DOCTORAL THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY (PH.D.) AT THE MEDICAL FACULTY

OF THE LUDWIG MAXIMILIAN UNIVERSITY OF MUNICH

Nutritional Status, Muscle Health, and Sarcopenia: Evidence from Epidemiological Studies in Older Adults

Romy Adélaïde Marie-Thérèse Conzade

2019

From
The Institute of Epidemiology, Helmholtz Zentrum München
German Research Center for Environmental Health (GmbH)
Director: Prof. Dr. Annette Peters

DOCTORAL THESIS

Nutritional Status, Muscle Health, and Sarcopenia: Evidence from Epidemiological Studies in Older Adults

Submitted for the Degree of Doctor of Philosophy (Ph.D.)
in Medical Research: Epidemiology and Public Health
at the Medical Faculty of the Ludwig Maximilian University of Munich

by
Romy Adélaïde Marie-Thérèse Conzade

from
Paris, France

on
August 27, 2019

With approval of the Medical Faculty

of the Ludwig Maximilian University of Munich

Supervisors/Examiners:	Prof. Dr. Barbara Thorand, MPH Prof. Dr. Eva Grill, MPH
Co-Examiners:	Prof. Dr. Jakob Linseisen PD Dr. med. Michael Drey
Co-Supervisors:	Prof. Dr. med. Heike A. Bischoff-Ferrari, DrPH Prof. Dr. med. Gustavo Duque
Dean:	Prof. Dr. med. dent. Reinhard Hickel
Date of oral defense :	12.11.2019

Pour mes grands-parents

Table of contents

Abbreviations

25OHD	25-hydroxyvitamin D
ALM	Appendicular lean mass
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CI	Confidence interval
DAG	Directed acyclic graph
DEGS1	German Health Interview and Examination Survey for Adults
DXA	Dual-energy X-ray absorptiometry
ESPEN	The European Society for Clinical Nutrition and Metabolism
EWGSOP	European Working Group on Sarcopenia in Older People
FFC	Falls and fractures clinic
ICD-10-CM	International Classification of Diseases, Tenth Revision, Clinical Modification
ICFSR	International Conference on Frailty and Sarcopenia Research
ICOPE	Integrated Care for Older Adults
KORA	Cooperative Health Research in the Region of Augsburg
MONICA	Monitoring of Trends and Determinants in Cardiovascular Disease
MNA	Mini Nutritional Assessment
OR	Odds ratio
PTH	Parathyroid hormone
SMI	Skeletal muscle mass index
SPPB	Short Physical Performance Battery
TUG	Timed Up and Go
WHO	World Health Organization

I. Introductory summary

"*There is probably no decline in structure and function more dramatic than the decline in lean body mass or muscle mass over the decades of life*" Irwin H. Rosenberg [1]

1. General introduction

Worldwide, declined fertility and increased survival to older age* are leading to the rapid ageing of populations [2]. Most people aspire to live a long and healthy life. In 2015, the World Health Organization (WHO) proposed a new vision for healthy ageing. Rather than a disease-free concept, the updated concept of healthy ageing centers on the notion of functional ability: the combination of one's intrinsic capacity (all physical and mental capacities), the environment, and the interaction between them [3]. The 2015 *World report on ageing and health* acknowledges that key lifestyle factors such as diet and physical activity may strongly influence intrinsic capacity in older age. Their impact on intrinsic capacity may be essential to strategies aimed at preventing or slowing declines in functional ability, including conditions such as frailty [3].

1.1. Sarcopenia and age-related muscle changes

Sarcopenia, the progressive loss of muscle mass and function that occurs with age [4], is a core component of frailty [5]. Sarcopenia is a public health challenge due to the associated higher risk of falls, functional decline, frailty, and mortality [6]. It was first described in 1989 by Rosenberg as an age-related decline in lean body mass that affects mobility, nutritional status, and independence [1]. Subsequent definitions have included measures of muscle function, such as muscle strength or physical performance, because muscle function was consistently shown to be a stronger predictor of clinical outcomes than muscle mass alone [7]. Present in an estimated 1-29% of community-dwelling older adults [8], sarcopenia is best understood as a geriatric syndrome [9] and has since 2016 an own ICD-10-CM† code (M62.84) [10].

Muscle mass and muscle strength peak in young adulthood. Beginning around age 50, both start decreasing gradually, with a faster decline in muscle strength (Figure 1) [4]. Resulting predominantly from the reduction in size and number of type II muscle myofibers [11], loss of skeletal muscle might be perceived as a normal component of the ageing process. However, varying degrees of muscle mass and strength decline across the population [12,13] point to modifiable behavioral factors such as diet and lifestyle as important influencers of muscle changes [14], suggesting that these factors may play a role in both the prevention and treatment of sarcopenia.

* Often defined for research purposes as 65 years and beyond.

† ICD-10-CM: International Classification of Diseases, Tenth Revision, Clinical Modification.

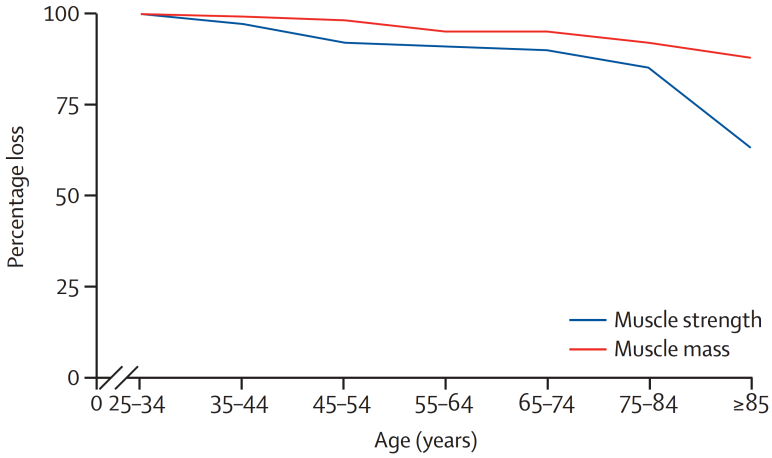

Figure 1. Percentage loss of muscle mass and strength with age in men [4]. Reproduced with permission from RightsLink®/Elsevier, obtained on 24 July 2019

1.2. Nutrition and ageing

A consistent finding from epidemiological studies is that poor diet is common in older adults [15]. Food and energy intakes decline with age [16], for a variety of reasons that range from physiological (decreased energy needs, appetite loss, poor dentition), psychological (depression, cognitive decline) to socioeconomic factors (loneliness, economic hardship) [17]. Moreover, abilities to absorb and utilize specific micronutrients become less efficient with age (e.g., vitamin B_{12} [18], vitamin D [19]).

Compromised nutrient intake and assimilation put older adults at risk of malnutrition* [20]. Estimates of the prevalence of malnutrition vary across definitions and subgroups studied but are consistently higher with increasing level of dependency. In a pooled analysis of data from 12 countries, 38% of older adults living in the community were malnourished or at risk for malnutrition, compared to 92% in rehabilitation settings [21]. Apart from general malnutrition, older adults are at risk of specific micronutrient deficiencies [15]. For example in Germany, the nationwide DEGS1 survey† uncovered a high prevalence of low vitamin D status, defined by serum 25-hydroxyvitamin D (25OHD) levels <50 nmol/L, in older women (69.9%) and men (62.6%) aged 65 to 79 [22], signaling a serious public health concern.

* The 2017 ESPEN (The European Society of Clinical Nutrition and Metabolism) terminologies for clinical nutrition define *malnutrition/undernutrition* as "a state resulting from lack of intake or uptake of nutrition that leads to altered body composition (decreased fat free mass) and body cell mass leading to diminished physical and mental function and impaired clinical outcome from disease". *Overnutrition* (overweight and obesity) and *micronutrient abnormalities* (deficiency or excess) represent other separate concepts [20]. This thesis focuses on nutrition-related disorders associated with a negative nutrient balance, i.e. malnutrition/undernutrition and micronutrient deficiencies.

† DEGS1 survey: German Health Interview and Examination Survey for Adults

1.3. Associations between nutrition, muscle health, and sarcopenia

The negative health consequences of malnutrition are well known. Earlier studies have focused on malnutrition in older adults in terms of clinical outcomes, such as morbidity and mortality [23,24]. More recently, the association of malnutrition with functional decline has become more evident. Declining food and energy intakes contribute to weight loss, with implications for muscle mass and function [25,26]. Thus, malnutrition has emerged as a potential important risk factor in the development of sarcopenia. However, much of the research exploring the effects of individual nutrients on sarcopenia and related muscle components is relatively young, as highlighted by Robinson et al. [14].
The nutrient that has received increased research attention in relation to muscle health and sarcopenia is vitamin D. Despite several reasons to consider how vitamin D may influence skeletal muscle [27], individual observational studies examining the associations of vitamin D levels with changes in muscle mass and function have yielded inconsistent results [28-33]. Moreover, very few prospective studies have investigated vitamin D status in relation to the incidence of sarcopenia defined by both muscle mass and muscle function as requested by actual definitions of sarcopenia [28,34].

Issues related to poor nutritional status are not impossible to overcome. Routine screening of nutritional status and early diagnosis of malnutrition are essential. However, this seems not to be prioritized in geriatric health care [35]. If we can understand which nutritional factors influence the rate of decline in muscle mass and muscle function in older age, and the underlying mechanisms involved, we can lay the foundation for targeted strategies directed to prevent or delay sarcopenia. Such a foundation would allow older adults to maintain a higher quality of life and promote healthy ageing.

2. Aim of this thesis and outline

In light of the growing ageing population and the high societal relevance of successful healthy ageing, this cumulative thesis aims to study the nutritional status of older adults and to clarify its associations with sarcopenia and changes in related muscle components. Figure 2 outlines the thematic structure of this thesis and highlights the analyzed relationship of the nutritional status with muscle components and sarcopenia in older adults. This thematic structure serves as a basis for the three publications included in this cumulative dissertation.

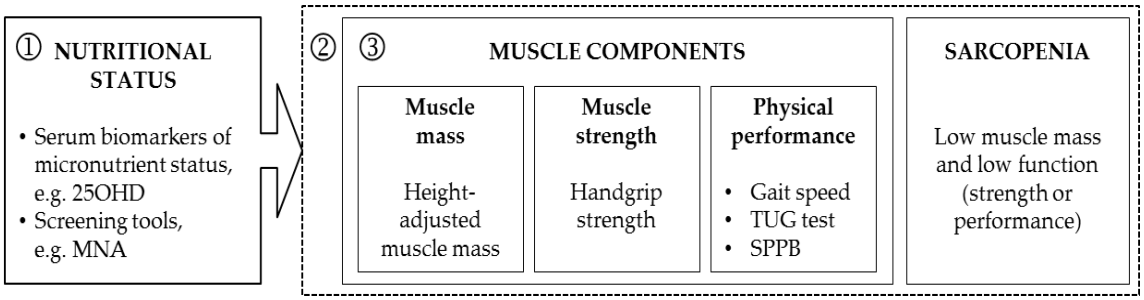

Figure 2. Thesis thematic structure: linkage between nutritional status, muscle health and sarcopenia in older adults. Each number corresponds to one of the three publications and indicates the respective theme and methods used.

In the first publication, a cross-sectional study, the focus lies on studying the nutritional and more specifically, the micronutrient status of older adults. We estimate the prevalence of vitamin D, folate, vitamin B_{12}, and iron deficiencies and describe socio-demographic, lifestyle, and health determinants associated with micronutrient deficiencies in older age. Among others, we consider the evidence that supports the importance of diets of adequate quantity and quality and discuss the possibility that regular and appropriately dosed micronutrient supplementation might aid older adults, otherwise unable to follow dietary guidelines, to satisfy nutritional needs.

Publication 1: Prevalence and Predictors of Subclinical Micronutrient Deficiency in German Older Adults: Results from the Population-Based KORA-Age Study. Conzade, R.; Koenig, W.; Heier, M.; Schneider, A.; Grill, E.; Peters, A.; Thorand, B. *Nutrients* 2017, 9.

In the second publication, we turn our attention to the public health concern of low vitamin D status in older adults and consider the evidence that links vitamin D deficiency to sarcopenia risk in older age. We describe the prospective associations of vitamin D status with 3-year changes in muscle mass, strength, and physical performance, and explore whether low baseline vitamin D levels are linked to a higher risk for developing incident sarcopenia, thereby considering competing risks such as losses to follow-up due to death. We also consider the potential mediating role of parathyroid hormone (PTH) in the relationship between vitamin D status and sarcopenia.

Publication 2: Vitamin D in Relation to Incident Sarcopenia and Changes in Muscle Parameters Among Older Adults: The KORA-Age Study. Conzade, R.; Grill, E.; Bischoff-Ferrari, H.A.; Ferrari, U.; Horsch, A.; Koenig, W.; Peters, A.; Thorand, B. *Calcif Tissue Int* 2019, 105.

In the third publication, we look at 6-month changes in overall nutritional status among older outpatients with a history of falling. We describe prospective associations of changes in nutritional status with changes in muscle strength and physical performance over six months and discuss how improvement in nutritional status may influence muscle/functional recovery after a fall. Lastly, we put our results in the broader context of patient-centered or personalized care for older adults.

Publication 3: Changes in Nutritional Status and Musculoskeletal Health in a Geriatric Post-Fall Care Plan Setting. Conzade, R.; Phu, S.; Vogrin, S.; Bani Hassan, E.; Sepúlveda-Loyola, W.; Thorand, B.; Duque, G. *Nutrients* 2019, 11.

3. A brief overview of methods

3.1. Study design and populations

For the present thesis, data were derived from two sources: the KORA-Age Augsburg study (KORA: Cooperative Health Research in the Region of Augsburg) in Germany, and a clinical study at a falls and fractures clinic (FFC) in Australia ('Australian FFC study').
The KORA-Age Augsburg study, which started in 2008/2009, is a population-based study of 9,197 older adults aged ≥65 years. All participants had previously been part of the MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease)/KORA health surveys S1–S4, conducted between 1984 and 2001 in the region of Augsburg. Data were collected via self-administered questionnaires and telephone interviews. Publications 1 and 2 of this thesis refer to an age- and sex-stratified random sample of the cohort, of whom 1,079 individuals took part in a baseline physical examination in 2009 (response 53.8%). In 2012, a follow-up physical examination took place, in which 822 men and women from the baseline examination participated (response 76.2%) [36].
The Australian FFC study consists of older adults aged ≥65 years who were assessed at the outpatient Falls and Fractures Clinic at Western Health-Sunshine Hospital in St Albans, Australia, after a history of falling in the previous year. Data were collected as part of standard care at this health service. Publication 3 of this thesis refers to a sample of 106 outpatients who had data from attendance at baseline (between October 2016 and December 2018) and from attendance at follow-up six months later.

3.2. Assessment of nutritional status

To evaluate nutritional status, we first zoomed in into single micronutrients using serum nutritional biomarkers in the KORA-Age Augsburg study, and then zoomed out into overall nutritional status using a nutrition screening and assessment tool in the Australian FFC study.

Micronutrient status: Nutritional biomarkers included concentrations biomarkers that reflect micronutrient status, namely serum 25-hydroxyvitamin D (25OHD), folate, vitamin B_{12}, and iron. The selection of cut-offs to classify subclinical micronutrient deficiency was informed by the literature (25OHD: <50 nmol/L [37]; folate: <13.6 nmol/L [38]; vitamin B_{12}: <221 pmol/L [39]; iron: men <11.6 μmol/L, women <9.0 μmol/L [40]). Compared to *clinical* deficiency, *subclinical* deficiency has no recognizable clinical signs and symptoms [41]. Concentration biomarkers were only measured at baseline.

Overall nutritional status: The Mini Nutritional Assessment (MNA®) is a validated screening and assessment tool for older adults in every healthcare setting. The MNA consists of 18 items capturing anthropometric measures, dietary intake, appetite, general health, and mobility. The final MNA score allows grading the overall nutritional status according to clearly defined thresholds: MNA score ≥24/30: "well-nourished"; 17≤ MNA score <24: "at risk of malnutrition"; MNA score <17: "malnourished" [42]. Repeated measurements of the MNA were available.

Furthermore, we also included data on anthropometry measurements (body weight, body mass index (BMI)) and dietary supplement use (mode, dosage, frequency).

3.3. Assessment of muscle health

Muscle mass: In the KORA-Age Augsburg study, body composition was assessed using bioelectrical impedance analysis (BIA). The prediction equation by Janssen et al. was then applied to estimate skeletal muscle mass [43]. The skeletal muscle mass index (SMI) was calculated as skeletal muscle mass/height^2. We used population-specific cut-offs that were previously published in KORA-Age to define low SMI: ≤8.72 kg/m^2 in men and ≤6.33 kg/m^2 in women [44]. In the Australian FFC study, body composition was assessed using dual-energy X-ray absorptiometry (DXA), which then automatically calculated height-adjusted appendicular lean mass* (ALM/height^2). We used cut-offs proposed by the European Working Group on Sarcopenia in Older People (EWGSOP) to define low ALM/height^2: ≤7.26 kg/m^2 in men and ≤5.5 kg/m^2 in women [45]. BIA and DXA rely on different technologies and assess slightly different aspects of muscle mass [46].

Muscle strength: Handgrip strength was assessed with a hydraulic handheld dynamometer. We applied EWGSOP cut-offs for low grip strength: <30 kg in men and <20 kg in women [45], in the KORA-Age Augsburg study and the Australian FFC study.

Physical performance: Usual gait speed was assessed with an electronic walkway on a 4.88-m distance, using the EWGSOP cut-off of ≤0.8 m/sec to define low physical performance [45]. The Timed Up and Go (TUG) test measured the time taken to stand up from a standard chair, walk a distance of 3 m, turn, walk back to the chair and sit down. We defined the cut-point of ≥13.74 sec in the KORA-Age Augsburg study [47], and used a previously published cut-off of ≥13.5 sec in the Australian FFC study [48]. The Short Physical Performance Battery (SPPB), a group of measures that combines results of standing balance tests, gait speed and repeated chair stands tests, was also assessed in the Australian FFC study, and the EWGSOP cut-off of SPPB score ≤8 points was used [45].

* Appendicular lean mass is the portion of skeletal muscle mass contained in the limbs, and represents about 75% of total skeletal muscle mass [46].

3.4. Sarcopenia definition

Sarcopenia status was determined using the diagnostic algorithm proposed in 2010 by the EWGSOP, namely low muscle mass combined with low muscle function (low muscle strength or low physical performance) [45].

3.5. Statistical methods

Publication 1 has a cross-sectional design, Publication 2 combines both cross-sectional and longitudinal analyses, and Publication 3 has a longitudinal design.
In all three publications, data exploration and visualization techniques were performed to calculate descriptive statistics and generate plots using means and standard deviations for continuous data and median and interquartile range for data that deviated from normal distribution. Categorical data were described using frequencies and percentages.
For the main analyses, multiple linear or logistic regression analyses were performed, depending on whether the dependent variable was continuous or categorical, respectively. All analyses were adjusted for relevant confounders identified in the literature or through directed acyclic graphs (DAGs). Statistical analyses were performed using SAS version 9.4. Results were considered statistically significant with a two-sided $p < 0.05$.

3.6. Author contributions

The three publications included in this thesis are published in either *Nutrients* or *Calcified Tissue International*, which are both international peer-reviewed scientific journals. *Nutrients* belongs to the top 20% of journals in the Category Nutrients & Dietetics according to Journal Citation Reports® 2018, whereas *Calcified Tissue International* belongs to the top 50% of journals in the Category Endocrinology & Metabolism.
In all three publications, I am the first author and was significantly involved in developing the research questions under the supervision of my doctoral supervisor, Prof. Barbara Thorand (Publications 1 and 2), or my co-supervisor in Australia, Prof. Gustavo Duque (Publication 3). I performed all statistical analyses and interpreted the results together with my supervisors and the members of my Thesis Advisory Committee. Moreover, I have written all manuscript drafts, incorporated co-authors' comments, finalized the manuscripts based on reviewers' comments, and coordinated the communication between co-authors and editors from each journal.
Specifically for the KORA-Age Augsburg study, I was involved in quality control of biomarker measurements, thereby working closely with the KORA study center in Augsburg and external laboratory partners. For the Australian FFC study, I wrote the ethics protocols for my study (Publication 3) and coordinated the ethics approval process under the supervision of Prof. Gustavo Duque.

4. Key findings

The overall purpose of this thesis was to study the nutritional status of older adults and unravel its associations with sarcopenia and changes in related muscle outcomes, including muscle mass, muscle strength, and physical performance. Nutritional factors of main interest were serum biomarkers of micronutrient status and overall nutritional status based on the MNA score.

The starting point of this research was to obtain a better understanding of the magnitude of poor nutritional among older adults, with a specific focus on micronutrients. In my first publication, we therefore estimated the prevalence of subclinical vitamin D, folate, vitamin B_{12}, and iron deficiencies among 1,079 older adults aged ≥65 years from the KORA-Age Augsburg study, and examined associated determinants.
We found that the prevalence of subclinical vitamin D and vitamin B_{12} deficiencies among KORA-Age individuals were high, with 52.0% and 27.3% of older adults having low 25OHD (<50 nmol/L) and low vitamin B_{12} concentrations (<221 pmol/L), respectively. Moreover, 11.0% had low iron (men <11.6 μmol/L, women <9.0 μmol/L) and 8.7% had low folate levels (<13.6 nmol/L). We identified four common determinants that were associated with subclinical micronutrient deficiency: age 85 years and above (ORs*: from 2.0 to 2.3, 95% CIs: from 1.2 to 4.3); physical inactivity (ORs: from 1.4 to 2.0, 95% CIs: from 1.0 to 3.4); frailty (ORs: from 1.6 to 4.2, 95% CIs: from 1.0 to 10.2); and no/irregular use of supplements containing the specific micronutrients examined (ORs: from 3.9 to 4.8, 95% CIs: from 1.4 to 16.1).

Key finding 1: There is a high prevalence of vitamin D and vitamin B_{12} deficiencies among older adults.

In view of the high prevalence of suboptimal vitamin D levels among KORA-Age older adults, the aim of my second publication was to clarify whether low baseline vitamin D levels (<50 nmol/L) were associated with 3-year changes in muscle mass, muscle strength, and physical performance, and ultimately, with the incidence of sarcopenia. The role of PTH therein was also investigated. We studied 702 older adults from the KORA-Age Augsburg study with data at 3-year follow-up.

* OR: odds ratio; CI: confidence interval.

We found that baseline 25OHD levels <25 vs. ≥50 nmol/L were associated with a 0.9% (95% CI: 0.3, 1.6) greater annual decrease in muscle mass and a 3.1% (95% CI: 0.6, 5.5) greater annual increase in time for the TUG test. Vitamin D status was not associated with changes in grip strength or gait speed. Low baseline vitamin D levels were not associated with the risk for developing incident sarcopenia, but a significant association was found with the combined endpoint of incident sarcopenia and death (OR: 3.19, 95% CI: 1.54, 6.57 for 25OHD <25 vs. ≥50 nmol/L). There was no evidence for a PTH-mediating effect.

Key finding 2: Low vitamin D status was associated with unfavorable changes in muscle mass and physical performance in older adults over three years, but not with incident sarcopenia.

Building on the findings that low vitamin D levels and more generally a poor nutritional status may be early risk factors for unfavorable muscle changes, we hypothesized that replenishing nutritional status may help to enhance muscle health in older age. In my third publication, we studied changes in nutritional status among 106 older outpatients aged ≥65 years from the Australian FFC study, who were provided with post-fall care plans as part of standard care. We tested whether changes in nutritional status over six months were associated with changes in muscle strength and physical performance.
Over six months, the prevalence of malnutrition or risk thereof decreased from 29% to 15% in older outpatients from the Australian FFC study, based on an MNA score <24/30. Specifically, 20 individuals (19%) improved, 7 (7%) deteriorated, and 73 (69%) maintained their nutritional status. A 1-point increase in MNA score over six months, indicating improvement in nutritional status, was associated with improvement in physical performance based on an increase of 0.2 points (95% CI: 0.1, 0.3) in the SPPB score.

Key finding 3: Improvement in overall nutritional status over six months was associated with improvement in physical performance among older adults with a history of falling.

5. Discussion

This work confirms the importance of maintaining nutritional adequacy in older age to ensure sufficient supply of essential nutrients and to preserve muscle mass and muscle function. It also builds on previous findings in the field of sarcopenia by thoroughly investigating the effects of low vitamin D status on the development of incident sarcopenia and on changes in related muscle components.
The findings suggest that the nutritional and more specifically the micronutrient status of older adults is suboptimal. The observed high prevalence of subclinical vitamin D and vitamin B_{12} deficiencies, defined by 25OHD levels <50 nmol/L and vitamin B_{12} levels <221 nmol/L, is a matter of concern. Low vitamin D status appears to be an early risk factor for loss in muscle mass and muscle function in older adults. The hypothesized association of low baseline vitamin D status with the incidence of sarcopenia could not be demonstrated, but a significant association persisted with the combined endpoint of incident sarcopenia and death. A particularly interesting and promising final result of this work is that an improvement in overall nutritional status seems to improve muscle function such as physical performance among older adults with a history of falling.
Results of the specific analyses included in this thesis have been discussed in the corresponding publications. This section addresses public health implications, reviews general methodological issues and provides recommendations for future research.

5.1. Public health implications

Overall, this thesis underpins the importance from a public health perspective of maintaining an adequate nutritional status in older age to secure a sufficient supply of nutrients and to preserve muscle health.

5.1.1. Ensuring an adequate nutritional status for older adults

The high prevalence of micronutrient deficiencies such as vitamin D and B_{12} observed among older adults from this thesis, but also from many other studies among older populations [39,49], highlights the immediate need to ensure that older adults are effectively supported to have sufficient dietary intakes and an adequate nutritional status.
Essential supporting strategies are routine screening of nutritional status and early diagnosis of malnutrition, both in the community and other healthcare settings. However, these strategies seem not always to be prioritized. In a postal survey among German geriatric hospital departments, only 40% of geriatric wards reported performing a standardized screening for malnutrition using a validated screening tool [35]. This finding highlights that malnutrition may often remain undiagnosed and hence, untreated. It appears that in many health services across Europe, neither routine screening for malnutrition nor standards of treatment exist [35].

However, recent aligned efforts from major global clinical nutrition societies will enhance the diffusion and implementation of malnutrition awareness in clinical practice. In 2018, the first evidence-based universal definition of malnutrition for adults in all healthcare settings was published by the Global Leadership Initiative on Malnutrition (GLIM) [50], followed in 2019 by a new ESPEN guideline for clinical nutrition and hydration, which offers 82 evidence-based recommendations to treat malnutrition in older adults [51].
For vitamin D we observed the opposite trend. Vitamin D screening and prescriptions have dramatically increased over the past decade. According to a recent pooled analysis of 6 countries, population-wide vitamin D screening has become commonplace, instead of targeted testing of subgroups at risk of low vitamin D levels, as recommended by authoritative bodies and professional societies [52]. Great variability in clinicians' knowledge and practices related to vitamin D, compounded by inconsistent cut-offs and conflicting recommendations/guidelines [52] calls for more consensus among parties to inform better and more consistent clinical guidelines in the field of vitamin D, including for older adults.

5.1.2. Maintaining an adequate nutritional status for preserving muscle health

This thesis advocates the potential benefits of maintaining an adequate nutritional status such as adequate intake of vitamin D for enhancing relevant sarcopenia-related muscle components, such as muscle mass and muscle function.
The reduced muscle mass and the impaired muscle function that define sarcopenia in older adults are associated with functional decline and physical disability [53], with implications for independence, mobility, and subsequent falls [54,55]. An increased risk of mortality has also been evidenced in individuals with sarcopenia [6]. In our analyses, low vitamin D levels were associated with changes in muscle mass and function, but not with the risk for developing sarcopenia. Nevertheless, a significant association was found with the combined endpoint of incident sarcopenia and death. Future well-designed prospective studies that consider the issue of competing risks such as mortality in older cohorts might be important to clarify the role of vitamin D in the management of sarcopenia.

The association found between overall improvement in nutritional status and improvement in physical performance in older outpatients provides a basis for interventional studies to evaluate nutritional models of care to preserve muscle health in older age. While the management of sarcopenia today focuses on resistance training [56], current research is also looking at the effect of exercise combined with diet. Several studies have demonstrated that physical activity together with nutritional supplements can improve muscle function [57-59]. Large-scale trials are also underway to address the individual or combined effects of nutritional and/or exercise interventions in healthy older adults (e.g. the DO-HEALTH study[*]), or in older adults with sarcopenia (e.g. the SPRINTT trial [60,61]). With the prospect of effective interventions, the assessment and identification of sarcopenia should be considered to prevent adverse health outcomes [62].

5.1.3. Bridging the gaps between research and clinical practice

New definitions of malnutrition and sarcopenia have been released in 2018 and 2019, respectively [50,63]. These updated definitions present an important crossroad for the diagnosis and treatment of both conditions, and an opportunity to bridge gaps between research and clinical practice. In 2015, the WHO launched the *World Report on Ageing and Health* [3], proposing a new framework for healthy ageing (see Introduction of this thesis). This report was followed in 2017 by new ICOPE[†] guidelines by the WHO [64], which provide community-level interventions for health care professionals to manage declines in intrinsic capacity. Mobility loss and malnutrition are specifically addressed.
Next, national public health agencies and health authorities should strengthen their efforts to develop and endorse adequate guidelines and campaigns to increase malnutrition and sarcopenia awareness in the older population. An example is the German campaign "Fit in later life", which was initiated in the framework of the National Action Plan "IN FORM: German national initiative to promote healthy diets and physical activity" in 2008 [65]. The campaign seeks to improve nutritional knowledge and dietary habits, and by extension, to promote health in later life for all older adults. Given global population ageing and the burden of sarcopenia that is associated with growing life expectancies around the world, it is essential to use optimal nutrition in later life as one important dimension of prevention for sarcopenia.

