NOVA MEDICAL SCHOOL FACULDADE DE CIÊNCIAS MÉDICAS

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PREVALENCE OF FUNCTIONAL LIMITATIONS IN PORTUGUESE OLDER ADULTS AND ITS ASSOCIATION WITH HIGH-PROTEIN FOODS: AN EPIDOC DATABASE ANALYSIS

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A dissertation submitted in partial fulfillment of the requirements for the Degree of Masters in Metabolism and Human Nutrition

Dissertação para obtenção do grau de Mestre em Nutrição Humana e Metabolismo

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ABBREVIATIONS

ADL/ADLs	Activities of Daily Living
ARS	Regional Health Authorities
B	unstandardized coefficient
BMI	Body Mass Index
BW	Body Weight
CI	Confidence Interval
climb	Able to climb up five steps
EAA	Essential Amino Acid
ELSA	English Longitudinal Study of Ageing
EpiDoC	Epidemiology of Chronic Diseases Cohort
EQ-5D-3L	European Quality of Life questionnaire with five dimensions and three levels
ESPEN	European Society for Clinical Nutrition and Metabolism
HAQ	Health Assessment Questionnaire
HAQ-DI	Health Assessment Questionnaire Disability Index
HBV	High Biologic Value
HLY	Healthy Life Years
HRS	Health and Retirement Study
IADL	Instrumental Activities of Daily Living
ICF	International Classification of Functioning, Disability and Health
ICIDH	International Classification of Impairments, Disabilities, and Handicaps
IOM	Institute of Medicine of the United States of America
IPI	Inadequate Protein Intake
ISCED	International Standard Classification of Education
IWL	Involuntary Weight Loss
mTOR	Mammalian Target Of Rapamycin
NA	Not applicable
NUTS	Nomenclature of Territorial Units for Statistics
OECD	Organisation for Economic Co-operation and Development
OR	Odds Ratio
p	p-value
RDA	Recommended Dietary Allowance
reach	Able to reach and get down a 2.5 kg object from above the head

ref	Reference category
SD	Standard deviation
SE	Standard Error
SHARE	Survey of Health, Ageing and Retirement in Europe
stand up	Able to stand up from a straight chair
t	t-value
t/w	Times per week
VIF	Variance Inflation Factor
walk	Able to walk outdoors on flat ground
WHO	World Health Organization
y	Years
β	Beta estimate

RESUMO

Introdução: O dramático aumento da esperança de vida é uma das maiores conquistas das sociedades modernas. No entanto, muitos desses últimos anos podem ser passados com incapacidade física e má qualidade de vida. A reduzida ingestão proteica associa-se à perda de massa muscular e de força nos idosos. Contudo, a evidência científica sobre a associação entre a ingestão proteica e limitação funcional em idosos é limitada. Assim, os objetivos deste estudo são: 1) determinar a prevalência de limitações funcionais na população idosa a viver na comunidade em Portugal; 2) estabelecer a associação entre a ingestão de alimentos com alto teor proteico e as limitações funcionais nesta população.

Métodos: Para responder aos objetivos foi utilizado um estudo transversal em 2393 indivíduos (≥ 65 anos) da segunda onda de avaliação da coorte EpiDoC, um estudo de base populacional representativo da população portuguesa. Os participantes responderam a um questionário estruturado durante uma entrevista por telefone. Foram recolhidos dados sociodemográficos, de saúde, de estilos de vida, de ingestão alimentar, dados antropométricos e função física. A limitação funcional foi definida como uma pontuação superior a 0 no *Health Assessment Questionnaire* (HAQ). Utilizou-se regressão logística binária e regressão linear para avaliar a associação entre os alimentos com alto teor proteico e a prevalência de limitações funcionais.

Resultados: A prevalência de limitação funcional entre os idosos portugueses foi de 81,0% (n = 2236). A prevalência aumentou com o grupo etário (76,9% para os indivíduos entre os 65 e os 75 anos vs. 93,1% nos indivíduos com mais de 85 anos) e foi mais elevada em indivíduos do sexo feminino (87,1%) e na região dos Açores (85,3%). Não houve associação entre o consumo de alimentos com alto teor proteico (carne, peixe e produtos lácteos) e as limitações funcionais [por exemplo, comer carne todos os dias vs. comer carne raramente ou nunca, em relação à presença de limitações funcionais (OR 0,75; IC a 95%: 0,32-1,78)]. Apesar deste resultado, verificou-se que a idade, o sexo, o índice de massa corporal, a multimorbilidade, a toma de medicação e o estado de saúde percebido estavam significativamente associados (p < 0,05) à existência de limitação funcional [por exemplo, índice de massa corporal (OR 1,08; IC a 95%: 1,03-1,12)], conforme já reportado na literatura científica.

Conclusão: Existe uma elevada prevalência de limitações funcionais nos idosos portugueses e as intervenções nutricionais que previnam o seu desenvolvimento são de extrema importância. Não foi identificada associação entre o consumo de alimentos com alto teor proteico e a prevalência de limitações funcionais em idosos portugueses. Estes resultados carecem de replicação noutros estudos em diferentes contextos.

Palavras-chave: Atividades da Vida Diária (AVD); Envelhecimento; Limitações Funcionais; Portugal; Proteína.

ABSTRACT

Introduction: The dramatic increase in life expectancy ranks one of the greatest achievements of the modern societies. However, many of these later years may be spent with increasing disability and compromised quality. Reduced protein intake has been associated with loss of muscle mass and strength in older adults. However, evidence on the association between protein intake and functional limitations among older adults is limited. We aimed to: 1) determine the prevalence of functional limitations among community-dwelling Portuguese older adults and 2) establish the association between the consumption of high-protein foods and functional limitations among Portuguese older adults in these population.

Methods: A cross-sectional analysis of 2393 adults (≥ 65 years), of the second wave of follow-up of the EpiDoC cohort - a population-based study representative of the Portuguese population, was performed. Subjects completed a structured questionnaire during a telephone interview. Sociodemographic, health data, lifestyle behaviours, dietary intake, anthropometric data and physical function were collected. Functional limitation was defined as a score > 0 in the Health Assessment Questionnaire. Binary logistic regression and linear regression were used to assess the effect of high-protein foods on functional limitations.

Results: Functional limitation prevalence among older adults was 81.0% (n = 2236), increased with age strata (76.9% for 65 – 75 years to 93.1% for > 85 years), and was highest in female individuals (87.1%) and in the Azores (85.3%). There was no association between the consumption of high-protein foods (meat, fish and dairy products) and functional limitation outcomes [e.g. the odds of having functional limitations (HAQ > 0) was similar for eating meat every day vs. eating meat rarely or never (OR 0.75; 95% CI: 0.32-1.78)]. Despite this result, we found that age, sex, body mass index, multimorbidity, medication use and perceived health status were significantly associated ($p < 0.05$) with functional limitation prevalence [e.g. body mass index (OR 1.08; 95% CI: 1.03-1.12)], as previously reported in scientific literature.

Conclusion: Community-dwelling Portuguese older adults have a high prevalence of functional limitations, suggesting the urge to address this situation with nutritional interventions that prevent the development of functional limitations. There was no association between the consumption of high-protein foods and the prevalence of functional limitations in Portuguese older adults. These findings need to be replicated in other studies in different settings.

Keywords: Activities of Daily Living (ADL); Aging; Functional Limitations; Portugal; Protein.

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1. INTRODUCTION

1.1 Ageing and functional limitations

1.1.1 *Ageing and functional decline*

The world's population is ageing and the proportion of older people (65+) is increasing across the globe (1). In the European Union 20.3% of people are older adults (2). This epidemiologic transition encompasses a broad set of changes that include a decline from high to low fertility rates; a steady increase in life expectancy at birth and at older ages; and a shift in the leading causes of death and illness from infectious and parasitic diseases to noncommunicable diseases and chronic conditions (3). Estimates show that the number of people over 60 are expected to double by 2050 (1). The rising life expectancy at 65 is increasing the number and proportion of people at very old ages. The "oldest old" (people aged 85 or older) represent 8% of the world's 65-and-over population: 12% in more developed countries and 6% in less developed countries. In developed countries, the "oldest old" are the fastest growing part of the total population (1, 3). Worldwide, the 85-and-over population is projected to increase 351% between 2010 and 2050, compared to a 188% increase for the population aged 65 or older and a 22% increase for the population under age 65 (3).

Portugal has one of the highest proportions of people aged 65 or older (21.8%)(4). Although the average life expectancy at birth exceeds 80 years, Portugal is one of the countries in the Organisation for Economic Co-operation and Development (OECD) with fewest years of healthy life after age 65 (4). The index of ageing in Portugal is currently of 163 older adults (≥ 65 years) for every 100 young people (0-14 years), with an average life expectancy of 80.9 years (5).

In fact, living longer does not necessarily equate to being healthy or living independently (6). Most adults will experience one or more health problems with lingering consequences. The impact of the disease or injury determines overall well-being and quality of life (6). Frailty, sarcopenia and malnutrition are common geriatric syndromes associated with functional decline, disability, hospitalization, institutionalization, and mortality (6). Frailty can be defined as a "biologic syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems, and causing vulnerability to adverse outcomes" (7). Frailty markers include age-related declines in lean body mass, strength, endurance, balance, walking performance, and low activity, which must be clinically present to constitute frailty. Many of these factors are related and can, in theory, be unified in a cycle of frailty associated with decreased energy and reserves (7). Fried et al. proposed a phenotypic model, based on five essential features: (a) unintentional weight loss; (b) muscle weakness; (c) self-reported fatigue; (d) impaired mobility, and (e) sedentary behaviour (7). This model is used as an operational definition of frailty in research and clinical practice: individuals with three or more factors should be identified as frail, whereas those with one or two features should be considered pre-frail (8).

Geriatric syndromes are not independent of each another and often overlap (6). Ageing and functional decline are inevitable factors. However, the coexistence of frailty, sarcopenia and malnutrition increase the pace and progression towards loss of independence in older adults (6). The coexistence of these syndromes may lead to a cycle of functional decline (**Figure 1**).

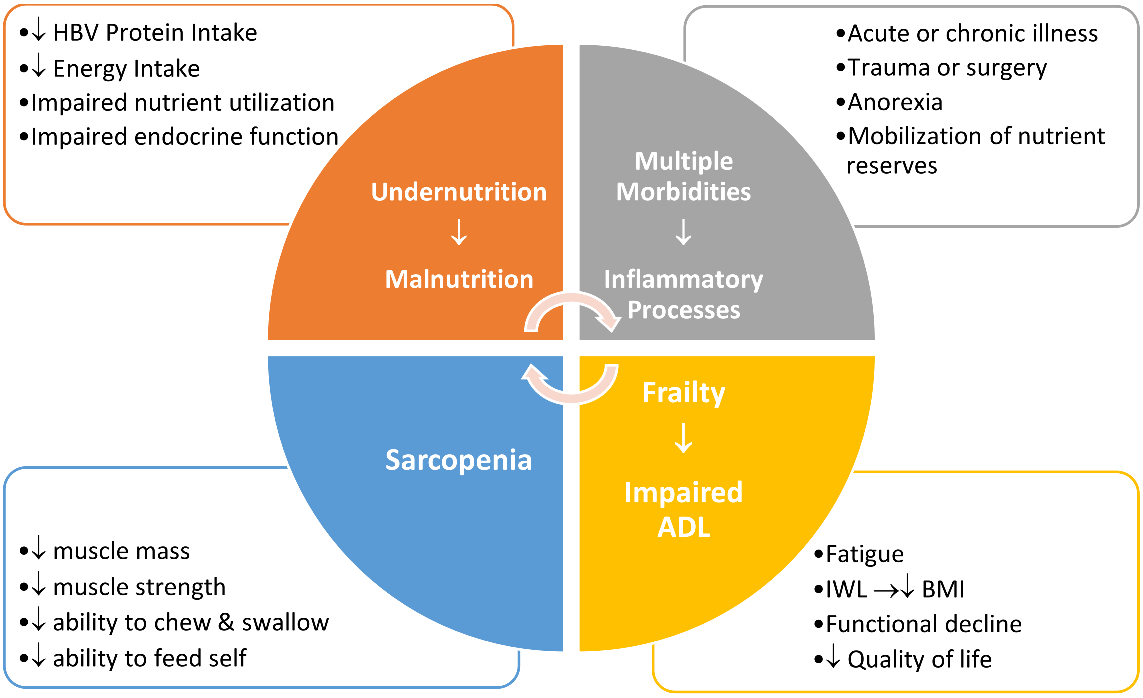

Figure 1. Cycle of functional decline (adapted from Litchford, 2014).

ADL, activities of daily living; BMI, body mass index; HBV, high biologic value; IWL, involuntary weight loss.

