determinants of cognitive status, such as dietary factors, as potential strategies to slow cognitive decline.

Several nutrients (177, 361, 362), foods (363, 364) and overall diet quality (142, 144, 365) have been related to cognition. Yet, limited consideration was given to dairy products and their plausible direct and indirect (e.g. via improved functions, such as vascular (366)) influence in modulating cognitive function. The potential beneficial effects of dairy products could pertain to their unique food matrix and content in specific bioactive peptides rich in tryptophan and cysteine, lactic acid bacteria in fermented products, fatty acids and peptides derived from the fermentation process, B vitamins, including vitamin B_{12}, vitamin D, vitamin K, calcium and other nutrients (29, 367).

Few studies specifically examined the relationship between dairy product intake and cognitive functions (180, 182, 183, 368). Observational studies published prior 2010 did not focus on dairy intake as the main exposure and showed mixed results (31). More recent studies of dairy product intake as the main exposure suggested positive cross-sectional associations with cognitive performance (180, 182), and a greater intake was associated with a decreased risk of Alzheimer's (369). Neutral (183) or negative associations (370) were found with milk intake prospectively. However, the current evidence suffers from methodological limitations. Indeed, most studies used global cognitive scores, clinical diagnosis of dementia or depression-oriented self-reported questionnaires, precluding the identification of potential associations with cognitive domains (31, 180, 369). Dairy intake assessment lacked reporting dairy fat content or distinct dairy products, or included dairy-based desserts, ice cream, cream or butter in the total dairy intake. Few studies accounted for diet quality, which may have interfered with observed results.

This study aimed to examine the association between total dairy intake and three cognitive domains in a large contemporary cohort of community-dwelling older Canadians. The secondary objective was to investigate associations with specific dairy types; milk, yogurt, cheese, regular-fat and low-fat, and fermented products.

6.3 Methods

Study population

The present study used baseline data (2011-2015) of the nationally representative Canadian Longitudinal Study on Aging (CLSA) comprehensive cohort, which includes 30, 097 community-dwelling men and women aged 45-86 years who were able to speak French or English and underwent in-depth neuropsychological assessments (302). Detailed description of the CLSA was reported (302).

The subsample used in the current analyses included participants aged ≥ 65 years, with complete cognitive and dietary assessments, who were free of multiple sclerosis, Alzheimer's disease, sequelae of stroke or transient ischemic attack, Parkinson's disease, surgery within the last 3 months, polio, unstable heart condition within the past 3 months, pulmonary embolism within the past 6 weeks, chemotherapy within the past 4 weeks, traumatic brain injury with memory problem, positive screen for post-traumatic stress disorder and receiving dialysis treatment. The final analytic sample was 7,945 participants (4,079 men and 3,866 women) (**Supplementary Figure** 6-1). The CLSA study was approved by the research site ethics boards (REB) and all participants provided informed consent (303). The present study was approved by the McGill University REB (REB 46-0618).

Cognitive function assessment

Participants underwent 10 different standardized cognitive tests assessing 3 cognitive domains: memory, executive functions and psychomotor speed as described previously (347, 348). Under the supervision of psychologists, the tests were administered by trained interviewers as part of the in-home and data collection site questionnaires and scored using computer algorithms for standardization. Briefly, memory was evaluated using the 15-word Rey auditory verbal learning test immediate recall (RAVLT-I) and 5-min delayed recall (RAVLT-II); executive functions using the mental alternation test (MAT), high interference of the Victoria Stroop test (interference/dot), event- and time-based prospective memory tests, and two verbal fluency tests, namely the animal fluency test (AFT) and the sum of the controlled oral word association of the letters F, A and S (COWAT F-A-S total score); psychomotor speed was assessed using the mean response time of the Choice Reaction Time (CRT), a computer-administered test.

Dietary assessment

The 36-item Short Diet Questionnaire (SDQ), a semi-quantitative validated food frequency questionnaire was administered as part of the 90-min in-home questionnaire (371, 372). Dairy product intake was assessed as the consumption frequency of 8 dairy products within the last 12 months: whole (3.25%), low-fat (2%, 1%, skimmed) and calcium-fortified milk (35% more calcium) for drinking, regular and low-fat yogurt and regular and low-fat cheese, and desserts including ice cream, ice milk, frozen yogurt, milk-based desserts (pudding, etc). Desserts were excluded from the total due to the different nature of these foods containing more refined sugar and fat. All dairy product frequencies of intake (per day, week, month and year) were converted to frequencies per day; servings were not available in this questionnaire. Total dairy intake, regular and low-fat dairy intake frequencies were calculated as the sum of each respective dairy products intake frequencies. Fermented dairy intake was calculated as the sum of cheese and yogurt intake frequencies.

Given the recognized association between diet quality, dietary patterns and cognitive function (373, 374) and because the SDQ does not permit to compute the validated diet quality Canadian Healthy Eating Index (C-HEI) we developed a diet quality score using available data for adjustment of our models. This score was compiled following 3 steps: (1) selecting SDQ food items to include in the score to obtain four food groups as per the Canada's Food Guide (CFG)

2007, i.e., fruit and vegetables, grains products, meats and alternatives, dairy and alternatives (375); (2) calculating the total frequencies of selected items per food group; (3) capping the four total intakes to the CFG servings recommendations for adults aged ≥51 years and for men and women respectively, and converting it to an equal maximal score of 10 for each food group as in the HEI 2015 (376). Six food items were included in the fruits and vegetables group, 8 items in the meat and alternatives group and two items in the grain products group (**Supplementary Table** 6-2). Dairy product intake frequency was not included in the total score to avoid over-adjustment; hence, the total diet quality score ranged from 0 to 30, a higher score representing better quality. Since this score is not validated, we performed a sensitivity analysis with fruits and vegetables, and whole grain intake frequency as alternative proxies for diet quality in our multivariate models 3.

Other covariates

At baseline, structured questionnaires administered over the phone or in person provided data on age (years), sex, language (English vs. French), province, education, ethnicity (Caucasian vs. non-Caucasian), household income, lifestyle characteristics and medical history. Body weight and standing height were measured following standard procedures and BMI was calculated by dividing the weight (kg) by the height (m) squared. The latter was divided into 6 categories (underweight, normal weight, overweight, obese class I, obese class II, obese class III) as per the International Classification. History of ischemic heart diseases, myocardial infarction or angina pectoris, and of type 2 diabetes was self-reported. Social participation was compiled as the number of social activities performed at least once per day out of 8 activities including family/friends, religious, sports, educational or cultural, clubs, volunteering or other recreational activities. Current smoking status was dichotomized, and level of alcohol consumption scored as almost every day, 4-5 times per week, 2-3 times per week, once per week, 2-3 times per month, once per month, less than once per month or never. Physical activity level was evaluated by the Physical Activity Scale for Elderly (PASE) (319), depression symptoms using the Center for Epidemiological Studies Short Depression Scale (CES-D10) (score 0-30), a higher score indicating more depressive symptoms (350) and the risk of poor nutritional state with the abbreviated version of the Seniors in the Community Risk Evaluation for Eating and Nutrition (SCREEN II-AB; score 0-48), a lower score indicating a greater risk (320).

Statistical analyses

Participants characteristics are shown as means ± SDs for continuous variables and as percentages for categorical variables across quartiles of total dairy intake frequency (Q1: ≤ 1.1 times/d, Q2: 1.2-1.7 times/d, Q3: 1.7-2.4 times/d, Q4: > 2.5 times/d). Differences in sample characteristics at baseline were tested by one-way ANOVA and Kruskal-Wallis tests, for normally and non-normally distributed variables, respectively, and chi-square tests for proportions. Participants were also classified by quartiles of milk, cheese and yogurt, regular-fat and low-fat dairy and fermented dairy intake for analyses of associations by dairy product types. Given its distribution of intake frequency, milk could not be ranked in four equal groups but rather in three, as Q2 and Q3 combined.

Cognitive tests measured in time units (s and ms), were natural log-transformed for normalization of the data distribution and were multiplied by –1 for dependent variables to have the same orientation, with a higher score indicating a better cognitive performance. All tests results were converted to the same scale for comparison using Z-scores; distributions had a mean of zero and SD of 1. Due to a ceiling effect, prospective memory tests were not included in analyses. Cross-sectional associations with quartiles of dairy intake frequency were examined using MANCOVA for memory and executive function domains, and univariate one-way ANCOVA for the psychomotor speed test. All assumptions were verified; multivariate outliers, were detected using Mahalanobis distance and removed as implausible data (n=43 for memory and n=46 for executive function). No violation of MANCOVA or ANCOVA assumptions were found. $P<0.05$ was considered statistically significant. Significant cognitive domain multivariate results were further analyzed in univariate ANCOVA for each of the cognitive test with Bonferroni correction for multiple comparisons.

Three models are presented per cognitive domain, for total and specific dairy product intake frequency. Model 1 was adjusted for age, gender, education, language and province; model 2, for model 1's covariates plus ethnicity, income, smoking, alcohol use, social participation, symptoms of depression, type 2 diabetes, heart conditions, physical activity level, BMI category and nutritional risk; model 3, for all of the above and diet quality.

A sensitivity analysis was carried out to test the stability and validity of the main models for executive function, memory and psychomotor speed; two sets of models were tested, one including both regular- and low-fat dairy within each model and the other including all dairy

product types (milk, cheese and yogurt) within each model. Also, the main models were performed with fruits and vegetables intake, and whole grains intake as an alternative to the diet quality score. Lastly, the main models were repeated with milk-based desserts intake as part of the total intake of dairy products. Analyses were conducted using IBM® SPSS® Statistics, version 24 (Chicago, Il, USA).

6.4 Results

A total of 7,945 participants (49% women) were included in the analyses (**Supplementary Figure** 6-1). Participants were 65-86 years with a mean age of 72.8±5.6, they were primarily White (97%) and highly educated (72.5% with post-secondary degree or diploma). Participants excluded for missing cognitive or dietary assessment were slightly older, had non-clinically relevant higher BMI, were marginally less educated and included 3% less women than the study sample.

The mean total dairy intake frequency was 1.9±1.1 times/d; 1.3±1.0 as low-fat and 0.6±0.6 as regular-fat products (data not shown). Milk contributed the majority of dairy intake with frequency of intake of 0.85±0.80 time/d followed by cheese (0.54±0.40) and yogurt intake (0.47±0.44). Participants had a mean diet quality score of 15.5±4.1 out of 30. The majority of participants were dairy product consumers (99%) with only 79 individuals not consuming any.

Participant characteristics are summarized by dairy intake frequency quartile in **Table** 6-1. The highest consumers of dairy products were older and included a higher proportion of women and French-speakers and a lower proportion of current smokers. They presented lower nutritional risk, greater diet quality, and lower BMI. Education, symptoms of depression, physical activity levels, heart disease and diabetes prevalence did not differ across quartiles of total dairy product intake.

Total dairy intake was associated with the executive function domain in adjusted model 1 and remained after adjustment for additional covariates (**Table** 6-2, model 2) including diet quality (model 3). Total dairy intake was not associated with memory and psychomotor speed cognitive domains. No interactions between total dairy intake and age, sex, education, or BMI were observed in any cognitive domain.

Within the executive function domain, analyses of individual tests revealed significance for the COWAT F-A-S score test after Bonferroni correction. Participants with a dairy product

intake (in frequency of intake) >2.5 times/d were able to name 1.2 more words compared to those with intakes ≤1.1 times/d (95% CI: -2.3, -0.02 words), and 1.4 more words than those with intakes between 1.2-1.7 times/d (95% CI: -2.6, -0.2), **Figure** 6-1. Significance for differences in the COWAT F-A-S score across dairy intake quartiles did not remain after adjustment for diet quality score (model 3: Q4 vs. Q1, p=0.291; Q4 vs. Q2, p=0.062).

Intake in all types of dairy products, except milk, were associated with the executive function domain in the adjusted model 1 (**Table** 6-3). In model 2, cheese, low-fat dairy and fermented dairy intake were significantly associated with the executive function domain and the association for cheese and low-fat dairy remained after further adjustment for diet quality. When executive function tests were considered separately in univariate ANCOVA, cheese was associated with both the COWAT F-A-S and animal naming tests and low-fat dairy, with the F-A-S test (Figures 1B, 1C - **Figure** 6-1 & 2A - **Figure** 6-2). Yogurt was the only dairy product to be significantly and independently associated with the memory domain (**Table** 6-3), but the single memory tests were not significant after Bonferroni correction (data not shown). We did not find any associations with the psychomotor speed domain.

Results from the sensitivity analysis were consistent with those of our main analyses. The effect sizes and significance of cheese (Wilks' λ =0.996, F=1.80 (12, 17450), p=0.043) and low-fat dairy product consumption (Wilks' λ =0.995, F=2.66 (12, 15398), p=0.001) as determinants of executive functions remained unchanged in the fully-adjusted models; and for yogurt as a determinant of memory (Wilks' λ =0.998, F=2.20 (6, 11636), p=0.040). Also, as an example, total dairy intake frequency remained significantly associated with executive functions when replacing the diet quality score with fruits and vegetables intake, and whole grains (Wilks λ =0.995, F=2.46 (12, 15578), p=0.003). The addition of milk-based desserts consumption (mean 0.14±0.25 time/d) to the total dairy intake resulted in a slight attenuation of associations with the executive function domain (model 3: Wilks λ =0.996, F=1.84 (12, 15404), p=0.037) and no change for the memory and psychomotor speed domains.

6.5 Discussion

This large national community-based study revealed a positive cross-sectional association between total dairy product, cheese and low-fat intake frequencies and the executive function domain, and between yogurt intake and the memory domain. These associations were

independent of important factors linked to cognitive function including age, income, education and diet quality, which suggest a role for dairy intake in specific cognitive domains.

Two previous studies examined the cross-sectional association between dairy product intake and cognitive function in populations that included younger and older adults, and both found favorable and neutral associations (180, 182). Crichton et al. reported positive relationships between consumption of dairy products at least once per day and scores of overall cognition, memory and executive functions in 972 American men and women aged 23-92 years. In contrast to our study, only tests of executive functions, including the COWAT, were no longer significantly associated with total dairy intake after adjusting for covariates (182). A possible explanation to attenuated associations may be the adjustment for plasma homocysteine. Dairy foods, a rich source of B-vitamins, inversely modulate homocysteine circulating levels which may improve cognition, especially executive/language functions (377). Park et al. found that non-consumers of dairy products, as assessed by a single 24-h recall, had lower digit symbol substitution test score (vs. other quartiles of dairy intake; n=2,189, ≥60 years 1999-2002) and short-term memory (vs. consumers; n=4,282, ≥60 years, 1988-1994) in older adults of the NHANES (180). While we also found a positive association with the COWAT test within the executive function domain, our analyses did not show an association between total dairy intake and short-term memory. Interestingly, these observational results are supported by those of an interventional study which showed improvement of working memory, a test of executive functions, after a 6-month intervention of high reduced-fat dairy intake (4 servings/d) compared to a low intake (1 serving/d) in low-dairy consumers middle-aged adults (179). We hypothesized that regular dairy consumers have a better overall diet quality which in turn, has been associated with better cognitive function (373, 374). In models adjusted for diet quality (models 3), the association between total dairy intake and the executive function domain was attenuated, yet remained significant (**Table** 6-2, p=0.012) pointing to a potential independent effect of dairy products. In univariate models, the association between total dairy intake and the COWAT F-A-S score was also attenuated (**Figure** 6-2, Q2 vs. Q4; model 2: p=0.012 vs. model 3: mean adjusted difference= -1.2 words, p=0.062, data not shown). The association between total dairy intake and the executive function domain may be attributed to specific dairy products, yet this evidence is very limited. Sensitivity analysis with milk-based desserts showing a slight attenuation of relationships suggests that higher sugar and fat content may counteract beneficial

dairy nutrients. But the association of total dairy with executive function remained, at least at this low, infrequent consumption of milk-based desserts.

To our knowledge, no cross-sectional study of cognitive functions and dairy product intake as the main exposure variable reported analyses by fat content (regular- vs. low- or reduced-fat). Similar to our observations with total dairy intake, we found independent associations between total low-fat dairy and the executive function domain and with the verbal fluency F-A-S score. Interestingly, low-fat dairy intake was driving the relationship between total dairy product and executive functions as evidenced by greater F statistics in multivariate and greater F-A-S total score differences between highest and lowest consumers, in univariate models. This is mirroring the popular choice of dairy products of low-fat content by this population, possibly influenced by national dietary recommendations (139). A systematic review showed a possible association between high intakes in full-fat dairy and/or saturated dairy fats and greater risk of MCI, Alzheimer's or dementia, poorer psychomotor speed and global cognitive function (31). However, the classification of full-fat dairy in these studies consisted in dairy desserts and ice cream, whole milk or fat from milk and spreads. In contrast, in our study, regular-fat dairy (milk, yogurt and cheese) intake was positively associated with executive functions in the first model adjusted for age, gender, language, education and province; significance was lost after full adjustment for other covariates supporting a neutral, but no negative association.

The role of dairy in cognition may be indirect and independent of fat content. Indeed, consistent evidence supports either favorable or neutral associations between both low-fat and whole-fat dairy and the risk of type 2 diabetes, stroke, hypertension and cardiovascular diseases (171, 172), and vascular diseases were shown to predict cognitive impairment (378). In our study, the relationship between low-fat dairy intake and executive functions was independent of type 2 diabetes and heart conditions suggesting that explanatory mechanisms may pertain to effects of dairy constituents other than fat content, such as alpha-lactalbumin and B-vitamins (30, 379). Our findings reinforce the importance of defining and grouping dairy products as per their nutrient profile for proper interpretation.