[*] http://do-health.eu/wordpress/

[†] ICOPE: Integrated Care for Older Adults

5.2. Methodological considerations

5.2.1. Study design and populations

The KORA-Age Augsburg study provides deep phenotyping of a wide range of health-related factors with participants being followed over several years. Its prospective design allows to clarify the temporal sequence between exposure and outcome, but its observational nature does not allow to ascertain causality [66]. Since participants had to visit the KORA study center, a selection bias is possible which may be responsible for somewhat higher rates of healthier individuals. The KORA-Age Augsburg study uses a large, broadly representative sample of community-dwelling older adults, which increases the external validity of our results.
The Australian FFC study has the advantages of providing a scientific basis for the development of future prospective or interventional studies, and an opportunity for outpatients to play an active role in their own health care. Moreover, the availability of repeated measurements of the nutritional exposure makes it possible to account for the effects of changes in nutritional status on changes in health-related outcomes. However, the examined study population consists of a rather small sample of older outpatients with a history of falling, who differ from a healthy or otherwise diseased population. Therefore, our findings might not be generalizable to other population subgroups.

5.2.2. Quality of the methodology

A common strength from both study populations relates to the careful and standardized evaluation of nutritional and muscle measurements by qualified medical staff. However, each assessment method has its own strengths and limitations.
Blood nutritional biomarkers can be more objectively and precisely measured than dietary intake using food questionnaires, though they may be affected by hydration status, inflammation, and diurnal variations. Concentration biomarkers do not necessarily reflect the amount of micronutrient intake from foods, but relate to the nutritional status of micronutrients after absorption, and can directly reflect deficiency at the blood level [67].
The use of cut-offs to determine micronutrient deficiency may be difficult [68]. Ideally, a cutoff accurately distinguishes individuals with optimal from those with suboptimal levels. However, in real-life situations, misclassification can occur. Prevalence estimates may be affected, with implications for preventive and therapeutic strategies [68].
The MNA® is the most widely used tool for nutritional screening and assessment for older adults in all healthcare settings due to its ease of use, feasibility, and high sensitivity. Structured in 18 questions grouped in five rubrics, it captures the potential multifactorial origin of malnutrition [69]. Changes in nutritional status upon reassessment are reflected by corresponding changes in MNA score [70], making the MNA useful to assess changes in nutritional status over six months in the Australian FFC study.

A broad spectrum of muscle components was used to assess sarcopenia. A common problem is the difficulty to measure muscle mass accurately. Bioelectrical impedance analysis (BIA) is inexpensive, quick, and easy to use; however, it is affected by hydration status, the device, and the equations used to predict muscle mass [46]. Dual-energy X-ray absorptiometry (DXA) is currently the most widely used method; however, it is relatively expensive, not portable, and can overestimate or underestimate muscle mass [46]. New promising techniques for the assessment of muscle mass are emerging, including one based on the dilution of oral D_3-creatine [71]. Muscle strength and physical performance measures are limited by pain, including arthritis or neurological disorders.
Of important note, the EWGSOP sarcopenia definition has been updated as EWGSOP2 in 2019, after the conception of the three publications comprising this thesis. The EWGSOP2 proposes a new diagnosis approach for sarcopenia, in which low muscle strength comes to the forefront as primary indicator of probable sarcopenia, rather than muscle mass [63]. These updated EWGSOP2 recommendations, which include clear cut-off points for measurements of variables, are expected to increase consistency of research design in the field of sarcopenia and facilitate early identification of sarcopenia in clinical practice [63].
Last, but not least, we have been able to include in our analyses a wide range of relevant confounders, thereby minimizing confounding in examining potential causal associations of the nutritional and the vitamin D status with sarcopenia and muscle-related outcomes. However, imperfectly measured or unmeasured confounders cannot be ruled out, and this can result in residual confounding.

5.3. Future research

Overall, our findings indicate the potential benefits of healthier dietary habits leading to adequate micro- and macronutrient intake to preserve muscle mass and function, with implications for sarcopenia prevention and treatment.

5.3.1. Nutritional interventions in sarcopenia

Evidence gathered throughout this thesis is observational and from two high-income countries (Germany and Australia). Hence, further high-quality randomized controlled trials in more diverse populations are needed to ascertain causality and better understand dose and duration effects of specific nutritional interventions on relevant muscle outcomes. Further mechanistic studies that explain mechanistic links are also required.
The advent of patient-centered or personalized care has increased attention to the fact that different settings and populations can require different management and treatment approaches for sarcopenia [72]. Hence, future work should also consider the role of tailored interventions in specific settings (e.g. in the community vs. in hospitals) and subgroups of the older population. Potential relevant subgroups are older adults who have specific phenotypic characteristics (e.g. falls), or who differ in either their usual diets (e.g. low protein diet) or in their nutritional status (e.g. low 25 OHD levels).

Moreover, nutritional research into sarcopenia needs to expand outside of protein and vitamin D research. Antioxidant nutrients and long-chain polyunsaturated fatty acids deserve research attention, as do specific foods (including dairy and nitrate-rich foods) and dietary patterns [14]. Promising novel nutritional interventions in sarcopenia were presented at the 2019 International Conference on Frailty and Sarcopenia Research (ICFSR), which I have attended with great interest. For example, omega-3 polyunsaturated fatty acid (fish oil) supplements with resistance training have shown interesting results in women with sarcopenia [73].

5.3.2. Life-course approach to sarcopenia

One novelty of the updated EWGSOP2 definition for sarcopenia is to highlight the potential role of sarcopenia in earlier stages of life [63]. The hypothesis is that muscle mass and strength achieved in later life are not only determined by the rate of skeletal muscle loss and the factors that influence this (e.g., diet, lifestyle) but also by the peak attained earlier in young adulthood (Figure 1 of this thesis). Future research into the prevention of sarcopenia needs to explore the potential effectiveness of nutritional interventions earlier in the life course [63].

This life-course approach to sarcopenia suggests that studies such as birth cohorts with longer follow-up and repeated measurements would be needed to examine associations between early life nutritional factors and changes in muscle outcomes over the life course. These follow-up studies would enable us to investigate to what extent nutritional factors impact later adulthood and how they affect the risk of developing sarcopenia in later life.

6. Conclusions

To develop strategies that prevent or delay sarcopenia, a better understanding of lifestyle behaviors that influence age-related changes in muscle mass and function is needed.
Findings from this thesis indicate the importance of maintaining nutritional adequacy in older age, to ensure sufficient supply of essential nutrients such as vitamin D, and to enhance muscle health. Vitamin D levels ≥50 nmol/L and an overall adequate nutritional status seem to be beneficial for relevant sarcopenia-related muscle components, including muscle mass and muscle function. Although this evidence is observational and the mechanisms are not fully understood, the high prevalence of subclinical vitamin D deficiency observed among older adults makes this a current concern.
Optimizing nutrition and ensuring an adequate nutritional status for older adults may be key to preventing or delaying sarcopenia, enabling mobility and independence into older age. Further randomized trials are needed to assess whether the observed associations are causal and to determine optimal nutrient levels for muscle health. My hope is that upcoming nutritional treatment options will lower the burden of both malnutrition and sarcopenia for older adults.

II. Publications

1. Publication: Prevalence and Predictors of Subclinical Micronutrient Deficiency in German Older Adults

Title:	Prevalence and Predictors of Subclinical Micronutrient Deficiency in German Older Adults: Results from the Population-Based KORA-Age Study
Authors:	Romy Conzade, Wolfgang Koenig, Margit Heier, Andrea Schneider, Eva Grill, Annette Peters and Barbara Thorand
Journal:	*Nutrients*
Year:	2017
Volume:	9
DOI:	10.3390/nu9121276
Supplements:	www.mdpi.com/2072-6643/9/12/1276/s1 (available online)
Impact factor:	4.171 (Journal Citation Reports® 2018)
Rank:	16/86 in Category Nutrition & Dietetics (Journal Citation Reports® 2018)

nutrients

MDPI

Article

Prevalence and Predictors of Subclinical Micronutrient Deficiency in German Older Adults: Results from the Population-Based KORA-Age Study

Romy Conzade [1], Wolfgang Koenig [2,3,4], Margit Heier [1,5], Andrea Schneider [1], Eva Grill [6], Annette Peters [1] and Barbara Thorand [1,*]

1 Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Epidemiology II, Ingolstädter Landtraße 1, 85764 Neuherberg, Germany; romy.conzade@helmholtz-muenchen.de (R.C.); heier@helmholtz-muenchen.de (M.H.); shneider@helmholtz-muenchen.de (A.S.); peters@helmholtz-muenchen.de (A.P.)

2 German Heart Centre Munich, Technical University of Munich, Lazarettstraße 36, 80636 Munich, Germany; koenig@dhm.mhn.de or wolfgang.koenig@uniklinik-ulm.de

3 DZHK (German Centre for Cardiovascular Research), Partner Site Munich Heart Alliance, 80802 Munich, Germany

4 Ulm Medical Center, Department of Internal Medicine II—Cardiology, University Hospital Ulm, Albert-Einstein-Allee 23, 89081 Ulm, Germany

5 Central Hospital of Augsburg, MONICA/KORA Myocardial Infarction Registry, Stenglinstraße 2, 86156 Augsburg, Germany

6 Institute for Medical Information Processing, Biometrics and Epidemiology, Ludwig-Maximilians-University Munich, Marchioninistraße 15, 81377 Munich, Germany; eva.grill@med.uni-muenchen.de

* Correspondence: thorand@helmholtz-muenchen.de; Tel.: +49-89-3187-4480

Received: 11 October 2017; Accepted: 20 November 2017; Published: 23 November 2017

Abstract: Subclinical micronutrient deficiency in older adults is associated with chronic age-related diseases and adverse functional outcomes. In Germany, the older population is at risk of insufficient micronutrient intake, but representative studies on micronutrient status in old and very old adults are scarce. This study's objectives were to estimate the prevalence of subclinical vitamin D, folate, vitamin B_{12} and iron deficiencies among older adults, aged 65 to 93, from the KORA-Age study in Augsburg, Germany (n = 1079), and to examine associated predictors, using multiple logistic regression. Serum concentrations of 25-hydroxyvitamin D (25OHD), folate, vitamin B_{12}, and iron were analyzed. The prevalence of subclinical vitamin D and vitamin B_{12} deficiencies were high, with 52.0% and 27.3% of individuals having low 25OHD (<50 nmol/L) and low vitamin B_{12} concentrations (<221 pmol/L), respectively. Furthermore, 11.0% had low iron (men <11.6 µmol/L, women <9.0 µmol/L) and 8.7% had low folate levels (<13.6 nmol/L). Common predictors associated with subclinical micronutrient deficiency included very old age, physical inactivity, frailty and no/irregular use of supplements. Subclinical micronutrient deficiency is a public health concern among KORA-Age participants, especially for vitamins D and B_{12}. The predictors identified provide further rationale for screening high-risk subgroups and developing targeted public health interventions to tackle prevailing micronutrient inadequacies among older adults.

Keywords: subclinical micronutrient deficiency; vitamin D; folate; vitamin B_{12}; iron; predictors; older adults; population-based study; Germany

1. Introduction

Deficiencies in essential vitamins, minerals and trace elements (micronutrients) affect an estimated 2 billion people, in both developing and developed countries. This 'hidden hunger' has negative health impacts among vulnerable groups in the population, especially older adults [1].

Epidemiological evidence suggests that subclinical micronutrient deficiencies in older adults are associated with chronic age-related diseases and adverse functional outcomes. Older adults with low 25-hydroxyvitamin D (25OHD) levels have a significantly higher risk for type 2 diabetes mellitus, cardiovascular disease (CVD) and osteoporosis-related fractures [2]. Age-associated declines in muscle mass and strength (sarcopenia), which in turn affect balance, gait, and overall independence [3], have been linked to low 25OHD levels as well [4]. Vitamin B_{12} and folate are necessary for one-carbon metabolism and DNA synthesis, and have been investigated in relation to degenerative diseases, including CVD, cognitive dysfunction and osteoporosis [5]. Iron deficiency is the most prevalent nutritional deficiency worldwide and its main consequence is anemia [6], which has been associated with an increased risk of developing dementia in old age [7].

Ageing is associated with physiological changes that can impact the nutritional and more specifically the micronutrient status of older adults. Importantly, energy requirements decrease due to a loss of lean body mass and a reduction in physical activity, resulting in decreasing energy intakes. At a low energy intake, older adults may be at increased risk for specific micronutrient deficiencies, unless their diets contain micronutrient-rich foods. Moreover, older adults' abilities to absorb and utilize specific micronutrients become less efficient (e.g., reduced gastric acid secretion preventing the release of vitamin B_{12} from foods [5]; decline in subcutaneous vitamin D synthesis capacity and lowered renal conversion to its active form [8]). Last, but not least, the likelihood of multiple chronic diseases, such as cardiovascular disease, type 2 diabetes mellitus and cancer, use of multiple medications and variable diet quality, associated with appetite loss, chewing/swallowing difficulties and socioeconomic barriers, may affect and even increase requirements for specific micronutrients [9,10]. All these factors make it difficult to ensure an optimal micronutrient supply for the older population.

Due to the role of micronutrient status in chronic disease and health prevention, it is important to quantify the magnitude of subclinical micronutrient deficiency and to identify subgroups at risk in the older population. Results from the second German National Nutrition Survey (NVS II) uncovered a high prevalence of insufficient dietary intake of vitamin D, folic acid and calcium in older adults aged 65 years and over. Vitamins B_1, B_2, B_{12} and iron were additional critical micronutrients in older women [11]. More recently, the national 'German Health Interview and Examination Survey for Adults' (DEGS1) revealed that 69.9% of women and 62.6% of men, aged 65 to 79, had low 25OHD <50 nmol/L [12], signaling a potentially serious public health issue. Nationally representative studies on the statuses of other micronutrients, in old and very old adults, in Germany, are scarce.

To support health care in developing purposive prevention measures that handle micronutrient inadequacies among older adults, factors associated with subclinical micronutrient deficiencies in older adults have to be identified. Besides insufficient dietary intake and impaired absorption, low micronutrient levels in older adults have been studied in relation to increasing age, female sex, poor eating habits, low physical activity, smoking, obesity, low kidney function, presence of chronic diseases, intake of drugs, non-use of supplements and, specifically for vitamin D, low sunlight exposure and winter season [13–18]. Despite recent concerns about the possible high prevalence of subclinical micronutrient deficiencies in the German older population, and apart from vitamin D [12,18], there is a lack of data on both micronutrient status and its predictors in older adults.

In light of the growing ageing population, as well as the high societal relevance of successful healthy ageing, this cross-sectional study aimed to provide insight into the magnitude of subclinical micronutrient deficiency in German older adults, aged 65 and over, from the population-based KORA (Cooperative Health Research in the Region of Augsburg)-Age study. Based on the micronutrients identified in the NVS II as possible public health concerns in the German older population, we selected four critical micronutrients with available nutritional biomarkers in the KORA-Age dataset.

Specific objectives were (i) to determine the prevalence of subclinical vitamin D, folic acid, vitamin B_{12} and iron deficiencies in this older population, using serum biochemical biomarkers; and (ii) to identify predictors of subclinical micronutrient deficiency using multiple logistic regression. Possible predictors examined included socio-demographic, lifestyle, and health factors, as well as the use of supplements containing specific micronutrients.

2. Materials and Methods

2.1. Study Design and Participants

Data for the present analysis was obtained from the population-based KORA-Age study, conducted in 2008/2009, which was a follow-up of all participants, aged 65 and over on 31 December 2008 (n = 9197), who took part in at least one of four cross-sectional MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease)/KORA health surveys S1–S4 conducted between 1984 and 2001, among the inhabitants of Augsburg and surrounding counties. Details about the general study design and participants have been described previously [19]. Briefly, a self-administered health questionnaire was mailed to all eligible participants of the KORA-Age cohort, i.e., those who were still alive and reachable in 2008/2009 (n = 5991). The response rate was 76.2% (n = 4565). Moreover, 68.9% of eligible participants took part in a standardized telephone interview (n = 4127). The present analysis refers to a sex and age-stratified random sample of n = 2005 eligible individuals of whom n = 1079 (537 men, 542 women) participated in an extensive physical examination in 2009 (response rate 53.8%). All examinations were performed by trained interviewers. A flowchart of the KORA-Age 2008/2009 recruitment and retention profile is shown in Supplementary Figure S1.

2.2. Ethical Considerations

Prior to their inclusion in the study, written informed consent was obtained from all participants or from the patient's caregiver when the participant was unable to make an informed decision. The Ethics Committee of the Bavarian Medical Association (Bayerische Landesärtzekammer) approved the study protocol (date of approval: 11 November 2008, reference number: 08064).

2.3. Blood Sample Processing

Non-fasting blood samples were collected between February and November 2009 at the KORA study center and drawn into serum gel S-Monovette tubes (Sarstedt, Nümbrecht, Germany). Blood was gently inverted twice and rested for 30 min at room temperature until complete coagulation. After centrifugation at 15 °C for 10 min, the serum obtained was aliquoted into Nunc cryotubes (Thermo Fisher Scientific, Waltham, MA, USA). For the analysis of iron status, serum probes were kept at 4 °C for a maximum of 6 h and directly analyzed at the central laboratory of Augsburg Hospital. For vitamin D, folic acid and vitamin B_{12} status, serum probes were frozen at −80 °C at the KORA study center, transported on ice and stored at a minimum of −80 °C until analysis, in partner laboratories, between August and September 2011. Months of blood collection were categorized according to calendar seasons: spring (February–May), summer (June–August) and autumn (September–November).

2.4. Biochemical Analyses of Nutritional Biomarkers

Serum concentrations of 25-hydroxyvitamin D (25OHD), folate and cobalamin (vitamin B_{12}) were measured by an electrochemiluminescence immunoassay (ECLIA, Elecsys 2010, Roche Diagnostics GmbH, Mannheim, Germany). The intra- and inter-assay coefficients of variations were 4.9% and <10% for 25OHD, 7.0% and <10% for folate and 5.3% and <10% for vitamin B_{12}. Iron levels were measured by photometric measurements, using the chromophore Ferene® (Dimension® Iron Flex® reagent cartridge, Dade Behring, Inc., Newark, DE, USA). The inter-assay coefficient of variation was <10%, and the maximal permissible imprecision and inaccuracy were 4% and 6%, respectively.

2.5. Cut-Off Points to Classify Subclinical Micronutrient Deficiency

Exact cut-off points for classifying subclinical micronutrient deficiencies remain debated. Recently, a serum 25OHD level of ≥50 nmol/L was recommended as an indicator of optimal vitamin D status by the critical review of the German Nutrition Society (DGE) for DACH countries (Germany, Austria and Switzerland) [20] as well as the last Nordic Nutrition Recommendations (NNR 2012) [21]. Accordingly, subclinical vitamin D deficiency was defined as a serum 25OHD level of <50 nmol/L. Subclinical folate and vitamin B_{12} deficiencies were defined as serum folate <13.6 nmol/L [22] and serum vitamin B_{12} <221 pmol/L [23], respectively. For serum iron, cut-offs were <11.6 µmol/L for men and <9.0 µmol/L for women [24].

2.6. Assesment of Predictors

The selection of potential predictors of subclinical micronutrient deficiencies was informed by the literature and their availability in the KORA-Age dataset. Variables were grouped into three categories (socio-demographic, lifestyle, health factors) plus the season of blood collection for vitamin D. Assessment methods and categorization of variables are described in Supplementary Table S1.

Briefly, the variables—sex, age groups and family status—were collected using the short form of the Demographics Standards of the German Society of Epidemiology [25]. Educational attainment was estimated by recording years of school completed. With the Geriatric Nutritional Risk Index (GNRI), the risk for malnutrition was assessed by measuring albumin, weight and height [26]. The Nutrition Score, indicating the risk of general malnutrition, was calculated using the German short form of the SCREEN II (Seniors in the Community: Risk Evaluation for Eating and Nutrition, version II) questionnaire [27]. The physical activity assessment included the frequency and duration of activity in summer and in winter [28]. Alcohol intake was assessed as daily average intake, based on the last weekend and the last weekday, according to a validated recall method [29]. Multi-morbidity was defined as suffering from two or more morbidities [30]. Information on smoking status was based on self-report and participants were classified as never smokers, former smokers, or current smokers [31]. The body mass index (BMI) was defined as the body mass (weight) measured in kilograms divided by the square of the body height measured in meters. Frailty was defined according to the five criteria proposed by Fried et al. [32] in a slightly modified way, depending on the availability of information [33]. The glomerular filtration rate (eGFR) was estimated from serum creatinine as an indicator of renal function [34]. Use of medications and supplements ingested in the last seven days was collected through a database supported computer software (IDOM, Instrument for Databased Assessment Of Medication) [35], together with the mode, dosage and frequency of ingestion [36]. The micronutrient composition of supplements was available from a database established by staff of the Helmholtz Zentrum München. Polypharmacy was defined as the use of ≥5 medications, taken regularly and prescribed (without herbal or homeopathic medications).

2.7. Statistical Analysis

Descriptive statistics for categorical variables were expressed as frequencies and percentages. Due to non-normally distributed concentrations, serum levels of nutritional biomarkers were expressed as median and interquartile range (IQR), from the first (Q1) to the third quartile (Q3). When biomarker levels fell below the cut-offs for subclinical micronutrient deficiency, micronutrient status was categorized as 'subclinical deficiency' for the respective micronutrient. Prevalence estimates of subclinical micronutrient deficiency were reported with weighted percentages, using the Bavarian population per 31 December 2015 for age and sex standardization (www.statistikdaten.bayern.de/genesis/). Pearson's chi-squared tests were conducted to assess sex and age differences in prevalence estimates.

To investigate predictors of subclinical micronutrient deficiencies, we defined subclinical micronutrient deficiency as the dependent variable and predictors as the categorical independent

variables. The category with the 'lowest risk' was considered as reference for each independent variable (e.g., male sex, 65–74 years, never smoker etc.). Participants with missing information in one or more variables were excluded, except for frailty, which had a high number of missing values (n = 84). To avoid deletion of numerous information-rich participants, all participants with missing frailty status were considered as a separate category of the frailty variable. The analysis was performed separately for 25OHD, folate, vitamin B_{12} and iron, according to the following scheme. We started with a binary logistic regression, for each of the potential predictors, by calculating unadjusted odds ratios (ORs) and 95% confidence intervals (CIs). Variables that were significant at $p < 0.25$ were considered for inclusion in the multiple logistic regression model. Sex and age groups were forced into every model. As simultaneous intake of multiple supplements is frequent among older adults, we considered, for each nutritional biomarker, only supplement intake of the corresponding micronutrient. The final model, with variables significant at $p < 0.05$, resulted from a stepwise selection procedure, which is a combination of the forward and backward selection techniques. Model estimates are presented as fully adjusted ORs along with 95% CIs. All analyses were performed using the statistical software package, SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Baseline Characteristics of Study Participants

A description of baseline characteristics is shown in Table 1, stratified by sex. A total of n = 1079 individuals, aged 65 to 93 years, participated in the study, including 542 (50.2%) women. Overall, 47.7% of participants were physically inactive and 30.2% had a BMI $\geq$ 30 kg/m^2, indicating obesity. Current smokers accounted for less than 5% of the population. Only 4.6% of all individuals were frail, but 37.9% were pre-frail. The prevalence of older adults having one or at least two diseases was 24.7% and 66.8%, respectively. Noticeable sex differences were observed for the variables, family status, educational level and alcohol consumption.

Table 1. Baseline characteristics of older adults in KORA-Age 2008/2009, stratified by sex.

Baseline Characteristics	All (n = 1079)		Men (n = 537)		Women (n = 542)	
	n	%	*n*	%	*n*	%
Season of blood collection						
Months of blood collection [a]						
February–May	440	42.3	242	46.2	198	38.4
June–August	360	34.6	173	33	187	36.2
September–November	240	23.1	109	20.8	131	25.4
Socio-demographic factors						
Age groups (years)						
65–74	457	42.4	233	43.4	224	41.3
75–84	486	45	243	45.3	243	44.8
85–93	136	12.6	61	11.4	75	13.8
Family status [b]						
Living with a partner	663	62.1	424	79.3	239	44.9
Living alone, divorced or widowed	404	37.9	111	20.8	293	55.1
Educational level (years)						
Medium to high (10 to 17)	854	79.2	490	91.3	364	67.2
Low (8 to 9)	225	20.9	47	8.8	178	32.8
Lifestyle factors						
Nutritional status						
Geriatric Nutritional Risk Index (GNRI) [c]						
No risk (>98)	960	92.3	482	92.3	478	92.3
Low risk (92 to 98)	59	5.7	29	5.6	30	5.8
Moderate or major risk (<92)	21	2	11	2.1	10	1.9
Nutrition Score (SCREEN II) [d]						
Low risk (41 to 48)	408	38.3	239	44.8	169	31.7
Medium risk (36 to <41)	377	35.4	181	34	196	36.8
High risk (<36)	281	26.4	113	21.2	168	31.5

Table 1. *Cont.*

Baseline Characteristics	All (n = 1079)		Men (n = 537)		Women (n = 542)	
	n	%	n	%	n	%
Physical activity [e]						
Very active or moderately active	564	52.3	304	56.6	260	48.1
Less active or inactive	514	47.7	233	43.4	281	51.9
Alcohol consumption (g/day) [f]						
0	393	36.6	126	23.5	267	49.7
>0 to <20	373	34.8	164	30.6	209	38.9
≥20	307	28.6	246	45.9	61	11.4
Smoking status						
Never smoker	617	57.2	204	38	413	76.2
Former smoker	413	38.3	303	56.4	110	20.3
Current smoker	49	4.5	30	5.6	19	3.5
Health factors						
Body mass index (BMI) (kg/m^2)						
Normal (18.5 to <25)	228	21.1	99	18.4	129	23.8
Overweight (25 to <30)	525	48.7	281	52.3	244	45
Obese (≥30)	326	30.2	157	29.2	169	31.2
Frailty [g]						
Non-frail	572	57.5	290	57.4	282	57.6
Pre-frail	377	37.9	194	38.4	183	37.4
Frail	46	4.6	21	4.2	25	5.1
Polypharmacy (≥5 medications)						
No	712	66	356	66.3	356	65.7
Yes	367	34	181	33.7	186	34.3
eGFR (mL/min/1.73 m^2)						
Normal (≥60)	709	65.7	366	68.2	343	63.3
Low (<60)	370	34.3	171	31.8	199	36.7
Multi-morbidity [h]						
No disease	91	8.5	56	10.5	35	6.6
One disease	263	24.7	138	25.9	125	23.4
Two or more diseases	712	66.8	338	63.5	374	70
Use of supplements						
Vitamin D						
Regular intake	140	13	36	6.7	104	19.2
No/irregular intake	939	87	501	93.3	438	80.8
Folic acid						
Regular intake	116	10.8	51	9.5	65	12
No/irregular intake	963	89.3	486	90.5	477	88
Vitamin B_{12}						
Regular intake	114	10.6	48	8.9	66	12.2
No/irregular intake	965	89.4	489	91.1	476	87.8
Iron						
Regular intake	36	3.3	15	2.8	21	3.9
No/irregular intake	1043	96.7	522	97.2	521	96.1

SCREEN II: Seniors in the Community Risk Evaluation for Eating and Nutrition, version II; eGFR: estimated glomerular filtration rate; number of missing values: [a] 39, [b] 12, [c] 39, [d] 13, [e] 1, [f] 6, [g] 84, [h] 13.

Seniors regularly using supplements containing vitamin D, folic acid, vitamin B_{12} and iron accounted for 6.7%, 9.5%, 8.9% and 2.8% of the male population, and 19.2%, 12.0%, 12.2% and 3.9% of the female population, respectively. In these regular users, the median amounts of vitamin D, folic acid, vitamin B_{12} and iron consumed from these supplements per day were, for men, 6.2 µg (IQR: 3.6–10.0), 400.0 µg (IQR: 200.0–714.3), 3.3 µg (IQR: 1.8–10.0) and 5.0 mg (IQR: 1.7–35.0), and for women 10.0 µg (IQR: 5.0–20.0), 300.0 µg (IQR: 200.0–400.0), 3.0 µg (IQR: 1.5–9.0) and 4.0 mg (IQR: 3.5–5.0), respectively.

Serum concentrations of 25OHD, folate, vitamin B_{12} and iron were measured in n = 1040, n = 1043, n = 1044 and n = 1050 KORA-Age individuals, respectively. Accordingly, a total of n = 39 (25OHD), n = 36 (folate), n = 35 (vitamin B_{12}) and n = 29 individuals (iron) had missing values in serum nutritional biomarker measurements. Using Pearson's chi-squared tests, KORA-Age individuals with missing micronutrient values were more likely to be very old (85–93 years); to live alone, to be divorced or widowed; to be less active or inactive; to be alcohol abstainers; to have a normal BMI

($18.5 \leq$ BMI < 25 kg/m^2) and to have a low kidney function (eGFR < 60 mL/min/1.73 m^2) (data not shown).

3.2. Prevalence of Subclinical Micronutrient Deficiency

Serum concentrations of nutritional biomarkers and total age- and sex-standardized prevalence of subclinical micronutrient deficiencies are presented in Table 2, stratified by sex.

Median concentrations of 25OHD, folate, vitamin B_{12} and iron were 48.3 nmol/L (IQR: 31.4–69.6), 24.5 nmol/L (IQR: 18.2–33.3), 277 pmol/L (IQR: 214–376) and 16.1 µmol/L (IQR: 13.1–20.0), respectively.

More than half of KORA-Age participants (52.0%) had low 25OHD concentrations. In season-specific analyses, the prevalence of a subclinical vitamin D deficiency was 60.9% in spring (February–May), 46.9% in summer (June–August) and 45.4% in fall (September–November). The prevalence of a subclinical vitamin B_{12} deficiency (27.3%) was also high. Approximately 10% of individuals had low levels of iron and folate. In sex-specific analyses, the prevalence of subclinical deficiencies were more common in women for 25OHD (59.0% vs. 44.4%) and folate (9.4% vs. 8.0%) and in men, for vitamin B_{12} (28.5% vs. 26.0%) and iron (13.5% vs. 8.4%). Using Pearson's chi-squared tests, these sex differences in prevalence were significant for 25OHD and iron ($p < 0.001$).