Reduced energy intake and insufficient high biologic value protein intake, combined with impaired nutrient utilization and impaired endocrine function, can contribute to undernutrition and consequently to malnutrition. Suboptimal intake of dietary protein compared with estimated physiological requirements contributes to gradual loss of muscle mass and diminished function and strength, evident in sarcopenia (6). Likewise, malnutrition results in skeletal muscle mass loss and involuntary weight loss. Reduced muscle strength leads to weakness, impaired functionality, and loss of nutrition-related activities of daily living (ADLs). Consequences of weakness and functional decline include a more sedentary and reclusive behaviour, affecting the ability of grocery shopping and meal preparation, which can result in the arise of food insecurity (6). Inactivity and age or disease-related inflammation lead to weakness and decreased lean body reserves, which combined with sustained protein and energy intakes below physiological needs, maintains a cycle of impaired functionality and reduced ability to perform nutrition-related ADLs (6). The worst consequences are evident when acute or chronic episodes of illness or injury demands hospitalization. These episodes can significantly reduce physical stamina and appetite, which results in a very low nutrient intake. However, inflammatory stress elevates resting energy requirements and reserves of lean body mass are mobilized to meet protein requirements. The following decline trajectory is exacerbated by inflammation effects aggravated by the iatrogenic effects of hospitalisation or institutionalisation and prolonged bed rest which further promote muscle mass loss. These geriatric syndromes can be prevented and/or attenuated through prevention strategies, including the increase of high-quality protein intake (6).

In a Portuguese cross-sectional study of 1454 older adults with 65 years or older (Nutrition UP 65 study), pre-sarcopenia and sarcopenia were diagnosed in 457 (31.4%) and 65 (4.5%) older adults, respectively, while pre-frailty was identified in 791 (54.4%) and frailty in 310 (21.3%) individuals (9). Also 646 (44.4%) were classified as overweight (25.0 - 29.9 kg/m^2) and 568 (39.1%) as obese (30.0 kg/m^2 or above). Undernutrition was present in 18 (1.2%) older adults and 211 (14.5%) were at risk of undernutrition (9).

Although life expectancy at birth has increased by approximately 3 years over the past decade, disability-free life expectancy [i.e., the healthy life year (HLY) expectancy] has not increased over this same time period (10). This occurrence is not consistent across European countries, since in 12 countries out of 28 the HLY expectancy at 65 years decreased from 2010 to 2014 (11). Furthermore, the gap between life expectancy and healthy life expectancy is increasing (10). HLY expectancy is determined primarily by progressive impairment in performing ADLs, a measure of functional decline that is associated with frailty (12, 13). Frailty is associated with loss of autonomy in performing ADLs as well as health-related problems, institutionalization, and/or hospitalization, with negative impacts on quality of life (10). From a public health perspective, frailty is a multidimensional issue resulting from changes in physical and mental health and functional status as well as lack of social and economic resources (10). There is evidence that functional decline is associated with lower psychosocial status, namely social isolation, malnutrition, and comorbidity (14-18), which are all determinants of frailty (10).

The World Health Organization (WHO) estimates that around 15% of the world population lives with some sort of disability and that between 2% and 4% of people aged 15 and over have functional limitations, with an increase in the rates of disability and chronic health problems, partly due to ageing population (19). A major problem faced by older adults is the decline of their functional capacity (20) and consequently, having to resign themselves to a more dependent way of life (21). Thus, estimating older adults' health involves the assessment of both their physical and functional status (21, 22). The degree of functionality is considered an important determinant of older people quality of life, as it constitutes a strong indicator of aging and independent living (22, 23), as well as a strong indicator of disability (24) and, as a consequence, of mortality

(25), institutionalization (26) and increased use of health care services (27, 28). Therefore, the ability of societies to continue to meet the needs of their oldest members may be increasingly challenged by an increase in the rates of physical impairment due to advanced aging (29). However, it has been noted that such a decline in health may not be inevitable and is certainly not experienced equally by all older adults (30).

Understanding the factors that contribute to functional limitation may help all the older adults' health care workers in the development of efficient preventive strategies (21). Several studies have addressed the risk factors for functional decline, which were summarized in biological, psychological and social factors such as comorbidity, body mass index (BMI), health related behaviours such as drinking and smoking, demographical factors, family and social contacts, cognitive impairment, depression and stress (21, 28).

1.1.1.1 Sarcopenia: a closer look

The ageing process is associated with gradual and progressive loss of muscle mass, accompanied by a decrease in muscle strength and physical endurance, which is known as sarcopenia (31). Sarcopenia is a progressive and generalized skeletal muscle condition associated with increased likelihood of adverse outcomes including falls, fractures, physical disability and mortality (32, 33). It is common among adults of older age but can also occur earlier in life, namely among sedentary adults (31, 32). Sarcopenia definition has suffered updates over time (32, 34). Recently, muscle strength has gained particular importance. In the revised European Guidelines on the Definition and Diagnosis of Sarcopenia (2019), muscle strength comes to the forefront, as it is recognized that strength is better at predicting adverse outcomes than muscle mass (32). Muscle quality is also impaired in sarcopenia and this term describes micro- and macroscopic aspects of muscle architecture and composition (32). Muscle quantity and muscle quality remain problematic as primary parameters to define sarcopenia due to technological limits (32). The detection of low physical performance predicts adverse outcomes and can be used to identify the severity of sarcopenia (32). Therefore, sarcopenia is now defined by low levels of measures for three parameters: (i) muscle strength, (ii) muscle quantity/quality and (iii) physical performance as an indicator of severity (32).

Sarcopenia can be prevented with regular resistance and aerobic exercise (31). In addition, good nutrition, in particular adequate energy and protein intake, can help stem the decline in muscle mass, strength and functional abilities (31).

1.1.2 *Concepts: from "active ageing" to "functional limitations"*

Active ageing is a multidimensional concept affected by several factors, including physical functionality, lifestyle, urban environment, and social inclusion (10, 35, 36). In 2015, the WHO defined active ageing as "the process of optimizing opportunities for health, participation and security in order to enhance quality of life as people age" and healthy ageing as "the process of developing and maintaining the functional ability that enables well-being in older age" (37). The World Health Report on ageing emphasized the role of public health strategies in building and maintaining health in older adults (10, 37). The concept of active aging is closely linked to functional capacity, defined by the maintenance of autonomy and independence in daily life, although there may coexist some physical, mental or social limitation (36, 38).

An operational definition of healthy ageing is still being debated, nevertheless, and agreement has not yet been achieved (10). McLaughlin and colleagues analysed the impact of different definitions of healthy ageing and concluded that a functional definition of health, i.e., free from symptomatic diseases and disabilities, may be acceptable (39), although they did not account for the social dimension of "active" ageing (10). This social dimension is considered to be crucial since its impact on developing and maintaining health at all ages (10). A more comprehensive approach to assess the process of active and healthy ageing at the population level should therefore consider several domains, including health status, income security, capability, and environment (10, 40). This concept is consistent with the approach recently proposed by WHO, describing healthy ageing as the result of the interaction between the physical and mental capacity of an individual (the intrinsic capacity), and the context of each individual's life (the environment) (10, 41).

Through the assessment of changes in pain and movement, and corresponding improvements that arise from these changes, the disablement process can be evaluated at the levels of impairment, functional limitation, and disability (42).

Impairment has previously been defined as "anatomical, physiological, mental or emotional abnormalities or loss", whereas functional limitation is defined as "limitation in performance at the level of the whole organism or person" (43). In fact, functional limitations constitute restrictions in performing fundamental physical and mental actions used in daily life by one's age-sex group (21, 43). In contrast, disability is considered a "limitation in performance of society defined roles and tasks within a sociocultural and physical environment" (43, 44) or more concisely as "any restriction or lack of ability to perform a task or an activity in the manner considered normal for a person" (45). The disablement model refers to "various impact(s) of chronic and acute conditions on the functioning of specific body systems, on basic human performance, and on people's functioning in necessary, usual, expected, and personally desired roles in society" (43, 44, 46). Therefore, this model is used to determine the consequences of disease and injury "both at the level of the person and at the level of society" (44).

In contrast to disablement models such as the Nagi model (44), the International Classification of Functioning, Disability and Health (ICF), published by WHO, does not discriminate between functional limitation and disability (45, 47). The domains described within the ICF model are classified from body, individual, and societal perspectives by means of two lists: (1) a list of body functions and structure; and (2) a list of domains of activity and participation (47). Activity and participation are influenced by contextual factors, including personal and environmental. The ICF differs most dramatically from the disablement model in that the definition of functioning (or activity) is highly complex and multi-dimensional, and likely differs from person to person (42, 47). For example, the WHO describes functioning as "an umbrella term for body functions, body structures, activities and participation"; "it denotes the positive aspects of the interaction between an individual (with a health condition) and that individual's contextual factors (environmental and personal factors)" (47). The ICF defines disability as a concept comprising impairments, activity limitations and participation restrictions (47). Impairment is considered a "loss or abnormality in body structure or physiological function (including mental functions)"; activity limitations are "difficulties an individual may have in executing activities", whereas participation restrictions are considered "problems an individual may experience in involvement in life situations" (47).

Figure 2 represents two conceptual schemes created in the 80's and early 90's that have informed discussions and research on disability and contributed to the Disablement Process Model (**Figure 3**) (43). The International Classification of Impairments, Disabilities, and Handicaps (ICIDH), now referred to as the ICF, provided an inventory of numerous specific titles about three central concepts: Impairment, Disability, Handicap (43). Despite political acceptance, scientific researchers had trouble using ICIDH as a basis for hypothesis development and study design, which resulted in an updated version in 2001 (43). The other scheme was conceived and developed by the sociologist Saad Nagi and had four central concepts: Active Pathology, Impairment, Functional Limitation and Disability (43). The concepts of Functional Limitation and Disability cover essentially the same scope as ICIDH's Disability (43). There is no parallel concept for Handicap. It was sociological theory that underpinned Nagi's work, not taxonomic interest (43). The Institute of Medicine of the United States of America (IOM) adopted Nagi's basic framework (43). The Disablement Process, published in 1994, has its main foundation in the Nagi scheme, but also draws in the scope and detail of ICIDH, being useful for research design (43).

International Classification of Impairments, Disabilities, and Handicaps (ICIDH)*

"DISEASE" →	IMPAIRMENT →	DISABILITY →	HANDICAP
The intrinsic pathology or disorder	Loss or abnormality of psychological, physiological, or anatomical structure or function at organ level	Restriction or lack of ability to perform an activity in normal manner	Disadvantage due to impairment or disability that limits or prevents fulfillment of a normal role (depends on age, sex, sociocultural factors) for the person

Nagi Scheme

ACTIVE PATHOLOGY →	IMPAIRMENT →	FUNCTIONAL LIMITATION →	DISABILITY
Interruption or interference with normal processes, and efforts of the organism to regain normal state	Anatomical, physiological, mental, or emotional abnormalities or loss	Limitation in performance at the level of the whole organism or person	Limitation in performance of socially defined roles and tasks within a sociocultural and physical environment

Figure 2. Conceptual schemes for disablement (adapted from Verbrugge & Jette, 1994).

*This version was revised and updated to the actual version: International Classification of Functioning, Disability and Health, World Health Organization 2001.

EXTRA-INDIVIDUAL FACTORS

MEDICAL CARE & REHABILITATION
surgery, physical therapy, speech therapy, counseling, health education, job retraining, etc.

MEDICATIONS & OTHER THERAPEUTIC REGIMENS
drugs, recreational therapy/aquatic exercise, biofeedback/meditation, rest/energy conservation, etc.

EXTERNAL SUPPORT
personal assistance, special equipment and devices, standby assistance/supervision, day care, respite care, meals-on-wheels, etc.

BUILT, PHYSICAL, & SOCIAL ENVIRONMENT structural modifications at job/home, access to buildings and to public transportation, improvement of air quality, reduction of noise and glare, health insurance & access to medical care, laws & regulations, employment discrimination, etc.

THE MAIN PATHWAY

PATHOLOGY → **IMPAIRMENTS** → **FUNCTIONAL LIMITATIONS** → **DISABILITY**

PATHOLOGY
diagnoses of disease, injury, congenital/ developmental condition.

IMPAIRMENTS
dysfunctions and structural abnormalities in specific body systems: musculoskeletal, cardiovascular, neurological, etc.

FUNCTIONAL LIMITATIONS
restrictions in basic physical and mental actions: ambulate, reach, stoop, climb stairs, produce intelligible speech, see standard print, etc.

DISABILITY
difficulty doing activities of daily life: job, household management, personal care, hobbies, active recreation, clubs, socializing with friends and kin, childcare, errands, sleep, trips, etc.

RISK FACTORS

Predisposing characteristics: demographic, social, lifestyle, behavioral, psychological, environmental, biological.

INTRA-INDIVIDUAL FACTORS

LIFESTYLE & BEHAVIOUR CHANGES
overt changes to alter disease activity and impact.