The highest quartile of cheese consumers scored higher in the F-A-S and animal naming score than the two lowest quartiles, a finding consistent with two other studies (380) that investigated individual dairy foods. However, two more studies did not find any cross-sectional

(182) or prospective (183) associations after adjusting for covariates. Emerging evidence is pointing to a potential role for fermented dairy in cognitive functions possibly explaining these associations. Indeed, we also observed positive and independent associations of total fermented dairy intake, i.e. cheese and yogurt, with the executive function domain before adjustment for diet quality. Mechanisms of action through which fermented dairy products may improve cognition could involve bioactive peptides that may increase brain dopamine levels (175) and through their high content in vitamin K2 that may contribute to higher vitamin K status (381), related to better memory (177).

Whether milk intake is specifically associated with cognitive function remains unclear. Previous groups observed associations between a higher intake of milk and lower verbal performance (183), greater cognitive decline (370) and impaired cognitive function (MMSE≤ 24) (142) in older adults, but others found no associations (180, 380, 382, 383). In our study, milk was the only dairy product not to emerge as a significant predictor of any cognitive domain. This may be attributed to the majority of participants reporting an intake between the 2nd and 3rd quartiles, resulting in a merge of the two quartiles.

Our findings are supported by several strengths namely the large and contemporary cohort, the sensitive and standardized cognitive assessment of three distinct cognitive domains, the robust multivariate statistical approach with adjustment for key covariates including diet quality, and reporting of associations as exposure-responses. Despite that dietary assessment using the SDQ was limited by the absence of portion size estimation, optimal categorization of dairy products was applied and analyses performed separately, providing further insight into potential association with specific matrices and/or fermented products. The cross-sectional nature prevents from inferring causality; residual confounding cannot be excluded, and a potential bias may occur by selecting participants with complete cognitive and dietary assessments.

In summary, we found positive and independent cross-sectional associations between total dairy products, low-fat dairy products, cheese intake and executive functions and between yogurt intake and memory in community-dwelling older adults. This study points to a potential preventive role for dairy product consumption as a possible modifiable factor of cognitive decline in aging, contributing to increase awareness of the health benefits of dairy products. The positive or neutral associations found between both low-fat and regular-fat dairy intake and

cognitive functions in this study, along with those regarding cardiovascular outcomes reported by others, do not wholly support the current Canadian recommendations for older adults to consume low-fat dairy products. Future research of longitudinal investigation of dairy product intake and its relationship with cognitive decline and onset of cognitive impairment and dementia is warranted.

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Conflict

The authors declare no conflict of interest.

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Table 6-1 Baseline characteristics of participants by baseline quartiles of dairy intake frequency

	Q1 ≤ 1.1 times/d	Q2 1.2-1.7 times/d	Q3 1.7-2.4 times/d	Q4 > 2.5 times/d	P
n	2110	1889	2132	1814	
Gender, n (%W)	873 (41)	878 (46)	1108 (52)	1007 (56)	< 0.001[a]
Age, y	72.6 ± 5.5	72.6 ± 5.5	72.9 ± 5.6	73.2 ± 5.7	0.005
White, n (%)	2006 (95)	1828 (97)	2072 (97)	1775 (98.0)	< 0.001 [a]
BMI, kg/m^2	27.7 ± 4.7	27.9 ± 4.8	27.7 ± 4.7	27.4 ± 4.5	0.017 [a]
Education, n (%)					0.091 [a]
Post-secondary degree/diploma	1483 (70.5)	1337 (70.9)	1575 (74.0)	1347 (74.5)	
Heart conditions, n (%)	369 (17)	304 (16)	370 (17)	312 (17)	0.642 [a]
Type 2 diabetes, n (%)	245 (12)	202 (11)	227 (11)	203 (11)	0.727 [a]
Physical activity (PASE)	119.5 ± 61.0	118.5 ± 55.9	119.2 ± 57.7	118.3 ± 55.0	0.991
Depression scale (CES-D 10; 0-30)	4.7 ± 4.3	4.6 ± 3.9	4.6 ± 4.1	4.7± 3.9	0.529
Current smoker, n (%)	136 (7)	79 (5)	88 (4)	68 (4)	< 0.001 [a]
Cognitive test scores					
Rey immediate recall, n words (0-15)	5.2 ± 1.8	5.3 ± 1.8	5.3 ± 1.8	5.3 ± 1.8	0.130[b]
Rey delayed recall, n words (0-15)	3.3 ± 1.9	3.4 ± 1.9	3.4 ± 2.0	3.4 ± 2.0	0.418 [b]
Animal naming, n words	17.9 ± 5.1	17.8 ± 5.1	18.0 ± 5.2	18.0 ± 5.1	0.618 [b]
MAT, (0-51)	24.6 ± 8.6	24.4 ± 8.3	25.1 ± 8.5	24.6 ± 8.5	0.064 [b]
F-A-S total, n words	37.0 ± 12.6	37.2 ± 12.9	37.6 ± 12.5	38.0 ± 12.8	0.073 [b]
Stroop's high interference, s	2.3 ± 0.7	2.3 ± 0.7	2.3 ± 0.7	2.3 ± 0.7	0.049 [b]
Choice reaction time, ms	870.1 ± 181.3	880.3 ± 195.8	877.6 ± 206.9	874.9 ± 193.0	0.476 [b]
Dietary intake frequencies, times/d					
Total dairy	0.70 ± 0.35	1.48 ± 0.16	2.12 ± 0.20	3.33 ± 0.81	< 0.001
Yogurt	0.17 ± 0.25	0.38 ± 0.36	0.61 ± 0.39	0.75 ± 0.48	< 0.001
Milk	0.21 ± 0.31	0.64 ± 0.42	0.90 ± 0.45	1.8 ± 0.95	< 0.001
Cheese	0.32 ± 0.27	0.46 ± 0.27	0.60 ± 0.35	0.80 ± 0.53	< 0.001
Low-fat dairy	0.36 ± 0.37	0.92 ± 0.45	1.39 ± 0.59	2.47 ± 1.04	< 0.001
Regular-fat dairy	0.34 ± 0.32	0.55 ± 0.43	0.72 ± 0.57	0.86 ± 0.77	< 0.001
Fermented dairy	0.49 ± 0.35	0.84 ± 0.42	1.21 ± 0.44	1.55 ± 0.74	< 0.001

Diet quality without dairy (0-30)	14.2 ± 4.1	15.0 ± 3.9	15.9 ± 3.8	16.9 ± 3.9	< 0.001[b]

Note. Values are mean ± SD unless otherwise indicated. P values are from Kruskal-Wallis test unless otherwise indicated. Q, quartile; PASE, Physical activity scale for elderly; CES-D 10, Center for epidemiological studies short depression scale; MAT, Mental alternation test.

[a] From chi-square test.

[b] From one-way ANOVA.

Table 6-2 Comparison of cognitive performance across quartiles of dairy intake frequency by analysis of covariance in the CLSA cohort

Cognitive domains	Model 1				Model 2				Model 3			
	Wilks' λ	F (df1, df2)	Partial η^2	P	Wilks' λ	F (df1, df2)	Partial η^2	P	Wilks' λ	F (df1, df2)	Partial η^2	P
Memory[a]	1.000	0.556 (6, 15730)	0.000	0.765	1.000	0.352 (6, 11946)	0.000	0.909	1.000	0.297 (6, 11646)	0.000	0.939
Executive functions[b]	0.996	2.413 (12, 20796)	0.001	0.004	0.995	2.546 (12, 15801)	0.002	0.002	0.996	2.132 (12, 15407)	0.001	0.012
Psychomotor speed[c]	-	0.526 (3, 7909)	0.000	0.665	-	0.352 (3, 6008)	0.000	0.788	-	0.487 (3, 5858)	0.000	0.691

Note. Model 1, adjusted for age, gender, language, province and education; model 2, adjusted for model 1 covariates and ethnicity, smoking, BMI, alcohol consumption, income, social participation, physical activity level, depression, nutritional risk, diabetes, heart conditions; Model 3, adjusted for model 2 covariates and diet quality score.

[a]From MANCOVA based on 2 tests: the Rey immediate recall and delayed recall.

[b]From MANCOVA based on 4 tests: the F-A-S oral word association, animal naming, mental alternation test, and Stroop high interference.

[c]From univariate ANCOVA based on 1 test: the choice reaction time.

Table 6-3 Comparison of cognitive performance across quartiles of cheese, yogurt and milk intake frequency by analysis of covariance

Cognitive domains	Model 1			Model 2			Model 3		
	Wilks' λ	F (df1, df2)	Partial η^2	Wilks' λ	F (df1, df2)	Partial η^2	Wilks' λ	F (df1, df2)	Partial η^2
Cheese									
Memory[a]	0.999	1.355 (6, 15730)	0.001	0.999	0.593 (6, 11946)	0.000	0.999	0.568 (6, 11646)	0.000
Executive functions[b]	0.993	4.729*** (12, 20796)	0.002	0.995	2.381** (12, 15801)	0.002	0.996	1.925* (12, 15407)	0.001
Psychomotor speed[c]	-	0.716 (3, 7909)	0.000	-	0.218 (3, 6008)	0.000	-	0.383 (3, 5858)	0.000
Yogurt									
Memory[a]	0.996	5.410** (6, 15730)	0.002	0.998	2.129* (6, 11946)	0.001	0.998	2.348* (6, 11646)	0.001
Executive functions[b]	0.996	2.859** (12, 20796)	0.001	0.997	1.666 (12, 15801)	0.001	0.996	1.706 (12, 15407)	0.001
Psychomotor speed[c]	-	0.847 (3, 7909)	0.000	-	0.539 (3, 6008)	0.000	-	0.563 (3, 5858)	0.000
Milk									
Memory[a]	1.000	0.365 (4, 15732)	0.000	1.000	0.578 (4, 11988)	0.001	1.000	0.499 (4, 11648)	0.000
Executive functions[b]	0.999	0.757 (8, 15722)	0.000	0.998	1.365 (8, 11946)	0.001	0.998	1.425 (8, 11648)	0.001
Psychomotor speed[c]	-	0.842 (2, 7910)	0.000	-	0.325 (2, 6009)	0.000	-	0.259 (2, 5859)	0.000
Fermented dairy									
Memory[a]	0.999	1.175 (6, 15730)	0.000	0.999	1.335 (6, 11946)	0.001	0.999	1.226 (6, 11646)	0.001
Executive functions[b]	0.994	3.840*** (12, 20796)	0.002	0.996	1.799* (12, 15811)	0.001	0.997	1.562 (12, 15417)	0.001
Psychomotor	-	1.023	0.000	-	1.160	0.001	-	0.776	0.000

speed[c]		(3, 7909)			(3, 6008)			(3, 5858)	
Low-fat dairy									
Memory[a]	0.999	1.282	0.000	0.999	0.751	0.000	0.999	0.784	0.000
		(6, 15730)			(6, 11946)			(6, 11646)	
Executive functions[b]	0.996	2.860*	0.001	0.994	2.804**	0.002	0.995	2.660**	0.002
		(12, 20796)			(12, 15801)			(12, 15407)	
Psychomotor speed[c]	-	0.740	0.000	-	1.544	0.001	-	1.609	0.001
		(3, 7909)			(3, 6008)			(3, 5858)	
Regular-fat dairy									
Memory[a]	0.999	1.208	0.000	0.999	1.015	0.001	0.999	1.095	0.001
		(6, 15730)			(6, 11946)			(6, 11646)	
Executive functions[b]	0.997	1.880*	0.001	0.997	1.515	0.001	0.997	1.547	0.001
		(12, 20796)			(12, 15801)			(12, 15407)	
Psychomotor speed[c]	-	1.808	0.001	-	0.802	0.000	-	0.955	0.000
		(3, 7909)			(3, 6008)			(3, 5858)	

Note. Model 1, adjusted for age, gender, language, province and education; model 2, adjusted for model 1 covariates and ethnicity, smoking, BMI, alcohol consumption, income, social participation, physical activity level, depression, nutritional risk, diabetes, heart conditions; model 3, adjusted for model 2 covariates and diet quality score.

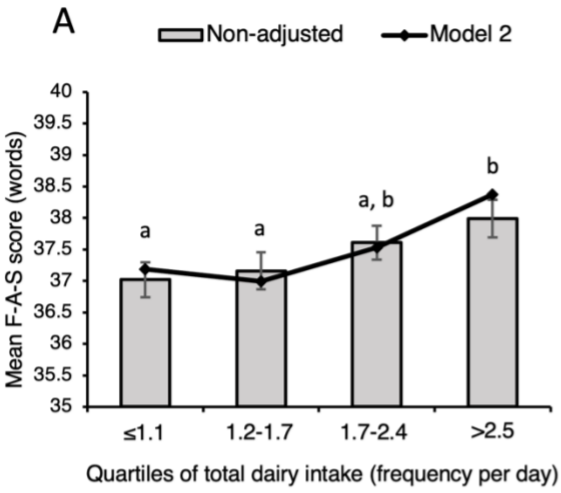
[a]From MANCOVA based on 2 tests: the RAVLT immediate recall and delayed recall.

[b]From MANCOVA based on 4 tests: the COWAT F-A-S total, animal naming, mental alternation test, and Stroop high interference.

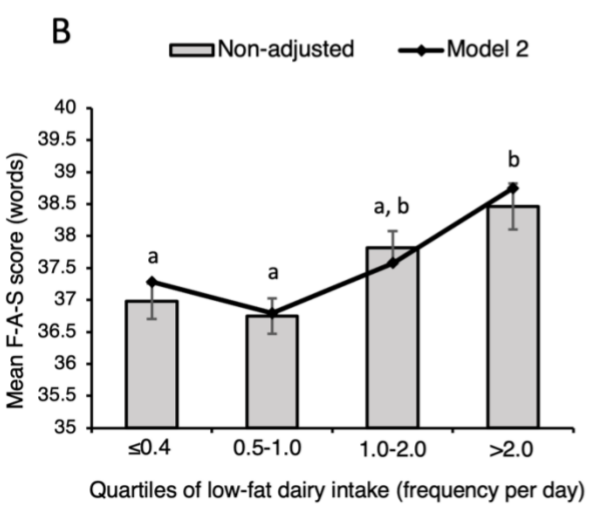
[c]From univariate ANCOVA based on 1 test: the choice reaction time.

*<0.05; **< 0.01; ***<0.001

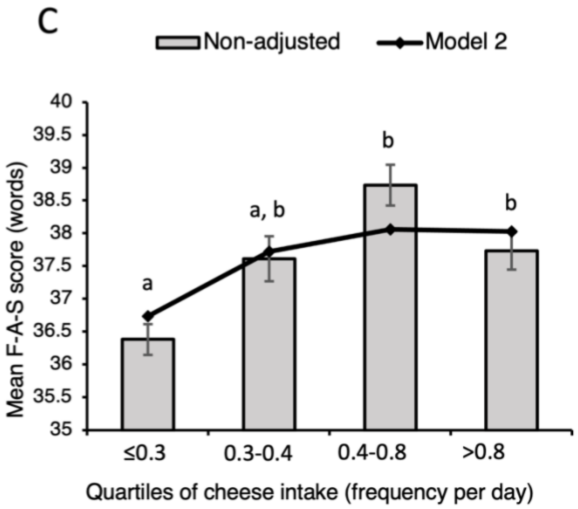
Figure 6-1 Executive function mean COWAT F-A-S total score across quartiles of total dairy and dairy categories intake in the CLSA cohort



Model 2, Q1 vs. Q4: aMD= -1.2 words, 95% CI: -2.3, -0.02; p=0.045;
Q2 vs. Q4: aMD= -1.4 words, 95% CI: -2.6, -0.2; p=0.012.
Model 3, Q1 vs. Q4; p=0.291; Q2 vs. Q4; p=0.062 (not shown).



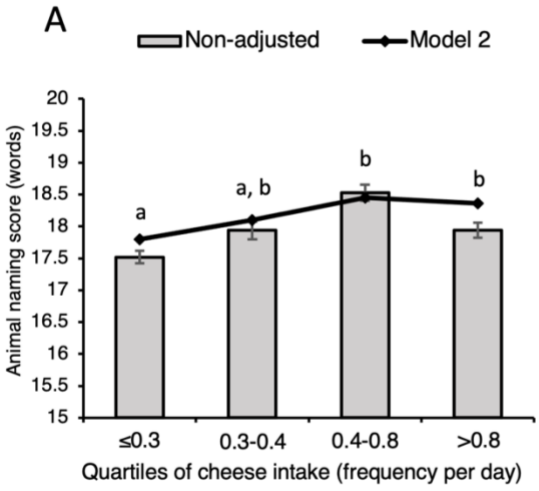
Model 2, Q1 vs. Q4: aMD= -1.5 words, 95% CI: -2.8, -0.0, p=0.016;
Q2 vs. Q4: aMD = -2.0 words, 95% CI: -3.2, -0.7, p<0.001.
Model 3, Q1 vs. Q4, p=0.089; Q2 vs. Q4: aMD = -1.8 words, 95% CI: -3.1, -0.5, p=0.002 (not shown).