The prevalence of subclinical micronutrient deficiencies, by age groups, is shown in Figure 1. The prevalence increased gradually with age for all micronutrients. Specifically, the proportion of participants with serum 25OHD <50 nmol/L increased from 43.8% in the age group 65–74 years, to 74.2% in the age group 85–93 years. In Pearson's chi-squared tests, divergences between age groups were significant for all biomarkers (25OHD: $p < 0.001$; folate: $p < 0.001$; vitamin B_{12}: $p = 0.008$; iron: $p = 0.001$).

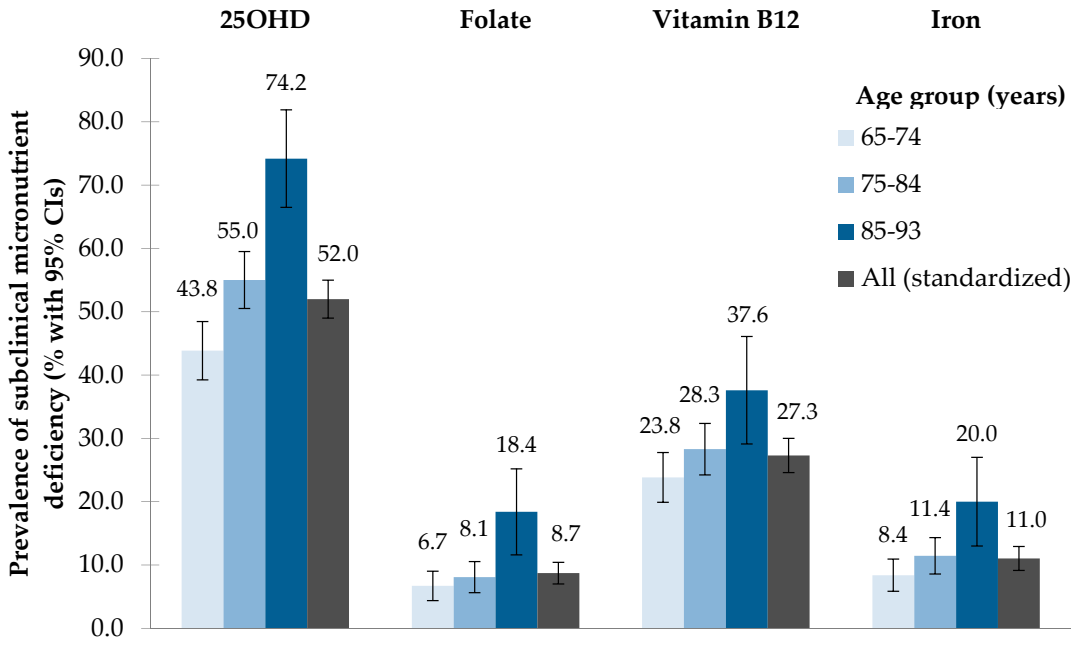

Figure 1. Prevalence of subclinical micronutrient deficiencies by age groups, based on serum biomarkers in KORA-Age 2008/2009 (25OHD = 25-hydroxyvitamin D; CI = confidence interval).

Table 2. Biomarker concentrations and prevalence of subclinical micronutrient deficiencies by sex in KORA-Age 2008/2009.

Nutritional Biomarkers	Biomarker Concentrations (Percentile Scale)										Prevalence of Subclinical Deficiency		
	n	Min	1st	10th	Q1	Median	Q3	90th	99th	Max	%	% (Standardized *)	95% CI
All													
25OHD [nmol/L]	1040	3.7	7.7	21.3	31.4	48.3	69.6	93.1	136.3	174.7	52.5	52.0	49.0–55.0
Folate [nmol/L]	1043	5.8	9.0	14.0	18.2	24.5	33.3	45.3	45.3	90.6	8.7	8.7	7.0–10.4
Vitamin B_{12} [pmol/L]	1044	66	104	173	214	277	376	523	1476	1476	27.5	27.3	24.6–30.0
Iron [µmol/L]	1050	3.4	5.2	10.0	13.1	16.1	20.0	22.8	32.9	40.5	11.1	11.0	9.1–12.9
Men													
25OHD [nmol/L]	524	3.7	3.7	22.5	36.2	51.8	75.0	96.8	145.0	174.7	46.4	44.4	40.2–48.6
Folate [nmol/L]	524	5.8	10.2	14.4	17.9	23.8	31.3	45.3	45.3	63.9	8.2	8.0	5.7–10.3
Vitamin B_{12} [pmol/L]	525	68	104	170	210	263	355	494	1441	1476	29.3	28.5	24.6–32.4
Iron [µmol/L]	527	3.4	5.7	10.7	13.6	16.8	19.9	24.2	33.7	40.5	14.2	13.5	10.6–16.4
Women													
25OHD [nmol/L]	516	3.7	8.7	20.6	27.7	45.3	65.9	86.4	119.8	156.3	58.7	59.0	54.8–63.2
Folate [nmol/L]	519	7.3	8.9	13.8	18.4	25.2	35.4	45.3	45.3	90.6	9.2	9.4	6.9–12.0
Vitamin B_{12} [pmol/L]	519	66	108	175	218	292	392	542	1476	1476	25.6	26.0	22.3–29.3
Iron [µmol/L]	523	3.6	5.0	9.3	12.4	15.6	18.8	22.0	28.6	38.0	8.0	8.4	6.0–10.8

25OHD = 25-hydroxyvitamin D; Q1: first quartile or 25th percentile; Q3: third quartile or 75th percentile; CI: confidence interval; * Ten-year-age-group and sex-standardized prevalence using the Bavarian population per 31/12/2015; cut-offs for subclinical micronutrient deficiency: <50 nmol/L (25OHD); <13.6 nmol/L (folate); <221 pmol/L (vitamin B_{12}); men: <11.6 µmol/L, women: <9.0 µmol/L (iron).

3.3. Predictors of Subclinical Micronutrient Deficiency

As illustrated in Supplementary Tables S2 and S3, 15 variables were tested in a binary logistic regression for the association between subclinical micronutrient deficiencies and each of the potential predictors, plus the season of blood collection for 25OHD. Besides sex and age groups, common potential predictors with *p*-values < 0.25 included educational level, nutritional status (Nutrition Score), physical activity, alcohol consumption, frailty and use of micronutrient-containing supplements.

The final results of the multiple logistic regression analyses are shown in Table 3. Common predictors that were significantly ($p < 0.05$) associated with low levels of several micronutrients were identified. Seniors aged 85 and over had two times higher odds of having low 25OHD (OR = 2.2, 95% CI 1.3–3.8, $p = 0.003$), low folate (OR = 2.3, 95% CI 1.2–4.3, $p = 0.011$) and low vitamin B_{12} levels (OR = 2.0, 95% CI 1.2–3.2, $p = 0.004$) compared to their 65–74-year old counterparts. Physical inactivity remained significantly associated with low 25OHD (OR = 1.6, 95% CI 1.2–2.2, $p = 0.001$), low folate (OR = 2.0, 95% CI 1.2–3.4, $p = 0.006$) and low vitamin B_{12} levels (OR = 1.4, 95% CI 1.0–1.8, $p = 0.042$). Both pre-frailty and frailty were strong independent correlates of subclinical 25OHD and iron deficiency. Frailty increased four-fold, the odds of a subclinical iron deficiency, compared to non-frail individuals (OR = 4.2, 95% CI 1.6–10.2, $p = 0.002$). Moreover, non/irregular users of supplements, containing specific micronutrients, had four to five times higher odds of having low 25OHD (OR = 4.8, 95% CI 3.1–7.6, $p < 0.001$), low folate (OR = 3.9, 95% CI 1.4–16.1, $p = 0.024$) and low vitamin B_{12} levels (OR = 4.7, 95% CI 2.5–10.2, $p < 0.001$), compared to regular users.

A few predictors remained independently associated with low levels of specific micronutrients only (Table 3). Springtime, female sex and obesity were significantly associated with a subclinical 25OHD deficiency. Former smokers had lower odds of having low 25OHD levels, compared to non-smokers. Polypharmacy was associated with better vitamin B_{12} levels. Regarding folate, drinkers of $\geq$20 g alcohol per day had lower odds of having low folate levels than non-drinkers. Finally, having a moderate/major and even low risk for malnutrition (as assessed by the GNRI), male sex and polypharmacy were associated with an increased risk for low iron levels.

Table 3. Fully adjusted ORs with 95% CIs for subclinical micronutrient deficiencies by categories of identified predictors: Final results from multiple logistic regression analyses in KORA-Age 2008/2009.

Predictor	Predictor Categories	Low 25OHD (n = 525)			Low Folate (n = 86)			Low Vitamin B_{12} (n = 283)			Low Iron (n = 106)		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Season of blood collection													
Months of blood collection	February–May vs. June–August	2.1	1.5–2.8	<0.001	.	.	.	.	.	.	.	.	.
Months of blood collection	September–November vs. June–August	0.8	0.5–1.1	0.135	.	.	.	.	.	.	.	.	.
Socio-demographic factors													
Sex	Women vs. men	1.9	1.4–2.5	<0.001	0.8	0.5–1.4	0.459	0.8	0.6–1.1	0.231	0.4	0.3–0.7	<0.001
Age groups (years)	75–84 vs. 65–74	1.3	1.0–1.8	0.058	0.9	0.6–1.6	0.818	1.3	0.9–1.8	0.110	1.0	0.6–1.7	0.911
Age groups (years)	85–93 vs. 65–74	2.2	1.3–3.8	0.003	2.3	1.2–4.3	0.011	2.0	1.2–3.2	0.004	1.2	0.6–2.3	0.564
Lifestyle factors													
Nutritional status	GNRI: Low (92 to 98) vs. no risk (>98)	-	-	-	-	-	-	-	-	-	2.7	1.3–5.4	0.005
Nutritional status	GNRI: Moderate/major (<92) vs. no risk (>98)	-	-	-	-	-	-	-	-	-	4.0	1.2–12.0	0.015
Physical activity	Less active or inactive vs. very active or moderately active	1.6	1.2–2.2	0.001	2.0	1.2–3.4	0.006	1.4	1.0–1.8	0.042	-	-	-
Alcohol consumption (g/day)	>0 to <20 vs. 0	-	-	-	1.0	0.6–1.6	0.876	-	-	-	-	-	-
Alcohol consumption (g/day)	≥20 vs. 0	-	-	-	0.4	0.2–0.8	0.017	-	-	-	-	-	-
Smoking status	Current smoker vs. never smoker	0.8	0.4–1.5	0.516	-	-	-	-	-	-	-	-	-
Smoking status	Former smoker vs. never smoker	0.6	0.4–0.8	0.002	-	-	-	-	-	-	-	-	-
Health factors													
BMI (kg/m^2)	Overweight (25 to <30) vs. normal (18.5 to <25)	0.9	0.7–1.4	0.763	-	-	-	-	-	-	-	-	-
BMI (kg/m^2)	Obese (≥30) vs. normal (18.5 to <25)	1.8	1.2–2.6	0.005	-	-	-	-	-	-	-	-	-
Frailty	Missing value vs. non-frail	1.1	0.6–2.2	0.736	-	-	-	-	-	-	3.4	1.3–8.0	0.008
Frailty	Pre-frail vs. non-frail	1.8	1.3–2.5	<0.001	-	-	-	-	-	-	2.7	1.7–4.5	<0.001
Frailty	Frail vs. non-frail	2.5	1.2–5.4	0.022	-	-	-	-	-	-	4.2	1.6–10.2	0.002
Polypharmacy	Yes vs. no	-	-	-	-	-	-	0.5	0.4–0.7	<0.001	1.6	1.0–2.4	0.045
Use of supplements	Vitamin D: No/irregular intake vs. regular intake	4.8	3.1–7.6	<0.001	.	.	.	.	.	.	.	.	.
Use of supplements	Folic acid: No/irregular intake vs. regular intake	.	.	.	3.9	1.4–16.1	0.024	.	.	.	.	.	.
Use of supplements	Vitamin B_{12}: No/irregular intake vs. regular intake	.	.	.	.	.	.	4.7	2.5–10.2	<0.001	.	.	.

25OHD = 25-hydroxyvitamin D; GNRI: Geriatric Nutritional Risk Index; BMI: body mass index; OR: odds ratio; CI: confidence interval; p: p-value; -: variable not significant at $p < 0.05$; .: not investigated (see Methods); cut-offs for subclinical micronutrient deficiency: <50 nmol/L (25OHD); <13.6 nmol/L (folate); <221 pmol/L (vitamin B_{12}); men: <11.6 µmol/L, women: <9.0 µmol/L (iron).

4. Discussion

The determination of the magnitude and predictors of subclinical micronutrient deficiency in KORA-Age older adults revealed that the prevalence of subclinical vitamin D and B_{12} deficiencies were high. Very old age, physical inactivity, frailty and no/irregular use of micronutrient-containing supplements were common predictors of subclinical micronutrient deficiencies.

4.1. Prevalence of Subclinical Micronutrient Deficiency

Direct comparison of our findings with other studies is hampered by the lack of international consensus as to which nutritional biomarker (biochemical and/or functional), which assay methodology, and which cut off point should be used to define a subclinical micronutrient deficiency in older adults [23,37]. Nevertheless, our population-based data is in line with previous studies that have suggested that subclinical vitamin D and B_{12} deficiencies are prevalent public health problems among older adults [12,15,38–42].

In the present study, low 25OHD levels were found in more than half (52.0%) of KORA-Age participants, and were more frequent in women (59.0%) than in men (44.4%). These findings lie in the range of national estimates from selected European countries that have used 25OHD < 50 nmol/L as the cut-off, including Germany (women 69.9%, men 62.9%) [12], Austria (women 62.3%, men 64.8%) [38], France (women 42.2%, men 36.5%) [39] and England (women 57.0%, men 49.0%) [15].

Around one out of four participants (27.3%) had a low vitamin B_{12} concentration in the KORA-Age study population. In line with our data, a subclinical vitamin B_{12} deficiency has been reported in 10–15% of older adults aged 60 and over in the USA [40,41], and even in 23–35% of older adults aged 80 and over in the USA [42].

The prevalence of subclinical deficiencies for both serum folate and serum iron (~10%) was less of a concern in the studied older adults. Recent studies in the USA [43] and in Brazil [44] pointed to a prevalence of <5% for folate deficiency among older adults aged 60 and over, though it is important to note that these countries have adopted national folic acid fortification policies. For serum iron, comparable studies in older adults are lacking; other studies have focused on identifying more advanced cases of iron deficiency or anemia, or they have used, in combination with serum iron or without, other functional nutritional biomarkers of iron status, such as transferrin saturation, serum ferritin, or blood hemoglobin measurements [45–47]. In these studies, the prevalence of iron deficiency or anemia was only moderate ($\leq$11%) in the older population.

Risk factors for subclinical micronutrient deficiency among older adults include: (i) decreased energy needs due to a loss of lean body mass and a decline in physical activity; (ii) age-associated physiological changes that affect absorption and utilization of micronutrients (e.g., decreased renal production of the active vitamin D form by the aging kidney [8]), and finally (iii) the presence of multiple chronic diseases, nutrient-drug interactions and variable diet quality, which may affect micronutrient requirements [9,10]. To identify older adults who would benefit most from subclinical micronutrient deficiency screening, we next discuss other factors that are associated with subclinical micronutrient deficiencies among older adults.

4.2. Predictors of Subclinical Micronutrient Deficiency

4.2.1. Common Predictors of Subclinical Micronutrient Deficiency

Very Old Age

Seniors aged 85 and over were two times more likely to have low 25OHD, low folate and low vitamin B_{12} levels compared to their younger counterparts. It is well known that micronutrient inadequacies increase beyond 65 years [37,48], and even more in octogenarians and older [15,42].

The efficiency of producing vitamin D in the skin decreases with age [8], and may be coupled to age-related factors that limit sunlight exposure, such as being more housebound. A subclinical

vitamin B_{12} deficiency in older adults is mainly caused by inadequate dietary intake and malabsorption. Chronic atrophic gastritis (a chronic inflammation causing loss of the gastric acid-producing cells, which is prevalent in ~30% of older adults) and the intake of drugs, such as proton pump inhibitors, histamine receptor 2 antagonists and biguanides, affect the secretion of gastric acid, thereby preventing the release of vitamin B_{12} from foods. Other contributing risk factors for low vitamin B_{12} levels in older adults include *Helicobacter pylori* infection and intestinal bacterial overgrowth [5,23]. An insufficient folate status in older adults seems to be mainly due to poor diet [49].

Physical Inactivity

Physical inactivity was associated with low 25OHD, folate and vitamin B_{12} concentrations. In epidemiological studies on vitamin D status, physical activity is often used as a proxy for time spent outdoors and indirectly for sunlight exposure. Accordingly, housebound seniors and those spending less time doing outdoor physical activity have decreased 25OHD levels [16]. Not surprisingly, being physically active was associated with higher 25OHD levels in our study. It has to be noted that our variable probably only gives a rough estimate of time spent outside, as it was not possible to distinguish outdoor from indoor activities.

Brock et al. found a positive association between both outdoor and indoor physical activities and 25OHD, suggesting that physical activity per se may also be a surrogate for better general health [50]. This may explain the findings for folate and vitamin B_{12}. Prospective studies are needed to confirm a potential physiological link between physical activity and micronutrient concentrations.

Frailty

Pre-frail and frail individuals had a higher risk for subclinical 25OHD and iron deficiency. Prior cross-sectional and prospective studies among KORA-Age participants found low 25OHD levels to be associated with prevalent (pre-)frailty [33], incident pre-frailty and pre-frailty/frailty combined [51]. To our knowledge, the relationship between low serum iron levels and frailty occurrence has not been extensively investigated, yet Pires Corona et al. found that anemic older adults with low hemoglobin levels were more likely to be frail [52].

Although our findings suggest that (pre-)frailty may be a predictor of a subclinical micronutrient deficiency, it has also been suggested that poor nutrition itself can favor the development of frailty [53].

No/Irregular Use of Supplements

No/irregular use of supplements was a strong common predictor of subclinical 25OHD and folate and vitamin B_{12} deficiencies. Prior studies in older adults have found that supplement use is a major predictor of vitamin D status [14,17] and is linked to higher vitamin B_{12} and folate levels [54,55]. Although 44.7% of KORA-Age participants used supplements containing vitamins or minerals [36], only around 10% took supplements containing vitamin D, folate and vitamin B_{12}, respectively. As shown previously [56,57], optimal serum micronutrient levels, such as vitamin D, folate and vitamin B_{12}, can be maintained by supplementation. The possibility that regular supplementation with these specific micronutrients might help older adults in satisfying their requirements, and prevent chronic diseases via the correction of low levels, as found earlier [56], is of major interest and could stimulate research on biological pathways that link supplement intake, micronutrient status and disease state.

No/irregular use of iron-containing supplements was not significantly associated with a subclinical iron deficiency in our study. It is important to note that there are concerns about the adverse effects of iron-containing supplements [6], including nausea, epigastric discomfort and constipation, all of which are dose-related. In case of iron deficiency anemia, it is recommended to seek consultation from a physician, in order to use appropriately dosed iron-containing supplements.

4.2.2. Further Predictors of Subclinical Micronutrient Deficiency

Vitamin D (Springtime, Female Sex, Obesity, Former Smoking)

In line with previous studies among older adults, we found that springtime, female sex and obesity were significantly associated with low 25OHD levels [15–17]. Seasonal variations in 25OHD status are well known. During cold and dark months, older adults may spend more daylight hours indoors, resulting in less sun exposure. Furthermore, in Germany, from October to March, UVB radiation is too low to induce vitamin D synthesis by the skin [58]. Sex differences may be due to unaccounted confounders, such as clothing style, use of sun protection, and to dietary intake. Concerning the inverse association with obesity, it has been suggested that vitamin D is accumulated in adipose tissue, resulting in decreased bioavailability and lower circulating 25OHD levels in the blood of obese persons [59].

The investigation of smoking status yielded inconsistent results. In studies classifying former smoking as a separate category [15,60], former smoking was not found to be a significant predictor of low 25OHD levels. In our data, former smoking was associated with higher 25OHD levels than never smokers. Schwab et al. showed that former smokers, among KORA-Age individuals, were two times more likely to ingest vitamin and mineral-containing supplements compared to never smokers [36]. It is therefore conceivable that smoking cessation went along with improved diet and lifestyle habits that increased vitamin D intake. These unaccounted factors, such as improved intake of foods rich in micronutrients or longer times spent outdoors, may have confounded the observed association. Furthermore, there is always the possibility for a type I error due to multiple comparisons.

Vitamin B_{12} (No Polypharmacy)

Some drugs reduce absorption or affect the metabolism of vitamin B_{12} via known mechanisms (such as proton pump inhibitors or histamine receptor 2 antagonists) or unknown mechanisms (such as metformin) [23]. Conversely, we found that polypharmacy was associated with sufficient vitamin B_{12} levels. Differentiating between the different types of medications ingested was outside the scope of this analysis, but it would be interesting to find out which medication(s) may positively impact on vitamin B_{12} status.

Folate (Alcohol Consumption)

Contra-intuitively, drinkers of $\geq$20 g alcohol per day had higher folate levels than abstainers. The consumption of $\geq$20 g of alcohol is equivalent to $\geq$0.5 L beer, $\geq$0.2 L wine or $\geq$3 small spirits of 2 cL [61], and was more frequent in men (n = 246) than women (n = 61) (Table 1). Differentiating between alcohol levels revealed that the majority of these female (n = 54) and male drinkers (n = 163) used between $\geq$20 and <40 g/day, which is still considered as moderate drinking for men. A lower proportion of men were high (between $\geq$40 to <60 g/day, n = 60) to heavy drinkers (>60 g/day, n = 23), respectively. Our variable category thus mostly covered moderate to high drinkers, and only a few heavy drinkers.

In observational epidemiological studies in humans, folate deficiency has been shown to be common in excessive alcohol drinkers and alcoholics [62], suggesting that alcohol consumption may affect folate status preponderantly at higher alcohol doses. Also, light to moderate alcohol consumption is known to be associated with a reduction in all-cause mortality [61]. It is thus possible that the dose at which alcohol was consumed in our study—mostly moderate amounts—was not harmful, but had a positive effect on health in general, thereby possibly affecting folate status. Possible mechanisms involved need further investigation. Nonetheless, caution is warranted as the observed association with alcohol use might be confounded by other factors that influence both traits. Another possible explanation for this counter-intuitive finding is the selection bias associated with the follow-up design of the KORA-Age study, whereby more health-interested individuals involved in former KORA studies, and probably fewer sick individuals with reduced alcohol consumption levels, participated.

Iron (Male Sex, GNRI, Polypharmacy)

Participants with a moderate/major and even low risk for malnutrition (GNRI) were at higher risk for low iron levels. The GNRI is a nutrition-related risk index that can predict the risk for morbidity and mortality in hospitalized older adults, in relation to malnutrition-associated pathologies. Our finding suggests that the GNRI may be a marker for subclinical iron deficiency in older adults. However, as the GNRI has primarily been established for use in hospitalized older adults, it may not necessarily be appropriate among community-dwelling older adults. Further research is warranted in this regard [26].

Men were at higher odds of having low iron levels, despite having higher median iron concentrations than women (Table 2). Sex divergence may be due to the use of a higher cut-off for men, as recommended in the literature [24]. Moreover, serum iron may increase after the ingestion of iron-containing foods [6], suggesting that sex differences may also be due to unequal dietary intakes. Finally, polypharmacy was inversely associated with iron status. Iron status in older adults is known to be affected by a number of medications [6], including antacids and proton pump inhibitors. Knowing which medication type may reduce iron levels is of interest from a prevention point of view and needs further research.

4.3. Strengths and Weaknesses

The KORA-Age study is singular in the sense that it includes a large (>1000 participants) and socio-demographically representative sample of old, and even very old, adults in Germany. Caution is warranted when generalizing findings on a national level, as possible regional differences in eating habits and latitude may exist. The assessment of multiple predictors of subclinical micronutrient deficiency was rendered possible by the extensive health assessment of participants and the quality of the KORA-Age data, which has been collected by trained interviewers and systematically controlled before entry into the KORA-Age database.

The use of biomarkers of micronutrient status is both a strength and a limitation of our study. Nutritional biomarkers may not necessarily reflect the amount of micronutrient intake from foods, and may be affected by multi-morbidity, inflammation and nutrient-drug interactions, especially in older adults. Nevertheless, biomarkers relate to the nutritional status of micronutrients after absorption in the body, and can directly reflect subclinical deficiency at the blood level.

Certain laboratory procedures, used for the assessment of micronutrient status were not the 'gold standard', e.g., the immunoassay procedure used in this study versus the commonly used *Lactobacillus rhamnosus* microbiological assay for measuring serum folate levels [22]. Also, the use of serum iron levels for the evaluation of iron status may be misleading because they are subject to diurnal rhythms and increase after the ingestion of iron-containing foods. However, serum iron concentrations indicate the adequacy of the iron supply to developing red blood cells [6], and may be used as a screening tool for a subclinical deficiency, as opposed to an established clinical deficiency. To identify more advanced cases of iron deficiency or anemia, additional measurements that use functional nutritional biomarkers are essential, including serum/plasma soluble transferrin receptor, serum/plasma ferritin, or blood hemoglobin measurements [22].

Another limitation arises from the cross-sectional design of this analysis, which precludes any direct cause-effect relationships between subclinical micronutrient deficiency and predictors. Also, causality cannot be definitively proven, owing to the possible correlation with other unknown or unmeasured predictors. Yet, a causal effect in one or the other direction is possible and has to be further examined in prospective studies.

5. Conclusions

Using serum nutritional biomarkers, determination of the magnitude of subclinical vitamin D, folate, vitamin B_{12} and iron deficiencies, among KORA-Age older adults, revealed that more than half of individuals had low 25OHD levels and more than a quarter had low vitamin B_{12} levels. The prevalence

of subclinical deficiencies for both folate and iron (~10%) were less of a concern. Very old age, physical inactivity, frailty and no/irregular use of micronutrient-containing supplements were identified as common predictors of subclinical micronutrient deficiencies in the studied older adults.

Our findings provide further rationale for screening subgroups at high-risk for subclinical micronutrient deficiencies. The possibility that regular and appropriately dosed micronutrient supplementation might help older adults otherwise unable to follow dietary guidelines in satisfying their requirements, and prevent chronic diseases via the correction of low micronutrient levels, is of major interest and could stimulate research on biological pathways that link supplement intake, micronutrient status and disease state.

Supplementary Materials: The following are available online at www.mdpi.com/2072-6643/9/12/1276/s1, Figure S1: Flowchart of the KORA-Age 2008/2009 recruitment and retention profile, Table S1: Description of data collection and categorization methods for the investigated predictors in KORA-Age 2008/2009, Table S2: Unadjusted ORs with 95% CIs for subclinical micronutrient deficiencies by categories of potential predictors: Results from binary logistic regression analyses in KORA-Age 2008/2009, Table S3: Predictors with $p < 0.25$ in binary logistic regression which were entered into each multiple logistic regression model.

Acknowledgments: The KORA-Age study was conducted with financial support from the German Federal Ministry of Education and Research (BMBF FKZ 01ET0713) as part of the 'Health in old age' program. Romy Conzade has received a PhD scholarship from the 'Studienstiftung des deutschen Volkes'. We thank Heike A. Bischoff-Ferrari, as member of the PhD Thesis Committee of Romy Conzade, for sharing her expertise and for helping in interpreting the results.

Author Contributions: R.C. and B.T. conceived and designed the present study; B.T., W.K., A.P., M.H. and A.S. were involved in data collection and quality control; R.C. analyzed the data and wrote the first draft of the paper; all authors contributed to the critical revision of the paper and approved the final version.