PSYCHOSOCIAL ATTRIBUTES & COPING
Positive affect, emotional vigor, prayer, locus of control, cognitive adaptation to one's situation, confidant, peer support groups, etc.

ACTIVITY ACCOMMODATIONS
changes in kinds of activities, procedures for doing them, frequency or length of time doing them.

Figure 3. The Disablement Process model (adapted from Verbrugge & Jette, 1994).

1.1.3 *Measuring functional limitations*

Function can be measured in a number of different ways, including through the use of impairment measures, self-report measures, and physical performance measures (42).

Functional limitations are restrictions in performing fundamental physical and mental actions used in daily life by one's age-sex group. These are generic actions, recruited in many specific circumstances (43). They indicate overall abilities of body and mind to do purposeful "work" (43). Fundamental physical (body) actions include overall mobility, discrete motions and strengths; examples are walking, lifting objects and climbing stairs (43). Tests of physical and mental actions have various formats: (i) self-reports or proxy reports (spouse, parent or personal physician, etc.) of difficulty doing an action (no difficulty, some, a lot, unable); (ii) an interviewer's observation of the subject doing an action, with a rating of her/his performance (fully able, partially able, unable) or sometimes counts (of steps, of repetitions before fatigue, etc.); and (iii) equipment-based evaluation of performance, including timed tasks. All these measure a person's ability to perform tasks "on their own" and without assistance (43).

Katz and Lawton developed the indexes of ADLs and instrumental activities of daily living (IADL) (13, 48). According to the National Research Council (2009), the purpose of measuring ADL has been to calculate the prevalence of functional limitation and disability, evaluate the service provision need of older populations, and to determine to what extent a given disabled group can participate in the activities of the broader population (49, 50).

The Health Assessment Questionnaire (HAQ), also referred to as the Health Assessment Questionnaire Disability Index (HAQ-DI) or the original HAQ (51), is one of the most widely used instruments for measuring functional disability (52). The HAQ was originally developed and validated for English-speaking populations in the United States and Canada (53). It has since been translated or culturally adapted into more than 60 different languages or dialects, often with only minor changes, including Portuguese language (53, 54). This instrument contains questions on functional limitations from which a valid, effective, and sensitive measure of disability, the HAQ-DI (range of scores 0-3; with a higher score representing worse functional ability), can be computed (52). Computation of a Disability Index is made possible by not considering questions regarding the use of aids/help (51). The cut-off value of HAQ-DI > 0 was proposed by Krishnan et al. (52). The decision of using this cut-off value for functional limitation is sustained by the previously used arguments in 2004 (52). The mentioned authors calculated disability rates as the proportion of individuals with a HAQ-DI > 0 as opposed to any other cut-off value, for the following reasons. Instead of asking how much disability is disability, from a clinical stand point they asked "How much disability is 'undisability' or having no disability?" (52). The answer to this question is simple when it comes to the HAQ – a HAQ-DI equal to 0 (52). They considered to be a less controversial method than using any other cut-off value for the HAQ-DI because once the individual expresses some difficulty in performing one or more activities (HAQ-DI > 0), then by definition the individual is disabled (52). This cut-off value was recommended to define disability, since it is intuitive, sensitive, valid, and is useful and meaningful in all settings (52). Furthermore, the other compelling reason presented for using a dichotomized measure of functional disability is the fact that the distribution of HAQ-DI in patient groups and in general population is not Gaussian (52). Consequently, any measures of central tendency, such as mean or median, are associated with imprecise measures of dispersion, such as standard deviation or interquartile range (52). Therefore, dichotomizing the HAQ-DI at 0 would result in a more robust measurement in certain situations and would supplement mean and median measurements (52).

1.2 Recommendations for protein intake in older adults

The WHO and the Institute of Medicine of the United States of America (IOM) recommend a protein intake (Recommended Dietary Allowance, RDA) of 0.8 g/kg body weight (BW)/d, regardless of age (> 18 years) (55, 56). In the United Kingdom, the recommendation is 0.75 g/kg BW/d (57), while in the Nordic Countries, Australia and New Zealand the value reaches 1.2 g/kg BW/d (58). Several authors and scientific associations consider the RDA of 0.8 g/kg BW/d insufficient to prevent sarcopenia and to compensate for the protein catabolism associated with aging (31, 59-63). The European Society for Clinical Nutrition and Metabolism (ESPEN) recommends the intake of 1.0 to 1.2 g protein/kg BW/d for healthy older people; 1.2 to 1.5 g protein/kg BW/d for older people who are malnourished or at risk of malnutrition, with even higher intake for individuals with severe illness or injury (31). ESPEN also recommends daily physical activity or exercise (resistance training, aerobic exercise) to older people, for as long as possible (31). These recommendations are in line with those of the PROT-AGE study group (64). Nordic nutrition recommendations suggest an even higher RDA, from 1.2 to 1.4 g/kg BW/d (65).

Although sarcopenia is multifactorial, protein intake, its dietary sources and timing of intake seem to be decisive in the aetiology and management of this ageing-related muscle and strength loss (61). Ensuring protein intake above 1.0 g/kg BW/d and the ingestion of ~25-30 g of protein per eating occasion across 3 main meals/d, in close temporal proximity to physical activity, may be the necessary strategy to effectively and efficiently stimulate muscle protein synthesis in older adults (66, 67). Experimental studies that compared muscle protein synthetic responses to protein ingestion in young and old adults suggest that a higher relative protein intake is necessary to maximally stimulate skeletal muscle protein synthesis in older adults (58). A large and comprehensive study with over 700 individuals aged 85 years old (Newcastle 85+ Study) found that 28% of the participants had protein intakes below the WHO recommendation (< 0.8 g /kg BW/d) (61). Low protein intake (< 0.8 g /kg BW/d) was less likely when participants had a higher percent contribution of meat and meat products to total protein intake (OR 0.97, 95% CI 0.95, 1.00) but more likely with a higher percent contribution of cereal and cereal products and non-alcoholic beverages (61). Having higher energy intake ($p < 0.001$), being a woman ($p < 0.001$) and higher tooth count ($p = 0.047$) was associated with higher protein intake in adjusted models (61).

Timing, distribution, and quality of dietary protein throughout the day are important factors in maximizing tissue accretion (67). The biological quality of dietary protein is determined by the distribution and quantity of essential amino acids (EAA) required for tissue accretion (68). Moreover, the timing of meals and the quantity of high-quality protein consumed are also important factors. One study in healthy older women found that providing 79% of the day's protein at the noon meal improved protein retention compared with a more even distribution of dietary protein across all 3 meals (69). Other researchers found that consuming 30 g of high-quality dietary protein at each meal maximized protein synthesis in non-exercising adults. The key was consuming sufficient amounts of the EAA leucine required to activate mammalian target of rapamycin (mTOR) signalling pathway that regulates protein synthesis and muscle tissue accretion (70, 71). Protein intake of 70 g in a mixed meal, which represents the manner in which dietary protein is normally consumed, induced a greater anabolic response in older adults, compared to an isocaloric meal containing 35 g of protein (72). The higher protein intake stimulated a greater protein synthetic response, both in the whole-body and skeletal muscle (72). Whole-body protein breakdown was also suppressed to a greater extent with the higher level of protein intake (72). These data indicate that the proposed "optimal" level of dietary protein in a meal of 30–35 g significantly underestimates the needs of older individuals (72). Offering up to 90 g high-quality dietary protein in a single serving did not stimulate further protein synthesis (71). Not all sources of dietary protein are nutritionally equivalent (68). Animal-based plant protein is expected to have a higher content of branched-chain amino acids, thus suggesting greater stimulation of anabolic pathways and muscle protein synthesis than plant-based protein (8). Additionally, animal-based protein have higher digestibility rates (~90%) compared to plant-based protein (~50%) (8). The benefit of enhancing a low-protein diet with a leucine supplement demonstrated an improved muscle synthetic response in a small non-random controlled cohort of community-dwelling elders at risk of muscle loss (73). More research is needed to clarify the long-term benefit of selected amino acid supplementation in modifying the trajectory of sarcopenia or as a secondary nutrition prevention to counteract the effects of sarcopenia (6). The Society for Sarcopenia, Cachexia and Wasting Disease recommendations for the prevention and management of sarcopenia serve as primary and secondary nutrition preventions to manage the concurrent occurrence of frailty, sarcopenia, and malnutrition. Selected recommendations include protein intakes 1.0–1.5 g/kg/d, leucine-enriched EAA supplements, and resistance exercise (74).

In a systematic review and meta-analysis including 29 studies, covering 2255 participants (mean age: 78.1 years), amino acids, creatine, β-hydroxy-β-methylbutyrate, and protein with amino acids supplementation significantly improved muscle mass (75). No effect was found for protein supplementation alone, protein and other components, and polyunsaturated fatty acids (75). High interstudy variability was observed regarding the dose, duration, and frequency, coupled with inconsistency in reporting timing and adherence (75). Overall, several nutritional interventions could be effective to improve muscle mass measures in older adults but, due to the substantial variability of the intervention factors among studies, the optimum profile is yet to be established (75).

In Portugal, regarding protein intake and according to the latest National Food and Physical Activity Survey (2015-2016), 48.6% of Portuguese older adults consume less than 1 g protein/kg BW/d (60.3% women; 38.5% men), being the "meat, fish and eggs" (46.0%) the group that contributes the most to protein intake, followed by "cereals, derivatives and tubers" (20.1%) and "dairy products" (15.9%) (76).

Considering there is no evidence that a reasonable increase in dietary intake adversely affects health outcomes and deductive reasoning suggests beneficial effects of a higher protein intake, it is logical to recommend an optimal dietary protein intake for older individuals greater than the RDA of 0.8 g/kg/day (33).

1.3 Protein intake and functional outcomes

Protein intake in combination with exercise are determinants for maintaining muscle function (31). Although all nutrients, in general, play an important role in maintaining muscle mass, it is the adequate and regular intake of protein that stands out as essential to stimulate protein synthesis (60, 77, 78). Reduced protein intake in the older adults has been associated with loss of muscle mass and strength, greater disability, loss of independence and mortality in several cohort studies (61, 79-82). For this reason, the prevention of sarcopenia is an important goal, given its association with the increased likelihood of falls and the inability to perform ADLs (65). Essential amino acids, particularly leucine, can directly stimulate muscle protein synthesis by activating translation initiation via the mTOR pathway (59). Adequate protein intake is critical to provide essential amino acids to replace those who are lost via catabolic pathways and support protein accretion and growth (59). Therefore, protein intake can play an important role in the maintenance of muscle mass which is determinant for adequate function and also for disease outcomes (59). Effective primary nutrition preventions for geriatric syndromes like sarcopenia, frailty and malnutrition include increasing intakes of high-quality protein with sufficient levels of leucine, optimizing vitamin D status with dietary supplements, and increasing daily resistance exercise (6). There is evidence for clinicians to encourage older adults to set a goal to consume 30 g of dietary protein per meal from foods and protein fortifiers or supplements (6). Foods rich in leucine include milk and other dairy products, eggs, seafood, and lean muscle meats. Leucine-rich protein supplements include whey protein isolate and soy protein isolate (6).

Scientific evidence on the association between protein intake and functional disability in older adults is limited (83). Some observational studies have found that higher protein intake was associated with a lower prevalence and lower incidence of functional disabilities in community-dwelling older adults (84-86). A more recent study concluded that protein intake ≥ 1.0 g/kg adjusted BW/d was associated with a lower probability of having functional limitations in the subsequent 5 years (83).

The Health, Aging and Body Composition study assessed the association between dietary protein and changes in lean body reserves over a 3-year period. Participants in the highest quintile of protein intake lost approximately 40% less lean body mass than those in the lowest quintile. The authors concluded that dietary protein may be a modifiable risk factor for sarcopenia in older adults (79).

The Newcastle 85+ Study revealed that a higher protein intake, especially ≥ 1.0 g/kg adjusted BW/d, in the oldest adults (aged 85 or more) was associated with fewer disabilities at baseline and shallower disability trajectories over the subsequent 5 years, after adjusting for covariates (83, 87). In turn, the intake of < 1.0 g of protein/kg adjusted BW/d can negatively affect future muscle strength and physical performance, especially in older women, regardless of important covariates (66).

Framingham Offspring Study participants (n = 1896), aged 50 and older, were followed for an average of 14.4 years to assess long-term effects of weight-adjusted animal and plant protein intakes on aging-related change in functional status and grip strength (88). Higher animal protein intake and higher levels of physical activity and skeletal muscle mass were independently associated with lower risks of functional impairment and greater preservation of grip strength (88). In this study, functional impairment was defined as failure to complete (or having a lot of difficulty completing) a given task (88). Another study with Framingham Offspring participants (n = 2917) observed favourable associations between protein intake and functional integrity over a greater than 20-year timeframe, notably in women. The approach relied on function-related questions, such as the ability to pull, lift, and do housework, asked repeatedly, and validated against objective physical performance as well as a well-characterized frailty phenotype (89).