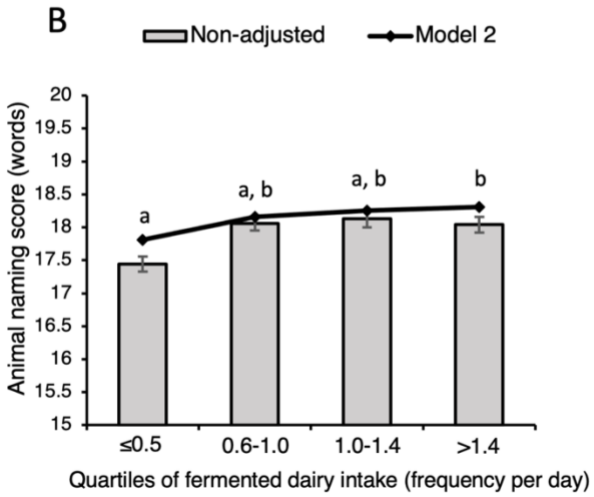
Model 2, Q1 vs. Q3 and Q4: aMD = -1.3 words, 95% CI: -2.4, -0.2, p=0.008.
Model 3, Q1 vs. Q3: aMD = -1.2 words, 95% CI: -2.3, -0.1, p=0.023; Q1 vs. Q4, p=0.057 (not shown).

Note. From univariate ANCOVA.
COWAT, controlled oral word association test; aMD, adjusted mean difference. Model 2, adjusted for gender, age, language, education, ethnicity, smoking, alcohol consumption, BMI, physical activity level, symptoms of depression, nutritional risk, income, social participation, diabetes and heart conditions. Model 3, adjusted for model 2 covariates and diet quality.
A, total dairy intake; B, low fat dairy intake; C, cheese intake.

Figure 6-2 Executive function mean animal naming score across quartiles of dairy intake categories in the CLSA cohort



Model 2, Q1 vs. Q3, aMD= -0.7 words, 95% CI: -1.1, -0.2, p<0.001;
Q1 vs. Q4, aMD= -0.6 words, 95% CI: -1.0, -0.1, p=0.003.
Model 3, Q1 vs Q3, aMD= -0.6 words, 95% CI: -1.0, -0.1, p=0.003;
Q1 vs. Q4, aMD= -0.5 words, 95% CI: -0.9, -0.1, p=0.016 (not shown).



Model 2, Q1 vs. Q4, aMD= -0.5 words, 95% CI: -1.0, -0.2, p=0.036.
Model 3, Q1 vs. Q4, p=0.330 (not shown).

Note. From univariate ANCOVA.
aMD, adjusted mean difference. Model 2, adjusted for gender, age, language, education, ethnicity, smoking, alcohol consumption, BMI, physical activity level, symptoms of depression, nutritional risk, income, social participation, diabetes and heart conditions. Model 3, adjusted for model 2 covariates and diet quality.
A, cheese intake; B, fermented dairy intake.

Supplementary Table 6-1 Quartiles of specific dairy products intake frequency (times/d) and by fat content

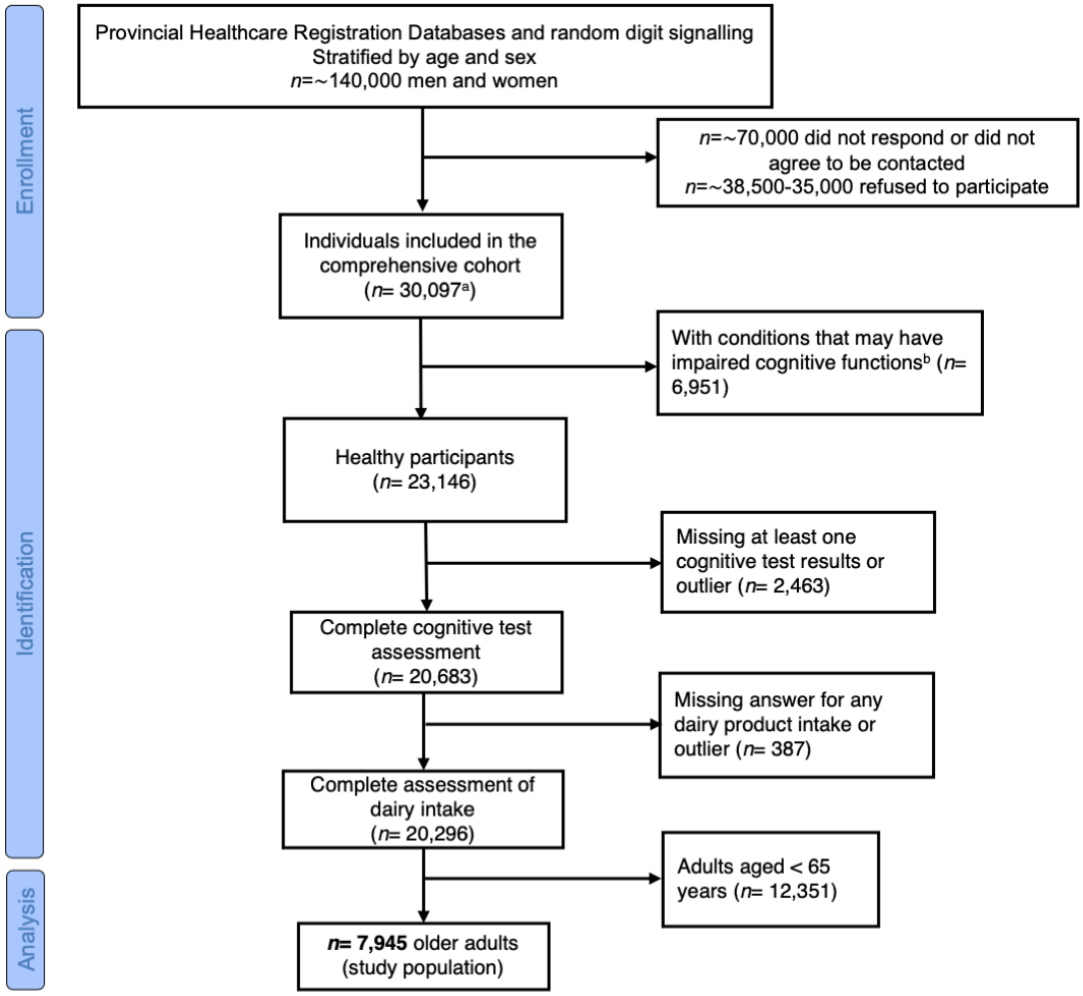
	Q1	Q2	Q3	Q4
Cheese	0.3	0.3-0.4	0.4-0.8	>0.8
Yogurt	0.0	0.0-0.4	0.4-0.9	>0.9
Milk	0.1	0.2-1.0		>1.0
Fermented dairy	0.5	0.6-1.0	1.0-1.4	>1.4
Regular-fat dairy	0.1	0.2-0.4	0.4-1.0	>1.0
Low-fat dairy	0.4	0.5-1.0	1.0-2.0	>2.0

Supplementary Table 6-2 Diet quality scoring from the Short Diet Questionnaire

	Men	Women
	Canada Food Guide 2007 servings for 51+[a]	
Fruits and vegetables (out of 10)	7	7
Fruit (fresh, frozen, canned)		
Calcium-fortified juices and 100% pure fruit juices		
Green salad		
Potatoes (boiled, mashed or baked)		
Carrots		
Other vegetables (except carrots, potatoes or salad)		
Meats and alternatives (out of 10)	3	2
Legumes (beans, peas, lentils)		
Beef, pork (ground, hamburgers, roast beef, steak, cubed…)		
Chicken, turkey		
Other meats (veal, lamb, game…)		
Omega-3 eggs		
Egg dishes except omega 3 eggs (eggs, omelette, quiche,...)		
Salmon, trout, sardines, herring, tuna, mackerel (fresh, frozen or canned)		
Nuts, seeds and peanut butter		
Whole grains (out of 10)	7	6
High fibre breakfast cereals		
Whole wheat breads, bran breads, multigrain breads, rye breads		

[a] Frequencies are treated as servings

Supplementary Figure 6-1 STROBE Diagram showing the flow of participants in the CLSA cohort



[a]Exclusion criteria are described by Raina et al. (302).
[b]Multiple sclerosis, Alzheimer's disease, effects from stroke or transient ischemic attack (TIA), Parkinson's disease, surgery within last 3 months, polio, unstable heart condition within last 3 months, pulmonary embolism within last 6 weeks, chemotherapy within last 4 weeks, traumatic brain injury with memory problem, positive screen for post-traumatic stress disorder, receiving dialysis treatment.

Connecting Statement 5

The results of the cross-sectional study in **Chapter 6** demonstrated that total dairy product, cheese and low-fat dairy intake were associated with executive functions in healthy older adults, but not with other domains, memory and psychomotor speed. Dairy product intake may also be related to muscle mass, strength and physical performance by its content in whey protein, vitamin D and other nutrients. Recent evidence supports a higher protein intake recommendation of 1.0-1.2 g/kg/d in healthy older adults to prevent sarcopenia, maintain physical function and optimal health (34, 35). It was also suggested that an evenly distributed mealtime protein intake or minimal protein per meal may be beneficial to muscle health (202). In addition, vitamin D (800-1,000 IU) and n-3 PUFA (~3 g/d; doses between 1 and 3 g/d were not tested) may be favorable for physical function, muscle mass and strength (36). Interventions of combined nutritional supplement including some or all of the above nutrients showed significant effects on chair stand performance (44) and total lean mass (46) after 13 weeks and 6 weeks, respectively. However, these studies were carried out in healthy or sarcopenic individuals. There may be further benefits of providing a multi-nutrient supplement to the most vulnerable, therefore I questioned if such a supplement would prove effective in older adults already experiencing a loss of autonomy. As an initial phase, I anticipated that a 16-week RCT of a multi-nutrient supplement (50-70 g/d of soluble milk protein, 6 g/d of leucine, 1,500 IU/d vitamin D and 2 g/d n-3 PUFA) to improve muscle mass, strength and physical performance in older adults participating in a rehabilitation program is feasible. This pilot RCT will provide groundwork for future large scale RCTs in vulnerable older adults, an understudied population.

CHAPTER 7: *Manuscript 4*: A pilot study of a multi-nutrient supplement to improve physical functions

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A 16-week randomized controlled trial of a fish oil and whey protein-derived supplement to improve physical performance in older adults losing autonomy – a pilot study

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7.1 Abstract

Background. Low functional capacity may lead to the loss of independence and institutionalization of older adults. A nutritional intervention within a rehabilitation program may attenuate loss of muscle function in this understudied population. **Objective.** This pilot study assessed the feasibility for a larger RCT of a nutritional supplementation in older adults referred to an outpatient assessment and rehabilitation program. **Methods.** Participants were randomized to receive a supplement (EXP: 2g fish oil with 1500 IU vitamin D3 1x/d + 20-30g whey protein powder with 3g leucine 2x/d) or isocaloric placebo (CTR: corn oil + maltodextrin powder) for 16 weeks. Handgrip and knee extension strength (using dynamometry), physical performance tests and plasma phospholipid n-3 fatty acids (using GCMS) were evaluated at weeks 0, 8 and 16; and lean soft tissue mass (using DXA), at weeks 0 and 16. **Results**. Over 2 years, 244 patients were screened, 46 were eligible (18.9%), 20 were randomized, 10 completed the study (6 CTR, 4 EXP). Median age was 87 years (77-94 y; 75% women) and gait speed was 0.69 m/s; 55% had low strength, and all performed under 420m on the 6-minute walk test, at baseline. Overall self-reported compliance to powder and oil was high (96% and 85%) but declined at 16 weeks for fish oil (55%). The EXP median protein intake surpassed the target 1.2–1.5 g/kg/d, without altering usual diet. Proportions of plasma phospholipid EPA and DHA increased significantly 3- and 1.5-fold respectively, at week 8 in EXP, with no change in CTR. Participants were able to complete most assessments with sustained guidance. **Conclusion**. Because of low eligibility, the pilot study was interrupted and deemed non-feasible; adherence to rigorous study assessments and to supplements was adequate except for long-term fish oil. The non-amended protocol may be applied to populations with greater functional capacity.

7.2 Introduction

Older adults represent the fastest-growing age group worldwide, and increasing life expectancy contributes to a burgeoning population of very old adults (384). Aging is associated with sarcopenia and functional decline; from the age of 50 y, 0.5-1% of muscle mass and 2-3% function are lost yearly (17). Especially in later life, this may lead to frailty, loss of independence, hospitalization and mortality (12). With multifactorial causes, i.e. physical, cognitive, mental and social, the loss of independence is commonly defined by difficulties in performing activities of daily living (385). It is often the leading reason for institutionalization.

To foster *aging well at home and in the community,* there is urgent need to identify and offer strategies such as nutritional interventions for improving functional capacity and maintenance of an independent life. Indeed, malnutrition is highly prevalent in the elderly population with up to ~30% being observed in rehabilitation settings (26). Poor nutritional status has been associated with impaired physical function capacity and frailty (28). Resistance-type training without (386) and with (387) protein supplementation was shown to promote gains in muscle mass and strength in younger and older adults. But, nutrition alone may represent an addressable risk factor and a suitable approach in older adults who cannot or do not want to engage in such exercises (36). The effect of protein supplementation on the muscular system may largely depend on protein quality, dose and timing of ingestion (36), and presence of anabolic resistance. Other nutrients are of interest for their potential role on muscle. Indeed, high-dose vitamin D supplements (800-1000 IU) were shown to have a favorable effect on balance, strength and physical performance in elderly populations. Improvements in physical performance and lean mass were also reported following supplementations with *n*-3 fatty acids in healthy older adults (36).

Few studies have tested the effect of combined-nutrient supplements and found modest but significant effects on total lean mass, strength and physical performance in sarcopenic older adults (44, 388) or healthy men (46). A nutrient-dense supplement may represent a practical approach for a short-term intervention as part of a geriatric rehabilitation program and may have additive beneficial effects on muscle parameters and physical function in very old adults with low functional capacity referred for assessment of loss of independence. This population is heterogenous, presenting with either sarcopenia, dynapenia, frailty, mobility limitations and low functional capacity, hence is challenging to study. Therefore, this pilot study aimed to assess

whether an RCT of a combined nutritional supplementation is feasible with regards to recruitment, compliance and completion of assessments. Our secondary outcome was to characterize the nutritional and functional status of this specific population of older adults.

7.3 Methods

Subjects

Participants were free-living older adults referred to an outpatient rehabilitation program that includes a comprehensive geriatric assessment to evaluate their level of independence and aims to improve it, to delay institutionalization. Participants were referred to the program either by the local community health center responsible for delivering home care assistance, or upon returning home after hospitalisation (commonly due to falls). The study was conducted at the Geriatric Day Hospitals (GDHs) of the McGill University Health Centre (MUHC) Montreal General Hospital and of the *Institut universitaire de gériatrie de Montréal*, QC. All new patients admitted to the GDHs were screened and approached if they met eligibility criteria. Eligible patients were able to read and speak English or French and had a MMSE >22/30. Patients with a BMI >35 kg/m^2, presence of kidney (eGFR <30 mL/min/1.73 m^2), liver or heart failure, stroke in the last 6 months, Parkinson's disease, severe neuropathy, active malignancies, acute inflammation (CRP >10 mg/L), diagnostic of hyperparathyroidism, recent acute weight loss (>10% in 3 months, unless stabilized), allergy to milk and/or fish, long-term use of corticosteroids or anti-neoplastic medication were excluded. Eligible patients were invited to review study procedures and sign the consent form. For the duration of the study, vitamin D supplementation dose was changed or kept at 400 IU/d for all participants one week prior to the study and those taking n-3 PUFA supplements were asked to cease it. This study was approved by the McGill University Health Centre Ethics Board (REB 15-633-MUHC), was registered on ClinicalTrials.gov (NCT04454359) and followed the Consolidated Standards of Reporting Trials (CONSORT checklist, **Supplementary Table** 7-1). This trial was registered after participant recruitment began due to the pilot nature of the study. The authors confirm that there are no ongoing or related trials for this intervention which required further registration.

This pilot study was not intended to evaluate the efficacy of the intervention and is not powered to detect significant differences in outcomes. Sample sizes between 10-40 participants per group have been recommended for pilot trials (389). We aimed to recruit 40 within 1 year

based on the expected rate of GDH patient annual turnover to provide confidence intervals (CI) to establish sample size for the large RCT.

Study design

Participants attended the rehabilitation program for 8 weeks on average; they would come at the GDH for half a day, twice per week, to meet with health professionals for general health and functional status assessment. Depending on individual needs, they performed few exercises determined by the physiotherapist during their consultation and were recommended individualized exercises to perform at home. Participants were randomly assigned to receive either the multi-nutrient supplement (EXP) or the placebo (CTR) for 16 weeks, combined with the rehabilitation program for the first 8 weeks and after program completion for 8 weeks. Simple randomization coded for the EXP and CTR group (1:1) was generated by a staff member external to the study using www.randomization.com. The study coordinator, the investigators and participants were all blinded to group allocation.

Visits for assessments of participants before the study, at weeks 8 and 16 were performed at the Research Institute of the MUHC during which physical tests, body composition assessment and questionnaires were completed. Overnight fasting blood was drawn during study visits or at home by a registered nurse or physician at all study time points. Adapted transport, or taxi was planned, and expenses covered. Supplements were planned to be delivered at the GDHs fortnightly by a research staff who would also collect used containers from participants. Participants were called by phone at least weekly for monitoring.

Intervention

The EXP group supplement consisted of 25 g, 30 g or 35 g flavored whey protein isolate (New Zealand Whey Protein Isolate, The Protein Company ATW Inc, QC, Canada) scaled to the participant's weight (65 kg, 65-75 kg, and 75 kg respectively) with a fixed 3 g of added leucine. The CTR group supplement was an isocaloric placebo of 25 g, 30 g or 35 g maltodextrin (**Supplementary Table** 7-4). Both products were vanilla-flavoured powders and were provided as individual pre-weighted doses in opaque containers. Participants were instructed to mix the powder with 125 mL water before ingestion and to consume it twice daily, once before breakfast for a more even mealtime distribution of protein intake (390) and the other before bedtime to potentially reduce overnight protein catabolism (391). The total amount of protein supplemented aimed to increase participants protein intake to 1.2-1.5 g/kg/d (34). The n-3 PUFA with vitamin

D supplement was provided as a fruit-flavored oil (NutraSea Liquid®, Ascenta, NS, Canada). Participants received a dosing cup, pre-marked to measure 7.5 mL, providing 1,500 IU vitamin D_3 and 1,125 mg eicosapentaenoic acid (EPA)+750 mg docosahexaenoic acid (DHA) as triglycerides and were instructed to consume this amount once daily. An isocaloric corn oil placebo was provided to the CTR group (**Supplementary Table** 7-4). Both the treatment and placebo oils were provided in amber bottles.