Conflicts of Interest: The authors declare no conflict of interest. The funding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

Abbreviations

25OHD	25-hydroxyvitamin D
BMI	Body Mass Index
CI	Confidence interval
CVD	Cardiovascular disease
DEGS1	German Health Interview and Examination Survey for Adults
DGE	German Nutrition Society
eGFR	Estimated Glomerular Filtration Rate
GNRI	Geriatric Nutritional Risk Index
IDOM	Instrument for Databased Assessment Of Medication
IQR	Interquartile range
KORA	Cooperative Health Research in the Region of Augsburg
MONICA	Monitoring of Trends and Determinants in Cardiovascular Disease
NNR	Nordic Nutrition Recommendations
NVS II	Second German National Nutrition Survey
OR	Odds ratio
Q1	First quartile or 25th percentile
Q3	Third quartile or 75th percentile
SCREEN II	Seniors in the Community Risk Evaluation for Eating and Nutrition, version II
WHO	World Health Organization

References

1. Bailey, R.L.; West, K.P., Jr.; Black, R.E. The epidemiology of global micronutrient deficiencies. *Ann. Nutr. Metab.* **2015**, *66* (Suppl. 2), 22–33. [CrossRef] [PubMed]
2. Wacker, M.; Holick, M.F. Vitamin D—Effects on skeletal and extraskeletal health and the need for supplementation. *Nutrients* **2013**, *5*, 111–148. [CrossRef] [PubMed]

3. Trombetti, A.; Reid, K.F.; Hars, M.; Herrmann, F.R.; Pasha, E.; Phillips, E.M.; Fielding, R.A. Age-associated declines in muscle mass, strength, power, and physical performance: Impact on fear of falling and quality of life. *Osteoporos. Int.* **2016**, *27*, 463–471. [CrossRef] [PubMed]
4. Visser, M.; Deeg, D.J.; Lips, P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): The Longitudinal Aging Study Amsterdam. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 5766–5772. [CrossRef] [PubMed]
5. Porter, K.; Hoey, L.; Hughes, C.F.; Ward, M.; McNulty, H. Causes, Consequences and Public Health Implications of Low B-Vitamin Status in Ageing. *Nutrients* **2016**, *8*, 725. [CrossRef] [PubMed]
6. Fairweather-Tait, S.J.; Wawer, A.A.; Gillings, R.; Jennings, A.; Myint, P.K. Iron status in the elderly. *Mech. Ageing Dev.* **2014**, *136–137*, 22–28. [CrossRef] [PubMed]
7. Hong, C.H.; Falvey, C.; Harris, T.B.; Simonsick, E.M.; Satterfield, S.; Ferrucci, L.; Metti, A.L.; Patel, K.V.; Yaffe, K. Anemia and risk of dementia in older adults: Findings from the Health ABC study. *Neurology* **2013**, *81*, 528–533. [CrossRef] [PubMed]
8. Gallagher, J.C. Vitamin D and Aging. *Endocrinol. Metab. Clin. N. Am.* **2013**, *42*, 319–332. [CrossRef] [PubMed]
9. Chernoff, R. Micronutrient requirements in older women. *Am. J. Clin. Nutr.* **2005**, *81*, 1240s–1245s. [PubMed]
10. Ter Borg, S.; Verlaan, S.; Hemsworth, J.; Mijnarends, D.M.; Schols, J.M.; Luiking, Y.C.; de Groot, L.C. Micronutrient intakes and potential inadequacies of community-dwelling older adults: A systematic review. *Br. J. Nutr.* **2015**, *113*, 1195–1206. [CrossRef] [PubMed]
11. Rubner-Institut, M. Nationale Verzehrsstudie II, Ergebnisbericht Teil 2. Die Bundesweite Befragung zur Ernährung von Jugendlichen und Erwachsenen. 2008. Available online: https://www.mri.bund.de/fileadmin/MRI/Institute/EV/NVSII_Abschlussbericht_Teil_2.pdf (accessed on 14 November 2017).
12. Rabenberg, M.; Scheidt-Nave, C.; Busch, M.A.; Rieckmann, N.; Hintzpeter, B.; Mensink, G.B. Vitamin D status among adults in Germany—Results from the German Health Interview and Examination Survey for Adults (DEGS1). *BMC Public Health* **2015**, *15*, 641. [CrossRef] [PubMed]
13. McNeill, G.; Vyvyan, J.; Peace, H.; McKie, L.; Seymour, G.; Hendry, J.; MacPherson, I. Predictors of micronutrient status in men and women over 75 years old living in the community. *Br. J. Nutr.* **2002**, *88*, 555–561. [CrossRef] [PubMed]
14. Hill, T.R.; Granic, A.; Davies, K.; Collerton, J.; Martin-Ruiz, C.; Siervo, M.; Mathers, J.C.; Adamson, A.J.; Francis, R.M.; Pearce, S.H.; et al. Serum 25-hydroxyvitamin D concentration and its determinants in the very old: The Newcastle 85+ Study. *Osteoporos. Int.* **2016**, *27*, 1199–1208. [CrossRef] [PubMed]
15. Hirani, V.; Tull, K.; Ali, A.; Mindell, J. Urgent action needed to improve vitamin D status among older people in England! *Age Ageing* **2010**, *39*, 62–68. [CrossRef] [PubMed]
16. Van Dam, R.M.; Snijder, M.B.; Dekker, J.M.; Stehouwer, C.D.; Bouter, L.M.; Heine, R.J.; Lips, P. Potentially modifiable determinants of vitamin D status in an older population in the Netherlands: The hoorn study. *Am. J. Clin. Nutr.* **2007**, *85*, 755–761. [PubMed]
17. McCarroll, K.; Beirne, A.; Casey, M.; McNulty, H.; Ward, M.; Hoey, L.; Molloy, A.; Laird, E.; Healy, M.; Strain, J.J.; et al. Determinants of 25-hydroxyvitamin D in older Irish adults. *Age Ageing* **2015**, *44*, 847–853. [CrossRef] [PubMed]
18. Jungert, A.; Neuhauser-Berthold, M. Sex-specific determinants of serum 25-hydroxyvitamin D3 concentrations in an elderly German cohort: A cross-sectional study. *Nutr. Metab. Lond.* **2015**, *12*, 2. [CrossRef] [PubMed]
19. Peters, A.; Doring, A.; Ladwig, K.H.; Meisinger, C.; Linkohr, B.; Autenrieth, C.; Baumeister, S.E.; Behr, J.; Bergner, A.; Bickel, H.; et al. Multimorbidity and successful aging: The population-based KORA-Age study. *Z. Gerontol. Geriatr.* **2011**, *44* (Suppl. 2), 41–54. [CrossRef] [PubMed]
20. German Nutrition Society. New reference values for vitamin D. *Ann. Nutr. Metab.* **2012**, *60*, 241–246. [CrossRef]
21. Lamberg-Allardt, C.; Brustad, M.; Meyer, H.E.; Steingrimsdottir, L. Vitamin D—A systematic literature review for the 5th edition of the Nordic Nutrition Recommendations. *Food Nutr. Res.* **2013**, *57*. [CrossRef] [PubMed]
22. Sauberlich, H. *Laboratory Tests for the Assessment of Nutritional Status*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 1999; p. 512.
23. Green, R.; Allen, L.H.; Bjorke-Monsen, A.L.; Brito, A.; Gueant, J.L.; Miller, J.W.; Molloy, A.M.; Nexo, E.; Stabler, S.; Toh, B.H.; et al. Vitamin B12 deficiency. *Nat. Rev. Dis. Prim.* **2017**, *3*, 17040. [CrossRef] [PubMed]

24. Kaplan, L.; Pesce, A. *Clinical Chemistry: Theory, Analysis, and Correlation*, 3rd ed.; Mosby, Inc.: St. Louis, MO, USA, 1996; p. 699.
25. Ahrens, W.; Bellach, B.M.; Jöckel, K.H. Measurement and Quantification of Sociodemographic Characteristics in Epidemiological Studies. German Society for Medical Informatics, Biometry and Epidemiology (GMDS), 1998. Available online: https://dgepi.de/fileadmin/pdf/leitlinien/11_MessungUndQuantifizierungSoziodemographischerMerkmale_pdf2.pdf (accessed on 14 November 2017).
26. Bouillanne, O.; Morineau, G.; Dupont, C.; Coulombel, I.; Vincent, J.P.; Nicolis, I.; Benazeth, S.; Cynober, L.; Aussel, C. Geriatric nutritional risk index: A new index for evaluating at-risk elderly medical patients. *Am. J. Clin. Nutr.* **2005**, *82*, 777–783. [PubMed]
27. Keller, H.H.; Goy, R.; Kane, S.L. Validity and reliability of SCREEN II (Seniors in the community: Risk evaluation for eating and nutrition, Version II). *Eur. J. Clin. Nutr.* **2005**, *59*, 1149–1157. [CrossRef] [PubMed]
28. Meisinger, C.; Lowel, H.; Thorand, B.; Doring, A. Leisure time physical activity and the risk of type 2 diabetes in men and women from the general population. The MONICA/KORA Augsburg Cohort Study. *Diabetologia* **2005**, *48*, 27–34. [CrossRef] [PubMed]
29. Doring, A.; Filipiak, B.; Stieber, J.; Keil, U. Trends in alcohol intake in a southern German population from 1984–1985 to 1989–1990: Results of the MONICA project augsburg. *J. Stud. Alcohol* **1993**, *54*, 745–749. [CrossRef] [PubMed]
30. Kirchberger, I.; Meisinger, C.; Heier, M.; Zimmermann, A.K.; Thorand, B.; Autenrieth, C.S.; Peters, A.; Ladwig, K.H.; Doring, A. Patterns of multimorbidity in the aged population. Results from the KORA-Age study. *PLoS ONE* **2012**, *7*, e30556. [CrossRef] [PubMed]
31. Strobl, R.; Muller, M.; Emeny, R.; Peters, A.; Grill, E. Distribution and determinants of functioning and disability in aged adults–results from the German KORA-Age study. *BMC Public Health* **2013**, *13*, 137. [CrossRef] [PubMed]
32. Fried, L.P.; Tangen, C.M.; Walston, J.; Newman, A.B.; Hirsch, C.; Gottdiener, J.; Seeman, T.; Tracy, R.; Kop, W.J.; Burke, G.; et al. Frailty in older adults: Evidence for a phenotype. *J. Gerontol. A Biol. Sci. Med. Sci.* **2001**, *56*, M146–M156. [CrossRef] [PubMed]
33. Pabst, G.; Zimmermann, A.K.; Huth, C.; Koenig, W.; Ludwig, T.; Zierer, A.; Peters, A.; Thorand, B. Association of low 25-hydroxyvitamin D levels with the frailty syndrome in an aged population: Results from the KORA-age Augsburg study. *J. Nutr. Health Aging* **2015**, *19*, 258–264. [CrossRef] [PubMed]
34. Inker, L.A.; Schmid, C.H.; Tighiouart, H.; Eckfeldt, J.H.; Feldman, H.I.; Greene, T.; Kusek, J.W.; Manzi, J.; Van Lente, F.; Zhang, Y.L.; et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N. Engl. J. Med.* **2012**, *367*, 20–29. [CrossRef] [PubMed]
35. Mühlberger, N.; Behrend, C.; Stark, R.; Holle, R. Database-supported identification and entry of drug data in health studies–experience with the IDOM software. *Inform. Biom. Epidemiol. Med. Biol.* **2003**, *34*, 601–611.
36. Schwab, S.; Heier, M.; Schneider, A.; Fischer, B.; Huth, C.; Peters, A.; Thorand, B. The use of dietary supplements among older persons in southern Germany–results from the KORA-age study. *J. Nutr. Health Aging* **2014**, *18*, 510–519. [CrossRef] [PubMed]
37. Mithal, A.; Wahl, D.A.; Bonjour, J.P.; Burckhardt, P.; Dawson-Hughes, B.; Eisman, J.A.; El-Hajj Fuleihan, G.; Josse, R.G.; Lips, P.; Morales-Torres, J. Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos. Int.* **2009**, *20*, 1807–1820. [CrossRef] [PubMed]
38. Austrian Nutrition Report. Österreichischer Ernährungsbericht 2012. Bundesministerium für Gesundheit, Wien. 2012. Available online: https://www.bmgf.gv.at/cms/home/attachments/4/5/3/CH1048/CMS1348749794860/oeb12.pdf (accessed on 14 November 2017).
39. Unité de Surveillance et D'Epidémiologie Nutritionnelle (Usen). Étude Nationale Nutrition Santé (ENNS, 2006)–Situation Nutritionnelle en France en 2006 Selon les Indicateurs D'Objectif et les Repères du Programme National Nutrition Santé (PNNS). Institut de Veille Sanitaire, Université de Paris 13, Conservatoire National des Arts et Métiers. 2007. Available online: http://opac.invs.sante.fr/doc_num.php?explnum_id=3481 (accessed on 14 November 2017).
40. Bailey, R.L.; Carmel, R.; Green, R.; Pfeiffer, C.M.; Cogswell, M.E.; Osterloh, J.D.; Sempos, C.T.; Yetley, E.A. Monitoring of vitamin B-12 nutritional status in the United States by using plasma methylmalonic acid and serum vitamin B-12. *Am. J. Clin. Nutr.* **2011**, *94*, 552–561. [CrossRef] [PubMed]

41. Carmel, R.; Sarrai, M. Diagnosis and management of clinical and subclinical cobalamin deficiency: Advances and controversies. *Curr. Hematol. Rep.* **2006**, *5*, 23–33. [PubMed]
42. Johnson, M.A.; Hausman, D.B.; Davey, A.; Poon, L.W.; Allen, R.H.; Stabler, S.P. Vitamin B12 deficiency in African American and white octogenarians and centenarians in Georgia. *J. Nutr. Health Aging* **2010**, *14*, 339–345. [CrossRef] [PubMed]
43. Pfeiffer, C.M.; Sternberg, M.R.; Hamner, H.C.; Crider, K.S.; Lacher, D.A.; Rogers, L.M.; Bailey, R.L.; Yetley, E.A. Applying inappropriate cutoffs leads to misinterpretation of folate status in the US population. *Am. J. Clin. Nutr.* **2016**, *104*, 1607–1615. [CrossRef] [PubMed]
44. Steluti, J.; Selhub, J.; Paul, L.; Reginaldo, C.; Fisberg, R.M.; Marchioni, D.M.L. An overview of folate status in a population-based study from Sao Paulo, Brazil and the potential impact of 10 years of national folic acid fortification policy. *Eur. J. Clin. Nutr.* **2017**, *71*. [CrossRef] [PubMed]
45. Corona, L.P.; Duarte, Y.A.; Lebrao, M.L. Prevalence of anemia and associated factors in older adults: Evidence from the SABE Study. *Rev. Saude Publica* **2014**, *48*, 723–731. [CrossRef] [PubMed]
46. Hinds, H.E.; Johnson, A.A.; Webb, M.C.; Graham, A.P. Iron, folate, and vitamin B12 status in the elderly by gender and ethnicity. *J. Natl. Med. Assoc.* **2011**, *103*, 870–877. [CrossRef]
47. Milman, N.; Pedersen, A.N.; Ovesen, L.; Schroll, M. Iron status in 358 apparently healthy 80-year-old Danish men and women: Relation to food composition and dietary and supplemental iron intake. *Ann. Hematol.* **2004**, *83*, 423–429. [CrossRef] [PubMed]
48. Allen, L.H. How common is vitamin B-12 deficiency? *Am. J. Clin. Nutr.* **2009**, *89*, 693s–696s. [CrossRef] [PubMed]
49. Wolters, M.; Strohle, A.; Hahn, A. Age-associated changes in the metabolism of vitamin B(12) and folic acid: Prevalence, aetiopathogenesis and pathophysiological consequences. *Z. Gerontol. Geriatr.* **2004**, *37*, 109–135. [CrossRef] [PubMed]
50. Brock, K.; Cant, R.; Clemson, L.; Mason, R.S.; Fraser, D.R. Effects of diet and exercise on plasma vitamin D (25(OH)D) levels in Vietnamese immigrant elderly in Sydney, Australia. *J. Steroid Biochem. Mol. Biol.* **2007**, *103*, 786–792. [CrossRef] [PubMed]
51. Vogt, S.; Decke, S.; de Las Heras Gala, T.; Linkohr, B.; Koenig, W.; Ladwig, K.H.; Peters, A.; Thorand, B. Prospective association of vitamin D with frailty status and all-cause mortality in older adults: Results from the KORA-Age Study. *Prev. Med.* **2015**, *73*, 40–46. [CrossRef] [PubMed]
52. Pires Corona, L.; Drumond Andrade, F.C.; de Oliveira Duarte, Y.A.; Lebrao, M.L. The Relationship between Anemia, Hemoglobin Concentration and Frailty in Brazilian Older Adults. *J. Nutr. Health Aging* **2015**, *19*, 935–940. [CrossRef] [PubMed]
53. Yannakoulia, M.; Ntanasi, E.; Anastasiou, C.A.; Scarmeas, N. Frailty and nutrition: From epidemiological and clinical evidence to potential mechanisms. *Metabolism* **2017**, *68*, 64–76. [CrossRef] [PubMed]
54. Fabian, E.; Bogner, M.; Kickinger, A.; Wagner, K.H.; Elmadfa, I. Vitamin status in elderly people in relation to the use of nutritional supplements. *J. Nutr. Health Aging* **2012**, *16*, 206–212. [CrossRef] [PubMed]
55. Bor, M.V.; Lydeking-Olsen, E.; Moller, J.; Nexo, E. A daily intake of approximately 6 microg vitamin B-12 appears to saturate all the vitamin B-12-related variables in Danish postmenopausal women. *Am. J. Clin. Nutr.* **2006**, *83*, 52–58. [PubMed]
56. Whiting, S.J.; Bonjour, J.P.; Payen, F.D.; Rousseau, B. Moderate amounts of vitamin D3 in supplements are effective in raising serum 25-hydroxyvitamin D from low baseline levels in adults: A systematic review. *Nutrients* **2015**, *7*, 2311–2323. [CrossRef] [PubMed]
57. Eussen, S.J.; de Groot, L.C.; Clarke, R.; Schneede, J.; Ueland, P.M.; Hoefnagels, W.H.; van Staveren, W.A. Oral cyanocobalamin supplementation in older people with vitamin B12 deficiency: A dose-finding trial. *Arch. Intern. Med.* **2005**, *165*, 1167–1172. [CrossRef] [PubMed]
58. O'Neill, C.M.; Kazantzidis, A.; Ryan, M.J.; Barber, N.; Sempos, C.T.; Durazo-Arvizu, R.A.; Jorde, R.; Grimnes, G.; Eiriksdottir, G.; Gudnason, V.; et al. Seasonal Changes in Vitamin D-Effective UVB Availability in Europe and Associations with Population Serum 25-Hydroxyvitamin D. *Nutrients* **2016**, *8*, 533. [CrossRef] [PubMed]
59. Wortsman, J.; Matsuoka, L.Y.; Chen, T.C.; Lu, Z.; Holick, M.F. Decreased bioavailability of vitamin D in obesity. *Am. J. Clin. Nutr.* **2000**, *72*, 690–693. [PubMed]

60. Shirazi, L.; Almquist, M.; Malm, J.; Wirfalt, E.; Manjer, J. Determinants of serum levels of vitamin D: A study of life-style, menopausal status, dietary intake, serum calcium, and PTH. *BMC Womens Health* **2013**, *13*, 33. [CrossRef] [PubMed]
61. Keil, U.; Chambless, L.E.; Doring, A.; Filipiak, B.; Stieber, J. The relation of alcohol intake to coronary heart disease and all-cause mortality in a beer-drinking population. *Epidemiology* **1997**, *8*, 150–156. [CrossRef] [PubMed]
62. Halsted, C.H.; Villanueva, J.A.; Devlin, A.M.; Chandler, C.J. Metabolic interactions of alcohol and folate. *J. Nutr.* **2002**, *132*, 2367s–2372s. [PubMed]

2. Publication: Vitamin D in Relation to Incident Sarcopenia and Changes in Muscle Parameters Among Older Adults

Title:	Vitamin D in Relation to Incident Sarcopenia and Changes in Muscle Parameters Among Older Adults: The KORA-Age Study
Authors:	Romy Conzade, Eva Grill, Heike A. Bischoff-Ferrari, Uta Ferrari, Alexander Horsch, Wolfgang Koenig, Annette Peters and Barbara Thorand
Journal:	*Calcified Tissue International*
Year:	2019
Volume:	105
DOI:	10.1007/s00223-019-00558-5
Supplements:	https://static-content.springer.com/esm/art%3A10.1007%2Fs00223-019-00558-5/MediaObjects/223_2019_558_MOESM1_ESM.docx (available online)
Year:	2019
Impact factor:	3.265 (Journal Citation Reports® 2018)
Rank:	67/145 in Category Endocrinology & Metabolism (Journal Citation Reports® 2018)

Calcified Tissue International
https://doi.org/10.1007/s00223-019-00558-5

ORIGINAL RESEARCH

Vitamin D in Relation to Incident Sarcopenia and Changes in Muscle Parameters Among Older Adults: The KORA-Age Study

Romy Conzade[1] · Eva Grill[2] · Heike A. Bischoff-Ferrari[3] · Uta Ferrari[4] · Alexander Horsch[5] · Wolfgang Koenig[6,7,8] · Annette Peters[1] · Barbara Thorand[1]

Received: 29 January 2019 / Accepted: 25 April 2019

Abstract

Effects of low serum 25OHD on age-related changes in muscle mass and function remain unclear. Our aims were to explore associations of baseline 25OHD levels with prevalent and incident sarcopenia and changes in muscle parameters, and to examine the role of parathyroid hormone (PTH) therein. Cross-sectional ($n = 975$) and prospective analyses ($n = 702$) of older adults aged 65–93 years participating in the KORA-Age study. Sarcopenia was defined using the 2010 European Working Group on Sarcopenia in Older People (EWGSOP) criteria as low muscle mass combined with low grip strength or low physical performance. Associations with baseline 25OHD were examined in multiple regression analyses. Low vitamin D status was linked to increased odds of prevalent sarcopenia. Over three years, low baseline 25OHD < 25 vs. ≥ 50 nmol/L were associated with greater loss of muscle mass and increased time for the Timed Up and Go test. The risk for developing incident sarcopenia was not significantly elevated in individuals with low baseline 25OHD but when including death as combined outcome alongside incident sarcopenia, there was a strong positive association in multivariable analysis [OR (95% CI) 3.19 (1.54–6.57) for 25OHD < 25 vs. ≥ 50 nmol/L]. There was no evidence for a PTH-mediating effect. Low baseline 25OHD levels were associated with unfavorable changes in muscle mass and physical performance, but not with incident sarcopenia. Future randomized trials are needed to assess causality and to address the issue of competing risks such as mortality in older cohorts.

Keywords Vitamin D · Sarcopenia · Muscle changes · Prospective · Older adults

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00223-019-00558-5) contains supplementary material, which is available to authorized users.

✉ Barbara Thorand
thorand@helmholtz-muenchen.de

[1] Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Epidemiology, 85764 Neuherberg, Germany

[2] Institute for Medical Information Processing, Biometrics and Epidemiology, Ludwig Maximilian University of Munich, 81377 Munich, Germany

[3] Department of Geriatrics and Aging Research, University of Zurich and University Hospital Zurich, 8091 Zurich, Switzerland

[4] Department of Medicine IV, University Hospital, Ludwig Maximilian University of Munich, 80336 Munich, Germany

[5] Department of Computer Science, UiT - The Arctic University of Norway, 9037 Tromsø, Norway

[6] Deutsches Herzzentrum München, Technische Universität München, 80636 Munich, Germany

[7] DZHK (German Centre for Cardiovascular Research), Partner Site Munich Heart Alliance, 80802 Munich, Germany

[8] Institute of Epidemiology and Biostatistics, University of Ulm, 89081 Ulm, Germany

Published online: 08 May 2019

Introduction

Conceptualized as a geriatric syndrome of the gradual loss of muscle mass and function, sarcopenia becomes increasingly common in older age and often occurs simultaneously with low serum 25-hydroxyvitamin D (25OHD) levels [1]. Both conditions are linked to common clinical geriatric outcomes, including muscle weakness, falls and frailty [2].

There are several reasons to consider how vitamin D may have a beneficial effect on muscle health, yet individual observational studies examining the relationship of 25OHD levels with sarcopenia and related muscle parameters have yielded inconsistent results and the number of prospective studies is limited. We are aware of only two prospective studies that have examined 25OHD in relation to the incidence of sarcopenia defined by both low muscle mass and low muscle function. While the first study did not observe a significant association between vitamin D status and incident sarcopenia [3], low levels of 25OHD were associated with a significant increased risk of incident sarcopenia in the second one [4]. Furthermore, low 25OHD levels were not linked to change in muscle mass over time [3, 5, 6]. In some prospective studies, lower 25OHD levels were associated with loss of muscle function such a muscle strength or physical performance [5–8], while others did not find respective associations [3, 9–11].

The mechanisms by which vitamin D status may affect muscle metabolism and function are not fully elucidated [12]. They may be both direct, via activation of a vitamin D receptor on muscle tissue by the biologically active form of vitamin D [13], and indirect, via effects of low serum calcium [14], low serum phosphate [15] or increased serum parathyroid hormone (PTH) levels [6].

We used cross-sectional and prospective data from the population-based KORA (Cooperative Health Research in the Region of Augsburg)-Age study to determine the associations of baseline 25OHD levels with prevalent and incident sarcopenia as well as changes in related muscle parameters in German older adults. The role of PTH as a potential mediator was also examined.

Methods

Study Design and Participants

We used data from two time points of the KORA-Age study (baseline: 2008/2009; follow-up: 2012). Full details about the study design and participants have been published previously [16]. Briefly, the cohort includes 1079 eligible participants aged ≥ 65 years on 31.12.2008, who participated in a multidimensional health assessment at baseline. A total of 975 individuals with complete health assessment and without hypercalcemia at baseline (serum calcium levels > 2.6 mmol/L) were included in cross-sectional analyses. After exclusion of individuals with sarcopenia at baseline, without follow-up data or with missing information on sarcopenia at follow-up, a total of 702 participants were included in prospective analyses. Of those without follow-up data ($n = 179$), 57 died and 122 did not participate due to other reasons. The Ethics Committee of the Bavarian Medical Association approved the study protocol (reference number: 08064), and written informed consent was obtained from all participants.

Sarcopenia and Related Muscle Parameters

Sarcopenia status was assessed at baseline and follow-up using the European Working Group on Sarcopenia in Older People (EWGSOP) 2010 criteria: low muscle mass combined with either low muscle strength or low physical performance [17].

Muscle mass was assessed using bioimpedance values measured with a body impedance analyzer (BIA 2000-S; Data Input GmbH, Frankfurt, Germany) [18]. Calibration of the BIA device occurred every day before and after the last use of the device with the test resistor. Results of the calibration were systematically documented and compared to target values of resistance: $R = 500\ \Omega$ (± 4) and reactance $Xc = 144\ \Omega$ (± 4). The multiple regression prediction equation developed by Janssen et al. was applied to estimate skeletal muscle mass [19]. Muscle mass index (kg/m^2) was then calculated as skeletal muscle mass divided by height-squared. For *low muscle mass index*, we used cut-offs previously published in KORA-Age: ≤ 8.72 kg/m^2 in men and ≤ 6.33 kg/m^2 in women [20].

Handgrip strength (kg) was assessed using the JAMAR handheld dynamometer (Saehan Corp., Masan, Korea). The mean value of three measurements in the participant's dominant hand was taken for analyses. We applied the EWGSOP cut-offs for *low grip strength* < 30 kg in men and < 20 kg in women [17].

Gait speed (m/sec), assessed using the GAITRite system (CIR Systems, Havertown, PA, USA) [21], was used as the primary measure of physical performance. The standard cut-off of ≤ 0.8 m/s of the normal walking speed was applied for defining *slow gait speed* [17]. We also repeated all analyses using the time to complete the Timed Up and Go (TUG) test as an alternative measure of physical performance. The TUG test (sec) measured the time taken to stand up from a standard chair, walk a distance of 3 m, turn, walk back to the chair and sit down. *More time needed to complete the TUG test* was defined as completing the test in ≥ 13.74 s. This

cut-point corresponds to the same proportion of individuals from the total baseline sample below ≤ 0.8 m/s for the gait speed (14.3%).

Serum 25OHD and PTH Levels

Non-fasting blood samples were taken at baseline between February and November 2009 at the KORA study center, usually between 07:30 am and 11:00 am, and drawn into serum gel S-Monovette tubes (Sarstedt, Nümbrecht, Germany). Blood was gently inverted twice and rested for 30 min at room temperature until complete coagulation. After centrifugation at 15 °C for 10 min, the serum obtained was aliquoted into Nunc cryotubes (Thermo Fisher Scientific, Waltham, MA, USA). For the analysis of both vitamin D and PTH status, serum probes were frozen at − 80 °C at the KORA study center, transported on ice and stored at a minimum of − 80 °C until analysis in partner laboratories. Serum concentrations of 25OHD and PTH were measured by an electrochemiluminescence immunoassay (ECLIA) using the Vitamin D total test (Elecsys 2011, Roche, Germany) and the intact PTH (1–84) test (Elecsys 2011, Roche, Germany), respectively. Intra- and inter-assay coefficients of variations were < 5% and < 10% for 25OHD, and < 5% and < 5% for PTH, respectively. Vitamin D status was categorized as deficiency (< 25 nmol/L), insufficiency (25 to < 50 nmol/L) and sufficiency (≥ 50 nmol/L, reference) [22]. Due to the lack of consistent reference ranges [23], PTH levels were categorized as 1st tertile (< 2.8 pmol/L, reference), 2nd tertile (2.8 to < 3.8 pmol/L) and 3rd tertile (≥ 3.8 pmol/L).

Confounders

To avoid bias by adding covariates to the model that are intermediate factors or common effects, we constructed a directed acyclic graph (DAG) to identify relevant confounders in the association of vitamin D status with sarcopenia [24]. Using the graphical tool DAGitty [25], we identified a minimal adjustment set of covariates needed to estimate the causal effect without confounding: sex, age, Nutrition Score (SCREEN II), physical activity, BMI and use of vitamin D supplements. The variables—sex and age—were collected using the short form of the Demographics Standards of the German Society of Epidemiology [26]. The Nutrition Score, indicating risk of general malnutrition, was calculated using the German short form of the SCREEN II (Seniors in the Community: Risk Evaluation for Eating and Nutrition, version II) questionnaire [27]. Participants were classified as "low risk" (score 41–48), "moderate risk" (score 36 to < 41) or "high risk of malnutrition" (score < 36). Physical activity assessment included frequency and duration of activity in summer and winter [28]. Possible answers were (1) > 2 h/week, (2) 1–2 h/week, (3) < 1 h/week and (4) none. Participants, who had a total score < 5, obtained by summing the numbers (1)–(4) relating to winter and summer, were classified to be "physically active", all others were classified as "inactive". Body mass index (BMI) was defined as body weight (kg) divided by height-squared (m^2). Use of supplements ingested in the last 7 days was collected through a database supported computer software (IDOM, Instrument for Databased Assessment Of Medication) [29], together with the mode, dosage and frequency of ingestion [30]. Vitamin D supplement use was classified as "regular intake" or "no/irregular intake". The DAG with methodology and references can be seen in the Online Resource Fig. 1 (available in *Calcified Tissue International* online). Methods used to collect and categorize other baseline—not DAG confounders—variables (e.g. alcohol intake, smoking status, polypharmacy) have been described elsewhere [31].

Statistical Analysis

Baseline descriptive characteristics were expressed as mean ± standard deviation (SD), median (1st quartile–3rd quartile) or proportion (%) by sarcopenia status at baseline and were compared across categories using one-way ANOVA or Kruskal–Wallis tests for continuous variables and Pearson's χ^2 tests for categorical variables.

In cross-sectional analyses ($n = 975$), multiple logistic regression analyses tested the associations of categorized baseline 25OHD levels with the prevalence of sarcopenia and related muscle parameters, and in prospective analyses ($n = 702$) with the incidence of sarcopenia and related muscle parameters in individuals without the respective condition at baseline. We repeated the prospective analyses using a combined endpoint of incident sarcopenia or death, assuming that many of those who died before the follow-up examination developed sarcopenia prior to death. Results were expressed as odds ratios (OR) with 95% confidence interval (CI). Annual change (%) in each muscle parameter was calculated as: [(follow-up-baseline)/baseline)*(100/time)]. By visual inspection of normal plots (histograms) and Q–Q plots, annual change in each muscle parameter was normally distributed. Multiple linear regression analyses tested the associations of baseline 25OHD with annual changes in muscle parameters. Results were expressed as β-coefficients with 95% CIs.