A study examined whether adherence to a higher protein diet while participating in a resistance-based exercise program promoted more favourable changes in body composition, markers of health, and/or functional capacity in 54 overweight and obese older females in comparison to following a traditional higher carbohydrate diet or exercise training alone with no diet intervention (90). All groups experienced significant improvements in muscular strength, muscular endurance, aerobic capacity, markers of balance and functional capacity, and several markers of health (90). However, increasing the proportion of dietary protein during the diet phase did not promote greater improvement in fitness, strength, and/or functional capacity than those in the high carbohydrate or exercise only groups (90). A higher protein diet while participating in a resistance-based exercise program promoted more favourable changes in body composition compared to a higher carbohydrate diet in older females (90). This study supports recommendations that older individuals should participate in resistance-training and consume a higher proportion of protein in their diet in order to maintain muscle mass (91-95).

In a more recent cross-sectional study (2019), protein intake and functionality of IADL and ADL were assessed in 190 middle to older aged Mexican adults. It was found a high proportion of participants with inadequate protein intake (IPI) (< 1.2 g/kg/day, < 30 g/meal, or < 0.4 g/kg/meal) per day, being dinner the meal with the highest proportion of IPI. IPI at lunch was a significant risk factor for impaired functionality in ADL when assessed with the 30 g/meal criterion (96).

However, in a study with 97 healthy community-dwelling adults without functional limitations aged 75–85 years, aspects of protein intake were not associated with muscle mass, strength, or power (97). The study investigated single and combined associations of daily protein intake, evenness of protein distribution across the three main meals, number of meals providing ≥ 0.4 g protein/kg BW, and number of meals providing ≥ 2.5 g leucine, with muscle mass, strength, and power in healthy older adults (97).

Higher protein intake from vegetable source was associated with reduced muscle loss in Chinese community-dwelling older adults (≥ 65 years; n = 2726), whereas no association between total and animal protein intake and subsequent decline in muscle mass or physical performance measures was observed (98).

Giving the scarce and controversial evidence on the subject, it is clear the need to further investigate the relationship between protein intake, namely high-protein foods intake, and functional limitations in this age group.

The hypothesis of this study is that Portuguese older adults who consume more animal protein-source foods, like meat, fish and dairy products, would have greater preservation of muscle strength and better functional capacity, which would translate into less functional limitations (less difficulty with ADL). Considering the existence of potential confounders in this relationship, such as sex, education level, BMI and multimorbidity, statistical models were adjusted to these important variables. The rationale of this hypothesis is based on the beneficial effect of increased dietary protein intake, namely the maintenance of muscle mass and improvement of strength capacity (33). Increased dietary protein and subsequent increased availability of plasma amino acids stimulates muscle protein synthesis, which therefore leads to improved muscle mass, strength and function (33). Increased muscle mass, strength and function is related to improved health outcomes in older individuals (33). Then, it is reasonable to presume that a higher protein intake will translate to improved health outcomes in older adults (33). Although cause-effect relationship between muscle mass, strength and function and health outcomes is subject to debate, it is clear that increased muscle mass and function in older adults translates to an improved ability to perform ADLs and therefore quality of life (33).

2. AIMS

This study aims to (1) determine the prevalence of functional limitations, through the ability to perform ADLs, in community-dwelling Portuguese older adults, using the Epidemiology of Chronic Diseases Cohort (EpiDoC) database and (2) establish the association between high-protein food consumption and functional limitations among these population.

3. METHODOLOGY

3.1 Study design and participants

This study is a cross-sectional evaluation of the second wave of the EpiDoC Cohort (i.e., the EpiDoC 2 study). EpiDoC study is an observational prospective closed cohort study that started in 2011 and includes health information on 10661 adults (of whom 22% were ≥ 70 years old) (99). The sample is representative of the Portuguese population (≥ 18 years old) and the participants were followed for five years (99, 100). The participants were non-institutionalized or living in private residences in the Portuguese Mainland and Islands (Madeira and Azores) (99). Population recruitment was conducted by *Centro de Estudos e Sondagens de Opinião da Universidade Católica Portuguesa*, and multistage random sampling was used for participant selection (99). The study sample was stratified by administrative territorial units (NUTS II: Norte, Centro, Lisboa, Alentejo, Algarve, Azores and Madeira) (99).

The EpiDoC study is comprised of three waves: EpiDoC 1, EpiDoC 2 and EpiDoC 3 (99). For the purpose of this study data from the second wave (EpiDoC 2) was analyzed. The EpiDoC 2 enrolled 7591 participants (out of the initial 10 153 eligible participants), resulting in a response rate of 71.2% from EpiDoC 1 (99). Data collection occurred between March 2013 and July 2015 (99).

The EpiDoC 1, or EpiReumaPt, was the first cross-sectional evaluation, performed between September 2011 and December 2013 (100). This study primarily assessed the prevalence of rheumatic and musculoskeletal diseases and their impact on health-related quality of life, physical function, and mental health in Portugal (100, 101). In this wave, face-to-face interviews were conducted at the residences of the 10 661 participants, followed by a medical appointment one or two weeks later at the nearest primary health care centre (100).

The EpiDoC 2 expanded the scope of EpiDoC 1 and focused essentially on lifestyle behaviours and their determinants, with the secondary objective of identifying innovative solutions for patients with disabilities (99). It included more detailed questions, such as physical activity, dietary habits, tobacco and alcohol use and sleeping habits (99). A structured questionnaire was administered during a telephone interview conducted by trained research assistants, using a computer-assisted personal interview system (99). When the initial contact attempt was not successful, additional attempts were made (morning, afternoon, evening, and weekends), to a total of six attempts (100). Data collection included information on the sociodemographic and economic situation, chronic diseases and functional limitations (99). Data were recorded on a standardized form, and database access was restricted by use of a unique username and password for each research team member (100).

EpiDoC 2 included 7591 adult participants, of whom 2393 were older adults (65 years and older) (**Figure 4**). The population of this study consisted of Portuguese older adults from the EpiDoC cohort. Participants were included in the analysis if they were 65 years and older at the moment of data collection and if they presented data regarding the variables included in the analysis. The variables of interest comprised (i) independent variables, like the consumption of high-protein food groups (meat, fish and dairy frequency consumption), (ii) dependent variables (outcomes), namely the HAQ final score and four single scores of the HAQ regarding the ability to perform four different ADLs and also (iii) covariates, as the educational level, sex, physical activity and BMI. Participants were excluded from the analysis if they had less than 65 years of age (n = 5198) and if they had missing or invalid dietary data, missing covariates or had missing or invalid data on the outcome variables (n = 1064 to 1067). For the participants' characterization analysis all the participants with 65 years and older (n = 2393) were included in the analysis, despite some of them missing information regarding one or more variables of interest. The analytic study population used in regression models varied because of missing data (n = 1326 to 1329) (**Figure 4**).

3.2 EpiDoC cohort measurements

3.2.1 *Sociodemographic data*

Information regarding sociodemographic factors (sex, age, years of education, Nomenclature of Territorial Units for Statistics [NUTS] II region) was collected in the EpiDoC 1 study. During the EpiDoC 2 interview, subjects were asked if any of these characteristics had changed (102).

For this study, the educational level was defined based on the years of education of each participant. Education level was classified according to the UNESCO's International Standard Classification of Education (ISCED), a reference classification for organizing education programmes and related qualifications by education levels and fields (103). Primary education (ISCED levels 0-2) refers or 9 or less years of education; secondary education (ISCED levels 3-4) corresponds to 10 to 12 years of education and tertiary education (ISCED levels 5-8) to more than 12 years of education (103).

3.2.2 *Health data*

In the EpiDoC 1 study, individuals were asked whether they had been previously diagnosed with the following chronic diseases: hypercholesterolemia, hypertension, rheumatic disease, allergy, gastrointestinal disease, mental disease, cardiac disease, diabetes, thyroid or parathyroid disease, pulmonary disease, hyperuricemia, cancer, or neurologic disease (102). This information was updated in the EpiDoC 2 study interview. Multimorbidity was defined as the coexistence of two or more of these self-reported chronic diseases (104). Participants were also questioned about use of medication. Health-related quality of life was assessed using the European Quality of Life questionnaire with five dimensions and three levels (EQ-5D-3L) (105), which included perceived health status self-reported in a scale from 0 (the worst) to 100 (the best).

3.2.3 *Lifestyle data*

The EpiDoC 2 study included several questions concerning lifestyle habits, such as frequency of alcohol intake, smoking habits, frequency and type of physical activity (102). Physical activity level was classified according to self-reported weekly frequency of physical activity (102).

Smoking habits included the following options “never smoked”, “smoked in the past”, “smokes daily” and “smokes occasionally”. The last two options were merged in the final variable, due to limited frequencies, and originated one single category: “smokes daily or occasionally”. Alcohol intake was classified according to the following categories: “never consumed”, “daily intake”, and “occasional intake”. Regarding physical exercise, participants were asked if they were regularly active.

3.2.4 *Dietary intake*

Dietary intake was assessed through food frequency questions regarding the following foods and beverages: soup, vegetables, fresh fruit, milk, and other dairy products. Participants also reported the usual number of meals per day.

The intake of high-protein food sources was assessed through the frequency of fish, meat and milk and dairy products consumption reported in the questionnaire on dietary intake and behaviours.

Initially the high-protein foods variables had six categories for the consumption frequency, but they were transformed into four categories. The categories “Rarely” and “Never” were merged due to limited frequencies. The “Every day” and “7-10 times/week” or “6 times/week” categories were also merged as they represented an approximated food frequency value. The food frequency variables regarding fruit, vegetables, soup and milk and dairy products consumption assumed the following values: “Every day or 6 times/week”, “3-5 times/week”, “1-2 times/week”, “Rarely or Never”. The food frequency variables regarding fish and meat consumption had the following categories: “Every day or 7-10 times/week”, “4-6 times/week”, “1-3 times/week”, “Rarely or Never”.

3.2.5 *Anthropometric data*

Self-reported height and weight were collected in the EpiDoC study. Based on these data, BMI (weight/height^2 in kg/m^2) was calculated and initially categorized according to the World Health Organization (WHO) classification system (106, 107). For the purpose of this study some adjustments were made since our population are older adults and the WHO classification system may be inappropriate (108-111). Thus, the BMI values were re-categorized according to the BMI ranges for older adults proposed by Lipschitz in 1994 [underweight: BMI < 22 kg/m^2, normal weight: BMI 22 - 27 kg/m^2, overweight: BMI > 27kg/m^2)] (112). The BMI was included in the analysis both as a continuous variable and as a categorical variable.

3.2.6 *Physical function*

Physical function was evaluated using the HAQ (range of scores 0-3), with a higher score representing worse functional ability. The HAQ contained eight categories, reviewing a total of 20 specific functions evaluate patient difficulty with ADLs over the past week (51). There were 41 total items: 20 specific ADLs are assessed on a 4-point Likert scale where 0 = without any difficulty, 1 = with some difficulty, 2 = with much difficulty, and 3 = unable to do; 13 additional questions assessing use of assistive devices, and 8 additional questions assessing help received from another. The 20 activities were grouped into 8 functional categories with each category given a single score equal to the maximum value of their component activities (0, 1, 2, or 3). Categories include dressing and grooming, arising, eating, walking, hygiene, reaching, gripping, and errands and chores. It also identified specific aids or devices utilized for assistance, as well as help needed from another person (aids/help) (51). A Portuguese translated version of the HAQ was administrated during the telephone call by a trained interviewer. Portuguese validated versions of the assessment scale were used.

The prevalence of functional limitation was calculated using the HAQ DI > 0 cut-off value already proposed in literature by Krishnan et al. (52). Thereby, the presence of functional limitation was considered when participants had a final score > 0 in the HAQ.

The HAQ final score or HAQ-DI (range of scores 0-3) was considered an outcome variable to determine if there was an association between high-protein food consumption and functional limitations in the study population. The other outcome variables considered in this analysis consisted of four individual scores regarding the difficulty with four ADLs: 1) *Able to stand up from a straight chair, 2) Able to walk outdoors on flat ground*, 3) *Able to climb up five steps*, and 4) *Able to reach and get down a 5-pound object (such as a bag of sugar) from above your head*. The four mentioned ADLs were abbreviated in the results, respectively, as following: *stand up, walk, climb, reach*.

The choice of these specific ADLs relies on the fact that they are the ones that demand more from the large muscle groups in the body. Thus, considering the purpose of this study, these ADLs should be more suitable as outcome variables once they can better reflect functional limitations caused by insufficient protein consumption by older adults.