Outcome measures

The main outcome measures of this pilot study were feasibility as evaluated by the recruitment, eligibility, consent, attrition rates and completion of assessments by the participants, and acceptability as measured by the self-reported and objective adherence rates. Specific thresholds were established for each criterion prior to commencing the study: recruitment ≥50%, eligibility ≥30%, consent ≥50%, and attrition rates <10%, completion of study outcome assessments ≥80% and adherence ≥80%.

A logbook was provided to participants to report the amount of powder and oil supplements taken daily. All powder and oil containers were collected back at the GDH or at the participants' home for convenience, at the time of biweekly supplement provision. Empty and full containers were compiled and used to define self-reported adherence. N-3 PUFA proportions in the plasma phospholipid fraction was used as an objective measure of fish oil adherence (284).

Blood was collected in SST and EDTA-K2 vacutainers, kept on ice, and centrifuged within 1 hour of collection. Serum and plasma were aliquoted and kept at -80ºC until analysis. Serum albumin, pre-albumin, creatinine, CRP were measured by the MUHC Central Biochemistry Laboratory using standard and certified methods and serum 25(OH)D, by chemiluminescence immunoassay (CLIA; Beckman Dxl 800 Series, USA). Plasma glucose was measured by the glucose oxidase method (GM9 Glucose Analyzer, Analox™) and serum insulin by ELISA (Mercodia™). Insulin-like growth factor I was measured by ELISA (R&D Systems™).

Plasma phospholipid fatty acid profile and quantitative analyses were performed as described in (392). Briefly, following a modified Folch method, plasma lipids were extracted and the phospholipid fraction was separated by thin-layer chromatography. The internal standard C17:0 was added for quantification followed by methylation and analysed by gas

chromatography-flame ionization (Varian Instruments, Canada). Mean fatty acid amounts were calculated from duplicates; ≤5% variation between duplicates was considered acceptable.

Total lean soft tissue (lean mass) and fat mass were measured by dual-energy X-ray absorptiometry (DXA; GE Lunar iDXA, GE Healthcare). Appendicular lean mass (ALM) was calculated as the sum of the four limbs lean mass and ALM index, as ALM over height squared (kg/m^2). Calibration verification throughout the reportable range was completed on the morning of scheduled scans by a trained technician. All images were inspected for proper delimitation of limbs and re-analysed as needed. DXA scans were performed at baseline and week 16. Participants were classified as sarcopenic if their ALM index fell <7.31 kg/m^2 for men and <5.43 kg/m^2 for women, as per Canadian cut-points for sarcopenia (343). Body weight was measured using a digital scale (Scale-Tronix USA; nearest 0.1 kg) in light clothing and at the same time of day. Standing height was measured to the nearest 0.1 cm using a stadiometer as per standard procedures. BMI and waist circumference were measured according to standard procedures at all 3 study time points.

Maximal handgrip strength was evaluated by hand-held dynamometry (Jamar hydraulic, USA) (305). Participants were seated in a chair without armrests, elbow flexed at a 90-degree angle and were instructed to squeeze the device as hard as they could for 5 s; 3 measurements were performed with each hand, alternating sides. The highest measure was used for analysis and the maximal results from the same hand were compared over time. Participants were classified as having low handgrip strength according to the sex-specific Canadian cut-points for dynapenia, <33 kg for men and <20 kg for women (343). Maximal leg strength was measured using isometric knee extension test (Biodex System 4 Pro, Biodex Medical Systems Inc.) (393). Three 5-s contractions were performed with leg placed at a 60-degree angle, alternating with a 5-s rest. The peak torque was recorded in Newton-meters (Nm).

The timed up and go (TUG) test was used to assess mobility (308). Participants were instructed to stand up from an armchair, walk a 3-meter distance, turn around, walk back to the chair and sit. The test was repeated twice and the average time in seconds used in analyses. The 6-minute walk test (6MWT) (394) was performed in a 30-meter-labeled corridor. Participants walked back and forth this distance within a timed 6-minutes. The distance covered was recorded to the nearest 0.5 meter. The test was stopped if the participant needed to sit. Gait speed was

measured within the 6MWT to avoid exhaustion. To eliminate acceleration and deceleration, a tape was placed at 4 and 8 meters from the starting line. The time (seconds) required to cover the 4-meter distance was recorded; gait speed was reported in m/s (395). The use of usual daily living assistive devices was permitted for the 6MWT/gait speed and TUG. The 30-s chair-stand was used to assess lower limbs strength and balance (396). With arms crossed on their chest, participants had to rise from a chair to a full standing position and sit back as many times as they could within 30 seconds. The number of complete stands were recorded.

Dietary intake was assessed with three-day food diaries (3DFD) and analysed using the Food Processor software (ESHA®; Canadian Nutrient File v2015). Participants received instructions to record food intake and estimate portion sizes using measuring cups. Diaries were verified by a dietitian.

Daily average step counts were calculated using accelerometers (ActiGraph, GT3x) that participants wore during 4 consecutive days. Participants kept an activity log to verify concordance with the accelerometer results. Nutritional status and symptoms of depression were evaluated through the Mini-Nutritional Assessment-Short Form (MNA-SF) (26) and Geriatric Depression Scale (GDS), respectively. Global cognitive status was assessed using the MMSE and the Montréal Cognitive Assessment (MoCA) (397). Frailty was determined as per the Fried criteria (398).

Statistical analysis

For the primary outcomes including recruitment, eligibility, consent and attrition rates, 95% CI for a population proportion were calculated and compared to expected rates. Medians and 95% CI were used to evaluate adherence rates. Medians and ranges were reported for continuous variables and raw counts for nominal data. All statistical analyses were performed in Python 3.0 using Pandas and NumPy libraries.

7.4 Results

Of 244 patients screened, 61 were approached and further assessed for eligibility. Between August 2016 and August 2018, 23 agreed to participate, 20 were randomized and 13 received the allocated group intervention. Ten participants completed the study which ended in September 2018. **Figure** 7-1 shows the CONSORT flow diagram of participants as per the extension statement for pilot and feasibility trials (399). The recruitment rate was 1 participant

every two months (95% CI: 0.6, 1.4), 14% of the expected monthly recruitment rate; over 2 years, the eligibility rate was 19% (95% CI: 14, 24), consent rate, 46% (95% CI: 32, 60) and attrition rate, 23% (95% CI: 0, 46). Reasons for non-eligibility were mostly neuropathy, impaired cognitive function, corticosteroids use and early abandon of the rehabilitation program. Of the 23 patients who consented to participate, 2 were excluded shortly after baseline assessments and before starting the supplementation, for high serum CRP level and need for wheelchair; data were included in baseline characteristics.

The population studied (**Table** 7-1) had a median age of 87 (77-94) and predominantly comprised women (75%). At baseline, 26% had low lean mass, 55% had low handgrip strength, 26% were frail, 85% had mobility limitations requiring a walking aid on a daily basis. All participants had low functional capacity based on the 6-minute walk test, below 415 m (400). The median 25-hydroxyvitamin D was 74.0 nmol/l (44.0-159.0), above the sufficiency level of 50 nmol/L (401). **Table** 7-2 shows baseline and follow-up characteristics and outcome measures data by group. Based on 95% CI (not shown), there were no differences in baseline characteristics between groups. One participant had a BMI slightly >35 kg/m^2 but was included in the study to benefit from the study intervention and increase recruitment.

Adherence and adverse events

The overall median adherence to the powder and oil supplements as evaluated by leftover count was 95.6% (mean: 87.2%, 95%CI: 74.9, 99.6) and 85.1% (mean: 67.4%, 95%CI: 45.1, 89.7), respectively. When analyzing groups separately, adherence to powder was 99.1% (mean: 96.0%, 95%CI: 90.3, 101.7) in CTR compared to 93.3% (mean: 92.6%, 95%CI: 87.6, 97.7) in EXP and 94.0% (mean: 82.8%, 95%CI: 58.9, 106.8) compared to 70.2 % (mean: 68.8%, 95%CI: 40.1, 97.4) to oil; oil supplement intake decreased from 85.7% at week 8 to 54.8% at week 16 in EXP.

Proportions of plasma phospholipid EPA and DHA significantly increased in EXP at week 8 by 3 and 1.5 folds, respectively (**Figure** 7-2; **Supplementary Table** 7-2). Only the DHA change remained significant at week 16 (median: 1.5% of total phospholipid fatty acids, mean: 1.9%, 95%CI 0.35, 2.23) with no change in EPA and DHA proportions in CTR (**Figure** 7-2). Total plasma phospholipid fatty acids increased at week 16 in CTR (mean: +130.4 μg/mL, 95%CI: 3.1, 257.6) and tended to decrease in EXP (mean: -180.2 μg/mL, 95%CI: -364.8, 4.5). Serum 25(OH)D did not significantly change within groups (**Figure** 7-2).

One participant in EXP reported recurring diarrhea and therefore ceased taking the oil between week 0 and 8 and was later hospitalised for gastroenteritis which confirmed no adverse effect to the supplement. One EXP participant dropped out of the study complaining of low appetite caused by the powder supplement and another reported gastroesophageal reflux at bedtime which seemed to have been related to their eating pattern. One CTR participant developed pneumonia, not related to the supplement and another had high unexplained CRP value at the end of the study.

Physical and body composition assessments

All participants were able to complete the DXA scans, though most found it uncomfortable to lie flat on the scanner bed. Only one participant was not able to perform the TUG test at baseline (9.1%) and all were able to complete 6MWT, gait speed, handgrip and knee extension strength assessments at all 3 time points. Six (54.5%) participants were unable to execute at least one chair stand.

Table 7-3 reports data on physical performance, strength and **Figure** 7-3 illustrates the changes in these outcomes. Based on the 95% confidence intervals, no changes in TUG, 6MWT, chair stand, leg strength and ALM were observed in either group. In CTR, we observed a slight increase in gait speed (median: +0.19 m/s; mean: +0.12, 95%CI: 0.01, 0.23) and no change in EXP (median: +0.09 m/s; mean: +0.03, 95% CI: -0.16, 0.22). Handgrip strength decreased by 3.0 kg (mean: -2.5 kg, 95%CI: -4.3, -0.6) in EXP and did not change in CTR. While body weight increased in both groups (mean: +1.3-1.4 kg, 95%CI: 0.3, 2.3), total lean mass increased in EXP (median: +663.0 g; mean: +508.3 g, 95%CI: 142.3, 874.2) with no change in CTR.

Dietary data

Two participants did not complete all 3DFDs because it was deemed too demanding. At baseline, dietary intake in energy, protein, carbohydrate, fat, EPA, DHA and vitamin D were not different between groups (**Supplementary Table** 7-2). Dietary ALA intake tended to be higher in CTR. When including the supplement, the median daily total protein intake increased by 47.9 g (mean change: 45.6 g, 95%CI: 31.0, 60.3) in EXP at week 16 and reached a median intake of 1.94 g/kg/d (range: 1.55-2.09); total energy intake increased by 520 kcal (mean: +452 g, 95%CI: 240, 664). Median total protein intake of CTR was 1.09 g/kg/d (0.30-1.17) at the end of the trial and energy intake did not change when accounting for the supplement.

At baseline, two participants in EXP and two in CTR reported a daily protein intake below the recommended 1.2 g/kg for older adults (402). At week 16, all EXP participants reported daily protein intake >1.5 g/kg, and all CTR participants were <1.2 g protein/kg/d.

7.5 Discussion

This 16-week pilot randomized controlled trial of a nutritional supplementation in very old adults with low functional capacity was stopped after 2 years. It was deemed non-feasible because of exceedingly low recruitment rate resulting from a limited number of patients admitted at rehabilitation centers and low eligibility rate. Notwithstanding these issues, the adherence to rigorous study assessments and supplements was good, except for the drop in fish oil adherence at 16 weeks; the consent rate was adequate, and no major adverse events occurred.

Primary outcomes: feasibility and potential amendments to study design

Given that nutrition counseling was not an integral part of the rehabilitation programs, the objective of an eventual larger RCT was to test the impact of a multi-nutrient supplement when embedded in such a program on muscle mass, strength and physical performance. The expected rate of recruitment of 3.3 participant per month was noticeably above the observed 95%CI: 0.3, 0.7. The main limitation was the low number of patients admitted to the program reflective of limited resources. After one year of recruitment at one site, a second site was opened but resulted in only a modest increase in recruitment. Access to additional sites would have been necessary to reach the required sample size within a decent timeframe. Consequently, the proposed amendments to the trial include: liberalization of eligibility criteria to increase recruitment; the choice of multi-component endpoints or adjustments of statistical models to improve power; and home visits for provision of supplements, collection of food diaries, activity logs and blood tests, to reduce participant burden and promote retention. The latter strategy has time and financial resource implications to be considered in designing a larger RCT. Exclusion criterion could be more liberal but are nonetheless associated with potential confounding effects on measured outcomes. Hence, the most influential criteria, i.e., renal failure, impaired cognitive function that would prevent an individual from consuming the protein supplement and providing informed consent, respectively, should be retained. Contrastingly, the highly prevalent peripheral neuropathy may be removed as an exclusion criterion as it accurately represents the studied population. Given recruitment challenges and high variability in outcome measures, using a

multi-component endpoint as the primary outcome, i.e., combining TUG, 6MWT, gait speed test results, followed by an analysis of specific endpoints controlled for Type I error, or adjustment for prognostic variables should be considered with small sample sizes (403).

High short-term adherence to supplements was confirmed by a marked increase in plasma phospholipid EPA and DHA fatty acid proportion following a 1.9 g fish-oil EPA+DHA supplementation at week 8 (3- and 1.5-fold respectively). This supports effective incorporation into phospholipid, a marker of dietary intake and endogenous fatty acid metabolism. Comparably, Patterson et al. demonstrated a ~85% increase in plasma phospholipid proportion of total EPA+DHA for each additional 1 g EPA+DHA intake in young adults (404).

Serum 25(OH)D levels were adequate at baseline likely due to prevailing vitamin D supplementation in all but one participant. Usual supplements were ceased one week prior to the start of the study which may likely explain the declining trend in circulating levels in the CTR group. The 1500 IU/d dose provided in EXP did not significantly increase serum levels above already high levels (80 nmol/L). Because vitamin D was part of fish oil, the significant increase in EPA and DHA at week 8 and the subsequent decrease at week 16 corroborate self-reported adherence to the oil supplement and reflect a decline in adherence overtime. Self-reported compliance to powder was very good in both groups.

Participants required strong guidance and monitoring from the research team throughout their assessment visit. Assistance to position on the DXA bed was needed and most found challenging to remain lying flat on their back for the scan. Most participants were not able to complete one chair stand without using their arms, making it impossible to discriminate performance and detect changes overtime. Such difficulties must be considered in study design to avoid a floor effect. The modified 30-s chair stand allowing the use of hands to stand up is a valid and reliable alternative that may be used instead (405). Handgrip strength and gait speed (+/- assistive device) were feasible and easy to assess in this population and are generally not subject to a floor effect.

Food diaries collected were, for most, incomplete and memory challenges arose when revising diaries with participants. Functional losses in older adults may impede accuracy of dietary assessment, therefore specific approaches tailored to the characteristics of the population may be required (406). We provided participants with an iPod to capture pictures of their meals

as a complement to food diaries. Pictures were used to prompt recall of consumed food items, e.g., cooking method, preparation, and to corroborate reported portion sizes.

Potential effects on secondary outcomes

Protein, vitamin D and long-chain n-3 PUFA have shown potential effects on muscle mass and function separately and were provided together for potential additional or synergistic effects (36). While studies reported gains in whole-body lean mass (mean: +0.7 kg; duration: 6 weeks; n=38 older men)(46) and ALM (mean: +0.17 kg; n=259 older adults with sarcopenia)(44) from a whey protein and vitamin D-based supplement, another did not (6-month duration, n=60 older adults with sarcopenia) (47). A mean difference of 508 g in this study is clinically meaningful assuming a total lean mass DXA measurement error of 0.5% (407). A minimal increment of 180 g had to be detected in our population. Still, it is possible that this result occurred due to shifts in body fluids. Indeed, the larger-scale RCT would be warranted to confirm these results. While knee extension strength did not change in either groups, the median handgrip strength decreased in EXP but not in CTR. This test has shown excellent test-retest reliability (305) and was performed by the same administrator, for all participants throughout the study. This unexpected result may be explained by a decrease in upper body activities during the study, variable motivation during assessment and plausible within-subject variability.

Protein supplementation may have beneficial effects on clinical outcomes including weight, rates of complications and hospitalization, particularly in frail or malnourished/at risk individuals (36, 198). Thought a recent meta-analysis showed no added benefits from protein supplementation on lean mass and muscle strength (196), a dietary protein intake higher than the RDA (1.6 vs. 0.8 g/kg/d) may preserve lean mass with aging (197). In the current study, the EXP median protein intake surpassed the recommended 1.2–1.5 g protein/kg/d for older adults (34) without displacing habitual dietary intake, indicating a valuable approach to enhance protein – and vitamin D and n-3 PUFA – intake when needs are high but appetite low, in older adults with normal renal function.