In all analyses, three adjusted models were fitted: Model 1 was adjusted for age and sex, Model 2 was fully adjusted for DAG confounders: age, sex, Nutrition Score (SCREEN II), physical activity, BMI and use of vitamin D supplements, and Model 3 was fully adjusted plus additional inclusion of PTH tertiles to test the potential mediating effect of PTH. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using the statistical

software package, SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Participant Characteristics

Baseline characteristics of the 975 individuals (49.2% female, mean age 75.7 ± 6.5 years) included in cross-sectional analyses are shown in Table 1 stratified by sarcopenia status.

Overall, 6.7% of individuals had prevalent sarcopenia (2.3% in men, 4.4% in women). When using the TUG test as an alternative to gait speed, the prevalence of sarcopenia minimally decreased to 6.6% (2.3% in men, 4.3% in women). Sarcopenic individuals were more likely to be female, older, to live alone, be divorced or widowed, to be physically inactive, to have a lower BMI and lower 25OHD levels.

Cross-sectional Results

At baseline, individuals with low baseline 25OHD < 25 vs. ≥ 50 nmol/L tended to have higher odds of prevalent sarcopenia in fully adjusted analysis [OR (95% CI) 2.14 (0.98–4.63)]. Additional inclusion of PTH tertiles strengthened the association, which became significant [OR (95% CI): 2.64 (1.16–5.98)]. Vitamin D deficiency was associated with higher odds of low grip strength, slow gait speed and more time needed for the TUG test after multivariable adjustment (Table 2).

Prospective Results

Annual changes in muscle parameters are shown in Table 3 stratified by baseline 25OHD levels. In fully adjusted analysis, individuals with low baseline 25OHD < 25 vs. ≥ 50 nmol/L had a 0.94% greater annual decrease in muscle mass index and a 3.06% greater annual increase in time to complete the TUG test. Vitamin D status was not significantly associated with change in grip strength or gait speed.

After 2.9 ± 0.1 years of follow-up, 4.3% of individuals without sarcopenia at baseline developed incident sarcopenia (2.8% in men; 5.8% in women). The incidence of sarcopenia remained unchanged when using the TUG test as an alternative to gait speed. Lower baseline 25OHD levels were not significantly associated with the risk for developing incident sarcopenia. Adjustment for PTH tertiles slightly attenuated the observed trends (Table 4). When repeating analyses using a combined endpoint of incident sarcopenia or death, 11.5% of individuals developed the outcome. The presence of both vitamin D deficiency and insufficiency were strongly associated with a higher risk for incident sarcopenia or death [deficiency: OR (95% CI) 3.19 (1.54–6.57); insufficiency: OR (95% CI) 1.84 (1.06–3.22)]. Vitamin D insufficiency was associated with a higher risk for developing low muscle mass index after multivariable adjustment (Table 4).

Discussion

In this 3-year prospective study among German older adults, low baseline 25OHD < 25 vs. ≥ 50 nmol/L were significantly associated with changes in muscle mass and physical performance as assessed by the time to complete the TUG test. Vitamin D status was not associated with the risk for developing incident sarcopenia. However, when including death as a combined outcome alongside incident sarcopenia, there was a strong positive association with low baseline 25OHD < 25 vs. ≥ 50 nmol/L. There was no evidence for a mediating effect of PTH.

25OHD and Changes in Muscle Mass and Physical Performance

In previous prospective studies, low 25OHD levels were not linked to change in muscle mass [3, 5, 6]. Some studies have shown, however, an association with loss of muscle strength [5, 6, 8]. Findings on the association between 25OHD and decline in gait speed have relatively consistently shown no significant association [3, 10, 11]. In our study, gait speed increased with a mean annual gain of 2.95 ± 8.12%, possibly due to the selection bias in KORA-Age, whereby more healthy individuals participated at follow-up [32]. However, caution is warranted when interpreting our gait speed variable because a comparable effect was not seen for the TUG test. Studies investigating vitamin D in relation to change in other performance tests, including the TUG test, have shown either no association [9–11] or a greater decline in physical performance among those with low baseline 25OHD [7, 33].

Meta-analyses of randomized controlled trials (RCTs) have shown heterogeneous effects of vitamin D supplementation on muscle parameters. One meta-analysis of 13 trials in older adults aged ≥ 60 years has shown evidence of decreased time to complete the TUG test and gains in lower extremity strength, but no beneficial effect on gait speed [34]. Another larger meta-analysis of 30 trials in individuals with mean age 61.1 years found a small positive effect on muscle strength but not on muscle mass [35]. More recently, a meta-analysis of 15 studies in individuals aged ≥ 65 years demonstrated no improvement in muscle strength after vitamin D administration [36]. Further, RCTs that compare effects of vitamin D supplementation in sarcopenic versus non-sarcopenic participants are needed to confirm the place of vitamin D supplementation in the management of

Table 1 Participant characteristics by sarcopenia status at baseline, $n=975$

	All $n=975$		No sarcopenia $n=910$		Sarcopenia $n=65$		p value
Socio-demographic factors							
Women (%)	480	(49.2)	437	(48.0)	43	(66.2)	**0.005**
Age (years)	75.7	±6.5	75.3	±6.4	81.0	±6.4	**< 0.001**
Categorized (%)							**< 0.001**
65–74	434	(44.5)	422	(46.4	12	(18.5)	
75–84	435	(44.6)	403	(44.3	32	(49.2)	
85 +	106	(10.9)	85	(9.3	21	(32.3)	
Family status (%)							**< 0.001**
Living with a partner	617	(63.8)	590	(65.2)	27	(43.6)	
Living alone, divorced or widowed	350	(36.2)	315	(34.8)	35	(56.5)	
Low educational level (%)	195	(20.0)	183	(20.1)	12	(18.5)	0.748
Lifestyle factors							
Nutrition Score (SCREEN II), %							0.317
Low risk (41 to 48)	381	(39.1)	360	(39.6)	21	(32.3)	
Medium risk (36 to < 41)	350	(35.9)	327	(35.9)	23	(35.4)	
High risk (< 36)	244	(25.0)	223	(24.5)	21	(32.3)	
Physically inactive (%)	442	(45.3)	404	(44.4)	38	(58.5)	**0.028**
Alcohol consumption[a] (%)							**0.008**
Abstainer	340	(34.9)	306	(33.7)	34	(52.3)	
Light-moderate drinker	500	(51.3)	477	(52.5)	23	(35.4)	
High drinker	134	(13.8)	126	(13.9)	8	(12.3)	
Smoking (%)							0.286
Never smoker	555	(56.9)	512	(56.3)	43	(66.2)	
Ex-smoker	373	(38.3)	353	(38.8)	20	(30.7)	
Current smoker	47	(4.8)	45	(5.0)	2	(3.1)	
Health factors							
BMI (kg/m^2)	28.5	±4.3	28.7	±4.2	24.9	±3.1	**< 0.001**
Polypharmacy (≥ 5 medications) (%)	304	(31.2)	284	(31.2)	20	(30.8)	0.941
eGFR < 60 mL/min/1.73 m^{2b} (%)	381	(39.1)	351	(38.6)	30	(46.2)	0.229
Multimorbidity[c] (%)							0.259
No disease	89	(9.2)	86	(9.5)	3	(4.7)	
One disease	251	(25.9)	237	(26.2)	14	(21.9)	
Two or more diseases	629	(64.9)	582	(64.3)	47	(73.4)	
Vitamin D supplements (%)							0.500
Regular use	132	(13.5)	125	(13.7)	7	(10.8)	
No/irregular use	843	(86.5)	785	(86.3)	58	(89.2)	
Calcium supplements (%)							0.642
Regular use	139	(14.3)	131	(14.4)	8	(12.3)	
No/irregular use	836	(85.7)	779	(85.6)	57	(87.7)	
Biological measures							
Season of blood collection (%)							0.278
Jun–Aug	341	(35.0)	324	(35.6)	17	(26.2)	
Sep–Nov	215	(22.1)	200	(22.0)	15	(23.1)	
Feb–May	419	(43.0)	386	(42.4)	33	(50.8)	
25OHD (nmol/L)	48.9	31.9–69.9	49.0	32.7–70.6	41.4	24.4–67.1	**0.025**
Categorized (%)							**0.002**
Sufficiency (≥ 50)	468	(48.0)	443	(48.7)	25	(38.5)	
Insufficiency (25- < 50)	369	(37.9)	348	(38.2)	21	(32.3)	
Deficiency (< 25)	138	(14.2)	119	(13.1)	19	(29.2)	
PTH, pmol/L	3.3	2.5-4.1	3.3	2.5–4.1	3.1	2.5–4.3	0.923

Table 1 (continued)

	All $n=975$		No sarcopenia $n=910$		Sarcopenia $n=65$		p value
Categorized, %							0.466
1st Tertile (< 2.8)	326	(33.4)	302	(33.2)	24	(36.9)	
2nd Tertile (2.8 to < 3.8)	323	(33.1)	306	(33.6)	17	(26.2)	
3rd Tertile (≥ 3.8)	326	(33.4)	302	(33.2)	24	(36.9)	
Hyperparathyroidism (≥ 6.8) (%)	55	(5.6)	50	(5.5)	5	(7.7)	0.458
Sarcopenia-related muscle parameters							
Muscle mass index[d] (kg/m^2)	8.8	± 1.7	8.9	± 1.6	6.6	± 1.3	**< 0.001**
Grip strength (kg)	26.8	± 9.7	27.5	± 9.6	17.9	± 6.6	**< 0.001**
Gait speed[e] (m/s)	1.1	± 0.2	1.1	± 0.2	1.0	± 0.2	**0.0311**
Walking aid[f] (%)	45	(5.0)	43	(5.1)	2	(3.9)	0.695
Time to complete the TUG test[g] (s)	9.8	8.5–11.9	9.7	8.5–11.9	10.6	9.2–12.5	0.060

Statistically significant values are highlighted in bold ($p < 0.05$)

Results expressed as mean ± SD, median (1st quartile-3rd quartile) or proportion (%)

Number of missing values: [a]1; [b]1; [c]6; [d]18; [e]74; [f]74; [g]62

25OHD 25-hydroxyvitamin D, *PTH* parathyroid hormone, *TUG test* Timed Up and Go Test, *SD* standard deviation

Table 2 Cross-sectional associations of baseline 25OHD levels with the prevalence of sarcopenia and related muscle parameters, $n=975$

Outcome at baseline (dichotomized)	25OHD	Prevalence of outcome (%)	n	OR (95% CI)		
				Model 1: Age and sex	Model 2: Fully-adjusted	Model 3: Model 2 + PTH
Sarcopenia	All	6.7	975			
	Deficiency	13.8	138	1.52 (0.76–2.99)	2.14 (0.98–4.63)	**2.64 (1.16–5.98)***
	Insufficiency	5.7	369	0.84 (0.45–1.55)	0.96 (0.49–1.85)	0.99 (0.50–1.93)
	Sufficiency	5.3	468	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)
Low muscle mass index	All	10.3	957			
	Deficiency	15.2	138	1.02 (0.55–1.83)	1.70 (0.82–3.44)	**2.12 (1.00–4.45)***
	Insufficiency	9.1	362	0.76 (0.46–1.23)	0.96 (0.55–1.64)	1.02 (0.56–1.77)
	Sufficiency	9.9	457	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)
Low grip strength	All	38.4	975			
	Deficiency	57.3	138	1.52 (0.99–2.35)	**1.59 (1.00–2.52)***	1.52 (0.95–2.43)
	Insufficiency	38.5	369	1.02 (0.75–1.39)	1.04 (0.75–1.43)	1.02 (0.74–1.41)
	Sufficiency	32.7	468	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)
Slow gait speed	All	13.8	901			
	Deficiency	27.3	110	**2.77 (1.55–4.9)****	**2.33 (1.26–4.30)****	**2.36 (1.26–4.41)****
	Insufficiency	16.6	349	**1.93 (1.23–3.06)****	**1.89 (1.16–3.12)***	**1.90 (1.16–3.14)**
	Sufficiency	8.1	442	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)
More time needed to complete the TUG test	All	13.6	913			
	Deficiency	28.0	118	**3.05 (1.71–5.42)****	**2.31 (1.25–4.28)****	**2.13 (1.13–3.99)***
	Insufficiency	16.5	351	**2.12 (1.34–3.42)****	**1.94 (1.18–3.23)***	**1.97 (1.19–3.29)***
	Sufficiency	7.4	444	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)

Statistically significant values are highlighted in bold ($p < 0.05$)

Results of multiple logistic regression analyses expressed as OR (95% CI): *Model 1* adjusted for sex and age, *Model 2* fully-adjusted for DAG confounders: sex, age, Nutrition Score (SCREEN II), physical activity, BMI and use of vitamin D supplements; *Model 3* fully-adjusted plus PTH tertiles; *$p < 0.05$ versus *ref*, **$p < 0.01$ versus *ref*

25OHD 25-hydroxyvitamin D categorized as deficiency (< 25 nmol/L), insufficiency (25 to < 50 nmol/L) and sufficiency (≥ 50 nmol/L, *ref*), *PTH* parathyroid hormone, *TUG test* Timed Up and Go test, *DAG*, directed acyclic graph, *OR* odds ratio, *CI* confidence interval

Table 3 Prospective associations of baseline 25OHD levels with annual changes in related muscle parameters, $n = 702$

Outcome at follow-up (continuous)	25OHD	% change per year of outcome, mean (±SD)	*n*	β (95% CI)		
				Model 1: age and sex	Model 2: fully adjusted	Model 3: Model 2 + PTH
Muscle Mass Index	All	−0.17% (±2.28)	681			
	Deficiency	−0.89% (±2.98)	75	**−1.03 (−1.61, −0.45)****	**−0.94 (−1.55, −0.34)****	**-0.92 (−1.54, −0.30)***
	Insufficiency	−0.40% (±2.29)	262	**−0.52 (−0.89, −0.15)****	**−0.47 (−0.86, −0.09)***	**−0.46 (−0.84, −0.07)***
	Sufficiency	0.15% (±2.04)	344	0.00 (*ref*)	0.00 (*ref*)	0.00 (*ref*)
Grip strength	All	−0.42% (±7.16)	702			
	Deficiency	−1.10% (±7.38)	76	0.03 (−1.79, 1.85)	0.34 (−1.58, 2.25)	0.46 (−1.48, 2.41)
	Insufficiency	−0.12% (±7.76)	267	0.57 (−0.57, 1.72)	0.71 (−0.48, 1.91)	0.72 (−0.48, 1.93)
	Sufficiency	−0.50% (±6.64)	359	0.00 (*ref*)	0.00 (*ref*)	0.00 (*ref*)
Gait speed	All	2.95% (±8.12)	590			
	Deficiency	2.20% (±6.75)	54	−0.65 (−3.07, 1.76)	−0.71 (−3.23, 1.80)	−0.54 (−3.08, 2.00)
	Insufficiency	3.27% (±8.77)	218	0.58 (−0.84, 2.00)	0.64 (−0.83, 2.11)	0.75 (−0.73, 2.23)
	Sufficiency	2.86% (±7.86)	318	0.00 (*ref*)	0.00 (*ref*)	0.00 (*ref*)
Time to complete the TUG test	All	−0.19% (±8.36)	612			
	Deficiency	3.29% (±13.28)	58	**3.47 (1.14, 5.81)****	**3.06 (0.63, 5.49)***	**3.06 (−0.02, 2.85)****
	Insufficiency	0.68% (±8.05)	229	**1.49 (0.11, 2.88)***	1.42 (−0.02, 2.85)	**1.53 (0.09, 2.98)***
	Sufficiency	−1.42% (±7.14)	325	0.00 (*ref*)	0.00 (*ref*)	0.00 (*ref*)

Statistically significant values are highlighted in bold ($p < 0.05$)

Results of multiple linear regression analyses expressed as β (95% CI). ß coefficient is the mean difference in percentage change per year. *Model 1* adjusted for sex and age; *Model 2* fully adjusted for DAG confounders: sex, age, Nutrition Score (SCREEN II), physical activity, BMI and use of vitamin D supplements; *Model 3* fully adjusted plus PTH tertiles; *$p < 0.05$ versus *ref*, **$p < 0.01$ versus *ref*

25OHD 25-hydroxyvitamin D categorized as deficiency (< 25 nmol/L), insufficiency (25 to < 50 nmol/L) and sufficiency (≥ 50 nmol/L, *ref*), *PTH* parathyroid hormone; *TUG test* Timed Up and Go test, *DAG*, directed acyclic graph, *OR* odds ratio, *CI* confidence interval, *SD* standard deviation

sarcopenia, and to clarify optimal 25OHD levels for muscle health.

25OHD and Incident Sarcopenia or Death

Very few prospective studies have examined vitamin D status in relation to incident sarcopenia. In one study conducted among 433 men aged ≥ 60 years, the risk for developing incident sarcopenia over 4.3 years was not significantly related to baseline 25OHD in multivariable analysis [3]. Another study following a cohort of 709 men aged ≥ 70 years over 5 years showed that baseline 25OHD < 40 vs. ≥ 68.9 nmol/L were significantly associated with increased odds of incident sarcopenia with an OR (95% CI) of 2.53 (1.14–5.64) [4]. Comparison of our results with the findings of these prospective studies are limited due to differences in study populations (only men), sarcopenia definitions, length of follow-up, cut-off values to define low baseline 25OHD levels, and due to diverging confounding adjustment.

A large problem of prospective studies among older cohorts are competing risks, with numerous losses to follow-up due to death. In a previous KORA-Age analysis, low vitamin D status was associated with higher mortality [37]. Assuming that older individuals may often develop sarcopenia before death [38, 39], we tried to address the problem of competing risks by means of a combined endpoint, which included incident cases of sarcopenia and all losses to follow-up due to death. We found a strong positive association of low baseline 25OHD < 25 vs. ≥ 50 nmol/L with this combined outcome, highlighting the importance of considering competing risks such as mortality in older cohorts. Future longitudinal studies should include repeated follow-up examinations after short time periods to identify individuals who develop incident sarcopenia before death.

Possible Mechanisms, Including PTH

Mechanistically, a direct effect of the biologically active form of vitamin D on muscle has been suggested following the localization of a vitamin D receptor expressed on human muscle tissue [13]. Other studies have explored the well-known inverse relationship between serum 25OHD and PTH, showing effects of increased PTH levels on skeletal muscle mass and function [6], and suggesting that hyperparathyroidism secondary to vitamin D deficiency may mediate the effect of vitamin D on muscle [40].

Table 4 Prospective associations of baseline 25OHD levels with the incidence of sarcopenia (or death) and related muscle parameters, $n = 702$

Outcome at follow-up (dichotomized)	25OHD	Incidence of outcome (%)	*n*	OR (95% CI)		
				Model 1: age and sex	Model 2: fully adjusted	Model 3: Model 2 + PTH
Sarcopenia	All	4.3	702			
	Deficient	5.3	76	1.10 (0.29–3.44)	2.38 (0.54–8.99)	1.91 (0.43–7.24)
	Insufficiency	5.2	267	1.35 (0.61–3.05)	2.10 (0.89–5.04)	1.98 (0.83–4.83)
	Sufficiency	3.3	359	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)
Sarcopenia or death	All	11.5	759			
	Deficiency	23.4	94	**2.44 (1.25–4.69)****	**3.19 (1.54–6.57)****	**2.95 (1.40–6.18)****
	Insufficiency	12.5	289	1.51 (0.89–2.57)	**1.84 (1.06–3.22)***	**1.77 (1.01–3.12)***
	Sufficiency	7.7	376	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)
Low Muscle Mass Index	All	7.8	681			
	Deficiency	8.0	75	0.97 (0.34–2.42)	2.52 (0.76–7.59)	2.39 (0.72–7.30)
	Insufficiency	9.2	262	1.24 (0.67–2.27)	**2.01 (1.02–4.01)***	1.94 (0.98–3.90)
	Sufficiency	6.7	344	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)
Low grip strength	All	34.2	702			
	Deficiency	44.7	76	1.14 (0.63–2.01)	1.07 (0.58–1.95)	0.99 (0.53–1.82)
	Insufficiency	38.2	267	1.21 (0.84–1.74)	1.17 (0.80–1.71)	1.13 (0.77–1.66)
	Sufficiency	29.0	359	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)
Slow gait speed	All	6.8	590			
	Deficiency	13.0	54	2.05 (0.68–5.64)	1.67 (0.53–4.85)	1.54 (0.51–4.91)
	Insufficiency	8.7	218	1.66 (0.80–3.52)	1.53 (0.71–3.40)	1.50 (0.68–3.38)
	Sufficiency	4.4	318	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)
More time needed to complete the TUG test	All	10.3	612			
	Deficiency	20.7	58	2.71 (1.15–6.16)	2.02 (0.82–4.83)	1.94 (0.77–4.70)
	Insufficiency	13.1	229	1.83 (1.01–3.39)	1.57 (0.82–3.02)	1.53 (0.79–2.97)
	Sufficiency	6.5	325	1.00 (*ref*)	1.00 (*ref*)	1.00 (*ref*)

Statistically significant values are highlighted in bold ($p < 0.05$)

Results of multiple logistic regression analyses expressed as OR (95% CI): *Model 1*, adjusted for sex and age; *Model 2* fully-adjusted for DAG confounders: sex, age, Nutrition Score (SCREEN II), physical activity, BMI and use of vitamin D supplements; *Model 3* fully adjusted plus PTH tertiles; *$p < 0.05$ versus *ref*, **$p < 0.01$ versus *ref*

25OHD 25-hydroxyvitamin D categorized as deficiency (< 25 nmol/L), insufficiency (25 to < 50 nmol/L) and sufficiency (≥ 50 nmol/L, *ref*), *PTH* parathyroid hormone, *TUG test* Timed Up and Go test, *DAG* directed acyclic graph, *OR* odds ratio, *CI* confidence interval

In the present study, adjustment for PTH tertiles strengthened the association of 25OHD with sarcopenia in cross-sectional analyses, but only slightly attenuated it in prospective analyses. This suggests that a meaningful mediating effect of PTH was unlikely, though the number of participants with hyperparathyroidism was very small. Of note, there may be other indirect biological pathways mediating the effect of vitamin D on muscle, including hypocalcemia [14] or hypophosphatemia [15]. Further mechanistic studies are required to better understand the mechanisms by which 25OHD levels may influence sarcopenia and its onset.

Strengths and Limitations

Strengths include the use of a large, broadly representative, sample of community-dwelling German older adults with prospective data. Because reasons for non-participation were systematically recorded, we were able to incorporate withdrawal due to death as a combined outcome with incident sarcopenia. We have included 25OHD levels in addition to a wide range of covariates trough DAG modeling, thereby minimizing confounding in examining the causal association between vitamin D status and sarcopenia. Nonetheless residual confounding cannot be entirely excluded. Furthermore, we could not account for the effects of change in 25OHD levels on change in muscle parameters, because 25OHD levels were only measured at baseline. The power of the present study was limited especially regarding analyses on incident sarcopenia due to the low incidence in our rather healthy study population. While the EWGSOP allows use of BIA in conjunction with the Janssen multiple regression prediction equation for estimation of the skeletal muscle mass [17], we recognize that this approach should be used with caution because estimates of skeletal muscle mass can be artificially

elevated due to altered hydration, including fluid accumulation and hypohydration [41]. Furthermore, PTH levels are subject to diurnal rhythms and influenced by the ingestion of calcium-containing foods, hence PTH results may have been affected by the non-fasting status of KORA-Age participants and possible batch effects associated with sampling across extended time periods. Accurate interpretation and comparison of PTH results across studies may also be hampered by the lack of standardization and robust reference ranges due to variable analytical methods used for its determination [23].

Conclusion

In conclusion, our finding suggests that a low vitamin D status may be an early risk factor for changes in muscle mass and physical performance in older adults. Replenishing 25OHD levels may be important for the preservation of specific sarcopenia-related muscle parameters but further randomized trials are needed to assess whether the observed associations are causal and to determine optimal 25OHD levels for muscle health. We could not demonstrate a statistically significant association of low vitamin D status with incident sarcopenia, but a significant association was found with the combined endpoint of sarcopenia and death. This highlights the need for future well-designed prospective studies that address the issue of competing risks such as mortality in older cohorts.

Acknowledgements We thank all participants of the KORA-Age study for their extraordinary commitment and goodwill. The KORA study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. The KORA-Age Project was financed by the German Federal Ministry of Education and Research (BMBF FKZ 01ET0713 and 01ET1003A) as part of the 'Health in old age' program. RC is supported by a PhD scholarship from the 'Studienstiftung des deutschen Volkes'. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions RC and BT conceived and designed the study. RC analyzed the data and wrote the first draft of the paper. EV, HBF and BT, as members of the PhD Thesis Committee of RC, helped in interpreting the data. All authors revised the paper critically for intellectual content and approved the final version. All authors agree to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

Compliance with Ethical Standards

Conflicts of interest Romy Conzade, Eva Grill, Heike A. Bischoff-Ferrari, Uta Ferrari, Alexander Horsch, Wolfgang Koenig, Annette Peters and Barbara Thorand declare that they have no conflict of interest.

Human and Animal Rights The KORA-Age was approved by the Ethics Committee of the Bavarian Medical Association (Reference Number 08064).

Informed Consent Written informed consent has been obtained from the participants and all investigations have been conducted according to the principles expressed in the Helsinki Declaration.

References

1. Sayer AA, Robinson SM, Patel HP, Shavlakadze T, Cooper C, Grounds MD (2013) New horizons in the pathogenesis, diagnosis and management of sarcopenia. Age Ageing 42:145–150
2. Bischoff-Ferrari HA (2012) Relevance of vitamin D in muscle health. Rev Endocr Metab Disord 13:71–77
3. Gielen E, O'Neill TW, Pye SR, Adams JE, Wu FC, Laurent MR, Claessens F, Ward KA, Boonen S, Bouillon R et al (2015) Endocrine determinants of incident sarcopenia in middle-aged and elderly European men. J Cachexia Sarcopenia Muscle 6:242–252
4. Hirani V, Cumming RG, Naganathan V, Blyth F, Le Couteur DG, Hsu B, Handelsman DJ, Waite LM, Seibel MJ (2017) Longitudinal associations between vitamin D metabolites and sarcopenia in older australian men: the concord health and aging in men project. J Gerontol A 73:131–138
5. Scott D, Blizzard L, Fell J, Ding C, Winzenberg T, Jones G (2010) A prospective study of the associations between 25-hydroxyvitamin D, sarcopenia progression and physical activity in older adults. Clin Endocrinol (Oxf) 73:581–587
6. Visser M, Deeg DJ, Lips P (2003) Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. J Clin Endocrinol Metab 88:5766–5772
7. Wicherts IS, van Schoor NM, Boeke AJ, Visser M, Deeg DJ, Smit J, Knol DL, Lips P (2007) Vitamin D status predicts physical performance and its decline in older persons. J Clin Endocrinol Metab 92:2058–2065
8. Granic A, Hill TR, Davies K, Jagger C, Adamson A, Siervo M, Kirkwood TB, Mathers JC, Sayer AA (2017) Vitamin D status, muscle strength and physical performance decline in very old adults: a prospective study. Nutrients 9:379
9. Bartali B, Frongillo EA, Guralnik JM, Stipanuk MH, Allore HG, Cherubini A, Bandinelli S, Ferrucci L, Gill TM (2008) Serum micronutrient concentrations and decline in physical function among older persons. JAMA 299:308–315
10. Houston DK, Tooze JA, Neiberg RH, Hausman DB, Johnson MA, Cauley JA, Bauer DC, Cawthon PM, Shea MK, Schwartz GG et al (2012) 25-hydroxyvitamin D status and change in physical performance and strength in older adults: the Health, Aging, and Body Composition Study. Am J Epidemiol 176:1025–1034
11. Verreault R, Semba RD, Volpato S, Ferrucci L, Fried LP, Guralnik JM (2002) Low serum vitamin d does not predict new disability or loss of muscle strength in older women. J Am Geriatr Soc 50:912–917
12. Ceglia L, Harris SS (2013) Vitamin D and its role in skeletal muscle. Calcif Tissue Int 92:151–162
13. Ceglia L, da Silva Morais M, Park LK, Morris E, Harris SS, Bischoff-Ferrari HA, Fielding RA, Dawson-Hughes B (2010) Multistep immunofluorescent analysis of vitamin D receptor loci and myosin heavy chain isoforms in human skeletal muscle. J Mol Histol 41:137–142
14. Wassner SJ, Li JB, Sperduto A, Norman ME (1983) Vitamin D Deficiency, hypocalcemia, and increased skeletal muscle degradation in rats. J Clin Invest 72:102–112
15. Schubert L, DeLuca HF (2010) Hypophosphatemia is responsible for skeletal muscle weakness of vitamin D deficiency. Arch Biochem Biophys 500:157–161