3.3 Statistical analysis

A binary logistic regression model and a linear regression model were used to assess the effect of high-protein food sources on functional limitations. The dependent variable (functional limitations) was nominal. The independence of the observations was assumed and the categories of the dependent variable were mutually exclusive. Multicollinearity was kept to a minimum by examining the variance inflation factor. The variables were chosen based on their theoretical value for the research question and the statistical significance for the model in question.

In the descriptive analysis, absolute frequencies (n) and proportions (%) were used to summarize categorical variables, whereas continuous variables were described by mean values ± standard deviations (SD). The ADLs' outcome variables were transformed in order to merge two of the four initial categories, resulting in three new categories: 0 = without any difficulty, 1 = with some difficulty, 2 = with much difficulty or unable to do. This transformation was necessary given the small frequency in some categories. The HAQ final score was included in this analysis as a continuous variable. To access if there was consumption frequency difference between groups, Chi-squared test was used for categorical variables, ANOVA test for continuous variables and Kruskal-Wallis test for HAQ final score variable (homogeneity of variances was not observed).

The effect of high-protein foods on functional limitations was assessed through binary logistic regression and linear regression models.

To conduct the binary logistic regression there was the need to binarize the outcome variables. The HAQ final score variable was recoded into a binary variable: 0 = absence of functional limitation and 1 = presence of functional limitation. The other four outcome variables, regarding the difficulty with four ADL's were recoded as follows: 0 = without any difficulty, and 1 = with difficulty (any degree) or unable to do. Estimates of the binary logistic regression were presented as Odds Ratio (OR) and 95% Confidence Interval (CI).

Linear regression was conducted using the HAQ final score as a continuous outcome variable. Estimates for linear regression were Beta estimates (β) and Standard Error (SE).

In the final regression models, the considered reference category of food frequency questions was "Rarely or Never". The models were also analysed using the "Every day or 6 times/week" or the "Every day or 7-10 times/week" as the reference category.

Potential confounders of the relationship between high-protein foods consumption and functional limitations were included in both models (binary logistic and linear regression) as covariates. Models were adjusted as follows: the first model was not adjusted for any covariate (unadjusted model); socioeconomic model was adjusted for age, sex, geographical location (NUTS II) and education level; lifestyle model was adjusted as for socioeconomic model, plus variables of BMI, smoking status, alcohol intake, physical activity and vegetables consumption; health model was further adjusted for self-rated health (perceived health status scale), multimorbidity and pharmacological treatment.

To access if there was high-protein foods consumption difference between groups, Chi-squared test was used for categorical variables and ANOVA test for continuous variables. For meat and fish consumption, Kruskal-

Wallis test was used for HAQ final score variable (homogeneity of variances was not observed in the Levene's test: $p \leq 0.001$).

Statistical analyses were performed with SPSS Version 25 (IBM SPSS Statistics, Chicago, IL, USA) and $p < 0.05$ was considered to be statistically significant.

3.4 Ethical issues and data confidentiality

The EpiDoC study was performed according to the principles established by the Declaration of Helsinki and revised in 2013 in Fortaleza (113). The study obtained ethical approval from the National Committee for Data Protection (*Comissão Nacional de Proteção de Dados*) and by the NOVA Medical School Ethics Committee. Ethical Committees of Regional Health Authorities also reviewed and approved the study (100).

Participants' confidentiality was safeguarded by the lack of identifiers in the database (only a unique identification code was used for each participant) (102). The name and contact information for each subject were stored separately from the study data transmitted to the coordinating centre (102). All data were kept anonymously and securely by authorized EpiDoC staff. Written informed consent was signed during EpiDoC 1 by participants agreeing to be followed and participate in the EpiDoC 2 study (102).

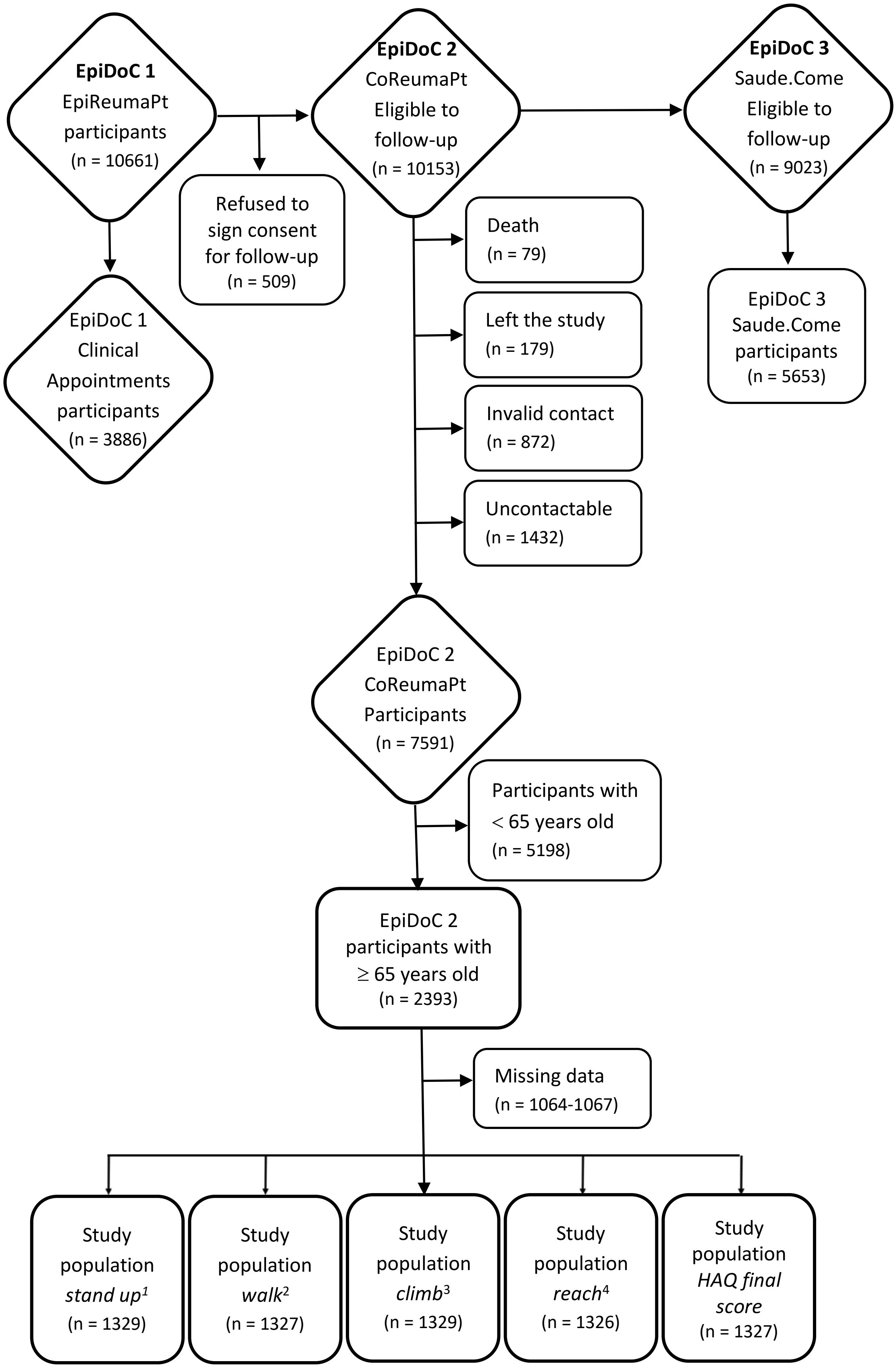

Figure 4. Flowchart describing the study population.

[1]*stand up*: stand up from a straight chair; [2]*walk*: walk outdoors on flat ground; [3]*climb*: climb up five steps; [4]*reach*: reach and get down a 2.5 kg object from above the head.

4. RESULTS

4.1 Prevalence of functional limitations

The overall prevalence of functional limitation among older adults was high (81.0%). In a total of 2236 individuals, 1812 had functional limitations (**Table 1**). This prevalence increased with age strata, ranging from 76.9% for individuals aged 65 to 69 years to 93.1% among adults ≥ 85 years. When analysed by sex groups, female participants had more prevalence of functional limitation compared to men (87.1% vs. 70.0%). Azores (85.3%) and Centro (83.0%) were the regions with the highest prevalence of functional limitation (**Table 1**). The lowest prevalence was identified in Norte (79.0%).

Table 1. Prevalence of functional limitation in the study population, stratified by age, sex and NUTS II region.

		Functional limitation (HAQ score > 0)		**No functional limitation** (HAQ score = 0)	
		n	**%**	**n**	**%**
Age strata	65 – 75 y	1064	76.9	319	23.1
	76 – 85 y	640	86.8	97	13.2
	+ 85 y	108	93.1	8	6.9
Sex	Female	1257	87.1	186	12.9
	Male	555	70.0	238	30.0
NUTS II region	Norte	481	79.0	128	21.0
	Centro	421	83.0	86	17.0
	Lisboa	349	79.1	92	20.9
	Alentejo	129	81.6	29	18.4
	Algarve	80	81.6	18	18.4
	Azores	168	85.3	29	14.7
	Madeira	184	81.4	42	18.6
Total		**1812**	**81.0**	**424**	**19.0**

Notes: n = 2236. y, years; HAQ, Health Assessment Questionnaire; NUTS II, Nomenclature of Territorial Units for Statistics.

When stratified by education level, the highest prevalence of functional limitation (82.6%) was identified among the older adults with primary education, equivalent to ≤ 9 years of education (**Table 2**). Secondary educated participants, on the other hand, presented the lowest value of functional limitation (67.5%).

Table 2. Prevalence of functional limitation in the study population, stratified by education level.

		Functional limitation (HAQ score > 0)		No functional limitation (HAQ score = 0)	
		n	**%**	**n**	**%**
Education level[1]	Primary education	1638	82.6	344	17.4
	Secondary education	77	67.5	37	32.5
	Tertiary education	92	70.2	39	29.8
Total		**1807**	**81.1**	**420**	**18.9**

Notes: n = 2227. HAQ, Health Assessment Questionnaire. [1]Education level coded according to the International Standard Classification of Education (ISCED, 2011): Primary education = ISCED levels 0-2 (≤ 9 years of education); Secondary education = ISCED levels 3-4 (10-12 years of education); Tertiary education = ISCED levels 5-8 (> 12 years of education).

4.2 Characteristics of study population and high-protein foods consumption

High-protein food consumption, characterized by meat, fish and milk and/or dairy products consumption, among Portuguese older adults is presented in **Table 3**. Most participants consumed fish and meat 4 to 6 times a week, combined with a milk and dairy products consumption of every day or 6 times a week (n = 261, 15,5%). Overall, 1305 participants reported a milk and dairy consumption of every day or 6 times a week, which represents 77,3% of the study population (n = 1689) (**Table 3**).

Analysing consumption group by group, 643 (38.1%) of participants reported a meat frequency consumption of 1-3 times/week, 637 (37.7%) consumed fish 4-6 times/week and 1305 (77.3%) included milk or dairy products every day or 6 times/week in their meals (**Table 3**).

Table 3. Cross-tabulation of meat, fish and milk/dairy products consumption in the study population.