Characterization of the population

Limited information is available on older adults' n-3 PUFA status. This is the first study to report comprehensive profiling in such a high age group of elderly. Higher plasma, plasma phospholipid and erythrocyte long-chain n-3 PUFA levels were previously reported in older adults compared to younger (408). Participants in this study appeared to have higher baseline

plasma phospholipid EPA (mean: 1.2% EPA) and similar DHA (mean: 2.5%) proportions compared to younger women (0.6% EPA and 3.0% DHA) (409) and older Americans (mean age 74 ± 5 years; 0.5% EPA and 2.9% DHA for middle quintile individuals) (276) from other studies. Proportions of EPA and DHA, and DHA concentrations, were comparable to those of Canadian patients with advanced cancer close to death specifically (64 ± 10 years; BMI 23.4 ± 4 kg/m^2); EPA concentrations were ~25% higher in our population (410). Interestingly, total plasma phospholipid fatty acids were similarly low in the current study (456± 156 µg/mL vs. 442 ± 316 µg/mL in advanced cancer patients (410)) compared to those of healthy individuals. Both diet and metabolism can modify n-3 PUFA proportions. Older adults may have higher levels because they consume more n-3 PUFA sources than younger adults (278) and also due to age-dependent factors altering metabolism such as decreased utilization, greater competition with linoleic acid, higher apparent retroconversion of DHA (411, 412). The increase in total plasma phospholipid fatty acids was not expected, especially in the CTR group. While adipose tissue loss was previously associated with decreased total plasma phospholipid fatty acids (410), the change observed may be related to the gain in fat mass.

Compared to national data on community-dwelling older adults (aged 65+ years), our participants tended to be older with lower weight and BMI. The prevalence rates of sarcopenia and dynapenia were higher than in the Canadian Longitudinal Study on Aging cohort aged ≥ 75 years (35% vs. 24% and 55% vs. 35%, respectively) (343). As per TUG, gait speed and chair-stand tests, our population had mobility limitations and was prone to falls (413, 414). Median TUG of 21.9 s was higher, and gait speed of 0.7 m/s, lower, than means of 9.9-11.0 s at the TUG and 0.9-1.2 m/s gait speed observed in community-dwelling older adult populations (343) including adults >80 years (415). Performance measured in the present study was similar to that observed in another rehabilitation setting (416). Lastly, the median 6-min walk test of 218 m is clearly below reference standards of healthy older adults (400) indicating poor endurance and functional capacity.

Conducting an intervention study in this population was challenging. Life events, e.g., acute diseases, death of loved ones, being a spousal caregiver, are stressful especially at an old age and when social support is limited. Isolating the effect of a nutrition intervention may be difficult to achieve in this context. Other limitations include the nature of our study and limited sample size attained which prevents drawing formal conclusions with regards to changes in lean

mass, strength and physical performance. Nevertheless, this pilot study has numerous strengths: the robust study design including a nutrient-dense supplement carefully intended to promote muscle health and selected for its taste and ease of preparation; the use of valid and reliable methods to assess plasma phospholipid composition as an objective compliance measure, body composition, strength and physical performance; the characterization of plasma phospholipid n-3 PUFA in this understudied population. Maintaining high adherence to the fish oil supplement up to 16 weeks remains a challenge that could be addressed through other means of delivery, such as part of favorite foods, change of flavors, and more intense follow-up.

7.6 Conclusion

In conclusion, due to limited access to potential patients and low eligibility rate, the pilot study was interrupted as deemed non-feasible. However, adherence to the rigorous study assessments and supplements was adequate, except for fish oil at 16 weeks. This RCT pilot study provides the foundation to support the elaboration of a large-scale study needed to investigate potential benefits of a multi-nutrient supplement on lean mass, muscle strength and physical performance. Amendments proposed for a large RCT to be successful in this challenging-to-study population include a multi-center setting and/or larger scale rehabilitation programs, more liberal yet judiciously selected eligibility criteria, allocation of additional time and related resources for close monitoring of participants during and between study visits. The non-amended protocol may be applied to younger or populations with greater autonomy.

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Conflict of interest

None.

Table 7-1 Baseline descriptive characteristics of participants

Sex	5M/15W	
Age, y	87 (77, 94)	
Education, y	13 (2, 23)	
Height, m	1.57 (1.48, 1.75)	
Weight, kg	66.1 (49.6, 102.6)	
BMI, kg/m^2	25.4 (20.6, 36.9)	
Walking aid n (%)	17 (85)	
Sarcopenic, n (%)	5 (26)	
Dynapenic, n (%)	11 (55)	
Frail, n (%)	5 (26)	
MNA-SF, out of 14	12 (7, 14)	
Geriatric depression scale, out of 15	6 (2, 15)	
Average daily step count	1406 (121, 3344)	
Physical function		
TUG, s	21.9 (10.1, 53.3)	
6-minute walk test, m	218 (68, 415)	
Gait speed, m/s	0.7 (0.2, 1.4)	
Chair stand, number	0 (0, 9)	
Cognitive status		
MMSE, out of 30	27 (23, 30)	
MoCA, out of 30	23.5 (16, 29)	
Clinical markers		
25(OH)D, nmol/L	74.0 (44.0, 159.0)	
Albumin, g/L	40 (32, 48)	
Pre-albumin, mg/L	221 (135, 339)	
IGF-1, ng/mL	93.2 (79.8, 144.3)	
CRP, mg/L	1.4 (0.4, 56.8)	
Insulin, pmol/L	28.9 (8.8, 102.8)	
Glucose, mmol/L	5.1 (3.4, 7.1)	
	Men n=5	**Women n=15**
Waist circumference, cm	89.0 (83.0, 130.7)	83.5 (68.0,108.0)
Body composition	n=4	n=15
Total lean mass, kg	45.8 (36.9, 56.1)	38.0 (28.7, 50.5)
Appendicular lean mass, kg	20.0 (15.2, 25.9)	15.8 (11.8, 24.3)
Appendicular lean mass index, kg/m^2	7.04 (6.74, 8.43)	6.37 (4.85, 9.55)
Strength	n=5	n=15
Handgrip, kg	36.0 (22.0, 38.0)	20.0 (6.0, 27.0)
Knee extension, N-M	116.4 (71.8, 151.8)	59.0 (45.3, 84.2)

Values are medians (range), n=20. M, men; W, women; BMI, body mass index; MNA-SF, mini nutritional assessment - short form; TUG, timed up and go; MMSE, mini mental state examination; MoCA, Montreal cognitive assessment; 25(OH)D, 25-hydroxyvitamin D; IGF-1, insulin-like growth factor 1; CRP, c-reactive protein.

Table 7-2 Anthropometric measures, step count, and serum clinical markers of participants by group

	CTR			EXP		
	Week 0	**Week 8**	**Week 16**	**Week 0**	**Week 8**	**Week 16**
Sex	1M/5F	-	-	1M/4F	-	-
Age, y	88.5 (77.0, 91.0)	-	-	85.0 (81.0, 94.0)	-	-
BMI, kg/m^2	28.4 (20.5, 36.9)	28.3 (20.7, 37.6)	28.6 (21.3, 38.3)	22.5 (21.5, 25.4)	22.9 (21.2, 25.9)	22.8 (21.5, 25.9)
MNA-SF, out of 14	12 (9, 14)	-	13 (11, 14)	11 (7, 12)	-	13 (13, 14)
Average daily step count	2133 (121, 3304)	2088 (229, 3610)	1522 (496, 2509)	965 (352, 3344)	1754 (406, 2309)	1230 (418, 2174)
Clinical markers						
Albumin, g/L	41.5 (40.0, 44.0)	41.5 (39.0, 42.0)	39.0 (32.0, 40.0)	38.0 (32.0, 48.0)	39.0 (34.0, 45.0)	36.5 (35.0, 40.0)
Pre-albumin, mg/L	238 (219, 317)	262 (200, 305)	201 (163, 286)	194 (154, 246)	238 (132, 246)	181 (118, 246)
IGF-1, ng/mL	83.9 (65.4, 142.6)	119.9 (95.4, 144.3)	81.3 (45.7, 118.8)	74.3 (31.7, 104.2)	85.4 (79.8, 91.0)	91.4 (75.8, 97.3)
CRP, mg/L	1.5 (0.6, 10.1)	1.3 (0.8, 5.1)	2.0 (1.2, 11.8)	2.4 (0.5, 7.5)	2.0 (0.7, 8.9)	3.5 (1.0, 62.4)
Glucose, mmol/L	5.3 (4.5, 7.1)	5.5 (4.8, 7.6)	5.3 (4.8, 6.9)	4.6 (3.4, 5.2)	4.9 (4.4, 5.6)	5.4 (4.8, 5.7)

Values are median (range). CTR, control group; EXP, experimental group; M, men; W, women; BMI, body mass index; MNA-SF, mini nutritional assessment-short form; IGF-1, insulin-like growth factor 1; CRP, c-reactive protein.

Table 7-3 Physical function and body composition of participants by group

	CTR			EXP		
	Week 0	**Week 8**	**Week 16**	**Week 0**	**Week 8**	**Week 16**
Physical function						
TUG, s	22.2 (10.1, 53.3)	19.5 (10.6, 35.7)	18.3 (10.1, 44.5)	19.6 (14.4, 23.2)	19.8 (12.9, 29.3)	17.8 (11.4, 21.9)
6-minute walk test, m	199 (99, 415)	233 (126, 131)	201 (91, 423)	214 (172, 413)	220 (131, 428)	263 (228, 364)
Gait speed, m/s	0.6 (0.4, 1.3)	0.8 (0.5, 1.4)	0.8 (0.4, 1.5)	0.7 (0.6, 1.4)	0.7 (0.6, 1.3)	0.8 (0.7, 1.2)
Chair stand, n	3 (0, 8)	3 (0, 8)	0 (0, 9)	0 (0, 9)	0 (0, 11)	3 (0, 10)
Body composition						
Appendicular lean mass, M/W, kg	18.1 / 15.8 (12.9, 24.3)	-	16.6/ 15.8 (13.6, 25.0)	21.9 / 13.9 (11.8, 15.5)	-	22.3/ 13.7 (11.3, 14.9)
Appendicular lean mass index, M/W, kg/m^2	6.74 / 6.37 (5.37, 9.55)	-	6.19 / 6.53 (5.66, 9.83)	7.29 / 5.77 (4.85, 6.89)	-	7.43 / 5.93 (4.63, 6.79)

Values are median (range). CTR, control group; EXP, experimental group; TUG, timed up and go; M, men; W, women.

Figure 7-1 CONSORT flow diagram of the progress of participants through the study phases, conducted between August 2016 and August 2018

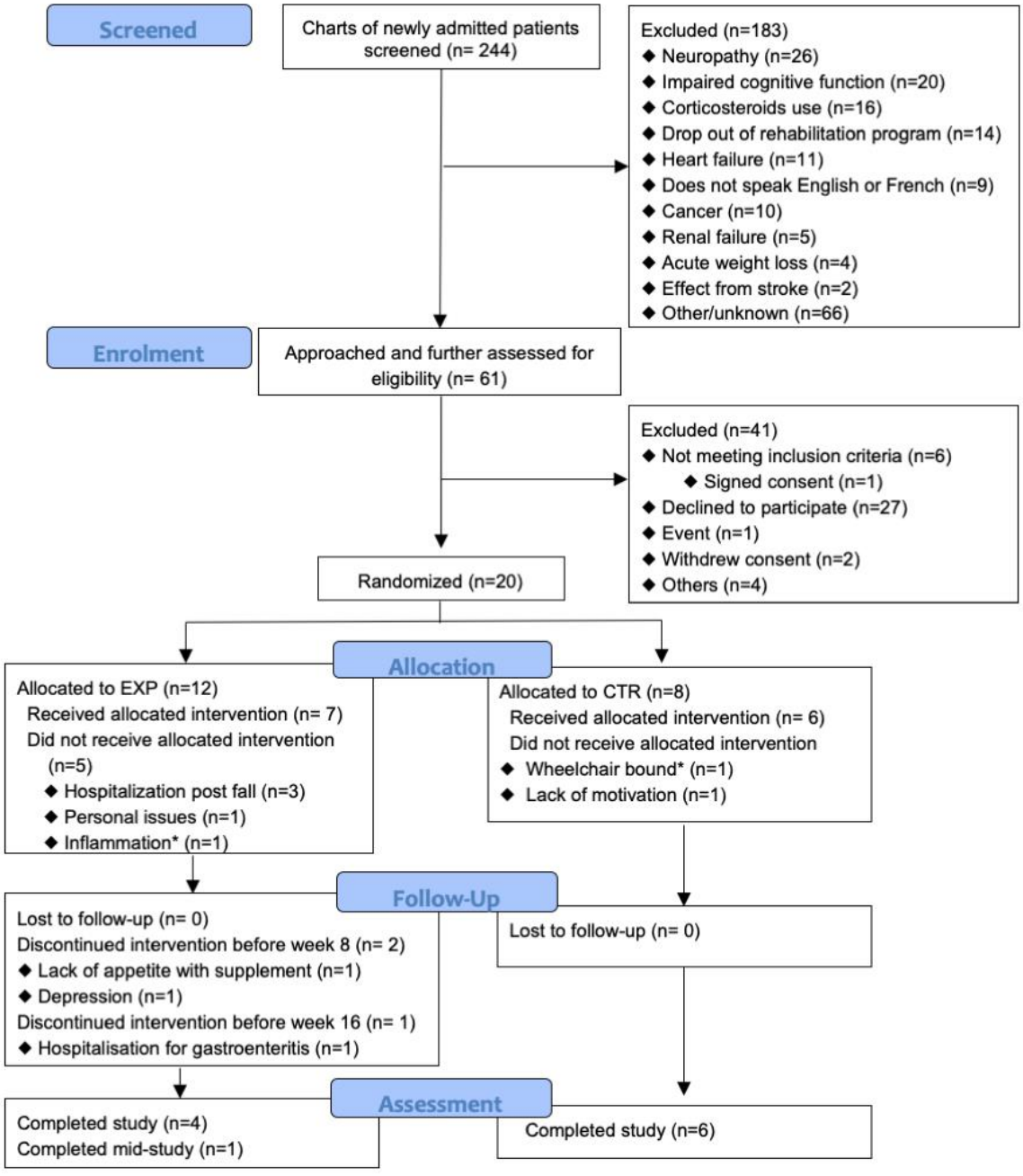
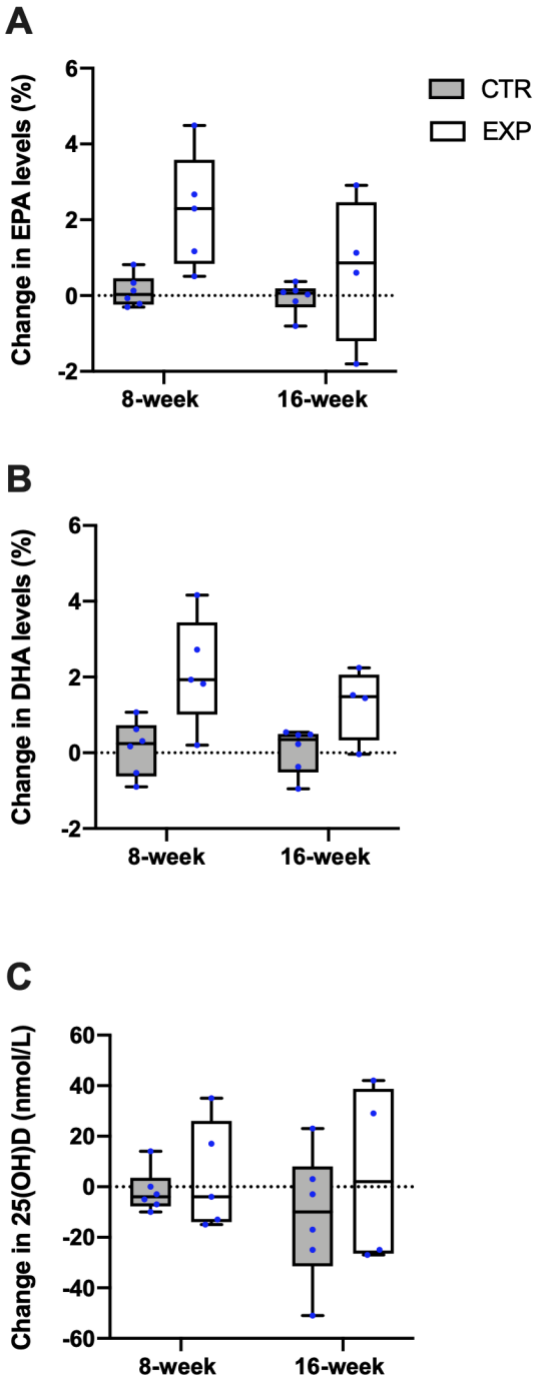
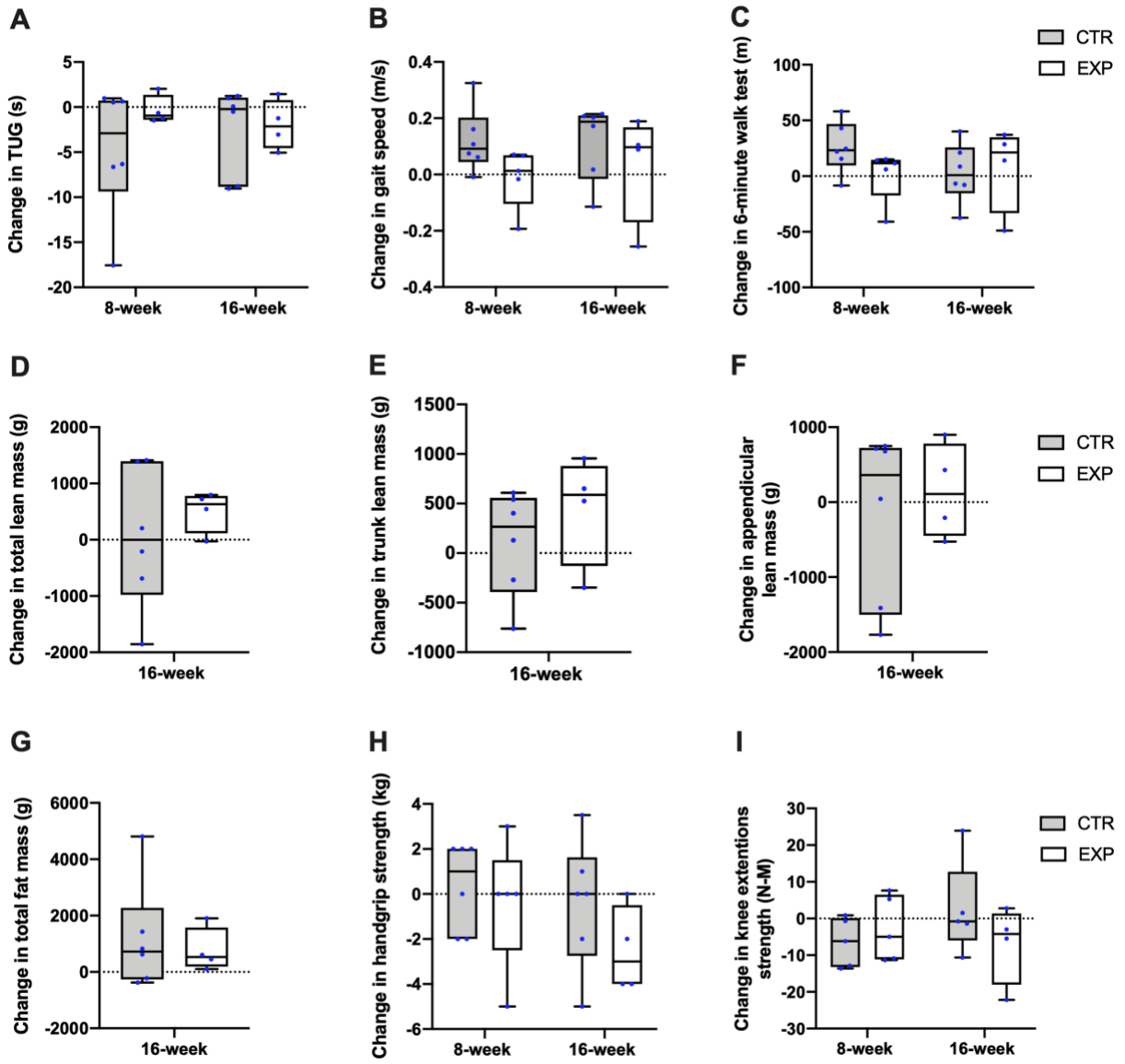