16. Peters A, Doring A, Ladwig KH, Meisinger C, Linkohr B, Autenrieth C, Baumeister SE, Behr J, Bergner A, Bickel H et al (2011) Multimorbidity and successful aging: the population-based KORA-Age study. Z Gerontol Geriatr 44(Suppl 2):41–54
17. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM et al (2010) Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on sarcopenia in older people. Age Ageing 39:412–423
18. Kyle UG, Genton L, Karsegard L, Slosman DO, Pichard C (2001) Single prediction equation for bioelectrical impedance analysis in adults aged 20–94 years. Nutrition 17:248–253
19. Janssen I, Heymsfield SB, Baumgartner RN, Ross R (1985) Estimation of skeletal muscle mass by bioelectrical impedance analysis. J Appl Physiol 2000(89):465–471
20. Phillips A, Strobl R, Vogt S, Ladwig KH, Thorand B, Grill E (2017) Sarcopenia is associated with disability status-results from the KORA-Age study. Osteoporos Int 28:2069–2079
21. CIR Systems Inc. (2006) The GAITRite electronic walkway measurements and definitions. *Haverton, CIR Systems*
22. German Nutrition Society (2012) New reference values for vitamin D. Ann Nutr Metab 60:241–246
23. Cavalier E, Delanaye P, Nyssen L, Souberbielle JC (2015) Problems with the PTH assays. Ann Endocrinol (Paris) 76:128–133
24. Greenland S, Pearl J, Robins JM (1999) Causal diagrams for epidemiologic research. Epidemiology 10:37–48
25. Textor J, Hardt J, Knuppel S (2011) DAGitty: a graphical tool for analyzing causal diagrams. Epidemiology 22:745
26. Ahrens W, Bellach B-M, Jöckel K-H (1998) Measurement and quantification of sociodemographic characteristics in epidemiological studies. German Society for Medical Informatics, Biometry and Epidemiology (GMDS), Osnabrück
27. Keller HH, Goy R, Kane SL (2005) Validity and reliability of SCREEN II (Seniors in the community: risk evaluation for eating and nutrition, Version II). Eur J Clin Nutr 59:1149–1157
28. Meisinger C, Lowel H, Thorand B, Doring A (2005) Leisure time physical activity and the risk of type 2 diabetes in men and women from the general population. The MONICA/KORA Augsburg Cohort Study. Diabetologia 48:27–34
29. Mühlberger N, Behrend C, Stark R, Holle R (2003) Database-supported identification and entry of drug data in health studies—experience with the IDOM software. Informatik Biometrie und Epidemiologie in Medizin und Biologie 34:601–611
30. Schwab S, Heier M, Schneider A, Fischer B, Huth C, Peters A, Thorand B (2014) The use of dietary supplements among older persons in southern Germany—results from the KORA-age study. J Nutr Health Aging 18:510–519
31. Conzade R, Koenig W, Heier M, Schneider A, Grill E, Peters A, Thorand B (2017) Prevalence and predictors of subclinical micronutrient deficiency in german older adults: results from the population-Based KORA-Age Study. Nutrients 9:1276
32. Holle R, Hochadel M, Reitmeir P, Meisinger C, Wichmann HE (2006) Prolonged recruitment efforts in health surveys: effects on response, costs, and potential bias. Epidemiology 17:639–643
33. Dam TT, von Muhlen D, Barrett-Connor EL (2009) Sex-specific association of serum vitamin D levels with physical function in older adults. Osteoporos Int 20:751–760
34. Muir SW, Montero-Odasso M (2011) Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. J Am Geriatr Soc 59:2291–2300
35. Beaudart C, Buckinx F, Rabenda V, Gillain S, Cavalier E, Slomian J, Petermans J, Reginster JY, Bruyere O (2014) The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. J Clin Endocrinol Metab 99:4336–4345
36. Rosendahl-Riise H, Spielau U, Ranhoff AH, Gudbrandsen OA, Dierkes J (2017) Vitamin D supplementation and its influence on muscle strength and mobility in community-dwelling older persons: a systematic review and meta-analysis. J Hum Nutr Diet 30:3–15
37. Vogt S, Decke S, de Las Heras Gala T, Linkohr B, Koenig W, Ladwig KH, Peters A, Thorand B (2015) Prospective association of vitamin D with frailty status and all-cause mortality in older adults: Results from the KORA-Age Study. Prev Med 73:40–46
38. Kelley GA, Kelley KS (2017) Is sarcopenia associated with an increased risk of all-cause mortality and functional disability? Exp Gerontol 96:100–103
39. Beaudart C, Zaaria M, Pasleau F, Reginster JY, Bruyere O (2017) Health outcomes of sarcopenia: a systematic review and meta-analysis. PLoS ONE 12:e0169548
40. de Souza Genaro P, de Medeiros Pinheiro M, Szejnfeld VL, Martini LA (2015) Secondary hyperparathyroidism and its relationship with sarcopenia in elderly women. Arch Gerontol Geriatr 60:349–353
41. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gomez J, Lilienthal Heitmann B, Kent-Smith L, Melchior JC, Pirlich M et al (2004) Bioelectrical impedance analysis-part II: utilization in clinical practice. Clin Nutr 23:1430–1453

3. Publication: Changes in Nutritional Status and Musculoskeletal Health in a Geriatric Post-Fall Care Plan Setting

Title: Changes in Nutritional Status and Musculoskeletal Health in a Geriatric Post-Fall Care Plan Setting

Authors: Romy Conzade, Steven Phu, Sara Vogrin, Ebrahim Bani Hassan, Walter Sepúlveda-Loyola, Barbara Thorand and Gustavo Duque

Journal: *Nutrients*

Year: 2019

Volume: 11

DOI: 10.3390/nu11071551

Supplements: https://www.mdpi.com/2072-6643/11/7/1551/s1 (available online)

Year: 2019

Impact factor: 4.171 (Journal Citation Reports® 2018)

Rank: 16/86 in Category Nutrition & Dietetics (Journal Citation Reports® 2018)

nutrients

MDPI

Article

Changes in Nutritional Status and Musculoskeletal Health in a Geriatric Post-Fall Care Plan Setting

Romy Conzade [1,2,3], Steven Phu [1,2], Sara Vogrin [1,2], Ebrahim Bani Hassan [1,2], Walter Sepúlveda-Loyola [1,2,4], Barbara Thorand [3] and Gustavo Duque [1,2,*]

1 Australian Institute for Musculoskeletal Science (AIMSS), The University of Melbourne and Western Health, St. Albans, Victoria 3021, Australia
2 Department of Medicine—Western Health, Melbourne Medical School, The University of Melbourne, St. Albans, Victoria 3021, Australia
3 Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), 85764 Neuherberg, Germany
4 Department of Physiotherapy, Londrina State University (UEL) and University North of Paraná (UNOPAR), Londrina, Paraná 86041-120, Brazil
* Correspondence: gustavo.duque@unimelb.edu.au; Tel.: +61-3-8395-8121

Received: 25 May 2019; Accepted: 5 July 2019; Published: 9 July 2019

Abstract: Understanding how changes in nutritional status influence musculoskeletal recovery after falling remains unclear. We explored associations between changes in nutritional status and musculoskeletal health in 106 community-dwelling older adults aged ≥65 years, who attended the Falls and Fractures Clinic at Sunshine Hospital in St Albans, Australia after falling. At baseline and after 6 months, individuals were assessed for Mini Nutritional Assessment (MNA®), grip strength, gait speed, Timed Up and Go (TUG) test, Short Physical Performance Battery (SPPB), and bone turnover marker levels. Associations were examined using multiple linear regression, adjusted for baseline covariates and post-fall care plans. Over 6 months, the prevalence of malnutrition or risk thereof decreased from 29% to 15% using MNA <24/30. Specifically, 20 individuals (19%) improved, 7 (7%) deteriorated, and 73 (69%) maintained nutritional status, including 65 (61%) who remained well-nourished and 8 (8%) who remained malnourished/at risk. A 1-point increase in MNA score over 6 months was associated with an increase of 0.20 points (95% confidence interval 0.10, 0.31, $p < 0.001$) in SPPB score. Improvement in nutritional status was associated with improvement in physical performance, providing a basis for interventional studies to ascertain causality and evaluate nutritional models of care for post-fall functional recovery in older adults.

Keywords: malnutrition; MNA; nutrition; physical performance; bone turnover; sarcopenia; osteosarcopenia; falls; elderly; prospective

1. Introduction

About a third of community-dwelling older adults aged ≥65 years in Western countries fall each year and the frequency of falls and fall-related injuries (fractures or head trauma) increase with age [1]. Falls increase the risk of hospitalization and nursing home admission, as well as morbidity and mortality [2]. Investigating modifiable risk factors of falls is a key priority area for healthcare systems, which strive to identify conditions that prevent falls.

Poor nutritional status has been considered an important modifiable risk factor for falls [3]. Compared to well-nourished older adults, risk of experiencing falls has been shown in a meta-analysis of prospective studies to be 45% higher in malnourished individuals or those at risk of malnutrition ($n = 9510$) [4], based on the validated Mini Nutritional Assessment (MNA®) tool [5]. In an interventional

study by Swanenburg et al., the combination of a three-month calcium/vitamin D supplementation plus protein/exercise was associated with a 89% reduction in the rate of falls over 12 months compared to only calcium/vitamin D supplementation in older women aged ≥65 years with low mineral density (n = 20) [6]. Altogether, these findings suggest that nutritional status should be properly considered when assessing the risk of falls in community-dwelling older adults; yet it is currently not included in most fall risk screening tools [7].

Poor nutritional status can be a consequence of underlying comorbid conditions [8], which may increase the risk of falls due to clinical and adverse effects on cognitive, functional, and physical performance [9]. Poor nutritional status due to inadequate nutritional intake, especially of proteins, can also be detrimental for maintaining the integrity and function of skeletal muscle and bone [10–12], possibly increasing the risk for sarcopenia, osteoporosis or both [13–16]. Sarcopenia-associated risk of falling and increased bone vulnerability have a synergistic impact on falls and fractures occurrence [17,18]. The impact of nutritional status on the risk of falls can thus be explored through the pathway of musculoskeletal health as an important contributor to falls risk [19,20].

Evidence suggests that malnutrition based on MNA is able to predict musculoskeletal decline in various healthcare settings [21–23], but the relationship between nutritional changes and musculoskeletal outcomes remains under-researched [24], in particular among those who fall. Improving knowledge about how nutritional changes may influence relevant musculoskeletal outcomes might be important to effective targeting of multidisciplinary post-fall interventions for older adults living in the community. This study aimed to investigate changes in nutritional status in older adults with a history of falling using the validated MNA®, and to determine associations between changes in nutritional status and relevant musculoskeletal outcomes. We hypothesized that improvement in nutritional status is associated with greater musculoskeletal recovery.

2. Materials and Methods

2.1. Study Design and Individuals

This retrospective observational study examined associations between changes in nutritional status and musculoskeletal outcomes among community-dwelling older adults who attended the Falls and Fractures Clinic at the Australian Institute for Musculoskeletal Science (Western Health-Sunshine Hospital) in St Albans, VIC, Australia. A multidisciplinary team at the clinic, including a geriatrician, a fracture liaison nurse, an accredited exercise physiologist, and a bone densitometrist, provides comprehensive care for older adults with a history of more than two falls in the previous year, or a single fall with established gait and/or balance problem, and/or clinical or radiological risk of falls and/or fractures. We analyzed information from baseline attendance between October 2016 and December 2018 and from follow-up attendance after a median time of 6 months (interquartile range (Q1–Q3) 6–8 months). All measurements obtained were part of standard care practices at this health service. The Western Health Low Risk Ethics Panel approved the registration of the Falls and Fractures Clinic Databank (DB2017.13, date of approval 23 October 2018) and the research protocol of the present study (QA2018.90_48118, date of approval 5 December 2018). Participant consent was waived due to use of de-identified data collected as part of standard care at the clinic and due to the low risk nature of the study beyond the initial consent to attend the clinic.

2.2. Demographic and Clinical Measures

Demographic data was obtained from the patient medical record including age, gender, and residential location. Comprehensive clinical assessment was performed by the geriatrician and the nurse as part of routine care practices on clinic attendance including comorbidities, family history, fracture history, osteoporosis risk assessment (e.g., hormone replacement therapy, menopause age, smoking, alcohol), falls risk (e.g., hearing and visual deficit, altered elimination, impaired mobility), assessment for postural drop, and list of current medications. For the purpose of this study,

a Charlson age-comorbidity index (CACI) was generated, with an index of ≥5 being suggestive of severe comorbidity [25]. The CACI calculation is explained in Supplementary Table S1. Polypharmacy was defined as use of ≥5 prescribed or regularly taken medications, including drugs and dietary supplements. Depression was screened using the Short Form Geriatric Depression Scale (GDS), with a score of ≥6/15 points considered as "suggestive of depression" [26].

2.3. Nutritional Status

Nutritional status was evaluated by the nurse using the Mini Nutritional Assessment (MNA®), which is a validated screening and assessment tool for older adults in community and hospital settings. The full MNA consists of 18 items (6 questions in the screening part, also called the MNA Short-Form (MNA-SF), and 12 questions in the assessment part) capturing anthropometric measures, dietary intake, appetite, general health, and mobility [5]. The screening part first identifies older adults as "well-nourished" (MNA-SF ≥ 12/14) or "at nutritional risk" (MNA-SF < 12/14), so that the full MNA is performed only if an individual is "at nutritional risk". The full categorized MNA then classifies individuals into "malnourished" (MNA < 17/30), "at risk of malnutrition" (17/30 ≤ MNA < 24/30) or "well nourished" (MNA ≥ 24/30) [5]. To calculate a full continuous MNA score, individuals with MNA-SF ≥ 12/14 in the screening part were adjusted into a full MNA score (MNA-SF + 16 points) to obtain a full score ranging 0–30 points. Weight and height were measured using standardized scales to the nearest 0.1 kg and 0.01 m, respectively.

2.4. Biochemical Measures

Fasting venous blood was collected for the measurement of serum albumin, 25-hydroxyvitamin D (25OHD), parathyroid hormone (PTH), hemoglobin, and C-terminal telopeptide of type 1 collagen (CTx). Serum albumin, and hemoglobin levels were determined using automated standard laboratory methods. Serum 25OHD levels were measured by chemiluminescence immunoassay on a LIAISON® XL analyzer (DiaSorin S.p.A., Saluggia, Italy). Circulating intact PTH was measured by immunochemoluminometric assay performed on ADVIA Centaur® (Siemens Healthcare Diagnostics, Deerfield, MA, USA). Serum CTx levels were measured by electrochemiluminescence immunoassay on a Cobas® 6000 analyzer (Roche Diagnostics International Ltd, Rotkreuz Switzerland). Cut-off values for subnormal levels were 25OHD < 75 nmol/L [27], PTH > 6.9 pmol/L, and hemoglobin <130 g/L (men), <120 g/L (women). Estimated-glomerular filtration rate (eGFR) was calculated from serum creatinine as an indicator of renal function (MDRD formula [28]), with subnormal cut-off values of <60 mL/min/1.73 m^2. All measurements were performed at the pathology networks affiliated with the Western Health-Sunshine Hospital in St. Albans, Australia.

2.5. Post-Fall Care Plan

After review of the results of the complete assessment of the individuals' risk of falls and fractures by the multi-disciplinary team, individuals were provided with individualized care plans that included pharmacological (e.g., osteoporosis treatment, vitamin D supplements, protein supplements), and non-pharmacological recommendations (e.g., nutrition advice, physical exercise), with a focus on preventing new or recurrent episodes of falls and/or osteoporotic fractures. The care plan was patient-centered through consideration of the risk assessment, individual patient circumstances, and preferences. In consultation with their local general practitioners, individuals were involved in the management of their respective care plan. The current study looks into changes in nutritional status and musculoskeletal components over a period of 6 months.

2.6. Musculoskeletal Outcome Measures

Musculoskeletal outcome measures were evaluated by the exercise physiologist as part of standard patient assessment on attendance at baseline and 6-month follow-up. Grip strength (kg) was measured with a handheld JAMAR hydraulic dynamometer (Sammons Preston Inc., Bolingbrook, IL, USA).

Individuals had to squeeze the device as hard as possible 3 times in each hand; the highest value was recorded. Gait speed (m/sec) was evaluated using a sensitive walkway (GAITRite system, 16' model, CIR Systems Inc., Havertown, PA, USA), which recorded spatiotemporal gait speed over 4.8 m with individuals walking at usual speed. The best result of two trials was considered. The Timed Up and Go (TUG) test (sec) measured the time taken to stand up from a standard chair, walk a distance of 3 m, turn, walk back to the chair, and sit down again [29]. The Short Physical Performance Battery (SPPB) is a group of measures that combines the results of the gait speed, chair stand, and balance tests [30]. We included serum CTx levels (assessment described under biochemical measures) as a measure of bone turnover.

2.7. Osteopenia/Osteoporosis and Sarcopenia

Body composition and areal bone mineral density (BMD) at three sites (lumbar spine, total hip, and femoral neck) were assessed by the bone densitometrist using a Horizon dual energy X-ray absorptiometry (DXA) machine (Hologic Inc., Bedford, MA, USA). DXA scans were only performed at baseline, and osteopenia/osteoporosis was defined as a BMD T-score <−1.0 SD on at least one of the three regions. As recommended by the Australian and New Zealand Society for Sarcopenia and Frailty Research (ANZSSFR) [31], sarcopenia was defined according to the EWGSOP 2010 definition by fulfillment of low height-adjusted appendicular lean mass (ALM/height^2) combined with low grip strength or slow gait speed [32]. (ALM/height^2) was calculated automatically by the DXA machine. We applied the EWGSOP cut-offs for low ALM/height^2: ≤7.26 kg/m^2 (♂), ≤5.5 kg/m^2 (♀); for low grip strength: <30 kg (♂), <20 kg (♀) and for slow gait speed: ≤0.8 m/sec [32]. Osteosarcopenia was defined as the simultaneous presence of osteopenia/osteoporosis and sarcopenia.

2.8. Statistical Analysis

Statistical analysis was performed using SAS, version 9.4 (SAS Institute Inc., USA). Statistical significance was based on a two-sided p-value <0.05. Normality was assessed using the Shapiro-Wilk test. Mean (standard deviation (SD)) or median (25th percentile (Q1), and 75th percentile (Q3)) were reported for continuous data, and number (percentage (%)) for categorical data. Change (Δ) in continuous variables was calculated as the difference between follow-up and baseline, e.g., ΔMNA = [MNA score (follow-up)—MNA score (baseline)]. To compare baseline and follow-up results, differences were tested using paired t-test or Wilcoxon signed-rank test for normally-distributed or not normally-distributed paired samples, respectively.

As proof of concept, multiple linear regression analyses were first performed to test the cross-sectional associations between baseline MNA score and baseline musculoskeletal outcomes. Analyses were adjusted for baseline variables (age, sex, GDS, CACI, and number of medications—all continuous except sex).

For main analysis, individuals were divided into four subgroups based on change in MNA category from baseline to follow-up: (1) Improved nutritional status from baseline to follow-up; (2) Deteriorated; (3) Maintained but remained malnourished or at risk of malnutrition; (4) Maintained and remained well-nourished (reference group). Comparison of clinical, biochemical and musculoskeletal outcome measures between the subgroups vs. the reference group was analyzed using t-test or Wilcoxon rank-sum test for normally-distributed or not normally-distributed variables, respectively.

To explore the longitudinal associations between changes in nutritional status and musculoskeletal outcomes, multiple linear regression analyses were performed for each of the musculoskeletal outcome. Change in nutritional status was considered as both continuous (ΔMNA) and categorized exposure. Analyses were adjusted for the baseline outcome, baseline variables (age, sex, GDS, CACI, and number of medications—all continuous except sex), and care plan variables (osteoporosis treatment, vitamin D supplements use, protein supplements use, and physical activity—all categorical). When change in nutritional status was used as continuous exposure (ΔMNA), analyses were additionally adjusted for baseline MNA score. To avoid deletion of information-rich participants, missing values for four

binary variables were coded as a separate category. Scatter and residual plots were examined to determine if ΔMNA was related to musculoskeletal changes in a linear manner and if the errors components were independent, homogenous with respect to the variance, and had a mean of zero. If these assumptions were violated, the outcome and/or independent variables were log-transformed to ensure good model fit.

To control for multiple testing, we ranked our hypotheses. Our primary hypothesis is that improvement in nutritional status is associated with greater musculoskeletal recovery. Our secondary hypotheses are that individuals who deteriorated and remained malnourished or at risk of malnutrition are associated with poorer musculoskeletal recovery. As such, *p*-values from multiple linear regression analyses were interpreted in the view of multiple comparisons. If the *p*-value was fairly large ($0.01 \leq p < 0.05$), we did not interpret them as definitely true, but considered that they may be likely false positive, while very small *p*-values ($p < 0.01$ and $p < 0.001$) were interpreted as likely real findings.

3. Results

3.1. Descriptive Characteristics, Including Change in Nutritional Status

Out of 254 patients screened at the Falls and Fractures Clinic between October 2016 and December 2018, 106 (76% female) consecutive individuals with median age of 79 (Q1, Q3 72, 82) years were re-assessed at 6-month follow-up. Table 1 presents descriptive characteristics of this study sample. On attendance at baseline, polypharmacy and severe comorbidity were quite prevalent (67% taking ≥5 medications and 45% with a CACI ≥5). The median number of reported falls in the past year was 2 falls. Most individuals (92%) were osteopenic/osteoporotic, and 22% were sarcopenic. On attendance at follow-up, the median number of reported falls in the past 6 months decreased to 0 falls. Moreover, 91 (86%) individuals reported using vitamin D supplements and 5 (5%) protein supplements, 70 (66%) reported having an osteoporosis treatment, and 51 (48%) reported being physically active, as part of the post-fall care plans recommended.

Table 1. Descriptive characteristics of the study sample, including change in nutritional status.

Characteristic	Descriptive Statistics	All (*n* = 106)
Baseline		
Age, year	Median (Q1, Q3)	79 (72, 82)
Female	*n* (%)	80 (75.5)
BMI at baseline, kg/m^2	Median (Q1, Q3)	27.8 (23.8, 31.9)
Weight at baseline, kg	Median (Q1, Q3)	69.6 (58.5, 85.0)
Height, m	Mean (SD)	1.59 (0.09)
Current smoker [g]	*n* (%)	11 (10.4)
Number of falls (past 12 months)	Median (Q1, Q3)	2 (1, 2)
Number of fractures (past 5 years)	Median (Q1, Q3)	1 (1, 1)
Severe comorbidity (CACI ≥5)	*n* (%)	48 (45.3)
Polypharmacy (≥5 medications)	*n* (%)	71 (67.0)
Suggestive of depression [a] (GDS ≥6/15)	*n* (%)	26 (24.5)
Osteopenia/osteoporosis [c]	*n* (%)	97 (91.5)
Sarcopenia [b]	*n* (%)	22 (20.8)
Osteosarcopenia [b]	*n* (%)	22 (20.8)
Nutritional status		
MNA at baseline, score	Median (Q1, Q3)	29 (23, 30)
Malnourished or at risk at baseline (MNA <24/30)	*n* (%)	31 (29.3)

Table 1. *Cont.*

Characteristic	Descriptive Statistics	All (n = 106)
Follow-up		
ΔBMI [h], kg/m^2	Median (Q1, Q3)	0.6 (−0.2, 1.5) *
Δweight [f], kg	Median (Q1, Q3)	0.6 (−1.0, 3.0) *
Number of falls [d] (past 6 months)	Median (Q1, Q3)	0 (0, 1)
Number of fractures [d] (past 6 months)	Median (Q1, Q3)	0 (0, 0)
Osteoporosis treatment [e]	*n* (%)	70 (66.0)
Vitamin D supplement use [e]	*n* (%)	91 (85.9)
Protein supplement use [e]	*n* (%)	5 (4.7)
Physically active [j]	*n* (%)	51 (48.1)
Nutritional status		
ΔMNA [d], score	Median (Q1, Q3)	0 (0, 3.3) *
Malnourished or at risk at follow-up [d] (MNA <24/30)	*n* (%)	16 (15.1)

Q1, Q3 = 25th, 75th percentile; CACI = Charlson age-comorbidity index; GDS = Geriatric Depression Scale; MNA= Mini Nutritional Assessment; BMI = body mass index; number of missing values: [a] 1, [b] 3, [c] 5, [d] 6, [e] 10, [f] 14 [g] 15, [h] 16, [j] 27; *: $p < 0.05$ for paired *t*-test or Wilcoxon signed-rank test for the difference between baseline and follow-up values.

The prevalence of malnutrition or risk of malnutrition based on an MNA score <24/30 was 29% at baseline and 15% at 6-month follow-up. Most individuals maintained or improved nutritional status with at least 75% not having a decrease in MNA score (median change of 0.0 (0.0, 3.3) points, $p = 0.001$) (Table 1). Specifically, 73 individuals (69%) maintained nutritional status, including 65 (61%) who remained well-nourished and 8 (8%) who remained malnourished or at risk of malnutrition. Moreover, 20 individuals (19%) improved and 6 individuals (7%) deteriorated nutritional status, as illustrated in Figure 1.

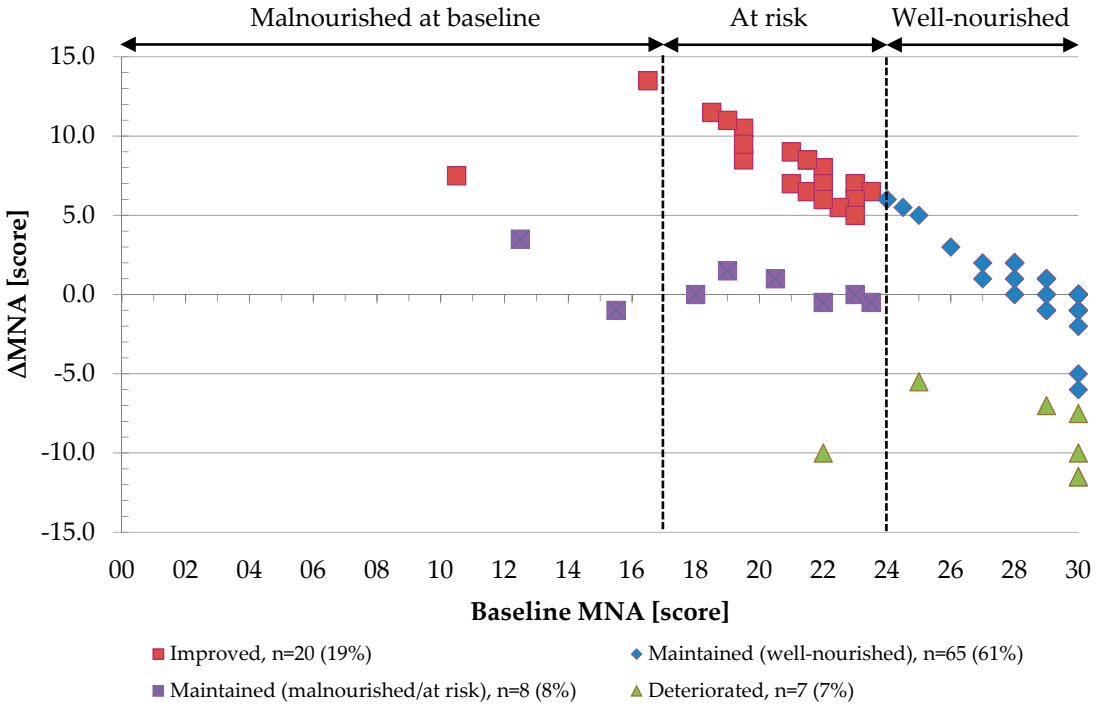

Figure 1. Change in MNA score (ΔMNA) by baseline MNA score across subgroups of nutritional status change. One dot represents one individual. Six participants could not be represented due to missing value in MNA score at follow-up.

3.2. Nutritional Status and Musculoskeletal Health at Baseline

In age-sex adjusted analyses, a 1-point increase in baseline MNA score was associated with an increase of 0.02 m/sec (95% CI 0.00, 0.03, $p = 0.022$) in gait speed and of 0.14 points (95% CI 0.05, 0.23, $p = 0.003$) in SPPB score. Additional adjustment for baseline variables weakened the associations, which became non-significant (Supplementary Table S2).

3.3. Changes in Nutritional Status and Musculoskeletal Health

Table 2 compares clinical, biochemical and musculoskeletal measures between subgroups of nutritional status change using the maintained (well-nourished) nutritional status group as reference group. The reference group was associated with significant increase in BMI ($p = 0.002$), weight ($p = 0.031$), 25OHD levels ($p = 0.013$), gait speed ($p < 0.001$), SPPB score ($p = 0.008$) and decrease in CTx levels ($p < 0.001$). Individuals who improved nutritional status were associated with greater increase in BMI ($p = 0.002$) and weight ($p = 0.006$) compared to the reference group, and similar increase in SPPB score ($p = 0.177$). They were not associated with significant improvement in gait speed ($p = 0.193$), but their TUG time significantly decreased ($p = 0.001$). Those who maintained (malnourished/at risk) or deteriorated nutritional status were not linked to significant improvement in any of the variables.

In multiple linear regression analyses (Table 3), change in nutritional status over 6 months showed the strongest associations with SPPB. After adjusting for baseline and care plan covariates, a 1-point increase in MNA score over 6 months was associated with an increase of 0.20 points (95% CI 0.10, 0.31, $p < 0.001$) in SPPB score. In subgroup analyses, individuals who improved nutritional status had for 3.30 sec (95% CI −6.34, −0.26, $p = 0.033$) a larger decrease in time for the TUG test compared to the reference group. Conversely, those who deteriorated in nutritional status had a larger decrease in SPPB score by 1.74 points (95% CI −3.29, −0.20, $p = 0.028$).

Table 2. Comparison of clinical, biochemical and musculoskeletal measures, stratified by subgroup of nutritional status change.