Dairy consumption			**Fish consumption**				
			Rarely or never	1-3 t/w	4-6 t/w	Every day or 7-10 t/w	**Total**
Rarely or never	**Meat consumption**	Rarely or never	**0.6** (1)	**1.3** (2)	**1.3** (2)	**2.5** (4)	**5.7** (9)
		1-3 t/w	**2.5** (4)	**17.7** (28)	**13.3** (21)	**7.0** (11)	**40.5** (64)
		4-6 t/w	**1.3** (2)	**8.9** (14)	**15.8** (25)	**0.6** (1)	**26.6** (42)
		Every day or 7-10 t/w	**3.8** (6)	**11.4** (18)	**2.5** (4)	**9.5** (15)	**27.2** (43)
	Total		**8.2** (13)	**39.2** (62)	**32.9** (52)	**19.6** (31)	**100** (158)
1-2 t/w	**Meat consumption**	Rarely or never	**1.1** (1)	**3.4** (3)	**1.1** (1)	**1.1** (1)	**6.8** (6)
		1-3 t/w	**0.0** (0)	**21.6** (19)	**11.4** (10)	**9.1** (8)	**42.0** (37)
		4-6 t/w	**0.0** (0)	**12.5** (11)	**18.2** (16)	**3.4** (3)	**34.1** (30)
		Every day or 7-10 t/w	**1.1** (1)	**10.2** (9)	**3.4** (3)	**2.3** (2)	**17.0** (15)
	Total		**2.3** (2)	**47.7** (42)	**34.1** (30)	**15.9** (14)	**100** (88)
3-5 t/w	**Meat consumption**	Rarely or never	**2.2** (3)	**0.7** (1)	**0.7** (1)	**0.0** (0)	**3.6** (5)
		1-3 t/w	**1.4** (2)	**17.4** (24)	**11.6** (16)	**10.1** (14)	**40.6** (56)
		4-6 t/w	**0.0** (0)	**9.4** (13)	**18.8** (26)	**3.6** (5)	**31.9** (44)
		Every day or 7-10 t/w	**2.9** (4)	**10.1** (14)	**0.7** (1)	**10.1** (14)	**23.9** (33)
	Total		**6.5** (9)	**37.7** (52)	**31.9** (44)	**23.9** (33)	**100** (138)
Every day or 6 t/w	**Meat consumption**	Rarely or never	**0.5** (6)	**1.5** (20)	**1.2** (16)	**1.2** (16)	**4.4** (58)
		1-3 t/w	**0.9** (12)	**15.3** (200)	**13.9** (181)	**7.1** (93)	**37.2** (486)
		4-6 t/w	**0.8** (10)	**8.7** (114)	**20.0** (261)	**4.4** (58)	**33.9** (443)
		Every day or 7-10 t/w	**0.8** (11)	**9.3** (121)	**4.1** (53)	**10.2** (133)	**24.4** (318)
	Total		**3.0** (39)	**34.9** (455)	**39.2** (511)	**23.0** (300)	**100** (1305)
Total	**Meat consumption**	Rarely or never	**0.7** (11)	**1.5** (26)	**1.2** (20)	**1.2** (21)	**4.6** (78)
		1-3 t/w	**1.1** (18)	**16.0** (271)	**13.5** (228)	**7.5** (126)	**38.1** (643)
		4-6 t/w	**0.7** (12)	**9.0** (152)	**19.4** (328)	**4.0** (67)	**33.1** (559)
		Every day or 7-10 t/w	**1.3** (22)	**9.6** (162)	**3.6** (61)	**9.7** (164)	**24.2** (409)
	Total		**3.7** (63)	**36.2** (611)	**37.7** (637)	**22.4** (378)	**100** (1689)

Notes: n = 1689. Participants with data regarding meat, fish and milk/dairy frequency consumption, simultaneously, were included in this analysis. Values presented in each cell: **%** (n). t/w, times per week.

4.2.1 *Sociodemographic data*

Of the 2393 older adults (≥ 65 years) included in this study, 1693 to 1711 participants had available information about high-protein foods consumption (meat, fish and dairy products). **Table 4**, **Table 5** and **Table 6** summarize participants' characteristics according to different food frequency groups.

Overall, participants had a mean age of 74.0 ± 7 years and 53.5 - 53.6% were female. Regarding meat (n = 1706) and fish (n = 1693) consumption, participants' mean age tended to decrease with higher frequency consumption (**Table 4** and **Table 5**). This tendency didn't occur in the dairy group (**Table 6**). Age and sex were significantly different between meat frequency categories ($p < 0.001$) (**Table 4**).

Primary education was the most prevalent education level among participants (88.3 - 88.4% had ≤ 9 years of education) (**Table 4**, **Table 5** and **Table 6**). Secondary education (10-12 years) was the least common (5.4 - 5.5%), followed by tertiary level (> 12 years of education) with 6.1 - 6.3% of participants. The "rarely or never" category had the highest proportion of primary educated participants amongst all food groups (meat, fish, and dairy).

Regarding geographic location (NUTS II classification), most individuals were from the Norte region (32.1 - 32.3%) and Centro region (25.2 - 25.4%) (**Table 4**, **Table 5** and **Table 6**).

4.2.2 *Health data*

The overall prevalence of multimorbidity among older adults was high (73.3 - 73.4%) (**Table 4**, **Table 5** and **Table 6**). Regarding high-protein foods consumption, the lowest prevalence was identified in the "rarely or never" meat category (68.3%) (**Table 4**), in the "4-6 times/week" fish category (71.5%) (**Table 5**) and in the "1-2 times/week" dairy category (68.9%) (**Table 6**).

Concerning pharmacological treatment, 92.2-92.3% of participants reported the use of medication (**Table 4**, **Table 5** and **Table 6**). The medication use prevalence was lowest among participants who reported to rarely or never consume meat (85.4%) and fish (90.3%) and among individuals who consume dairy products 1-2 times in a week (89.8%).

The overall mean self-perceived health status among Portuguese older adults was 65 ± 19, in a scale from 0 to 100 (**Table 4**, **Table 5** and **Table 6**).

4.2.3 *Lifestyle data*

Regarding lifestyle characteristics other than diet, 5.4 - 5.5% of individuals were smokers and 36.5 - 36.9% reported the daily intake of alcoholic beverages (**Table 4**, **Table 5** and **Table 6**). In the meat group, smokers were more prevalent (8.0%) in the highest consumption category (every day or 7-10 times/week) (**Table 4**).

The same occurred in the dairy group (**Table 6**), but not in the fish group (**Table 5**). In this last group, smokers were more prevalent (14.3%) in the lowest consumption category (rarely or never). In the fish (37.7%) and dairy (44.4%) groups, daily intake of alcohol was more common in the 1-2 or 1-3 times/week category (**Table 5** and **Table 6**). However, in the meat group daily intake of alcohol was more prevalent (43.0%) in the highest category of consumption (every day or 7-10 times/week) (**Table 4**).

Overall, 41.5 - 41.6% of participants reported to be physically active (**Table 4**, **Table 5** and **Table 6**). Meat and fish groups presented more physically active participants in the highest category of consumption: 43.1% and 51.5%, respectively, while dairy group had higher prevalence of physically active individuals in the 3-5 times/week category (47.5%). There were significant differences between frequency categories regarding physical activity in fish and dairy groups ($p \leq 0.001$) (**Table 5** and **Table 6**).

4.2.4 *Dietary intake*

Overall, 46.0 - 46.3% of participants consumed 3 meals per day and only 4.0 - 4.2% reported to do 2 meals per day (**Table 4**, **Table 5** and **Table 6**).

Regarding frequent vegetables consumption, 67.3 - 67.5% of all participants reported consuming vegetables every day or 6 times per week (**Table 4**, **Table 5** and **Table 6**). Frequent vegetable consumption prevalence was higher among individuals who consumed fish every day or 7-10/week (81.7%) and lower among older adults who rarely or never consumed fish (41.3%) (**Table 5**). The same was observed for frequent fresh fruit consumption (58.7% < 82.8% < 87.3% < 90.8%), showing an increased tendency of fruit and vegetables consumption with the rising fish frequency consumption ($p < 0.001$). Similar tendency occurred in the meat and milk/dairy groups, although in a less expressive way and not in all categories (**Table 4** and **Table 6**).

63.0% of Portuguese older adults reported frequent soup consumption (every day or 6 times/week) (**Table 4**, **Table 5** and **Table 6**). The prevalence of soup consumption was higher among individuals who consumed meat 1-3 times/week (67.4%), as for participants who included fish every day or 7-10 times/week (66.7%). Older adults who reported a 3-5 times/week consumption of dairy were the ones with higher frequent soup consumption (64.7%) (**Table 6**).

4.2.5 *Anthropometric data*

The mean BMI of all older adults was 27.2 $\pm$ 4.2 kg/m^2 (**Table 4**, **Table 5** and **Table 6**). The prevalence of overweight (BMI > 27 kg/m^2) was 47.8% in meat and dairy groups and 48.1% in the fish group. Underweight (BMI < 22 kg/m^2) represented 7.6% in the meat and fish groups and 7.7% in the dairy group. Overweight was more prevalent in the highest frequency category (every day or 7-10 times/week) of meat (49.6%) and fish (49.0%) consumption (**Table 4** and **Table 5**). This did not occur in the dairy group (**Table 6**).

4.2.6 *Physical function*

Regarding the ability to perform ADLs without any difficulty, 60.1 - 60.2% of all older adults reported being able to stand up from a straight chair; 60.8 - 61.1% were able to walk outdoors on flat ground; 53.0 - 53.1% had the ability to climb up five steps, and 56.4 - 56.7% could reach and get down a 2.5 kg object from above their head (**Table 4**, **Table 5** and **Table 6**). Although the prevalence's of stand up and walk abilities increased as meat frequency consumption raised, there were no significant differences between categories of consumption (**Table 4**).

The mean HAQ final score was 0.730 ± 0.722 in older adults when analysing meat frequency consumption (n = 1706); 0.724 ± 0.719 when looking at the fish frequency results (n = 1693) and 0.728 ± 0.722 when observing the dairy consumption alone (n = 1711). The HAQ final score showed a decreasing tendency with higher meat frequency consumption, excluding the latter category, and results were significantly different among meat frequency categories ($p < 0.001$) (**Table 4**). This was not observed for fish and dairy consumption (**Table 5** and **Table 6**).

Table 4. Characteristics of study population by meat consumption.

	all (n = 1706)	miss (%)	Meat consumption[1] Rarely or never (n = 82)	1-3 t/w (n = 650)	4-6 t/w (n = 561)	Every day or 7-10 t/w (n = 413)	*p**
Sociodemographic data							
Age, y (**mean** ± SD)	**74.0** ± 7	0.0	**75.0** ± 6	**75.0** ± 7	**74.0** ± 7	**73.0** ± 6	< 0.001
Female **%** (n)	**53.6** (914)	0.0	**65.9** (54)	**59.7** (388)	**52.4** (294)	**43.1** (178)	< 0.001
Education level[2] **%** (n)		0.4					0.364
Primary	**88.4** (1503)		**92.5** (74)	**90.2** (586)	**86.2** (482)	**87.8** (361)	
Secondary	**5.4** (92)		**2.5** (2)	**4.9** (32)	**6.3** (35)	**5.6** (23)	
Tertiary	**6.2** (105)		**5.0** (4)	**4.9** (32)	**7.5** (42)	**6.6** (27)	
NUTS II **%** (n)		0.0					0.001
Norte	**32.3** (551)		**22.0** (18)	**26.9** (175)	**35.8** (201)	**38.0** (157)	
Centro	**25.3** (432)		**29.3** (24)	**26.3** (171)	**27.1** (152)	**20.6** (85)	
Lisboa	**18.8** (321)		**19.5** (16)	**20.3** (132)	**19.3** (108)	**15.7** (65)	
Alentejo	**6.1** (104)		**4.9** (4)	**6.0** (39)	**5.3** (30)	**7.5** (31)	
Algarve	**3.5** (60)		**6.1** (5)	**4.3** (28)	**2.1** (12)	**3.6** (15)	
Azores	**5.5** (93)		**4.9** (4)	**6.8** (44)	**3.0** (17)	**6.8** (28)	
Madeira	**8.5** (145)		**13.4** (11)	**9.4** (61)	**7.3** (41)	**7.7** (32)	
Health							
Multimorbidity **%** (n)	**73.4** (1252)	0.0	**68.3** (56)	**76.6** (498)	**70.1** (393)	**73.8** (305)	0.050
Takes medication **%** (n)	**92.3** (1553)	1.3	**85.4** (70)	**94.4** (608)	**91.7** (506)	**91.1** (369)	0.049
Perceived health status scale (0-100) (**mean** ± SD)	**65** ± 19	12.8	**66** ± 20	**64** ± 20	**67** ± 18	**65** ± 18	0.035
Lifestyle							
Daily and occasional smokers **%** (n)	**5.5** (93)	0.1	**3.7** (3)	**4.3** (28)	**5.2** (29)	**8.0** (33)	0.002
Daily intake of alcohol **%** (n)	**36.5** (622)	0.2	**14.6** (12)	**32.6** (212)	**39.5** (221)	**43.0** (177)	< 0.001
Regular physical activity **%** (n)	**41.5** (708)	0.0	**40.2** (33)	**42.6** (277)	**39.2** (220)	**43.1** (178)	0.567
Dietary intake[3]							
Number of meals **%** (n)		0.5					0.013
2 meals per day	**4.1** (70)		**11.1** (9)	**3.1** (20)	**3.8** (21)	**4.9** (20)	
3 meals per day	**46.1** (783)		**49.4** (40)	**45.6** (295)	**45.2** (253)	**47.6** (195)	
4 meals per day	**33.7** (572)		**23.5** (19)	**33.1** (214)	**34.8** (195)	**35.1** (144)	
≥ 5 meals per day	**16.1** (273)		**16.0** (13)	**18.2** (118)	**16.3** (91)	**12.4** (51)	