Figure 7-2 Boxplots showing changes between baseline and week 8, and week 16 in (A) plasma phospholipid EPA, (B) DHA, (C) serum 25(OH)D by group



CTR, control group; EXP, experimental group; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid, 25(OH)D, 25-hydroxyvitamin D. Boxes are interquartile ranges and horizontal lines inside boxes are medians. Whiskers represent the minimal and maximal value, and the blue dots represent the participants' individual value. The dotted lines indicate a change of 0.

Figure 7-3 Boxplots showing changes between baseline and week 8, and week 16 in (A) TUG, (B) gait speed, (C) 6-minute walk test, (D) total lean mass, (E) trunk lean mass, (F) appendicular lean mass, (G) total fat mass, (H) handgrip and (I) knee extension strength by group



CTR, control group; EXP, experimental group; TUG, timed up and go; N-M, newton-metre. Boxes are interquartile ranges and horizontal lines inside boxes are medians. Whiskers represent the minimal and maximal value, and the blue dots represent the participants' individual value. The dotted lines indicate a change of 0.

Supplementary Table 7-1 CONSORT checklist of information to include when reporting a pilot trial.

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a pilot or feasibility randomised trial in the title	1
	1b	Structured summary of pilot trial design, methods, results, and conclusions (for specific guidance see CONSORT abstract extension for pilot trials)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale for future definitive trial, and reasons for randomised pilot trial	4
	2b	Specific objectives or research questions for pilot trial	5
Methods			
Trial design	3a	Description of pilot trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after pilot trial commencement (such as eligibility criteria), with reasons	11-12
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
	4c	How participants were identified and consented	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7
Outcomes	6a	Completely defined prespecified assessments or measurements to address each pilot trial objective specified in 2b, including how and when they were assessed	8-10
	6b	Any changes to pilot trial assessments or measurements after the pilot trial commenced, with reasons	15-17
	6c	If applicable, prespecified criteria used to judge whether, or how, to proceed with future definitive trial	8
Sample size	7a	Rationale for numbers in the pilot trial	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			

Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation(s); details of any restriction (such as blocking and block size)	6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12	Methods used to address each pilot trial objective whether qualitative or quantitative	11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were approached and/or assessed for eligibility, randomly assigned, received intended treatment, and were assessed for each objective	11 Fig. 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	11 Fig. 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	11
	14b	Why the pilot trial ended or was stopped	14
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 2.
Numbers analysed	16	For each objective, number of participants (denominator) included in each analysis. If relevant, these numbers should be by randomised group	12-14 Table 1 & 2.
Outcomes and estimation	17	For each objective, results including expressions of uncertainty (such as 95% confidence interval) for any estimates. If relevant, these results should be by randomised group	12-14 Table 1 & 2.
Ancillary analyses	18	Results of any other analyses performed that could be used to inform the future definitive trial	13
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	12

	19a	If relevant, other important unintended consequences	12
Discussion			
Limitations	20	Pilot trial limitations, addressing sources of potential bias and remaining uncertainty about feasibility	20
Generalisability	21	Generalisability (applicability) of pilot trial methods and findings to future definitive trial and other studies	20
Interpretation	22	Interpretation consistent with pilot trial objectives and findings, balancing potential benefits and harms, and considering other relevant evidence	15-19
	22a	Implications for progression from pilot to future definitive trial, including any proposed amendments	15
Other information			
Registration	23	Registration number for pilot trial and name of trial registry	N/A
Protocol	24	Where the pilot trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	21
	26	Ethical approval or approval by research review committee, confirmed with reference number	6

Supplementary Table 7-2 Proportions of fatty acids in plasma phospholipids of participants by group

	CTR			EXP		
Fatty acid (%)	**Week 0**	**Week 8**	**Week 16**	**Week 0**	**Week 8**	**Week 16**
C16:0	31.3 (28.9, 32.0)	30.4 (27.8, 32.1)	31.0 (28.7, 33.9)	29.0 (28.4, 31.1)	31.8 (28.4, 33.3)	31.0 (30.4, 31.7)
C18:0	15.1 (14.0, 15.9)	15.2 (14.5, 16.3)	15.3 (12.6, 17.3)	15.2 (14.0, 15.8)	15.1 (14.6, 16.9)	15.1 (14.7, 15.6)
C18:1 n-9	10.0 (9.4, 13.1)	11.6 (9.4, 12.9)	10.9 (10.1, 12.1)	11.7 (9.1, 15.0)	9.4 (7.8, 12.7)	9.9 (8.7, 11.8)
C18:2 n-6	18.5 (17.1, 23.1)	18.4 (15.5, 23.4)	18.2 (16.1, 19.6)	18.3 (14.8, 23.3)	14.6 (13.5, 19.4)	18.5 (17.6, 19.7)
C18:3 n-3	0.3 (0.1, 0.4)	0.3 (0.2, 0.6)	0.3 (0.3, 0.5)	0.3 (0.2, 0.6)	0.3 (0.2, 0.4)	0.4 (0.3, 0.5)
C20:3 n-3	0.1 (0.0, 0.9)	0.1 (0.0, 0.5)	0.1 (0.0, 0.3)	0.1 (0.0, 0.1)	0.1 (0.0,0.3)	0.2 (0.0, 0.2)
C20:4 n-6	9.3 (6.9, 12.3)	9.0 (6.8, 12.1)	9.7 (8.6, 11.1)	8.4 (6.9, 15.0)	7.1 (5.6, 8.4)	6.0 (5.4, 6.6)
C20:5 n-3	0.8 (0.5, 2.1)	1.2 (0.8, 1.8)	1.0 (0.5, 1.3)	1.1 (0.5, 4.3)	3.3 (1.6, 6.6)	2.4 (1.8, 3.6)
C22:5 n-3	0.7 (0.52, 1.04)	0.8 (0.7, 0.9)	0.8 (0.6, 1.0)	0.8 (0.6, 0.9)	1.1 (0.9, 1.3)	1.0 (0.7, 1.1)
C22:6 n-3	2.2 (1.6, 3.4)	2.3 (1.8, 3.2)	2.4 (1.7, 2.9)	3.0 (1.4, 4.2)	4.4 (3.3, 7.1)	4.0 (2.9, 4.7)
Total PUFA n-3	4.5 (3.4, 6.2)	5.2 (4.0, 5.5)	4.5 (3.7, 5.7)	5.3 (3.7, 9.2	9.0 (6.4, 14.5)	7.4 (6.63, 9.9)
Total PL fatty acids, µg/mL plasma	351.2 (269.3, 805.5)	383.8 (282.7, 967.7)	543.7 (344.7, 723.9)	555.7 (473.9, 702.9)	499.2 (343.6, 653.1)	367.9 (314.1, 545.6)

Week 0: CTR n=6/6, EXP n=5/5; Week 8: CTR n=6/6, EXP n=5/5; Week 16: CTR n=6/6; EXP n=4/5.
CTR, control group; EXP, experimental group; PL, phospholipid.

Supplementary Table 7-3 Dietary intake of participants by group

	CTR			EXP		
	Week 0	**Week 1**	**Week 2**	**Week 0**	**Week 1**	**Week 2**
Energy, M/W, kcal	2354 / 1859 (944, 2669)	1877 / 1644 (1034, 1731)	1531 / 1357 (771, 2672)	2250 / 1452 (1156, 2590)	n/a/1374 (814, 1730)	2550 / 1545 (1257, 1805)
Protein, g/kg	1.27 (0.66, 1.38)	1.08 (0.53, 1.37)	1.08 (0.30, 1.17)	1.21 (0.93, 1.36)	1.14 (0.40, 1.20)	1.17 (0.84, 1.38)
Carbohydrates, g	265 (106, 320)	179 (144, 247)	179 (120, 317)	169 (105, 311)	180 (108, 226)	217 (134, 363)
Fat, g	88 (31, 136)	64 (29, 101)	63 (21, 127)	68 (50, 80)	36 (31, 65)	63 (45, 82)
Vitamin D, IU	78.4 (12.51, 135.1)	70.2 (24.6, 228.8)	74.3 (46.3, 113.5)	163.7 (63.0, 369.3)	151.8 (32.1, 309.4)	146.0 (97.1, 210.0)
DHA, g	0.01 (0.00, 0.43)	0.04 (0.00, 0.70)	0.05 (0.02, 0.41)	0.04 (0.02, 0.36)	0.20 (0.01, 0.83)	0.03 (0.02, 0.56)
EPA, g	0.00 (0.00, 0.31)	0.02 (0.00, 0.23)	0.01 (0.00, 0.27)	0.01 (0.00, 0.17)	0.08 (0.00,0.40)	0.02 (0.00, 0.27)
ALA, g	3.14 (0.81, 4.31)	1.57 (0.22, 2.38)	0.60 (0.37, 6.57)	1.03 (0.56, 1.63)	0.77 (0.57, 0.89)	1.39 (0.27, 1.95)

Week 0: CTR *n*=5/6, EXP *n*=5/5; Week 8 : CTR *n*=5/6, EXP *n*=4/5; Week 16 : CTR *n*=5/6; EXP *n*=3/5
Daily average dietary intake without accounting for the supplement.
CTR, control group; EXP, experimental group; M, men; W, women.

Supplementary Table 7-4 Nutritional composition of the multi-nutrient supplement and placebo for a one-day provision

Component	CTR	EXP
Energy (kcal)	290	240
Carbohydrate (g)	58	0
Total protein (g)	0	51
Amino acid profile (g)	-	11.63
Leucine*	-	3.38
Isoleucine	-	3.15
Valine	-	0.90
Histidine	-	0.72
Lysine	-	0.63
Methionine	-	1.58
Phenylalanine	-	1.13
Threonine	-	0.90
Tryptophan	-	1.13
Alanine	-	1.35
Arginine	-	1.49
Aspartic acid	-	0.99
Cysteine	-	14.40
Glutamic acid	-	1.80
Glycine	-	2.48
Proline	-	2.03
Serine	-	1.35
Tyrosine		
Fat (g)	6.8	4.57
Omega 3 (g)	0.075	1.875
Eicosapentaenoic acid (EPA) (g)	-	1.125
Docosahexaenoic acid (DHA) (g)	-	0.750
Vitamin D (IU)	0	1583

These numbers are applicable for participants weighing 65-75 kg. A factor of 0.8 and 1.2 needs to be applied to the total protein and amino acid profile (except for leucine) to obtain the actual amount consumed for those weighing <65kg and >75kg respectively. All amino acids are "L" isomers.

*Each participant in the experimental group was given supplements containing both whey protein and leucine powder. Participants received 6g of leucine powder independently of their weight. Thus, the total amount of leucine per day for participants weighing <65kg was 10.5g, and 12.76g for those weighing >75kg.

CHAPTER 8: General discussion and conclusions

8.1 Main outcomes and hypotheses

The Canadian population is living longer, but unfortunately many individuals lose autonomy and quality of life with aging due to cognitive, muscle and physical function decline. Undoubtedly, the additional years older adults are gaining should be worth living.

The overall goal of this thesis was 2-fold, first to understand how low muscle mass, strength and physical functions are inter-related to clarify the definition of sarcopenia, and investigate whether sarcopenia is a predictor of cognitive decline; second, to identify potential dietary strategies to maintain these crucial functions known to be altered with aging, thus targeting muscle mass, physical and cognitive health. Two observational studies performed in the largest Canadian cohort of healthy older adults permitted to investigate the relationships between muscle mass, strength, physical functions and determine diagnostic cut-points for sarcopenia (Study 1), and to understand if it is a predictor of a 3-year cognitive decline (Study 2); one observational study allowed to investigate whether total and specific dairy products intake were determinants of cognition (Study 3); and a randomized controlled trial was designed to test the feasibility of a multi-nutrient supplement to improve muscle mass, strength and physical function in older adults experiencing a loss of autonomy (Study 4). Given the lack of consensus regarding the definition of sarcopenia and current arbitrary diagnosis, Study 1 adds strong and objective support to the existing literature and proposes the first empirical Canadian cut-points. No study previously looked at the relationship between sarcopenia (low appendicular lean soft tissue mass) and cognitive decline over time. Given that there is currently no treatment for MCI and dementia, Study 2 provides novel evidence on potential roles for muscle mass in cognitive function preservation. Lastly, considering the importance of nutrient-dense foods (i.e., dairy products) and specific nutrients (i.e., high-quality protein, leucine, vitamin D, n-3 PUFA) in cognitive, muscle and physical health, Study 3 (observational - dairy products) and 4 (multi-nutrient intervention) propose useful dietary solutions to slow down the loss of autonomy and add significant insight to both the research and clinical fields. A summary of results is provided in **Figure** 8-1.

- Study 1 showed modest associations between ALM index and strength and between strength and physical performance score in both men and women, and no association between ALM index and physical performance. This suggests that underlying

mechanisms and possibly health implications of presenting with low strength vs. low ALM differ.