Characteristic	Descriptive Statistics	Maintained (Well-Nourished) *n* = 65 (*ref*)	Improved *n* = 20	Maintained (Malnourished/at Risk) *n* = 8	Deteriorated *n* = 7
Clinical and biochemical					
Age, year	Median (Q1, Q3)	77 (71, 81)	79 (73, 83)	80 (72, 84)	81 (79, 82)
BMI at baseline, kg/m^2	Median (Q1, Q3)	29.4 (24.8, 34.2)	25.6 (20.8, 29.6) †	23.8 (18.9, 29.8) †	24.1 (21.4, 27.9) †
ΔBMI [l], kg/m^2	Median (Q1, Q3)	0.4 (−0.3, 1.3) *	1.2 (0.7, 2.5) *,†	−0.8 (−2.5, 0.6)	0.0 (−0.3, 1.5)
Weight at baseline, kg	Median (Q1, Q3)	74.0 (64.0, 87.7)	64.1 (51.0, 76.6) †	64.8 (42.3, 70.4) †	64.7 (50.4, 74.0)
Δweight [k], kg	Median (Q1, Q3)	0.5 (−0.9, 2.4) *	3.0 (1.0, 5.9) *,†	−2.0 (−9.3, 0.0) †	−2.1 (−4.0, 2.8)
Number of falls at follow-up [f]	Median (Q1, Q3)	0 (0, 0)	0 (0, 1)	1 (0, 2)	2 (0, 2) †
Albumin at baseline, g/L	Median (Q1, Q3)	38.0 (36.0, 40.0)	38.0 (36.5, 40.0)	34.0 (29.5, 39.5)	38.0 (34.0, 41.0)
Δalbumin [c], g/L	Median (Q1, Q3)	0.0 (−2.0, 2.0)	0.0 (−2.0, 2.0)	0.5 (−1.5, 4.5)	1.0 (−2.0, 2.0)
25OHD at baseline, nmol/L	Mean (SD)	65.5 (22.5)	74.7 (20.2)	66.0 (23.7)	77.1 (28.3)
Δ25OHD [a], nmol/L	Median (Q1, Q3)	4.0 (−7.0, 25.0) *	9.5 (−11.0, 17.5)	8.5 (−5.0, 24.5)	12.0 (−1, 23.0)
PTH at baseline [f], pmol/L	Median (Q1, Q3)	6.9 (5.3, 10.3)	7.5 (5.8, 11.2)	6.0 (4.7, 10.5)	5.5 (3.9, 5.7) †
ΔPTH [g], pmol/L	Median (Q1, Q3)	−0.1 (−2.0, 2.3)	0.8 (−1.4, 3.8)	1.4 (1.1, 4.9) †	−0.1 (−1.4, 2.6)
Calcium, mmol/L	Mean (SD)	2.4 (0.1)	2.4 (0.1)	2.5 (0.1)	2.5 (0.1)
Phosphate, mmol/L	Mean (SD)	1.2 (0.2)	1.2 (0.2)	1.2 (0.2)	1.2 (0.1)
Hemoglobin at baseline [a], g/L	Mean (SD)	130.1 (13.7)	131.5 (13.2)	135.4 (18.9)	130.6 (13.5)
Δhemoglobin [c], g/L	Median (Q1, Q3)	1.0 (−4.0, 5.0)	1.0 (−5.0, 5.0)	1.5 (−6.5, 10.0)	5.0 (−11.0, 15.0)
eGFR at baseline, mL/min/1.73 m^2	Median (Q1, Q3)	75.0 (59.0, 86.0)	67.0 (52.5, 84.5)	85.0 (79.5, 87.5)	59.0 (55.0, 68.0)
ΔeGFR [d], mL/min/1.73 m^2	Median (Q1, Q3)	0.0 (−4.0, 4.0)	−3.0 (−8.0, 1.0)	−3.0 (−13.0, 0.0)	8.0 (0.0, 14.0) †
Musculoskeletal					
ALM/height^2 at baseline, kg/m^2	Median (Q1, Q3)	6.8 (6.0, 8.1)	6.0 (5.2, 6.8) †	6.2 (5.5, 6.3) †	5.7 (5.3, 6.6) †
Grip strength at baseline [a], kg	Median (Q1, Q3)	22.0 (17.0, 28.0)	20.5 (18.0, 26.0)	19.0 (15.0, 24.5)	16.0 (10.0, 24.0)
Δgrip strength [d], kg	Median (Q1, Q3)	0.0 (−3.0, 2.0)	0.0 (−2.0, 0.5)	0.5 (−1.5, 1.5)	−3.0 (−4.0, 2.0)
Gait speed at baseline [e], m/sec	Median (Q1, Q3)	0.7 (0.5, 1.0)	0.6 (0.5, 0.7)	0.7 (0.5, 0.9)	0.8 (0.6, 0.9)
Δgait speed [h], m/sec	Median (Q1, Q3)	0.1 (−0.1, 0.2) *	0.1 (−0.1, 0.2)	−0.0 (−0.1, −0.0) †	−0.1 (−0.2, 0) †
TUG at baseline [f], sec	Median (Q1, Q3)	15.2 (10.2, 21.3)	19.4 (16.5, 24.0) †	15.5 (10.9, 19.9)	18.3 (12.6, 22.0)
ΔTUG [k], sec	Median (Q1, Q3)	−0.5 (−2.2, 1.3)	−3.2 (−7.4, −0.6) *,†	−1.2 (−2.9, 3.4)	−2.6 (−2.9, 1.1)
SPPB at baseline [b], score (/12)	Median (Q1, Q3)	7.0 (5.0, 10.0)	6.0 (5.0, 7.0)	6.0 (4.0, 9.0)	7.0 (4.0, 8.0)
ΔSPPB [g], score	Median (Q1, Q3)	1.0 (0.0, 2.0) *	1.0 (0.0, 2.0) *	0.0 (−1.5, 1.0)	−1.5 (−2.0, 0.0) †
CTx at baseline [j], ng/L	Median (Q1, Q3)	330 (245, 447)	284 (204, 604)	278 (180, 361)	290 (162, 308)
ΔCTx [m], ng/L	Median (Q1, Q3)	−127 (−224, −15) *	−116 (−268, −9)	75.0 (−151.0, 102.0)	130 (8, 149) †

SD = standard deviation; Q1, Q3 = 25th, 75th percentile; ALM/height^2 = height-adjusted appendicular lean mass; BMI = body mass index; 25OHD = 25-hydroxyvitamin D; PTH = parathyroid hormone; EGFR = estimated glomerular filtration rate; TUG test = Timed Up and Go test; SPPB = Short Physical Performance Battery; CTX = C-terminal telopeptide of type 1 collagen; number of missing values: [a] 1, [b] 2, [c] 3, [d] 4, [e] 5, [f] 6, [g] 9, [h] 11, [j] 12, [k] 14, [l] 16, [m] 19; *: $p < 0.05$ for *t*-test or Wilcoxon signed-rank test for the difference between baseline and follow-up values within each group; †: $p < 0.05$ for *t*-test or Wilcoxon rank-sum test for the difference between the improved, maintained (malnourished/at risk) or deteriorated group vs. the maintained (well-nourished) nutritional status group (*ref*), respectively.

Table 3. Results of multiple linear regression analyses testing the association between changes in nutritional status and musculoskeletal outcomes.

Change in Musculoskeletal Outcome	Change in Nutritional Status		*n*	Age-Sex Adjusted β (95% CI)	Multivariable Adjusted β (95% CI)
Δgrip strength, kg	Categorized [a]	Improved vs. *ref*	20	−0.17 (−1.98, 1.63)	0.34 (−1.70, 2.73)
		Maintained (malnourished/at risk) vs. *ref*	8	−1.13 (−3.78, 1.52)	−1.22 (−4.07, 1.63)
		Deteriorated vs. *ref*	7	−2.12 (5.00, 0.75)	−1.48 (−4.41, 1.44)
	Continuous [b]	1-point higher in ΔMNA	96	0.10 (−0.10, 0.30)	0.09 (−0.11, 0.30)
Δgait speed, m/sec	Categorized [a]	Improved vs. *ref*	16	−0.03 (−0.12, 0.07)	0.04 (−0.07, 0.15)
		Maintained (malnourished/at risk) vs. *ref*	8	−0.14 (−0.27, −0.01) [i]	−0.07 (−0.21, 0.07)
		Deteriorated vs. *ref*	4	−0.15 (−0.33, 0.02)	−0.14 (−0.31, 0.04)
	Continuous [b]	1-point higher in ΔMNA	89	0.01 (0.00, 0.03) [i]	0.01 (0.00, 0.02) [i]
ΔTUG, sec	Categorized [a]	Improved vs. *ref*	17	−3.41 (−5.82, −0.99) [ii]	−3.30 (−6.34, −0.27) [i]
		Maintained (malnourished/at risk) vs. *ref*	8	0.04 (-3.25, 3.33)	−0.28 (−4.13, 3.56)
		Deteriorated vs. *ref*	3	−1.87 (−7.09, 3.34)	−1.74 (−7.35, 3.87)
	Continuous [b]	1-point higher in ΔMNA	86	−0.18 (−0.46, 0.10)	−0.13 (−0.41, 0.15)
ΔSPPB, score	Categorized [a]	Improved vs. *ref*	18	0.40 (−0.54, 1.33)	1.05 (−0.06, 2.15)
		Maintained (malnourished/at risk) vs. *ref*	8	−1.18 (−2.47, 0.11)	−0.72 (−2.13, 0.69)
		Deteriorated vs. *ref*	6	−2.21 (−3.69, −0.72) [ii]	−1.74 (−3.29, −0.20) [i]
	Continuous [b]	1-point higher in ΔMNA	91	0.21 (0.11, 0.31) [iii]	0.20 (0.10, 0.31) [iii]
Δlog (CTx), ng/L	Categorized [a]	Improved vs. *ref*	15	0.17 (−0.32, 0.66)	0.00 (−0.56, 0.56)
		Maintained (malnourished/at risk) vs. *ref*	5	0.10 (−0.69, 0.89)	0.10 (−0.79, 1.00)
		Deteriorated vs. *ref*	5	0.60 (−0.19, 1.40)	0.69 (−0.09, 1.48)
	Continuous [b]	1-point higher in ΔMNA	81	−0.02 (−0.08, 0.04)	−0.04 (−0.09, 0.02)

MNA = Mini Nutritional Assessment; TUG test = Timed Up and Go test; SPPB = Short Physical Performance Battery; CTX = C-terminal telopeptide of type 1 collagen; [iii] $p < 0.001$, [ii] $p < 0.01$, [i] $p < 0.05$: *p*-value of multiple linear regression models testing the associations between changes in nutritional status and musculoskeletal outcomes with the maintained (well-nourished) nutritional status group as reference group (*ref*); [a] β coefficient is the change in musculoskeletal outcome associated with change in MNA category from baseline to follow-up. Age-sex model adjusted for the baseline outcome, sex, and age. Multivariable model adjusted for the baseline outcome, baseline variables (sex, age, GDS, CACI, number of medications—all continuous except sex), and care plan variables (osteoporosis treatment, vitamin D supplement use, protein supplement use, physical activity—all categorical); [b] β coefficient is the change in musculoskeletal outcome associated with a unit increase in nutritional status over 6 months (ΔMNA). Models also adjusted for baseline MNA score.

4. Discussion

Poor nutritional status is subject to intense discussion in geriatric research mainly due to its high prevalence in older adults with falls, associations with higher morbidity and mortality risk, and effects on increased healthcare spending [33]. We assessed change in nutritional status in older adults with a history of falling and how it is related to relevant musculoskeletal changes during post-fall recovery. We found that improvement in nutritional status, based on increase in the MNA score over 6 months, was associated with improvement in physical performance, based on increase in the SPPB score over time.

4.1. Changes in Nutritional Status

Approximately one-third (29%) of the studied 106 older adults were malnourished or at risk of malnutrition at baseline. This prevalence is comparable to other studies of community-dwelling older adults using the MNA® (6%–32%) [34]. The prevalence of malnutrition or risk thereof decreased to 15% at follow-up. Comparable observational studies in community-dwelling older adults are lacking, but studies within inpatient settings reported a similar reduction in malnutrition prevalence (10%–13%) based on MNA category change between admission and discharge. However, older adults receiving inpatient services differ significantly in nutritional status and health recovery goals post-discharge to the community, so that results cannot be compared to community-dwelling older adults with confidence [24,35,36].

Most individuals (89%) maintained or improved nutritional status. It is worth noting that 8% of them remained malnourished or at risk of malnutrition at follow-up. A smaller number (7%) deteriorated to an extent sufficient to downgrade MNA category. This implies that while improvement or stabilization of nutritional status is possible during post-fall recovery, a number of individuals may not reach a well-nourished state, despite provision of individualized care plans, which included education and prescription of protein supplements, when indicated. This may have long-term implications for musculoskeletal recovery and quality of life and highlights the need for adequate follow-up of nutritional assessment. Moreover, while it is possible that subtle improvement or deterioration occurred within the stable group, the degree of change may not have been sufficient to alter MNA category.

Our study further demonstrated that MNA change was consistent with significant anthropometric (weight and BMI) changes. This is an important finding, as it is valuable to have a validated nutrition assessment tool to monitor nutrition progress over time, rather than relying only on anthropometric or biochemistry measures such as albumin, which may be confounded by clinical factors such as inflammation [37,38].

4.2. Changes in Nutritional Status and Musculoskeletal Health

Over 6 months, there were significant improvements in physical performance (based on gait speed, SPPB score, and TUG test performance) and in CTx levels. For gait speed and SPPB score, improvements were within a range indicating clinically meaningful changes [39], supporting that performance measures may offer a powerful mechanism to act on healthcare needs of older adults at risk for falls.

The observational design of this study prevents us from attributing changes of nutritional status and musculoskeletal outcomes to specific post-fall recommendations or other causes. Care plans were individualized through consideration of patient circumstances and treatment preferences. Incorporation of patients' decisions about treatment choices and their active involvement in managing their own care plan forms an integrative part of patient-centered medicine [40].

Our research investigated whether changes in nutritional status were reflected by changes in relevant musculoskeletal outcomes post-fall recovery. Improvement in nutritional status, based on a 1-point increase in MNA score over 6 months, was strongly associated with improvement in physical performance, based on an increase of 0.20 points (95% CI 0.10, 0.31, $p < 0.001$) in SPPB score over

time. In subgroup analyses, the improved group was significantly associated with decrease in time to perform the TUG test and the deteriorated group with decrease in the SPPB score over time, compared to the reference group. These tools are interrelated and provide valid and reliable measurements of physical performance in community-dwelling older adults, incorporating elements of mobility and balance, with the addition of strength in both the SPPB and TUG test [41]. Nevertheless, caution is warranted when interpreting findings from subgroup analyses, because *p*-values were fairly large ($0.01 \leq p < 0.05$).

The impact of change in nutritional status on physical performance may be explained by direct or indirect mechanisms. First, increased adequacy of nutritional intake (in terms of quantity and quality) may contribute to recovery of muscle mass and function [42]. This affects physical performance, leading to functional and mobility improvements [10,11]. Improved nutritional status may also be an indicator of decreased comorbidity, which has positive effects on cognitive, functional, and physical performance [8]. Detailed data on changes in disease-related and medical factors could not be considered in this study and may have influenced changes between nutritional status groups and the time taken to recover musculoskeletal health. Finally, there was no association between changes in nutritional status and CTx levels. Physical performance may be more likely than bone turnover to improve alongside nutritional status due to recovery of muscle mass and function.

Our findings support the hypothesis that adequate nutritional follow-up support might increase relevant functional abilities during recovery from a fall. Two recent intervention studies, involving over 200 older adults aged ≥65 years each, showed that nutrition interventions (including enriched diets and/or oral nutritional supplements, home visits and/or telephone follow-ups) yielded significant improvements in weight and functional status over 3 months [43]. Another randomized control study involving over 150 geriatric patients aged >65 years at nutritional risk demonstrated the positive effect of individualized dietician counseling at home after discharge from hospital [44]. Perhaps this study design [44] can be used to conduct larger randomized controlled trials evaluating the effectiveness of specific nutritional interventions and models of care to improve nutritional and musculoskeletal measures in older adults at risk for falls.

4.3. Strengths and Limitations

Strengths of the study include the use of validated nutritional and musculoskeletal assessment tools, and the repeated measurements at two time points. The follow-up period of 6 months makes the study appropriate for documenting changes in nutritional status and musculoskeletal outcomes. There are also a number of limitations. The MNA lacks sensitivity to detect subtle changes in nutritional status [36]. As a result, the four nutritional status change subgroups are not evenly represented and are dominated by those who maintained well-nourished nutritional status. Another limitation arises from the selection bias associated with the follow-up design of this study, whereby only those willing to attend a follow-up session were assessed.

A control group was not feasible as routine geriatric care needed to be provided, which included individualized care plans for all patients. This limits the ability to attribute changes observed in musculoskeletal outcomes to specific recommendations. Importantly, it remains unclear whether change in nutritional status has a causal role in change in physical performance, or whether it is a case of reverse causation. The possibility of the temporal association between nutritional status and physical performance being due to residual confounding by unmeasured genetic, lifestyle or environmental factors cannot be ruled out. Finally, our findings may not be generalized because of the heterogeneous and convenience nature of the database.

5. Conclusions

Approximately one third of community-dwelling older adults with a history of falling were malnourished or at risk of malnutrition at baseline, and nearly one fifth improved nutritional status at 6-month follow-up. Improvement in nutritional status, based on increase in the MNA score over 6

months, was associated with improvement in physical performance, based on increase in the SPPB score over time. Larger intervention studies are required to ascertain causality and to evaluate specific nutritional interventions and models of care to improve nutritional status and functional recovery in older adults at risk for falls.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/7/1551/s1, Table S1: The Charlson age-comorbidity index (CACI), Table S2: Cross-sectional associations of baseline nutritional status with baseline musculoskeletal outcomes.

Author Contributions: R.C. and G.D. conceived and designed the study; S.P., E.G., and G.D. were involved in data collection and quality control; R.C. and G.D. coordinated the ethics application, R.C. analyzed the data, under the supervision of S.V., and wrote the first draft of the paper; W.S.-L. and B.T. helped interpreting the data; all authors contributed to the critical revision of the paper and approved the submitted version.

Funding: This study was funded by the Australian Institute for Musculoskeletal Science (AIMSS). Romy Conzade received a PhD scholarship from the 'Studienstiftung des deutschen Volkes'. The funding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

Acknowledgments: We thank all patients attending the Falls and Fractures Clinic for their extraordinary commitment and good will. We also thank Liz Liberts for her guidance on the ethics application process, Solange Bernardo for her assistance with the assessments of the participants, and Florian Schederecker for sharing his expertise on longitudinal data analysis.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

25OHD	25-hydroxyvitamin D
ALM	Appendicular lean mass
ANZSSFR	Australian and New Zealand Society for Sarcopenia and Frailty Research
BMD	Bone mineral density
BMI	Body mass index
CACI	Charlson age-comorbidity index
CI	Confidence interval
CTx	C-terminal telopeptide of type 1 collagen
DXA	Dual energy X-ray absorptiometry
EWGSOP	European Working Group on Sarcopenia in Older People
eGFR	Estimated glomerular filtration rate
GDS	Geriatric Depression Scale
MNA	Mini Nutritional Assessment
PTH	Parathyroid hormone
Q1	25th percentile
Q3	75th percentile
SD	Standard deviation
SPPB	Short Physical Performance Battery
TUG test	Timed Up and Go test

References

1. Gillespie, L.D.; Robertson, M.C.; Gillespie, W.J.; Lamb, S.E.; Gates, S.; Cumming, R.G.; Rowe, B.H. Interventions for preventing falls in older people living in the community. *Cochrane Database Syst. Rev.* **2009**. [CrossRef]
2. WHO (World Health Organization). WHO Global Report on Falls Prevention in Older Age. Available online: https://www.who.int/ageing/publications/Falls_prevention7March.pdf (accessed on 10 May 2018).
3. Torres, M.J.; Feart, C.; Samieri, C.; Dorigny, B.; Luiking, Y.; Berr, C.; Barberger-Gateau, P.; Letenneur, L. Poor nutritional status is associated with a higher risk of falling and fracture in elderly people living at home in France: the Three-City cohort study. *Osteoporos. Int.* **2015**, *26*, 2157–2164. [CrossRef]

4. Trevisan, C.; Crippa, A.; Ek, S.; Welmer, A.K.; Sergi, G.; Maggi, S.; Manzato, E.; Bea, J.W.; Cauley, J.A.; Decullier, E.; et al. Nutritional Status, Body Mass Index, and the Risk of Falls in Community-Dwelling Older Adults: A Systematic Review and Meta-Analysis. *J. Am. Med. Dir. Assoc.* **2018**. [CrossRef]
5. Vellas, B.; Villars, H.; Abellan, G.; Soto, M.E.; Rolland, Y.; Guigoz, Y.; Morley, J.E.; Chumlea, W.; Salva, A.; Rubenstein, L.Z.; et al. Overview of the MNA—Its history and challenges. *J. Nutr. Health Aging* **2006**, *10*, 456–463.
6. Swanenburg, J.; de Bruin, E.D.; Stauffacher, M.; Mulder, T.; Uebelhart, D. Effects of exercise and nutrition on postural balance and risk of falling in elderly people with decreased bone mineral density: randomized controlled trial pilot study. *Clin. Rehabil.* **2007**, *21*, 523–534. [CrossRef]
7. Park, S.H. Tools for assessing fall risk in the elderly: a systematic review and meta-analysis. *Aging Clin. Exp. Res.* **2018**, *30*, 1–16. [CrossRef]
8. Vellas, B.J.; Hunt, W.C.; Romero, L.J.; Koehler, K.M.; Baumgartner, R.N.; Garry, P.J. Changes in nutritional status and patterns of morbidity among free-living elderly persons: A 10-year longitudinal study. *Nutrition* **1997**, *13*, 515–519. [CrossRef]
9. Afrin, N.; Honkanen, R.; Koivumaa-Honkanen, H.; Lukkala, P.; Rikkonen, T.; Sirola, J.; Williams, L.J.; Kroger, H. Multimorbidity predicts falls differentially according to the type of fall in postmenopausal women. *Maturitas* **2016**, *91*, 19–24. [CrossRef]
10. Mithal, A.; Bonjour, J.P.; Boonen, S.; Burckhardt, P.; Degens, H.; El Hajj Fuleihan, G.; Josse, R.; Lips, P.; Morales Torres, J.; Rizzoli, R.; et al. Impact of nutrition on muscle mass, strength, and performance in older adults. *Osteoporos. Int.* **2013**, *24*, 1555–1566. [CrossRef]
11. Misu, S.; Asai, T.; Doi, T.; Sawa, R.; Ueda, Y.; Saito, T.; Nakamura, R.; Murata, S.; Sugimoto, T.; Yamada, M.; et al. Association between gait abnormality and malnutrition in a community-dwelling elderly population. *Geriatr. Gerontol. Int.* **2017**, *17*, 1155–1160. [CrossRef]
12. Bonjour, J.P. Dietary protein: an essential nutrient for bone health. *J. Am. Coll. Nutr.* **2005**, *24*, 526S–536S. [CrossRef]
13. Sanchez-Rodriguez, D.; Marco, E.; Ronquillo-Moreno, N.; Miralles, R.; Vazquez-Ibar, O.; Escalada, F.; Muniesa, J.M. Prevalence of malnutrition and sarcopenia in a post-acute care geriatric unit: Applying the new ESPEN definition and EWGSOP criteria. *Clin. Nutr.* **2017**, *36*, 1339–1344. [CrossRef]
14. Salminen, H.; Saaf, M.; Johansson, S.E.; Ringertz, H.; Strender, L.E. Nutritional status, as determined by the Mini-Nutritional Assessment, and osteoporosis: a cross-sectional study of an elderly female population. *Eur. J. Clin. Nutr.* **2006**, *60*, 486–493. [CrossRef]
15. Huo, Y.R.; Suriyaarachchi, P.; Gomez, F.; Curcio, C.L.; Boersma, D.; Gunawardene, P.; Demontiero, O.; Duque, G. Comprehensive nutritional status in sarco-osteoporotic older fallers. *J. Nutr. Health Aging* **2015**, *19*, 474–480. [CrossRef]
16. Reiss, J.; Iglseder, B.; Alzner, R.; Mayr-Pirker, B.; Pirich, C.; Kassmann, H.; Kreutzer, M.; Dovjak, P.; Reiter, R. Sarcopenia and osteoporosis are interrelated in geriatric inpatients. *Z. Gerontol. Geriatr.* **2019**. [CrossRef]
17. Zhang, X.; Huang, P.; Dou, Q.; Wang, C.; Zhang, W.; Yang, Y.; Wang, J.; Xie, X.; Zhou, J.; Zeng, Y. Falls among older adults with sarcopenia dwelling in nursing home or community: A meta-analysis. *Clin. Nutr.* **2019**. [CrossRef]
18. Hirschfeld, H.P.; Kinsella, R.; Duque, G. Osteosarcopenia: where bone, muscle, and fat collide. *Osteoporos. Int.* **2017**, *28*, 2781–2790. [CrossRef]
19. Tinetti, M.E.; Kumar, C. The patient who falls: "It's always a trade-off". *Jama* **2010**, *303*, 258–266. [CrossRef]
20. Deandrea, S.; Lucenteforte, E.; Bravi, F.; Foschi, R.; La Vecchia, C.; Negri, E. Risk factors for falls in community-dwelling older people: a systematic review and meta-analysis. *Epidemiology* **2010**, *21*, 658–668. [CrossRef]
21. Nuotio, M.; Tuominen, P.; Luukkaala, T. Association of nutritional status as measured by the Mini-Nutritional Assessment Short Form with changes in mobility, institutionalization and death after hip fracture. *Eur. J. Clin. Nutr.* **2016**, *70*, 393–398. [CrossRef]
22. Inoue, T.; Misu, S.; Tanaka, T.; Kakehi, T.; Ono, R. Acute phase nutritional screening tool associated with functional outcomes of hip fracture patients: A longitudinal study to compare MNA-SF, MUST, NRS-2002 and GNRI. *Clin. Nutr.* **2019**, *38*, 220–226. [CrossRef]
23. Vivanti, A.; Ward, N.; Haines, T. Nutritional status and associations with falls, balance, mobility and functionality during hospital admission. *J. Nutr. Health Aging* **2011**, *15*, 388–391. [CrossRef]

24. Whitley, A.; Skliros, E.; Graven, C.; McIntosh, R.; Lasry, C.; Newsome, C.; Bowie, A. Changes in Nutritional and Functional Status in Longer Stay Patients Admitted to a Geriatric Evaluation and Management Unit. *J. Nutr. Health Aging* **2017**, *21*, 686–691. [CrossRef]
25. Charlson, M.E.; Pompei, P.; Ales, K.L.; MacKenzie, C.R. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. *J. Chronic Dis.* **1987**, *40*, 373–383. [CrossRef]
26. Sheikh, J.I.; Yesavage, J.A. Geriatric Depression Scale (GDS): Recent evidence and development of a shorter version. *Clin. Gerontol. J. Aging Ment. Health* **1986**, *5*, 165–173.
27. Vitamin, D. Supplementation Guidelines-Osteoporosis Australia. Available online: https://www.osteoporosis.org.au/sites/default/files/files/oa_medical_vitd_2nd_ed.pdf (accessed on 4 July 2019).
28. Levey, A.S.; Bosch, J.P.; Lewis, J.B.; Greene, T.; Rogers, N.; Roth, D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann. Intern. Med.* **1999**, *130*, 461–470. [CrossRef]
29. Podsiadlo, D.; Richardson, S. The timed "Up & Go": A test of basic functional mobility for frail elderly persons. *J. Am. Geriatr. Soc.* **1991**, *39*, 142–148.
30. Guralnik, J.M.; Simonsick, E.M.; Ferrucci, L.; Glynn, R.J.; Berkman, L.F.; Blazer, D.G.; Scherr, P.A.; Wallace, R.B. A short physical performance battery assessing lower extremity function: Association with self-reported disability and prediction of mortality and nursing home admission. *J. Gerontol.* **1994**, *49*, M85–M94. [CrossRef]
31. Zanker, J.; Scott, D.; Reijnierse, E.M.; Brennan-Olsen, S.L.; Daly, R.M.; Girgis, C.M.; Grossmann, M.; Hayes, A.; Henwood, T.; Hirani, V.; et al. Establishing an Operational Definition of Sarcopenia in Australia and New Zealand: Delphi Method Based Consensus Statement. *J. Nutr. Health Aging* **2019**, *23*, 105–110. [CrossRef]
32. Cruz-Jentoft, A.J.; Baeyens, J.P.; Bauer, J.M.; Boirie, Y.; Cederholm, T.; Landi, F.; Martin, F.C.; Michel, J.P.; Rolland, Y.; Schneider, S.M.; et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* **2010**, *39*, 412–423. [CrossRef]
33. Agarwal, E.; Miller, M.; Yaxley, A.; Isenring, E. Malnutrition in the elderly: A narrative review. *Maturitas* **2013**, *76*, 296–302. [CrossRef]
34. Kaiser, M.J.; Bauer, J.M.; Ramsch, C.; Uter, W.; Guigoz, Y.; Cederholm, T.; Thomas, D.R.; Anthony, P.S.; Charlton, K.E.; Maggio, M.; et al. Frequency of malnutrition in older adults: a multinational perspective using the mini nutritional assessment. *J. Am. Geriatr. Soc.* **2010**, *58*, 1734–1738. [CrossRef]
35. Collins, J.; Porter, J.; Truby, H.; Huggins, C.E. How does nutritional state change during a subacute admission? Findings and implications for practice. *Eur. J. Clin. Nutr.* **2016**, *70*, 607–612. [CrossRef]
36. McDougall, K.E.; Cooper, P.L.; Stewart, A.J.; Huggins, C.E. Can the Mini Nutritional Assessment (MNA) Be Used as a Nutrition Evaluation Tool for Subacute Inpatients over an Average Length of Stay? *J. Nutr. Health Aging* **2015**, *19*, 1032–1036. [CrossRef]
37. Kruizenga, H.M.; Wierdsma, N.J.; van Bokhorst-de van der Schueren, M.A.; Haollander, H.J.; Jonkers-Schuitema, C.F.; van der Heijden, E.; Melis, G.C.; van Staveren, W.A. Screening of nutritional status in The Netherlands. *Clin. Nutr.* **2003**, *22*, 147–152. [CrossRef]
38. Cabrerizo, S.; Cuadras, D.; Gomez-Busto, F.; Artaza-Artabe, I.; Marin-Ciancas, F.; Malafarina, V. Serum albumin and health in older people: Review and meta analysis. *Maturitas* **2015**, *81*, 17–27. [CrossRef]
39. Perera, S.; Mody, S.H.; Woodman, R.C.; Studenski, S.A. Meaningful change and responsiveness in common physical performance measures in older adults. *J. Am. Geriatr. Soc.* **2006**, *54*, 743–749. [CrossRef]
40. Morley, J.E.; Vellas, B. Patient-Centered (P4) Medicine and the Older Person. *J. Am. Med. Dir. Assoc.* **2017**, *18*, 455–459. [CrossRef]
41. Mijnarends, D.M.; Meijers, J.M.; Halfens, R.J.; ter Borg, S.; Luiking, Y.C.; Verlaan, S.; Schoberer, D.; Cruz Jentoft, A.J.; van Loon, L.J.; Schols, J.M. Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: A systematic review. *J. Am. Med. Dir. Assoc.* **2013**, *14*, 170–178. [CrossRef]
42. Robinson, S.M.; Reginster, J.Y.; Rizzoli, R.; Shaw, S.C.; Kanis, J.A.; Bautmans, I.; Bischoff-Ferrari, H.; Bruyere, O.; Cesari, M.; Dawson-Hughes, B.; et al. Does nutrition play a role in the prevention and management of sarcopenia? *Clin. Nutr.* **2018**, *37*, 1121–1132. [CrossRef]