	Meat consumption[1]						
	all (n = 1706)	miss (%)	Rarely or never (n = 82)	1-3 t/w (n = 650)	4-6 t/w (n = 561)	Every day or 7-10 t/w (n = 413)	*p**
Frequent soup consumption **%** (n)	**63.0** (1075)	0.1	**64.6** (53)	**67.4** (438)	**62.0** (348)	**57.3** (236)	0.016
Frequent vegetables consumption **%** (n)	**67.3** (1147)	0.1	**61.0** (50)	**66.0** (429)	**67.9** (381)	**69.8** (287)	0.026
Frequent fresh fruit consumption **%** (n)	**85.3** (1455)	0.1	**84.1** (69)	**84.9** (552)	**87.0** (487)	**84.0** (347)	0.013
Frequent fish consumption **%** (n)	**22.4** (379)	0.9	**26.9** (21)	**19.7** (127)	**12.0** (67)	**40.1** (164)	< 0.001
Frequent dairy consumption **%** (n)	**77.2** (1316)	0.1	**74.4** (61)	**75.5** (489)	**79.3** (445)	**77.7** (321)	0.445
Anthropometric data[4]							
BMI, kg/m^2 (**mean** ± SD)	**27.2** ± 4.2		**26.3** ± 4.8	**27.3** ± 4.1	**27.2** ± 4.2	**27.3** ± 4.2	0.287
Underweight **%** (n)	**7.6** (115)	11.0	**13.2** (9)	**8.0** (45)	**6.6** (33)	**7.3** (28)	
Normal **%** (n)	**44.6** (678)		**54.4** (37)	**43.2** (243)	**46.1** (232)	**43.1** (166)	0.137
Overweight **%** (n)	**47.8** (726)		**32.4** (22)	**48.8** (275)	**47.3** (238)	**49.6** (191)	
Physical function[5]							
No difficulty to *stand up* **%** (n)	**60.1** (1024)	0.1	**54.9** (45)	**56.0** (364)	**62.3** (349)	**64.4** (266)	0.088
No difficulty to *walk* **%** (n)	**60.8** (1034)	0.3	**56.1** (46)	**59.3** (384)	**62.0** (346)	**62.5** (258)	0.240
No difficulty to *climb* **%** (n)	**53.0** (902)	0.2	**47.6** (39)	**49.2** (319)	**56.4** (315)	**55.4** (229)	0.055
No difficulty to *reach* **%** (n)	**56.4** (960)	0.3	**48.8** (40)	**51.2** (331)	**62.1** (347)	**58.6** (242)	< 0.001
HAQ final score (0-3) (**mean** ± SD)	**0.730** ± 0.722	0.3	**0.888** ± 0.819	**0.820** ± 0.741	**0.649** ± 0.688	**0.666** ± 0.695	< 0.001

Notes: SD, standard deviation; *p*, p-value; y, years; BMI, body mass index; HAQ, Health Assessment Questionnaire; NUTS II, Nomenclature of Territorial Units for Statistics; t/w, times per week. [1]How many meals, per week, include meat. [2]Education level coded according to the International Standard Classification of Education (ISCED, 2011): Primary education = ISCED levels 0-2 (≤ 9 years of education); Secondary education = ISCED levels 3-4 (10-12 years of education); Tertiary education = ISCED levels 5-8 (> 12 years of education). [3]For fruit, vegetables, soup and milk/dairy, frequent consumption is equivalent to every day or 6 times per week consumption; For meat and fish, frequent consumption refers to every day or 7-10 times per week fish consumption. [4]BMI categories: underweight (BMI < 22 kg/m^2), normal weight (BMI 22 - 27 kg/m^2), overweight (BMI > 27 kg/m^2). [5]Results regarding the activities of daily living refer to the ability to perform the following activities without any difficulty: *stand up* - stand up from a straight chair; *walk* - walk outdoors on flat ground; *climb* - climb up five steps; *reach* - reach and get down a 2.5 kg object from above the head. *To access if there was meat consumption frequency difference between groups, Chi-squared test was used for categorical variables, ANOVA test for continuous variables and Kruskal-Wallis test for HAQ final score variable (homogeneity of variances was not observed).

Table 5. Characteristics of study population by fish consumption.

	Fish consumption[1]						
	all (n = 1693)	miss (%)	Rarely or never (n = 63)	1-3 t/w (n = 613)	4-6 t/w (n = 638)	Every day or 7-10 t/w (n = 379)	*p**
Sociodemographic data							
Age, y (**mean** ± SD)	**74.0** ± 7	0.0	**75.0** ± 7	**75.0** ± 7	**74.0** ± 7	**74.0** ± 7	0.015
Female **%** (n)	**53.5** (905)	0.0	**57.1** (36)	**55.0** (337)	**53.3** (340)	**50.7** (192)	0.550
Education level[2] **%** (n)		0.4					0.002
Primary	**88.4** (1492)		**95.2** (60)	**91.5** (560)	**84.7** (538)	**88.6** (334)	
Secondary	**5.5** (92)		**1.6** (1)	**3.4** (21)	**8.2** (52)	**4.8** (18)	
Tertiary	**6.1** (103)		**3.2** (2)	**5.1** (31)	**7.1** (45)	**6.6** (25)	
NUTS II **%** (n)		0.0					< 0.001
Norte	**32.1** (544)		**41.3** (26)	**37.2** (228)	**28.8** (184)	**28.0** (106)	
Centro	**25.4** (430)		**19.0** (12)	**21.5** (132)	**29.5** (188)	**25.9** (98)	
Lisboa	**19.0** (321)		**6.3** (4)	**14.5** (89)	**24.5** (156)	**19.0** (72)	
Alentejo	**6.0** (102)		**4.8** (3)	**6.7** (41)	**4.4** (28)	**7.9** (30)	
Algarve	**3.5** (60)		**1.6** (1)	**3.3** (20)	**2.7** (17)	**5.8** (22)	
Azores	**5.5** (93)		**7.9** (5)	**4.4** (27)	**5.0** (32)	**7.7** (29)	
Madeira	**8.4** (143)		**19.0** (12)	**12.4** (76)	**5.2** (33)	**5.8** (22)	
Health							
Multimorbidity **%** (n)	**73.3** (1241)	0.0	**73.0** (46)	**73.2** (449)	**71.5** (456)	**76.5** (290)	0.377
Takes medication **%** (n)	**92.3** (1541)	1.4	**90.3** (56)	**92.0** (555)	**93.2** (589)	**91.4** (341)	NA
Perceived health status scale (0-100) (**mean** ± SD)	**65** ± 19	12.6	**64** ± 21	**63** ± 19	**67** ± 19	**65** ± 19	0.015
Lifestyle							
Daily and occasional smokers **%** (n)	**5.5** (93)	0.1	**14.3** (9)	**4.4** (27)	**5.5** (35)	**5.8** (22)	0.029
Daily intake of alcohol **%** (n)	**36.9** (623)	0.2	**30.2** (19)	**37.7** (231)	**37.4** (238)	**35.6** (135)	0.109
Regular physical activity **%** (n)	**41.6** (705)	0.0	**25.4** (16)	**36.2** (222)	**42.6** (272)	**51.5** (195)	< 0.001
Dietary intake[3]							
Number of meals **%** (n)		0.5					< 0.001
2 meals per day	**4.0** (67)		**9.7** (6)	**5.4** (33)	**2.2** (14)	**3.7** (14)	
3 meals per day	**46.3** (780)		**59.7** (37)	**51.9** (317)	**44.0** (279)	**38.9** (147)	
4 meals per day	**33.7** (568)		**24.2** (15)	**30.3** (185)	**35.5** (225)	**37.8** (143)	
≥ 5 meals per day	**16.0** (270)		**6.5** (4)	**12.4** (76)	**18.3** (116)	**19.6** (74)	

	Fish consumption[1]						
	all (n = 1693)	miss (%)	Rarely or never (n = 63)	1-3 t/w (n = 613)	4-6 t/w (n = 638)	Every day or 7-10 t/w (n = 379)	*p**
Frequent soup consumption **%** (n)	**63.0** (1066)	0.1	**57.1** (36)	**58.1** (356)	**66.1** (422)	**66.7** (252)	< 0.001
Frequent vegetables consumption **%** (n)	**67.5** (1141)	0.1	**41.3** (26)	**58.3** (357)	**70.4** (449)	**81.7** (309)	< 0.001
Frequent fresh fruit consumption **%** (n)	**85.4** (1445)	0.1	**58.7** (37)	**82.8** (507)	**87.3** (557)	**90.8** (344)	< 0.001
Frequent meat consumption **%** (n)	**24.2** (409)	0.1	**34.9** (22)	**26.5** (162)	**9.6** (61)	**43.3** (164)	< 0.001
Frequent dairy consumption **%** (n)	**77.2** (1306)	0.1	**61.9** (39)	**74.3** (455)	**80.3** (512)	**79.4** (300)	0.004
Anthropometric data[4]							
BMI, kg/m² (**mean** ± SD)	**27.2** ± 4.2		**27.0** ± 5.5	**26.9** ± 4.2	**27.4** ± 4.1	**27.4** ± 4.3	0.215
Underweight **%** (n)	**7.6** (114)		**18.8** (9)	**9.5** (50)	**5.2** (30)	**7.0** (25)	
Normal **%** (n)	**44.4** (669)	10.9	**39.6** (19)	**43.6** (230)	**45.8** (264)	**43.9** (156)	0.013
Overweight **%** (n)	**48.1** (725)		**41.7** (20)	**47.0** (248)	**49.0** (283)	**49.0** (174)	
Physical function[5]							
No difficulty to *stand up* **%** (n)	**60.2** (1018)	0.1	**50.8** (32)	**60.8** (373)	**61.9** (394)	**57.8** (219)	0.183
No difficulty to *walk* **%** (n)	**61.1** (1031)	0.4	**49.2** (31)	**60.7** (371)	**62.8** (398)	**60.9** (231)	0.052
No difficulty to *climb* **%** (n)	**53.1** (897)	0.2	**42.9** (27)	**52.2** (319)	**54.7** (348)	**53.6** (203)	0.063
No difficulty to *reach* **%** (n)	**56.7** (956)	0.4	**55.6** (35)	**55.2** (338)	**58.1** (368)	**56.7** (215)	0.409
HAQ final score (0-3) (**mean** ± SD)	**0.724** ± 0.719	0.3	**0.907** ± 0.856	**0.733** ± 0.728	**0.672** ± 0.672	**0.767** ± 0.749	0.153

Notes: SD, standard deviation; *p*, p-value; y, years; BMI, body mass index; HAQ, Health Assessment Questionnaire; NA, not applicable; NUTS II, Nomenclature of Territorial Units for Statistics; t/w, times per week. [1]How many meals, per week, include fish. [2]Education level coded according to the International Standard Classification of Education (ISCED, 2011): Primary education = ISCED levels 0-2 (≤ 9 years of education); Secondary education = ISCED levels 3-4 (10-12 years of education); Tertiary education = ISCED levels 5-8 (> 12 years of education). [3]For fruit, vegetables, soup and milk/dairy, frequent consumption is equivalent to every day or 6 times per week consumption; For meat and fish, frequent consumption refers to every day or 7-10 times per week meat consumption. [4]BMI categories: underweight (BMI < 22 kg/m²), normal weight (BMI 22 - 27 kg/m²), overweight (BMI > 27 kg/m²). [5]Results regarding the activities of daily living refer to the ability to perform the following activities without any difficulty: *stand up* - stand up from a straight chair; *walk* - walk outdoors on flat ground; *climb* - climb up five steps; *reach* - reach and get down a 2.5 kg object from above the head. *To access if there was fish consumption frequency difference between groups, Chi-squared test was used for categorical variables, ANOVA test for continuous variables and Kruskal-Wallis test for HAQ final score variable (homogeneity of variances was not observed). The Chi-square results for the variable regarding the use of medication may be invalid due to insufficient cell count, therefore the results were not considered.

Table 6. Characteristics of study population by dairy consumption.