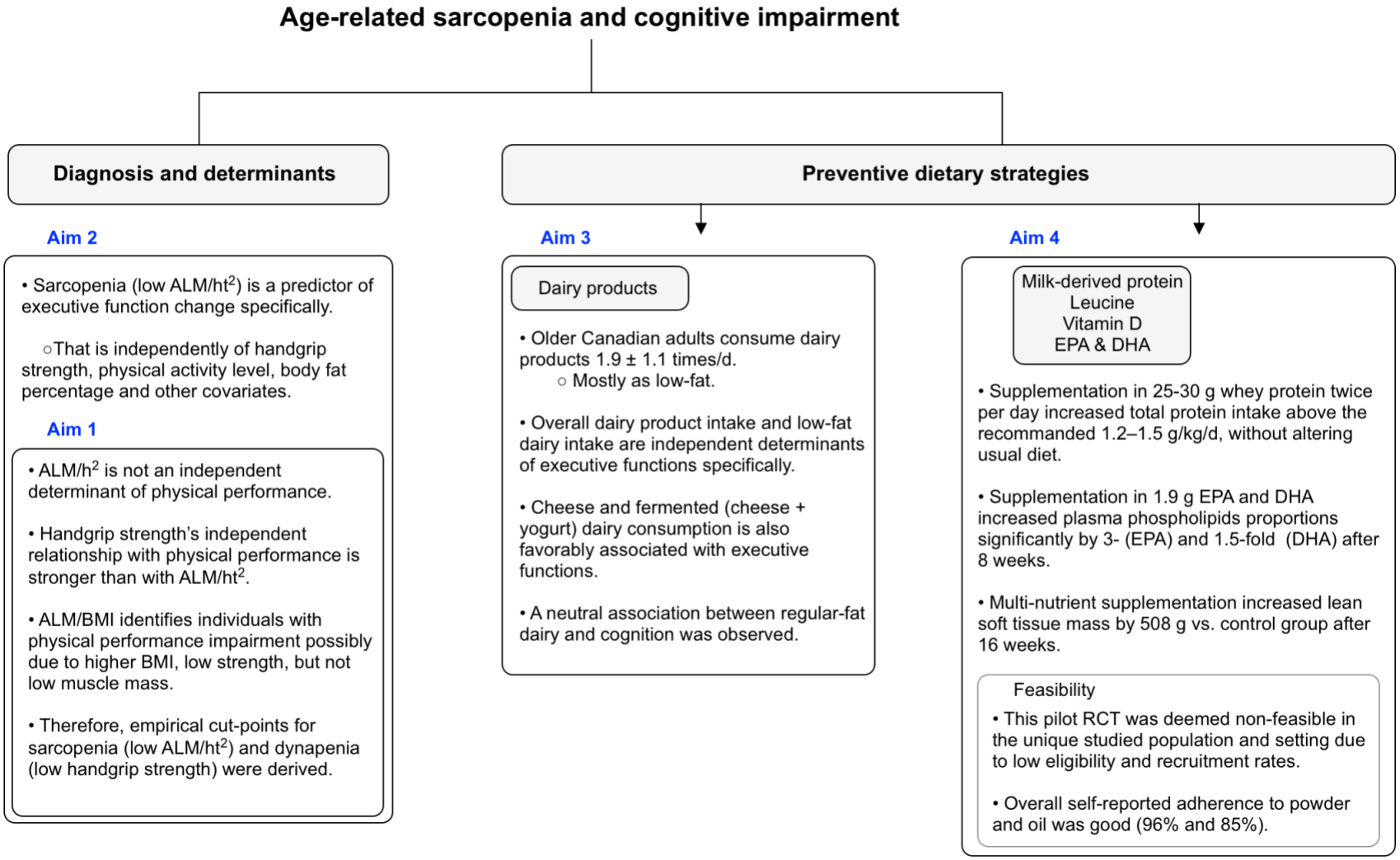
- As hypothesized, Study 2 found that sarcopenia (low ALM index; criteria as determined in Study 1) was a determinant of executive function decline over 3 years, independently of important factors including grip strength, percent body fat and physical activity. However, sarcopenia was not an independent predictor of memory and psychomotor speed decline.

- Study 3 showed a positive and independent cross-sectional association between higher total dairy product, low-fat and cheese intake frequency and greater executive functions, as well as between yogurt and memory.

- It was concluded from the pilot Study 4 that a 16-week randomized controlled trial of a carefully designed multi-nutrient supplement (protein, leucine, vitamin D and n-3 PUFA) to improve physical performance of older adults losing autonomy is not feasible on the basis of low eligibility and recruitment rate encountered at two Geriatric Day Hospitals in Montreal. Nevertheless, good adherence to the supplement showed a successful increase in overall protein intake and in plasma phospholipid n-3 PUFA without displacing intake from usual diet.

These studies established Canadian cut-points for the diagnosis of sarcopenia, and strongly support its link to cognitive decline, specifically of executive functions, over time. They also support that novel nutritional approaches are associated with higher executive functions (i.e., higher dairy product intake) and improve protein intake and n-3 PUFA status (multi-nutrient supplement consumed during 16 weeks) in older adults. However, these studies failed to prove associations with the other tested cognitive domains. Also, it is not clear from Study 4 whether the multi-nutrient supplement is effective at increasing muscle mass, strength and function which will require further research. Testing this supplement in a healthier population of older adults as a preventive strategy to the loss of muscle mass and strength would most likely be feasible and is warranted.

Figure 8-1 Summary of results from the studies included in this doctoral dissertation



ALM, appendicular lean soft tissue mass; h, height; BMI, body mass index; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RCT, randomized controlled trial

8.1 Should the definition of sarcopenia be rejuvenated?

Sarcopenia received an International Classification of Disease (ICD)-10 code in 2016 (21). The attribution of an ICD-10 code allows for sarcopenia to be recognized as a disease which typically promotes funding allocation and stimulates research on the disease; it aims to standardize the diagnosis; and it encourages sarcopenia diagnosis in clinical settings to offer treatment to those affected by the condition (417). However, the recognition of sarcopenia as a disease may have been premature given that there is still no consensus on its definition. Study 1 has contributed to better understand the relationships between ALM, body fat and muscle functional outcomes, which could aid in the refinement of an accepted definition.

Limited mobility and functional disabilities are of high concern during aging because of their recognized impact on quality of life; therefore, these have been frequently chosen as the main predicted outcomes of sarcopenia in recent research. Consequently, the definition of sarcopenia has evolved to include one or more functional parameters (strength and/or physical performance measure) to complement or even replace the measure of muscle mass to strengthen its predictive capacity of these outcomes (62). Indeed, owing to this reason, low muscle mass was excluded from the definition of sarcopenia in the recent (2020) definition of the Sarcopenia Definition and Outcome Consortium (418).

In contrast to the common assumption, but consistent with other groups' findings (325, 418, 419), this thesis confirmed that ALM is not independently associated with physical performance (Study 1). Indeed, only 1 in 8 men and 1 in 9 women with low ALM also had impaired physical performance. Higher ALM was initially associated with lower physical performance and the relationship was lost after adjustment for body fat mass (higher fat mass was negatively associated with lower physical performance; -0.25 in men and -0.28 women, $p<0.001$; **Figure** 4-1, **Chapter 4**). Similarly, two groups found the relationship between sarcopenia and physical limitations to be attenuated once body fat mass or BMI were considered (11, 326) and have suggested that ALM be normalized or adjusted for a measure or estimate of body fat in the identification of sarcopenia. However, Study 1 highlights that a cut-point normalized for BMI (FNIH criteria) identifies persons who do have impaired physical performance, but possibly due to the relative higher weight impeding weight-bearing movements such as rising from a chair and walking, whereas cut-points based on ALM/ht^2 identify older

adults with physiologically low ALM. For these reasons, BMI, fat mass or weight should not be considered in an ALM index; I posit that sex-specific ALM normalized for height squared should be used to clearly identify individuals with low lean mass and likely, muscle mass.

Furthermore, findings of this thesis (Study 1 and 3) suggested that although muscle mass and strength partly overlap, these components differ in predicting functional and cognitive outcomes pointing to unique roles of each muscle parameter. As covered in **Section 4.5**, given that muscle mass is the largest amino acid reservoir of the body (331), low muscle mass may have its particular health consequences in addition to activities of daily living dependency (420), namely lower metabolic rate (421) and impaired glucose metabolism (123, 422) (also related to cognition – **Section 2.4**), higher toxicity to chemotherapy in cancer patients (423, 424), poorer recovery post-surgery (423), falls and mortality post hospital discharge (425) and higher all-cause mortality (335-337). It is also possible that the association of muscle mass with health outcomes is relevant to particular populations such as hospitalized older adults and those living with chronic diseases. Lastly, consequences of low muscle mass may not be fully unveiled and understood at the moment. The recent focus on disability may have distracted research on sarcopenia (294) and exploratory research of health implications of low muscle mass alone is needed. Diverse indices for low ALM and any combination with another muscle parameter (strength and/or physical performance) introduces variability in the prevalence of sarcopenia, hinder harmonization of research findings with regards to determining treatments and adds confusion for clinical use. Accordingly, I would recommend that the term sarcopenia be restricted to its original definition and that the term attributed to low mobility or physical performance should be as stated. The latter should be directly evaluated using respective tests such as gait speed, chair stand, timed up and go or others.

Nonetheless, although modest, associations between handgrip strength and physical performance, and ALM index and strength observed in the CLSA justified the selected empirical approach to determine cut-points for the identification of low ALM index (as a determinant of low strength) and low strength (as a determinant of limited physical performance). The choice of the identification of binary cut-points stems from the fact that these are easy to understand, simple to apply in clinical practice, and it builds on previous sarcopenia research work (76, 77, 184). Using classification and regression trees, cut-points were to predict a binary outcome and the values obtained maximized purity in each group. Further, the performance of newly derived

cut-points was assessed and established using two techniques, first with an internal validation in an independent random sample of men and women for an unbiased evaluation, and second, by performing logistic regressions in different subgroups of the CLSA (at nutritional risk, with kidney and heart diseases, COPD and type 2 diabetes) (**Table** 4-2). The Canadian cut-points remain to be validated longitudinally, in diverse older adult populations.

Clinical implications

The diagnostic cut-points for sarcopenia elaborated in this thesis are simple to apply as they are sex-specific and binary. However, DXA instruments are not always easily accessible to clinicians for sarcopenia diagnosis purposes and are expensive (equipment and trained staff). Our results showed new Canadian cut-points for sarcopenia to predict executive function decline over 3 years, yet further research with longer follow-up period and repeated cognitive measures is needed to confirm this relationship. The economic burden attributable to both sarcopenia, as defined by low muscle mass, and dementia is substantial (18.5$ billion in the US (direct cost, in 2000) (188) and 10.4 billion in Canada (health care system and caregivers, in 2016) (426), respectively). Given that sarcopenia may play a role in cognitive decline and is associated with many other health outcomes, a cost-effectiveness analysis of the diagnosis should be performed before sarcopenia is diagnosed clinically. Also, the identification of cheaper and more accessible methods to evaluate muscle mass such as a segmental multifrequency BIA device (acceptable accuracy found in frail older women compared to DXA measurement (427, 428)), or novel and possibly more accurate methods such as D3-creatine dilution (429) would consist of other options. Upon diagnosis, appropriately targeted preventive or treatment measures such as the intervention proposed in Study 4 should be tested and implemented clinically if proven effective.

8.2 Unveiling the determinants of cognitive function and change in older age

An independent relationship between the presence of sarcopenia and greater decline in executive functions was observed in Study 2 and an independent association between dairy product intake and executive functions in Study 3. As observed in **Chapter 5** (Study 2), the strongest determinant of all 3 cognitive domain changes was baseline cognitive score, i.e., those who started with a higher score would experience a smaller decline after 3 years. Interestingly, following baseline cognitive score, the strongest determinants of cognitive domains were non-modifiable ones including age and education as expected, but also sex. Higher education

achieved in early life is a protective factor for dementia as it provides individuals with greater cognitive reserves (87). Biological sex and gender differences in cognitive function and decline have been reported previously (430). While the male sex appears advantageous for orientation tasks, the female sex seems favorable for verbal memory and object location tasks. In Study 2 all baseline cognitive scores differed between sexes; indeed, women had superior memory scores and were able to name more words at the COWAT F-A-S test (executive function), but men performed better at the animal naming and MAT test (executive function) and had higher psychomotor speed (data not shown). However, cognitive decline did not differ between sexes for most tests except memory (both immediate and delayed recall) and Stroop's high interference (executive function) for which men experienced greater declines. Also, sixty-five percent of dementia cases are observed in women probably because women live longer than men and therefore have more time to develop the disease (426); yet, it was reported that older women of the same age as older men would be more at risk of developing dementia possibly because of lower education and thus lower cognitive reserve (87). In Studies 2 (longitudinal) and 3 (cross-sectional), being a woman was protective of executive function and memory decline. This may be explained by our adjustment for both education and age, suggesting that independently of these factors, women experience a lower cognitive decline over time compared to men. The same strongest determinants, i.e., age, education and sex, of cognitive domains were identified in cross-sectional (Study 3) and longitudinal models, confirming robustness. Effect sizes of these covariates are reported in **Table** 8-1. The absence of a sex by sarcopenia interaction for all 3 cognitive domain changes (Study 2) and sex by dairy product intake interaction (Study 3) did not justify separate analyses, hence pooled analyses were conducted.

In the longitudinal model assessing cognitive decline, the effect size of sarcopenia was comparable to that of sex and was half the effect size of education. Although sarcopenia remained significantly associated with executive function change over 3 years, handgrip strength was also significant in the fully adjusted model and had a greater effect size than that of sarcopenia (std beta: 0.08 vs 0.03). Greater handgrip strength was protective of executive function decline ($p=0.001$), but also of memory ($p=0.027$) and psychomotor speed ($p<0.001$). Handgrip strength was in fact the third strongest predictor of psychomotor speed change among covariates included in the analysis. Similarly, other groups have reported associations between handgrip strength and cognitive change in older adults (111, 431-433), though none accounted

for ALM or low ALM in their models. Dynamometers are more practical and highly available in clinical settings compared to DXA. At the moment, it may be more realistic to screen older adults for low handgrip strength to identify those at risk of greater cognitive decline. Nonetheless, the relationship between strength and cognition may be related to the neural and motor function (54, 434) as the relationship between ALM and cognition may differ by being linked to vascular function (108) as discussed in **Section 2.4**. Future longitudinal studies should include longer follow-up period with additional repeated measures of cognitive functions, more sensitive memory tests, and measurement of low muscle mass as a unique criterion for sarcopenia for strong and unambiguous understanding of associations. Handgrip strength may be considered as a component in future tools to assess the risk of cognitive impairment.

Among the modifiable factors related to cognition, physical activity (by PASE score) was associated with memory only, but not executive function and psychomotor speed. It is possible that particular exercises such as aerobic exercise that increase cardiovascular fitness specifically relate to enhanced cognition (355). Despite the inclusion of such activities in the PASE, a relationship may not have been detected due to the large range of activities encompassed in this questionnaire (recreational activities, caring for others, exercise, housework, yard work); testing of separate exercise types performed by participants would be useful. Also, the duration and intensity of exercises may have different effects on cognitive performance (355). As a secretory organ, skeletal muscles release cytokines and other peptides together called myokines, which exert autocrine, paracrine and endocrine effects. These include IL-6, IL-8, IL-15, brain-derived neurotrophic factor (BDNF) and leukaemia inhibitory factor (352). In the systemic circulation, imbalances in cytokines may lead to an inflammatory state as observed with physical inactivity and metabolic diseases (435). Paradoxically, these factors also have anti-inflammatory activity namely when rapidly released into the circulation following exercise (435). BDNF is critical in the survival, growth and maintenance of nerve cells and synaptic plasticity, mainly of the hippocampus that is responsible for short-term and long-term memory (436). Though myokines were not measured in the current thesis due to cost and time limits, future work should consider the inclusion of these as part of statistical models.

Given that we found a cross-sectional link between lower dairy product intake frequency and poorer executive functions, low intake of particular foods or nutrients may be related to cognitive decline as well. A subsequent study should examine the association between dairy

products intake frequency and cognitive change in the CLSA, now that follow-up data are available (437).

Table 8-1 Effect size of some covariates included in fully adjusted models of the association between sarcopenia and the changes in executive functions, memory and psychomotor speed domains.

Std β	Executive functions	P-value	Memory	P-value	Psychomotor speed	P-value
Baseline cognitive composite score (continuous)	**-0.40**	<0.001	**-0.45**	<0.001	**-0.64**	<0.001
Age (continuous)	**-0.12**	<0.001	**-0.21**	<0.001	**-0.08**	<0.001
Education (categorical)	**0.06**	<0.001	0.04	0.004	0.05	<0.001
Sex (binary)	0.03	0.038	**0.24**	<0.001	0.04	0.082
Income (categorical)	0.05	0.005	0.07	<0.001	0.02	NS
Handgrip strength (continuous)	0.08	0.001	0.05	0.027	**0.08**	<0.001
Sarcopenia (low ALM index; binary)	-0.03	0.028	-0.01	NS	-0.01	NS
% body fat (continuous)	0.04	0.043	0.0	NS	0.01	NS
Physical activity (PASE; continuous)	0.0	NS	0.04	0.002	0.02	0.058
Nutritional risk (SCREEN II-AB; continuous)	0.0	NS	0.0	NS	0.0	NS

ALM, appendicular lean mass; PASE, physical activity scale for elderly; SCREEN II-AB, abbreviated seniors in the community risk evaluation for eating and nutrition version II.
These are part of the covariates included in the models. In bold are the 3 strongest predictors for that particular domain change over time (column). A positive sign means that an increase in the independent variable is associated with a smaller decline (change towards a positive value) in the domain. Models were adjusted for handgrip strength as a continuous variable to preserve the highest level of information in answering the objective of this study (Study 2).

8.3 Dairy products for brain health: public health implications

Dairy products are nutrient-dense food, yet 84% of women and 79% of men aged above 71 years do not meet the 2007 dairy intake recommendations in Canada (134). Our previous study on dairy product intake and lean mass performed in free-living older adult living in Québec (the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge) cohort, n=1,499, manuscript in preparation) revealed an average intake of 1.4 ± 0.8 servings/d, hence not even half of the recommended daily 3 servings (2007 recommendations). Similarly, the mean total dairy intake frequency of the CLSA senior population was 1.9 ± 1.1 times/d.
Although higher intakes of dairy products have shown several health benefits including stroke, hypertension, metabolic syndrome, type 2 diabetes and obesity (172), their representation in the new 2019-Canadian Food Guide is less predominant than in previous versions given that they are now included under "protein foods" along with meats and alternatives.

In this thesis, higher intake in total dairy products (>2.5 times/d vs. ≤1.7 times/d), cheese, low-fat and fermented dairy was cross-sectionally and independently associated with better executive functions (Study 3). These results are in part comparable and consistent with previous findings of positive associations between dairy product consumption and cognition (global cognition and memory) in young and older adults (180, 182). Improved performance at an executive function test in middle-aged adults was also observed following a 6-month intervention of high dairy intake (4 servings/d, reduced-fat) compared to a low intake (1 serving/d) (179). Evidence is accumulating, supporting that improving dairy intake among adults and older adults represents a potential target to preserve cognitive function with aging.