43. Neelemaat, F.; Bosmans, J.E.; Thijs, A.; Seidell, J.C.; van Bokhorst-de van der Schueren, M.A. Post-discharge nutritional support in malnourished elderly individuals improves functional limitations. *J. Am. Med. Dir. Assoc.* **2011**, *12*, 295–301. [CrossRef] [PubMed]
44. Beck, A.M.; Kjaer, S.; Hansen, B.S.; Storm, R.L.; Thal-Jantzen, K.; Bitz, C. Follow-up home visits with registered dietitians have a positive effect on the functional and nutritional status of geriatric medical patients after discharge: A randomized controlled trial. *Clin. Rehabil.* **2013**, *27*, 483–493. [CrossRef] [PubMed]

III. References

1. Rosenberg, I.H. Sarcopenia: origins and clinical relevance. *J Nutr* **1997**, *127*, 990s-991s.
2. United Nations. World Population Ageing. **2017**. Available online: https://www.un.org/en/development/desa/population/publications/pdf/ageing/WPA2017_Highlights.pdf (accessed on 16 August 2019).
3. World Health Organization. World report on ageing and health. **2015**. Available online: https://apps.who.int/iris/bitstream/handle/10665/186463/9789240694811_eng.pdf?sequence=1.pdf (accessed on 16 August 2019).
4. Cruz-Jentoft, A.J.; Sayer, A.A. Sarcopenia. *The Lancet* **2019**, *393*, 2636-2646.
5. Cesari, M.; Landi, F.; Vellas, B.; Bernabei, R.; Marzetti, E. Sarcopenia and physical frailty: two sides of the same coin. *Front Aging Neurosci* **2014**, *6*, 192.
6. Beaudart, C.; Zaaria, M.; Pasleau, F.; Reginster, J.Y.; Bruyere, O. Health Outcomes of Sarcopenia: A Systematic Review and Meta-Analysis. *PLoS One* **2017**, *12*, e0169548.
7. Newman, A.B.; Kupelian, V.; Visser, M.; Simonsick, E.M.; Goodpaster, B.H.; Kritchevsky, S.B.; Tylavsky, F.A.; Rubin, S.M.; Harris, T.B. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. *J Gerontol A Biol Sci Med Sci* **2006**, *61*, 72-77.
8. Cruz-Jentoft, A.J.; Landi, F.; Schneider, S.M.; Zuniga, C.; Arai, H.; Boirie, Y.; Chen, L.K.; Fielding, R.A.; Martin, F.C.; Michel, J.P., et al. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age Ageing* **2014**, *43*, 748-759.
9. Cruz-Jentoft, A.J.; Landi, F.; Topinkova, E.; Michel, J.P. Understanding sarcopenia as a geriatric syndrome. *Curr Opin Clin Nutr Metab Care* **2010**, *13*, 1-7.
10. Anker, S.D.; Morley, J.E.; von Haehling, S. Welcome to the ICD-10 code for sarcopenia. *J Cachexia Sarcopenia Muscle* **2016**, *7*, 512-514.
11. Riuzzi, F.; Sorci, G.; Arcuri, C.; Giambanco, I.; Bellezza, I.; Minelli, A.; Donato, R. Cellular and molecular mechanisms of sarcopenia: the S100B perspective. *J Cachexia Sarcopenia Muscle* **2018**, *9*, 1255-1268.
12. Dodds, R.M.; Syddall, H.E.; Cooper, R.; Benzeval, M.; Deary, I.J.; Dennison, E.M.; Der, G.; Gale, C.R.; Inskip, H.M.; Jagger, C., et al. Grip strength across the life course: normative data from twelve British studies. *PLoS One* **2014**, *9*, e113637.
13. Shaw, S.C.; Dennison, E.M.; Cooper, C. Epidemiology of Sarcopenia: Determinants Throughout the Lifecourse. *Calcif Tissue Int* **2017**, *101*, 229-247.
14. Robinson, S.M.; Reginster, J.Y.; Rizzoli, R.; Shaw, S.C.; Kanis, J.A.; Bautmans, I.; Bischoff-Ferrari, H.; Bruyere, O.; Cesari, M.; Dawson-Hughes, B., et al. Does nutrition play a role in the prevention and management of sarcopenia? *Clin Nutr* **2018**, *37*, 1121-1132.
15. ter Borg, S.; Verlaan, S.; Hemsworth, J.; Mijnarends, D.M.; Schols, J.M.; Luiking, Y.C.; de Groot, L.C. Micronutrient intakes and potential inadequacies of community-dwelling older adults: a systematic review. *Br J Nutr* **2015**, *113*, 1195-1206.

16. Otsuka, R.; Kato, Y.; Nishita, Y.; Tange, C.; Tomida, M.; Nakamoto, M.; Imai, T.; Ando, F.; Shimokata, H. Age-related Changes in Energy Intake and Weight in Community-dwelling Middle-aged and Elderly Japanese. *J Nutr Health Aging* **2016**, *20*, 383-390.
17. Clarke, D.M.; Wahlqvist, M.L.; Strauss, B.J. Undereating and undernutrition in old age: integrating bio-psychosocial aspects. *Age Ageing* **1998**, *27*, 527-534.
18. Porter, K.; Hoey, L.; Hughes, C.F.; Ward, M.; McNulty, H. Causes, Consequences and Public Health Implications of Low B-Vitamin Status in Ageing. *Nutrients* **2016**, *8*.
19. Gallagher, J.C. Vitamin D and aging. *Endocrinol Metab Clin North Am* **2013**, *42*, 319-332.
20. Cederholm, T.; Barazzoni, R.; Austin, P.; Ballmer, P.; Biolo, G.; Bischoff, S.C.; Compher, C.; Correia, I.; Higashiguchi, T.; Holst, M., et al. ESPEN guidelines on definitions and terminology of clinical nutrition. *Clin Nutr* **2017**, *36*, 49-64.
21. Kaiser, M.J.; Bauer, J.M.; Ramsch, C.; Uter, W.; Guigoz, Y.; Cederholm, T.; Thomas, D.R.; Anthony, P.S.; Charlton, K.E.; Maggio, M., et al. Frequency of malnutrition in older adults: a multinational perspective using the mini nutritional assessment. *J Am Geriatr Soc* **2010**, *58*, 1734-1738.
22. Rabenberg, M.; Scheidt-Nave, C.; Busch, M.A.; Rieckmann, N.; Hintzpeter, B.; Mensink, G.B. Vitamin D status among adults in Germany--results from the German Health Interview and Examination Survey for Adults (DEGS1). *BMC Public Health* **2015**, *15*, 641.
23. Visser, M.; Volkert, D.; Corish, C.; Geisler, C.; de Groot, L.C.; Cruz-Jentoft, A.J.; Lohrmann, C.; O'Connor, E.M.; Schindler, K.; de van der Schueren, M.A., et al. Tackling the increasing problem of malnutrition in older persons: The Malnutrition in the Elderly (MaNuEL) Knowledge Hub. *Nutrition Bulletin* **2017**, *42*, 178-186.
24. Sullivan, D.H.; Walls, R.C. Protein-energy undernutrition and the risk of mortality within six years of hospital discharge. *J Am Coll Nutr* **1998**, *17*, 571-578.
25. Newman, A.B.; Lee, J.S.; Visser, M.; Goodpaster, B.H.; Kritchevsky, S.B.; Tylavsky, F.A.; Nevitt, M.; Harris, T.B. Weight change and the conservation of lean mass in old age: the Health, Aging and Body Composition Study. *Am J Clin Nutr* **2005**, *82*, 872-878; quiz 915-876.
26. Ritchie, C.S.; Locher, J.L.; Roth, D.L.; McVie, T.; Sawyer, P.; Allman, R. Unintentional weight loss predicts decline in activities of daily living function and life-space mobility over 4 years among community-dwelling older adults. *J Gerontol A Biol Sci Med Sci* **2008**, *63*, 67-75.
27. Ceglia, L. Vitamin D and its role in skeletal muscle. *Curr Opin Clin Nutr Metab Care* **2009**, *12*, 628-633.
28. Gielen, E.; O'Neill, T.W.; Pye, S.R.; Adams, J.E.; Wu, F.C.; Laurent, M.R.; Claessens, F.; Ward, K.A.; Boonen, S.; Bouillon, R., et al. Endocrine determinants of incident sarcopenia in middle-aged and elderly European men. *J Cachexia Sarcopenia Muscle* **2015**, *6*, 242-252.
29. Scott, D.; Blizzard, L.; Fell, J.; Ding, C.; Winzenberg, T.; Jones, G. A prospective study of the associations between 25-hydroxy-vitamin D, sarcopenia progression and physical activity in older adults. *Clin Endocrinol (Oxf)* **2010**, *73*, 581-587.
30. Visser, M.; Deeg, D.J.; Lips, P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab* **2003**, *88*, 5766-5772.

31. Houston, D.K.; Tooze, J.A.; Neiberg, R.H.; Hausman, D.B.; Johnson, M.A.; Cauley, J.A.; Bauer, D.C.; Cawthon, P.M.; Shea, M.K.; Schwartz, G.G., et al. 25-hydroxyvitamin D status and change in physical performance and strength in older adults: the Health, Aging, and Body Composition Study. *Am J Epidemiol* **2012**, *176*, 1025-1034.
32. Verreault, R.; Semba, R.D.; Volpato, S.; Ferrucci, L.; Fried, L.P.; Guralnik, J.M. Low serum vitamin d does not predict new disability or loss of muscle strength in older women. *J Am Geriatr Soc* **2002**, *50*, 912-917.
33. Granic, A.; Hill, T.R.; Davies, K.; Jagger, C.; Adamson, A.; Siervo, M.; Kirkwood, T.B.; Mathers, J.C.; Sayer, A.A. Vitamin D Status, Muscle Strength and Physical Performance Decline in Very Old Adults: A Prospective Study. *Nutrients* **2017**, *9*.
34. Hirani, V.; Cumming, R.G.; Naganathan, V.; Blyth, F.; Le Couteur, D.G.; Hsu, B.; Handelsman, D.J.; Waite, L.M.; Seibel, M.J. Longitudinal Associations Between Vitamin D Metabolites and Sarcopenia in Older Australian men: The Concord Health and Aging in Men Project. *J Gerontol A Biol Sci Med Sci* **2017**, *73*, 131-138.
35. Volkert, D. Malnutrition in older adults - urgent need for action: a plea for improving the nutritional situation of older adults. *Gerontology* **2013**, *59*, 328-333.
36. Peters, A.; Doring, A.; Ladwig, K.H.; Meisinger, C.; Linkohr, B.; Autenrieth, C.; Baumeister, S.E.; Behr, J.; Bergner, A.; Bickel, H., et al. [Multimorbidity and successful aging: the population-based KORA-Age study]. *Z Gerontol Geriatr* **2011**, *44 Suppl 2*, 41-54.
37. New reference values for vitamin D. *Ann Nutr Metab* **2012**, *60*, 241-246.
38. Sauberlich, H. *Laboratory Tests for the Assessment of Nutritional Status*, 2nd ed.; CRC Press: 2012.
39. Green, R.; Allen, L.H.; Bjorke-Monsen, A.L.; Brito, A.; Gueant, J.L.; Miller, J.W.; Molloy, A.M.; Nexo, E.; Stabler, S.; Toh, B.H., et al. Vitamin B12 deficiency. *Nat Rev Dis Primers* **2017**, *3*, 17040.
40. Kaplan, L.; Pesce, A. *Clinical Chemistry: Theory, Analysis, and Correlation*, 3rd ed.; Mosby: 1996.
41. Bender, A.E. Subclinical and covert malnutrition. *Bull Schweiz Akad Med Wiss* **1976**, *31*, 279-290.
42. Vellas, B.; Villars, H.; Abellan, G.; Soto, M.E.; Rolland, Y.; Guigoz, Y.; Morley, J.E.; Chumlea, W.; Salva, A.; Rubenstein, L.Z., et al. Overview of the MNA--Its history and challenges. *J Nutr Health Aging* **2006**, *10*, 456-463; discussion 463-455.
43. Janssen, I.; Heymsfield, S.B.; Baumgartner, R.N.; Ross, R. Estimation of skeletal muscle mass by bioelectrical impedance analysis. *J Appl Physiol (1985)* **2000**, *89*, 465-471.
44. Phillips, A.; Strobl, R.; Vogt, S.; Ladwig, K.H.; Thorand, B.; Grill, E. Sarcopenia is associated with disability status-results from the KORA-Age study. *Osteoporos Int* **2017**, *28*, 2069-2079.
45. Cruz-Jentoft, A.J.; Baeyens, J.P.; Bauer, J.M.; Boirie, Y.; Cederholm, T.; Landi, F.; Martin, F.C.; Michel, J.P.; Rolland, Y.; Schneider, S.M., et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* **2010**, *39*, 412-423.
46. Buckinx, F.; Landi, F.; Cesari, M.; Fielding, R.A.; Visser, M.; Engelke, K.; Maggi, S.; Dennison, E.; Al-Daghri, N.M.; Allepaerts, S., et al. Pitfalls in the measurement of muscle mass: a need for a reference standard. *J Cachexia Sarcopenia Muscle* **2018**, *9*, 269-278.

47. Conzade, R.; Grill, E.; Bischoff-Ferrari, H.A.; Ferrari, U.; Horsch, A.; Koenig, W.; Peters, A.; Thorand, B. Vitamin D in Relation to Incident Sarcopenia and Changes in Muscle Parameters Among Older Adults: The KORA-Age Study. *Calcif Tissue Int* **2019**, *105*, 173-182.

48. Shumway-Cook, A.; Brauer, S.; Woollacott, M. Predicting the probability for falls in community-dwelling older adults using the Timed Up & Go Test. *Phys Ther* **2000**, *80*, 896-903.

49. Mithal, A.; Wahl, D.A.; Bonjour, J.P.; Burckhardt, P.; Dawson-Hughes, B.; Eisman, J.A.; El-Hajj Fuleihan, G.; Josse, R.G.; Lips, P.; Morales-Torres, J. Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos Int* **2009**, *20*, 1807-1820.

50. Cederholm, T.; Jensen, G.L.; Correia, M.; Gonzalez, M.C.; Fukushima, R.; Higashiguchi, T.; Baptista, G.; Barazzoni, R.; Blaauw, R.; Coats, A., et al. GLIM criteria for the diagnosis of malnutrition - A consensus report from the global clinical nutrition community. *Clin Nutr* **2019**, *38*, 1-9.

51. Volkert, D.; Beck, A.M.; Cederholm, T.; Cruz-Jentoft, A.; Goisser, S.; Hooper, L.; Kiesswetter, E.; Maggio, M.; Raynaud-Simon, A.; Sieber, C.C., et al. ESPEN guideline on clinical nutrition and hydration in geriatrics. *Clin Nutr* **2019**, *38*, 10-47.

52. Rockwell, M.; Kraak, V.; Hulver, M.; Epling, J. Clinical Management of Low Vitamin D: A Scoping Review of Physicians' Practices. *Nutrients* **2018**, *10*.

53. Janssen, I.; Heymsfield, S.B.; Ross, R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* **2002**, *50*, 889-896.

54. McLean, R.R.; Shardell, M.D.; Alley, D.E.; Cawthon, P.M.; Fragala, M.S.; Harris, T.B.; Kenny, A.M.; Peters, K.W.; Ferrucci, L.; Guralnik, J.M., et al. Criteria for clinically relevant weakness and low lean mass and their longitudinal association with incident mobility impairment and mortality: the foundation for the National Institutes of Health (FNIH) sarcopenia project. *J Gerontol A Biol Sci Med Sci* **2014**, *69*, 576-583.

55. Landi, F.; Liperoti, R.; Russo, A.; Giovannini, S.; Tosato, M.; Capoluongo, E.; Bernabei, R.; Onder, G. Sarcopenia as a risk factor for falls in elderly individuals: results from the ilSIRENTE study. *Clin Nutr* **2012**, *31*, 652-658.

56. Dent, E.; Morley, J.E.; Cruz-Jentoft, A.J.; Arai, H.; Kritchevsky, S.B.; Guralnik, J.; Bauer, J.M.; Pahor, M.; Clark, B.C.; Cesari, M., et al. International Clinical Practice Guidelines for Sarcopenia (ICFSR): Screening, Diagnosis and Management. *J Nutr Health Aging* **2018**, *22*, 1148-1161.

57. Lozano-Montoya, I.; Correa-Perez, A.; Abraha, I.; Soiza, R.L.; Cherubini, A.; O'Mahony, D.; Cruz-Jentoft, A.J. Nonpharmacological interventions to treat physical frailty and sarcopenia in older patients: a systematic overview - the SENATOR Project ONTOP Series. *Clin Interv Aging* **2017**, *12*, 721-740.

58. Yoshimura, Y.; Wakabayashi, H.; Yamada, M.; Kim, H.; Harada, A.; Arai, H. Interventions for Treating Sarcopenia: A Systematic Review and Meta-Analysis of Randomized Controlled Studies. *J Am Med Dir Assoc* **2017**, *18*, 553.e551-553.e516.

59. Denison, H.J.; Cooper, C.; Sayer, A.A.; Robinson, S.M. Prevention and optimal management of sarcopenia: a review of combined exercise and nutrition interventions to improve muscle outcomes in older people. *Clin Interv Aging* **2015**, *10*, 859-869.

60. Landi, F.; Cesari, M.; Calvani, R.; Cherubini, A.; Di Bari, M.; Bejuit, R.; Mshid, J.; Andrieu, S.; Sinclair, A.J.; Sieber, C.C., et al. The "Sarcopenia and Physical fRailty IN older people: multi-componenT Treatment strategies" (SPRINTT) randomized controlled trial: design and methods. *Aging Clin Exp Res* **2017**, *29*, 89-100.
61. Marzetti, E.; Cesari, M.; Calvani, R.; Msihid, J.; Tosato, M.; Rodriguez-Manas, L.; Lattanzio, F.; Cherubini, A.; Bejuit, R.; Di Bari, M., et al. The "Sarcopenia and Physical fRailty IN older people: multi-componenT Treatment strategies" (SPRINTT) randomized controlled trial: Case finding, screening and characteristics of eligible participants. *Exp Gerontol* **2018**, *113*, 48-57.
62. Beaudart, C.; McCloskey, E.; Bruyere, O.; Cesari, M.; Rolland, Y.; Rizzoli, R.; Araujo de Carvalho, I.; Amuthavalli Thiyagarajan, J.; Bautmans, I.; Bertiere, M.C., et al. Sarcopenia in daily practice: assessment and management. *BMC Geriatr* **2016**, *16*, 170.
63. Cruz-Jentoft, A.J.; Bahat, G.; Bauer, J.; Boirie, Y.; Bruyere, O.; Cederholm, T.; Cooper, C.; Landi, F.; Rolland, Y.; Sayer, A.A., et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* **2019**, 10.1093/ageing/afz046.
64. World Health Organization. Integrated care for older people: Guidelines on community-level interventions to manage declines in intrinsic capacity. **2017**. Available online: https://apps.who.int/iris/bitstream/handle/10665/258981/9789241550109-eng.pdf?sequence=1&isAllowed=y (accessed on 16 August 2019).
65. Federal Ministry of Food Agriculture and Consumer Protection; Federal Ministry of Health. IN FORM: German national initiative to promote healthy diets and physical activity. **2013**. Available online: https://www.bmel.de/SharedDocs/Downloads/EN/Publications/InForm.pdf;jsessionid=8BCA56BAE398035142357CDAE2F0E8E1.2_cid367?__blob=publicationFile (accessed on 16 August 2019).
66. Rothman, K.J. *Epidemiology An Introduction*, Second ed.; Oxford University Press: 2012.
67. Willett, W.C. *Nutritional Epidemiology*, Third ed.; Oxford University Press: 2012; Vol. 40.
68. Raghavan, R.; Ashour, F.S.; Bailey, R. A Review of Cutoffs for Nutritional Biomarkers. *Adv Nutr* **2016**, *7*, 112-120.
69. Vellas, B.; Guigoz, Y.; Garry, P.J.; Nourhashemi, F.; Bennahum, D.; Lauque, S.; Albarede, J.L. The Mini Nutritional Assessment (MNA) and its use in grading the nutritional state of elderly patients. *Nutrition* **1999**, *15*, 116-122.
70. Lauque, S.; Arnaud-Battandier, F.; Mansourian, R.; Guigoz, Y.; Paintin, M.; Nourhashemi, F.; Vellas, B. Protein-energy oral supplementation in malnourished nursing-home residents. A controlled trial. *Age Ageing* **2000**, *29*, 51-56.
71. Evans, W.J.; Hellerstein, M.; Orwoll, E.; Cummings, S.; Cawthon, P.M. D3 -Creatine dilution and the importance of accuracy in the assessment of skeletal muscle mass. *J Cachexia Sarcopenia Muscle* **2019**, *10*, 14-21.
72. Morley, J.E. Treatment of sarcopenia: the road to the future. *J Cachexia Sarcopenia Muscle* **2018**, *9*, 1196-1199.
73. Maltais, M.; Aubertin-Leheudre, M.; Dray, C.; Fielding, R.A.; Rolland, Y.; Cesari, M.; Vellas, B. Highlights from the 2019 International Congress on Frailty and Sarcopenia Research. *J Frailty Aging* **2019**, *8*, 117-119.

Acknowledgments

It all started when I spoke to my former colleague, Dr. Kersten Gutschmidt, at the World Health Organization in Geneva, about my ambition to pursue a Ph.D. in Epidemiology. He told me about his great experience as a Ph.D. student at the Helmholtz Zentrum München, and helped me to connect with the Institute of Epidemiology.

Almost three years have passed since I moved to Munich and started my Ph.D. at the Helmholtz Zentrum. Today, I am ready to submit my doctoral thesis. It is time to thank all the people who made my Ph.D. time a fulfilling, international and enjoyable experience.

First of all, I would like to thank my doctoral supervisor, Prof. Barbara Thorand, for the excellent guidance throughout the Ph.D., for giving constructive feedback, and for supporting my initiative to do the last project of my Ph.D. in Australia. Thank you for involving me in fascinating projects and for providing valuable advice. Your work style and ethics inspired me a lot.

I would like to thank the members of my Thesis Advisory Committee for their helpful contributions, expertise and time. Special thanks go to Prof. Eva Grill for introducing me to the fascinating world of epidemiology, and to Prof. Heike A. Bischoff Ferrari for sharing with me her expert clinical knowledge. I will never forget the spontaneous and enjoyable diner that we had together with her team during the ICFSR conference in Miami.

I am grateful to my co-supervisor in Australia, Prof. Gustavo Duque, for welcoming me as a visiting Ph.D. student at the Australian Institute for Musculoskeletal Science at Sunshine Hospital. Like the name of the hospital, I remember my 4 months in Melbourne as "sunshine" time during my Ph.D. I learned a lot from being involved in the different steps of a clinical study and from working in a stimulating and international work environment.

My deepest appreciation goes to the *Studienstiftung des deutschen Volkes* for the full-time doctoral scholarship, and for helping me to quickly integrate myself in Munich.

Moreover, thanks to all collaborators who have been involved in the planning and conduct of the KORA-Age study and the Australian FFC study, and to all co-authors and reviewers of my publications for their insightful comments and improvement suggestions. Many thanks to Andrea Schneider, Olga Lang, Steven Phu, and Sara Vogrin for answering all my questions concerning the data and for their helpful support.

I would like to thank Prof. Dr. Annette Peters for giving me the opportunity to work at the Helmholtz Zentrum München. My colleagues at the Institute of Epidemiology have contributed a lot to my personal and professional developement during the Ph.D., thank you all for the great work environment. Special thanks go to my officemate Dr. Jana Nano, for making our office so much better since day one, for all the hours spent together working, the great discussions and laughs. To Florian Schederecker, for the help in statistics, the talks and of course the fun lunches. To Dr. Birgit Linkohr, for giving me the interesting opportunity to work in data quality management. To Dr. Cornelia Huth, for the helpful input in my side job.

My Ph.D. time was complemented by Ph.D. courses offered by the LMU Munich and the HELENA Graduate School at Helmholtz Zentrum, with memorable Ph.D. annual retreats and with great running sessions with my colleague Dr. Regina Pickford and Susanne Demmeler.

I would like to thank my colleagues and friends from the ENLP (European Nutrition Leadership Platform) for the inspiring and eye-opening experience at the ENLP 2019.

Thanks to my wonderful friends at home, in Munich, in Switzerland, in the US and elsewhere, for the great moments in the past three years. I am especially thankful to my friend Dr. Sophia Doll from the Studienstiftung, for the great support in all aspects of the Ph.D. and life, and for enjoying together the French and German cultures. To my dear friend Dr. Iris Bardgett, for motivating me to do a Ph.D. and for always being by my side. To my childhood friend Dr. med. Vanina Nicol, for inspiring me to stay in the medical field and for always sending me positive energy from home. Last, but not least, I would like to thank my friends Dr. Einat Schmutz, Dr. Karianne Svendsen and Dr. med. James O'Donovan for their insightful advice before my oral defense.

Thank you to my 'family-in-law' for being my family in Munich, and for the constant support.

Lastly, I would like to thank my wonderful family. I can't thank enough my mother for all the support during my studies and for what I have become today. My dad, for all the encouragement in everything I pursue. My sisters Jill and Anouk, for their huge support. Finally, and above all, my biggest thank you goes to Julian, for always being there for me and for coming with me to Australia - I am so thankful and happy to have you.

I dedicate this thesis to my grandparents, for passing on the values that define me today, and for inspiring me to age healthily just as you do: merci, danke!

Curriculum vitae

Education

2016-2019	**LMU Munich, Ph.D. student in Epidemiology & Public Health**
Munich, Germany	Ph.D. thesis on nutritional status and sarcopenia in older age, doctoral scholarship by the *Studienstiftung des deutschen Volkes*
2012-2014	**ETH Zurich, M.Sc. in Food Science**
Zurich, Switzerland	Major Food Quality and Safety, minor Public Health Nutrition
2013-2014	**Harvard School of Public Health, Master thesis**
Boston, USA	Master thesis on iron-overload disease prevention
2012	**Chinese University of Hong Kong, Exchange Master student**
Hong Kong, China	Master courses in Global Health, Biostatistics, Chinese language
2008-2011	**ETH Zurich, B.Sc. in Food Science**
Zurich, Switzerland	Bachelor thesis on childhood obesity prevention

Professional experience

2016-2019	**Helmholtz Zentrum München, Ph.D. researcher**
Munich, Germany	Epidemiological data analysis using SAS, result publication and presentation at congresses, data quality management
2018	**Australian Institute of Musculoskeletal Science, Research stay**
Melbourne, Australia	Planning of a clinical research study (scientific/protocols, regulatory/ethics), data analysis, publication of results
2014-2016	**World Health Organization (WHO), Consultant in Food Safety**
Geneva, Switzerland	Global crisis management of large-scale and international food safety emergencies, analysis of animal and foodborne exposures of MERS-CoV cases, publication of results

Personal experience

2019	European Nutrition Leadership Platform (ENLP), Luxembourg
2016	Summer School in Nutritional Epidemiology (DIfE), Germany

Languages and interests

Languages	Native: **French** & **Swiss-German**, full professional proficiency: **German** & **English**, limited working proficiency: **Spanish**
Interests	Mountain sports and yoga, discover food cultures and cooking

List of all scientific publications to date

Conzade, R.; Koenig, W.; Heier, M.; Schneider, A.; Grill, E.; Peters, A.; Thorand, B. Prevalence and Predictors of Subclinical Micronutrient Deficiency in German Older Adults: Results from the Population-Based KORA-Age Study. *Nutrients* **2017**, *9*.

Conzade, R.; Grant, R.; Malik, M.R.; Elkholy, A.; Elhakim, M.; Samhouri, D.; Ben Embarek, P.K.; Van Kerkhove, M.D. Reported Direct and Indirect Contact with Dromedary Camels among Laboratory-Confirmed MERS-CoV Cases. *Viruses* **2018**, *10*.

Conzade, R.; Grill, E.; Bischoff-Ferrari, H.A.; Ferrari, U.; Horsch, A.; Koenig, W.; Peters, A.; Thorand, B. Vitamin D in Relation to Incident Sarcopenia and Changes in Muscle Parameters Among Older Adults: The KORA-Age Study. *Calcif Tissue Int* **2019**, *105*, 173-182.

Conzade, R.; Phu, S.; Vogrin, S.; Bani Hassan, E.; Sepúlveda-Loyola, W.; Thorand, B.; Duque, G. Changes in Nutritional Status and Musculoskeletal Health in a Geriatric Post-Fall Care Plan Setting. *Nutrients* **2019**, *11*, 1551.

Sepúlveda-Loyola, W.; Phu, S.; Bani Hassan, E.; Brennan-Olsen, SL.; Zanker, J.; Vogrin, S.; Conzade, R.; Kirk, B.; Al Saedi, A.; Probst, V.; Duque, G. The Joint Occurrence of Osteoporosis and Sarcopenia (Osteosarcopenia): Definitions and Characteristics. *J Am Med Dir Assoc* **2019**.

Affidavit

I hereby declare, that the submitted thesis entitled:

Nutritional Status, Muscle Health, and Sarcopenia: Evidence from Epidemiological Studies in Older Adults

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

Place, date: Munich, 16.08.2019
Signature: Romy Conzade

Confirmation of congruency

I hereby declare, that the electronic version of the submitted thesis entitled:

Nutritional Status, Muscle Health, and Sarcopenia: Evidence from Epidemiological Studies in Older Adults

is congruent with the printed version both in content and format.

Place, date: Munich, 16.08.2019
Signature: Romy Conzade