Whereas additional evidence regarding dairy intake and cognitive function is warranted, other dietary patterns, food groups and nutrients have shown a strong collection of evidence of beneficial associations with the incidence of cognitive impairment, as previously discussed in **Chapter 2, Section 2.6** (140). Though the elaboration of the Canada's Food Guide is based on a comprehensive review of emerging evidence and follows a well-structured cycle (the Evidence Review Cycle for Dietary Guidance), the outcomes of concern evaluated regrettably excluded that of dementia (438), a debilitating condition that affects 1 in 3 persons aged above 85 years in Canada (426). The outcomes were restricted to cardiovascular disease, type 2 diabetes, certain types of cancer, osteoporosis, and obesity (438). Reflective of the general food guide, the

resource destined to older adults – Canada's Food Guide: Healthy Eating for Seniors – focuses on maintenance of a healthy weight, lower risk of heart disease and type 2 diabetes and prevention of muscle and bone loss and risk of falls. Additionally, the recommendations are to choose lower fat dairy products. Our results showed a positive association between regular-fat dairy products (excluding dairy-based desserts) and executive functions in our first model (adjusted for age, sex, language, education and province) and a neutral association in the fully adjusted model. Also, the cheese frequency of intake was significantly associated with better executive functions after controlling for all covariates. As discussed in **Chapter 6**, other studies also showed neutral associations between whole-fat dairy products and the risk of chronic diseases (171, 172). However, it was not clear from all studies whether dairy-based desserts were included in this category, which would potentially have negatively affected the results given the different nature of these foods.

Study 3 contributes to increase awareness of the health benefits of dairy products, in addition to known ones, specifically addressing an age-related issue, cognitive performance that declines with aging and may lead to MCI and dementia. Future public health guidelines may consider greater representation of dairy products given their excellent sources in protein, calcium, vitamin B_{12} and many others, particularly for older adults who have increased needs and may experience decreased appetite. Longitudinal studies of the association between dairy intake and cognitive decline, MCI and dementia incidence are needed to strengthen this thesis findings. Of high importance, future studies should avoid pooling of dairy-based desserts, butter and fat from spreads into the regular-fat dairy category to prevent confusion in associations.

8.4 Strengths and limitations

The CLSA is a large (n=51,338) nationally representative, contemporary prospective cohort (recruitment 2011-2015) of adults aged 45-85 at baseline (302). Participants were recruited across Canada by province (7 included), age and sex strata. Out of the 51,338 individuals, 30,097 living within a 25-50 km radius from one of the 11 data collection site across provinces were selected at random to constitute the comprehensive cohort, all of which participants went through in-depth physical examination and provided biological samples. The CLSA is one of the largest cohorts to include such an extensive assessment of cognitive functions and physical parameters. In this thesis, studies 1 to 3 were performed using the older

adults aged ≥65 years (439) subgroup data of the comprehensive cohort as our objectives were focused on late life age-related outcomes and dietary strategies. The cohort included relatively healthy older adults at baseline and excluded those most vulnerable and the ones institutionalized; yet, the sample sizes were large, and province of participants and sampling weights were carefully considered in our analyses where applicable such that results have good generalizability to the Canadian community-dwelling older adult population.

A key strength to this thesis is the robust empirical method applied to determine cut-points for low strength and low ALM (Study 1) through the use of sophisticated statistical models (structural equation modelling, classification and regression trees and factor analysis for physical performance score creation). Compared to previous arbitrary approaches to the elaboration of cut-points (60, 62, 63, 72, 184, 300, 440), our method permitted to define strong cut-points that are determinants of clinically relevant outcomes. Indeed, the assessment of body composition (by DXA, the reference method for measuring muscle mass (67, 293), strength (by dynamometry, a proxy for overall body strength (441)) and physical performance (four valid and reliable tests (442)) allowed for an exhaustive evaluation of the associations between ALM, strength, physical performance (Study 1) and cognitive decline (Study 2). Also, the 7 cognitive tests used in Studies 2 and 3 allowed to assess 3 cognitive domains typically affected by aging (348). These tests enabled to shed light on potential mechanisms and link factors (sarcopenia and dairy products) investigated in this thesis to specific clinically relevant areas of cognitive functioning.

Compared with other cohort studies that measured cognitive function or diagnosed MCI at baseline only, Study 2 included repeated assessments of 3 cognitive domains, using 7 distinct tests at baseline and 3 years later. This has enabled us to investigate the relationship between sarcopenia and cognitive decline in multiple cognitive domains over time for the first time. Nonetheless, the studied population was found to have maintained good cognitive health between baseline and follow-up 1. The change in cognitive functions after 3 years was modest (Animal naming: -0.4 ± 4.4 words; MAT: -1.1 ± 6.6 counts; Stroop's high interference: 0.03 ± 0.73 s), and population mean actually increased for some tests after 3 years (RAVLT immediate recall: 0.4 ± 1.9 words; RAVLT delay immediate: 0.3 ± 1.9 words; F-A-S total: 0.4 ± 8.0 words; Choice reaction time: -5.3 ± 180.4 ms). Also, few individuals reported having received a

diagnosis of AD at follow-up 1 (n=34, 0.4% after 3 years). All of the above may explain the unique significant associations with executive function change in this study.

Unfortunately, due to release of follow-up data after completion of Study 3, only cross-sectional analyses of the relationship between dairy product intake and cognitive functions were possible. Therefore, reverse causality, i.e., low cognitive function leads to a lower consumption of dairy products in older adults, is plausible. A potential explanation to this may be difficulties in managing grocery shopping (443), resulting in shopping less often, buying minimal, easy to carry and cheaper products. Further, prospective design of Study 2 strengthens the direction of associations. Although observational studies have limitations compared to RCTs which allow to control from confounders prior to the start of the study (among others), Study 2 and 3 statistical models provide strong evidence as they were adjusted for important confounders and several covariates while maintaining excellent statistical power owing to the high sample size (n=9,000).

The main limitation of the CLSA data is the use of the Short Diet Questionnaire (SDQ; a semi-quantitative FFQ without specified quantities of food items consumed) which was chosen because of time constraints. Though FFQs are appropriate to capture intake in specific dairy products as they are typically consumed sporadically, the lack of reported portion sizes decreases accuracy of measured intake. I extrapolated the age- and sex-specific average portion sizes of each dairy product type consumed in the NuAge cohort to the frequency intakes in the CLSA, yet it did not improve the results due to negligible differences in portion sizes across strata; this approach was not retained in our methods. Data from the SDQ also resulted in multimodal distributions of dairy product frequency intake as a continuous variable, with many individuals reporting the same frequency. To palliate this issue, I ranked participants into quartiles of dairy product consumption frequency and used MANCOVA models to compare groups which permitted better interpretation/reporting of results and allowed for a dose-response analysis. It was also not possible to compute a validated diet quality score such as the Healthy Eating Index, from the SDQ. Since it was important to control for the potential effect of diet quality, we created a score using available data from the SDQ that was accounted for in our models. Our sensitivity analysis testing the adjustment for fruits and vegetables, and whole grain intake frequency as alternative proxies for diet quality provided consistent results.

The population studied in Study 4 (clinical trial) constitutes both a major strength and a limitation. Indeed, this understudied and growing segment of the population merits further

investigation to better orient treatment strategies, but recruitment and retention of vulnerable individuals proved difficult. The robustness of the study design was another strength. Plasma phospholipid fatty acids were used as biomarkers of compliance to the oil supplement; given they are stable to transient postprandial fluctuation in triacylglycerol pool and reflect cell membrane fatty acids incorporation, they permitted objective assessment of adherence at both mid- and post-intervention (284). By using DXA at baseline and at 4 months, it was possible to measure appendicular lean soft tissue mass and other regions (whole and trunk) lean soft tissue mass accrual. However, some older adults found it uncomfortable to lay down straight and still on the table during 10 min potentially due to age-associated kyphosis. The positioning, possible movement, but also hydration status may have impacted the ALM measurements (444). Strength of both the upper and lower body was measured using handgrip and knee extension dynamometers for good representation and measure of overall body strength over time (441). Similarly, additional physical tests (timed up and go, 30-s chair stand, gait speed and 6-min walking test) provided a comprehensive evaluation of physical performance of participants pre- and post- intervention. Dietary intake under-reporting using 24-hour recalls and food diaries has been documented in older adults (445, 446). Indeed, it was difficult to collect complete and accurate dietary information from participants. During in-home visits, they would show me the exact food items they consumed. As well, an iPod touch was also provided for them to take photographs of their meals and food packages helping to address the potential memory bias, and facilitate the estimation of portion sizes and overall revision of the 3-day food diaries. As a result, only 4 food diaries (from 2 different individuals, one who completed the study) out of 30 were identified as under-reported (energy intake:basal metabolic rate <0.9 (447)) throughout the study. Lastly, but most importantly, the eligibility and recruitment rates were poor which regrettably prevented us from considering the study as feasible.

Previous similar studies observed greater recruitment rates and achieved their required sample size timely (44, 448). Although their intervention was similar to ours and their population included sarcopenic older adults, several factors including more liberal eligibility criteria, overall healthier (44), more homogenous population may have facilitated recruitment. In Rondanelli et al.'s study (448), participants were taking part in an in-patient rehabilitation program which may also have facilitated recruitment and adherence to the protocol, as opposed to out-patient clinics. Our unique population was highly challenging to study not only due to their physical condition,

but also because of several life events such as acute diseases, death of loved ones, being a spousal caregiver, and others and psychological distress they were coping with at the time of their participation. Though our participants were still living in the community or assisted living, they were soon-to-be institutionalized older adults. Of course, the sample size was too small to draw formal conclusions with regards to changes in measured outcomes. Nevertheless, Study 4 is one of its kind as it comprehensively characterizes profiling of plasma phospholipid n-3 PUFA of this particular population for the first time to our knowledge (further discussed in **Section 7.5**).

8.5 Important considerations for future research

This thesis has advanced understanding with regards to the relationship between muscle mass, strength, dairy product intake and cognitive performance in older Canadian adults. Conversely, it has generated several new questions which remain to be answered. Future epidemiological work should investigate the link between dairy product intake and cognitive decline over a time, and the risk of MCI and dementia. Examination of sarcopenia in statistical models should also be performed given the significant association found with executive function change (Study 2). Now that follow-up data are available, the natural prolongation of this thesis would be to study this relationship in the CLSA cohort. To complement these results, analyses should be performed in the NutCog cohort, an embedded study of 428 French-speaking participants from the NuAge cohort (2006-2008) who underwent a thorough and similar cognitive evaluation as in the CLSA (mean follow-up ± SD: 3.0 ± 0.6 years) and which includes accurate and precise repeated 24-hour recalls (3 collected yearly). To my knowledge, only one intervention study tested the effect of high vs. low intake of reduced-fat dairy product on cognition, in middle-aged adults and not older ones. Since the study only tested low-fat products, the potential effects of regular-fat dairy intake remain to be tested. A trial of dairy product intake in healthy older adults including measures of cognitive performance, positron emission tomography (PET) scans of the brain, body composition, physical performance would greatly inform public health guidelines and shed additional light on potential mechanisms (179). The examination of the link between sarcopenia and cognitive decline over a longer follow-up period and including myokine measures (449) in mediation analyses would add understanding with regards to the potential role of muscle mass in cognitive functioning.

Lastly, epidemiological analyses should be replicated in other populations such as in the UK Biobank as it would give added strength and broader applicability of results. In fact, the use of harmonized data from multiple cohorts should be considered for cross-study comparison. The Cross-cohort Harmonization Project for Tomorrow Generic Harmonization Project population is an example of harmonized cohorts (includes the CLSA and UK Biobank) which regroups data from 2 million participants aged 18 years or older from Canada, the United States and across Europe (450).

Randomized controlled trials in the oldest and most vulnerable adults

The recruitment period for the pilot trial study (Study 4) was 2 years; only 46 patients were eligible out of 244 screened and 10 completed the study. After one year of recruitment at the MGH, the IUGM, offering a similar rehabilitation program, was opened for this research. However, it resulted in only a modest increase in recruitment again because of the strict eligibility criteria, refusal to participate and drop out of the rehabilitation program. In addition, the demand on staff resources to accompany older adults in this study was tremendous. It included the extensive assessments (3/4 of a day per participant), visits at home and at the GDHs to collect and review food diaries and accelerometers, and calls for follow-up and motivation; preparation of supplements in individual containers; and delivery of supplements to participants' home every 2 weeks.

Regrettably, the oldest and most vulnerable adults are underrepresented in studies including lifestyle and clinical trials (451, 452) in spite of their growing population. The older adults population is highly heterogenous; consequently, it may be desirable from a researcher's perspective to use strict eligibility criteria thereby to minimize variability in measured outcomes. However, this may lead to the exclusion of several older adults who may benefit from the intervention and it introduces recruitment issues as observed in our pilot study. In the context of an effectiveness trial (pragmatic trial), unlike an efficacy trial (explanatory trial), strict eligibility criteria may not be justifiable (453). Instead, the intervention should be elaborated to be well accepted by the inclusive targeted population. Our supplement appeared to be accepted by most of our participants, yet future studies should limit eligibility criteria to those known to be counterindications to the supplement use (or intervention) for safety.

From a participant's perspective, health problems, limited autonomy, self-inflicted ageism may all be factors for them to refuse to participate. It is important that the research team

provides dedicated support to patients from recruitment and throughout the study. Assessments comprised in our trial (physical performance, strength, body composition, anthropometry, blood tests, questionnaires) were numerous. Selecting fewer, sensitive, yet simple to perform ones for the target population may shorten visits duration and ease participation consent. It further reduces resources needed which are overall greater for studies in such populations.

Dietary assessment in older adults

The quality of dietary data represented an important limitation of the CLSA and to a lesser extent, of the pilot study included in this dissertation. High quality, comprehensive dietary assessment is crucial to establish strong diet-health associations or effects in nutrition studies. Nevertheless, this can be challenging to achieve in large cohort studies due to time burden (faced in the CLSA), necessity of qualified staff, e.g., a dietitian, to review and analyze collected data, and therefore associated costs. Novel computer-based 24-hour recall such as the automated self-administered 24-hour (ASA24©) allowing remote and automated dietary data collection has been trialed in the CLSA, but unfortunately showed poor reported usability (372). The administration of multiple 24-hour recalls or that combined with a FFQ (454) in a stratified representative subsample of the cohort should be considered to enable more robust and calibrated prospective analyses, as leveraged in CCHS 2004. Other particular challenges arose in our pilot study in vulnerable older adults, namely the lack of details reported in 3-day pen and paper food diary and memory burden encountered during revision of diaries with participants. However, the use of meal photographs captured by participants was helpful and the low overall under-reporting rate observed supports the potential of technology to assist with data collection in a vulnerable population.

Given the rising use of smartphones (equipped with cameras) in the general population including middle-aged adults (45-64 years: 87%) and older adults (> 65 years: 60%) in Canada (455), user-friendly novel strategies to dietary assessment such as image-assisted mobile applications (456, 457) may be able to address time burden during visits and memory bias at low costs, while maintaining or improving the degree of dietary measurement error. Also, smartphones and tablets have larger sizes and built-in accessibility settings to address possible age-related visual impairment. The use of these tools could be adapted to the specific needs and technological aptitudes of older adults. The most recent version of the image-assisted food diary Keenoa© connected to a web application for researchers is used in our ongoing COVIDiet study

including participants over 65 years (refer to Clinicaltrial.gov, NCT04407533). To support the oldest adults (+75 years), a promising strategy is that a staff member helps to review and complete their diary together over the phone, from pictures taken of their meals and available on the researcher's web interface, simultaneously in a short time. The investigation of the use of such a tool in older adults with potentially limited technology skills has not been done to date and is needed.

8.6 Conclusions

In conclusion, this thesis has confirmed that muscle mass is modestly associated with strength and is not associated with physical performance after adjustment for age and fat mass suggesting that muscle mass and strength are distinct constructs. Also, it highlighted that different indices for low ALM do not identify concordant individuals as having sarcopenia. These findings guided the elaboration of the empirical Canadian sex-specific cut-points to identify low muscle mass and low muscle strength as separate conditions. These new cut-points may be used in research and clinical settings where DXA and dynamometers are available, for a uniform and population-tailored diagnosis. Further, sarcopenia (low ALM) as defined by these cut-points was shown to be a marker of executive function decline over 3 years implying a potential role for muscle mass in cognitive decline. This thesis further demonstrated that total dairy product (≥2.5 times/d), low-fat and cheese intake were all independently associated with executive functions cross-sectionally, and yogurt intake with memory suggesting that the food matrix or specific nutrients of these products are beneficial for cognitive health. Lastly, it was concluded that a 16-week randomized controlled trial of a combined nutritional supplement to improve physical performance of a particular population of vulnerable older adults attending a rehabilitation program was not feasible due to low eligibility and recruitment. Nevertheless, good adherence to the supplement showed to effectively increase overall protein and leucine intake, and n-3 PUFA status without displacing intake from usual diet. A more liberal design of this trial in vulnerable older adults should be tested. This thesis will contribute to orienting research in sarcopenia, functional disability and cognitive impairment preventive treatment. In addition, this thesis lays groundwork for future longitudinal observational studies and intervention studies which will be needed before establishing recommendations with regards to dairy product intake

and nutrient supplementation to promote muscle mass, physical and cognitive functions preservation in aging.

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Appendix

Short diet questionnaire (SDQ)

- How often do you usually drink whole milk 3.25% m.f.?
- How often do you usually drink 2%, 1%, skim milk?
- How often do you usually eat all low-fat cheeses?
- How often do you usually eat all regular cheeses?
- How often do you usually eat yogurt (low-fat)?
- How often do you usually eat yogurt (regular)?

The above are questions related to dairy products intake frequencies used in **Chapter 6**, Study 3 analyses. Answers could be reported per day, per week, per month or per year. The full questionnaire is available in (458).