			Dairy consumption[1]				
	all (n = 1711)	miss (%)	Rarely or never (n = 162)	1-2 t/w (n = 90)	3-5 t/w (n = 139)	Every day or 6 t/w (n = 1320)	*p**
Sociodemographic data							
Age, y (**mean** ± SD)	**74.0** ± 7	0.0	**75.0** ± 6	**73.0** ± 7	**74.0** ± 7	**74.0** ± 7	0.499
Female **%** (n)	**53.6** (917)	0.0	**50.0** (81)	**53.3** (48)	**48.9** (68)	**54.5** (720)	0.467
Education level[2] **%** (n)		0.4					0.072
Primary	**88.3** (1506)		**93.1** (148)	**91.1** (82)	**85.5** (118)	**87.9** (1158)	
Secondary	**5.4** (92)		**1.3** (2)	**6.7** (6)	**8.7** (12)	**5.5** (72)	
Tertiary	**6.3** (107)		**5.7** (9)	**2.2** (2)	**5.8** (8)	**6.7** (88)	
NUTS II **%** (n)		0.0					0.004
Norte	**32.3** (552)		**38.3** (62)	**38.9** (35)	**33.1** (46)	**31.0** (409)	
Centro	**25.2** (432)		**17.3** (28)	**26.7** (24)	**20.9** (29)	**26.6** (351)	
Lisboa	**18.9** (324)		**17.3** 28)	**11.1** (10)	**20.1** (28)	**19.5** (258)	
Alentejo	**6.1** (105)		**4.9** (8)	**3.3** (3)	**5.0** (7)	**6.6** (87)	
Algarve	**3.5** (60)		**1.2** (2)	**1.1** (1)	**4.3** (6)	**3.9** (51)	
Azores	**5.4** (93)		**4.9** (8)	**7.8** (7)	**8.6** (12)	**5.0** (66)	
Madeira	**8.5** (145)		**16.0** (26)	**11.1** (10)	**7.9** (11)	**7.4** (98)	
Health							
Multimorbidity **%** (n)	**73.3** (1254)	0.0	**77.2** (125)	**68.9** (62)	**79.9** (111)	**72.4** (956)	0.127
Takes medication **%** (n)	**92.2** (1557)	1.3	**93.1** (148)	**89.8** (79)	**92.1** (128)	**92.3** (1202)	NA
Perceived health status scale (0-100) (**mean** ± SD)	**65** ± 19	12.9	**63** ± 20	**63** ± 22	**62** ± 17	**66** ± 19	0.050
Lifestyle							
Daily and occasional smokers **%** (n)	**5.4** (93)	0.1	**4.3** (7)	**5.6** (5)	**4.3** (6)	**5.7** (75)	0.859
Daily intake of alcohol **%** (n)	**36.5** (624)	0.2	**37.3** (60)	**44.4** (40)	**40.3** (56)	**35.5** (468)	0.318
Regular physical activity **%** (n)	**41.5** (710)	0.0	**33.3** (54)	**26.7** (24)	**47.5** (66)	**42.9** (566)	0.001
Dietary intake[3]							
Number of meals **%** (n)		0.5					< 0.001
2 meals per day	**4.2** (71)		**8.1** (13)	**7.8** (7)	**5.8** (8)	**3.3** (43)	
3 meals per day	**46.0** (784)		**53.8** (86)	**60.0** (54)	**46.4** (64)	**44.1** (580)	
4 meals per day	**33.6** (573)		**28.1** (45)	**22.2** (20)	**32.6** (45)	**35.2** (463)	
≥ 5 meals per day	**16.1** (275)		**10.0** (16)	**10.0** (9)	**15.2** (21)	**17.4** (229)	

	Dairy consumption[1]						
	all (n = 1711)	miss (%)	Rarely or never (n = 162)	1-2 t/w (n = 90)	3-5 t/w (n = 139)	Every day or 6 t/w (n = 1320)	*p**
Frequent soup consumption **%** (n)	**63.0** (1078)	0.1	**63.0** (102)	**56.7** (51)	**64.7** (90)	**63.3** (835)	0.371
Frequent vegetables consumption **%** (n)	**67.3** (1051)	0.1	**56.8** (92)	**55.6** (50)	**64.7** (90)	**69.7** (919)	0.001
Frequent fresh fruit consumption **%** (n)	**85.3** (1458)	0.1	**74.1** (120)	**75.6** (68)	**84.2** (117)	**87.4** (1153)	NA
Frequent meat consumption **%** (n)	**24.2** (413)	0.4	**27.5** (44)	**16.9** (15)	**23.7** (33)	**24.4** (321)	0.445
Frequent fish consumption **%** (n)	**22.4** (378)	1.2	**19.5** (31)	**15.9** (14)	**23.9** (33)	**23.0** (300)	0.004
Anthropometric data[4]							
BMI, kg/m^2 (**mean** ± SD)	**27.2** ± 4.2		**26.9** ± 4.0	**27.1** ± 4.3	**28.1** ± 5.3	**27.1** ± 4.1	0.073
Underweight **%** (n)	**7.7** (117)	11.0	**8.3** (11)	**8.1** (6)	**6.2** (8)	**7.8** (92)	
Normal **%** (n)	**44.5** (678)		**42.9** (57)	**47.3** (35)	**41.9** (54)	**44.8** (532)	0.954
Overweight **%** (n)	**47.8** (728)		**48.9** (65)	**44.6** (33)	**51.9** (67)	**47.4** (563)	
Physical function[5]							
No difficulty to *stand up* **%** (n)	**60.2** (1028)	0.1	**54.9** (89)	**56.7** (51)	**65.5** (91)	**60.5** (797)	0.014
No difficulty to *walk* **%** (n)	**60.9** (1038)	0.4	**57.8** (93)	**54.4** (49)	**60.9** (84)	**61.7** (812)	0.135
No difficulty to *climb* **%** (n)	**53.0** (905)	0.2	**50.9** (82)	**42.2** (38)	**55.1** (76)	**53.8** (709)	0.198
No difficulty to *reach* **%** (n)	**56.5** (963)	0.4	**55.6** (90)	**53.3** (48)	**58.4** (80)	**56.6** (745)	0.083
HAQ final score (0-3) (**mean** ± SD)	**0.728** ± 0.722	0.3	**0.810** ± 0.759	**0.826** ± 0.771	**0.693** ± 0.690	**0.715** ± 0.716	0.219

Notes: SD, standard deviation; *p*, p-value; y, years; BMI, body mass index; HAQ, Health Assessment Questionnaire; NA, not applicable; NUTS II, Nomenclature of Territorial Units for Statistics; t/w, times per week. [1]How many meals, per week, include dairy products. [2]Education level coded according to the International Standard Classification of Education (ISCED, 2011): Primary education = ISCED levels 0-2 (≤ 9 years of education); Secondary education = ISCED levels 3-4 (10-12 years of education); Tertiary education = ISCED levels 5-8 (> 12 years of education). [3]For fruit, vegetables, soup and milk/dairy, frequent consumption is equivalent to every day or 6 times per week consumption; For meat and fish, frequent consumption refers to every day or 7-10 times per week consumption. [4]BMI categories: underweight (BMI < 22 kg/m^2), normal weight (BMI 22 - 27 kg/m^2), overweight (BMI > 27 kg/m^2). [5]Results regarding the activities of daily living refer to the ability to perform the following activities without any difficulty: *stand up* - stand up from a straight chair; *walk* - walk outdoors on flat ground; *climb* - climb up five steps; *reach* - reach and get down a 2.5 kg object from above the head. *To access if there was milk consumption frequency difference between groups, Chi-squared test was used for categorical variables and ANOVA test for continuous variables. The Chi-square results for the variables regarding the use of medication and frequent fresh fruit consumption may be invalid due to insufficient cell count, therefore the results were not considered.

4.3 High-protein foods consumption and functional limitations

Table 7 show the binary logistic regression models used to examine the association between the prevalence of functional limitation to stand up from a straight chair in relation to high-protein foods, with the lowest consumption frequency as a reference.

In the unadjusted model, the odds of having functional limitation to stand up was similar between participants who eat meat every day compared to the ones who rarely or never eat meat (OR 0.90; 95% CI: 0.49-1.65) (**Table 7**). Same results were observed on the adjusted models and for other meat consumption frequencies. Regarding fish consumption, the odds of having functional limitations to stand up did not differ between eating fish every day and eating fish rarely or never, in all models (e.g. health model - OR 1.27; 95% CI: 0.56-2.88) (**Table 7**). For dairy, results were similar to meat and fish consumption. However, there was an exception for consuming dairy 3-5 t/w compared to rarely or never: the odds of having functional limitation to stand up were 0.52 times lower in the health model (OR 0.52; 95% CI: 0.28-0.94) (**Table 7**).

Table 7. Association between functional limitation to stand up and high-protein foods consumption.

Stand up				
	Meat consumption, OR (95% CI)			
	Rarely or never	1-3 t/w or 1-2 t/w	4-6 t/w or 3-5 t/w	Every day or 7-10 t/w or 6 t/w
n	51	493	445	340
Unadjusted model	1.00 (ref)	1.21 (0.66, 2.21)	0.95 (0.52, 1.75)	0.90 (0.49, 1.65)
Socioeconomic model	1.00 (ref)	1.30 (0.70, 2.42)	1.06 (0.57, 2.00)	1.10 (0.60, 2.10)
Lifestyle model	1.00 (ref)	1.33 (0.70, 2.53)	1.05 (0.55, 2.03)	1.12 (0.58, 2.16)
Health model	1.00 (ref)	1.13 (0.56, 2.27)	0.96 (0.47, 1.96)	0.93 (0.46, 1.91)
	Fish consumption, OR (95% CI)			
n	35	444	514	336
Unadjusted model	1.00 (ref)	0.89 (0.43, 1.82)	0.81 (0.39, 1.66)	1.12 (0.54, 2.30)
Socioeconomic model	1.00 (ref)	0.88 (0.42, 1.84)	0.87 (0.41, 1.83)	1.16 (0.55, 2.43)
Lifestyle model	1.00 (ref)	1.02 (0.47, 2.21)	1.02 (0.46, 2.22)	1.40 (0.64, 3.06)
Health model	1.00 (ref)	0.94 (0.42, 2.13)	0.94 (0.42, 2.15)	1.27 (0.56, 2.88)
	Dairy consumption, OR (95% CI)			
n	108	64	118	1039
Unadjusted model	1.00 (ref)	0.83 (0.44, 1.56)	0.61 (0.36, 1.05)	0.76 (0.51, 1.13)
Socioeconomic model	1.00 (ref)	0.83 (0.43, 1.60)	0.62 (0.35, 1.08)	0.74 (0.49, 1.13)
Lifestyle model	1.00 (ref)	0.78 (0.40, 1.53)	0.58 (0.32, 1.02)	0.73 (0.48, 1.12)
Health model	1.00 (ref)	0.73 (0.35, 1.49)	0.52 (0.28, 0.94)	0.76 (0.48, 1.20)

Notes: n = 1329; OR, Odds Ratio; CI, Confidence Interval; t/w, times per week; ref, reference category. Binary logistic regression was the statistic model used. Reference category of the outcome variable was not having functional limitations in standing up. The first model was not adjusted for any covariate; socioeconomic model was adjusted for age, sex, geographical location (NUTS II) and education level; lifestyle model was adjusted as for socioeconomic model, plus variables of BMI, smoking status, alcohol intake, physical activity and vegetables consumption; Health model was further adjusted for self-rated health (perceived health status scale), multimorbidity and pharmacological treatment.

Table 8 present the binary logistic regression models used to assess the association between the prevalence of functional limitation to walk outdoors on flat ground in relation to high-protein foods, with the lowest consumption frequency as a reference.

The models did not show association between functional limitation to walk and high-protein foods consumption in Portuguese older adults (**Table 8**). For example, the odds of having functional limitations to walk did not vary between participants who reported to eat meat every day and the ones who reported to rarely or never eat meat (e.g. health model - OR 1.27; 95% CI: 0.59-2.72).

Table 8. Association between functional limitation to walk and high-protein foods consumption.

Walk				
	Meat consumption, OR (95% CI)			
	Rarely or never	1-3 t/w or 1-2 t/w	4-6 t/w or 3-5 t/w	Every day or 7-10 t/w or 6 t/w
n	51	491	445	340
Unadjusted model	1.00 (ref)	1.07 (0.58, 1.97)	1.00 (0.54, 1.85)	1.07 (0.58, 1.99)
Socioeconomic model	1.00 (ref)	1.15 (0.61, 2.17)	1.17 (0.62, 2.24)	1.40 (0.73, 2.69)
Lifestyle model	1.00 (ref)	1.19 (0.60, 2.37)	1.13 (0.56, 2.27)	1.46 (0.72, 2.95)
Health model	1.00 (ref)	1.00 (0.47, 2.12)	1.05 (0.49, 2.24)	1.27 (0.59, 2.72)
	Fish consumption, OR (95% CI)			
n	35	443	513	336
Unadjusted model	1.00 (ref)	0.90 (0.44, 1.84)	0.83 (0.40, 1.72)	0.91 (0.44, 1.88)
Socioeconomic model	1.00 (ref)	0.89 (0,43, 1.85)	0.94 (0.45, 1.98)	0.93 (0.44, 1.96)
Lifestyle model	1.00 (ref)	0.99 (0.45, 2.20)	1.08 (0.48, 2.43)	1.14 (0.51, 2.57)
Health model	1.00 (ref)	0.96 (0.42, 2.21)	1.05 (0.45, 2.45)	1.06 (0.45, 2.46)
	Dairy consumption, OR (95% CI)			
n	108	64	117	1038
Unadjusted model	1.00 (ref)	0.82 (0.43, 1.56)	0.95 (0.55, 1.62)	0.79 (0.53, 1.19)
Socioeconomic model	1.00 (ref)	0.83 (0.42, 1.62)	1.00 (0.57, 1.76)	0.79 (0.52, 1.21)
Lifestyle model	1.00 (ref)	0.76 (0.37, 1.54)	1.02 (0.56, 1.85)	0.81 (0.51, 1.27)
Health model	1.00 (ref)	0.74 (0.35, 1.59)	0.94 (0.51, 1.75)	0.85 (0.52, 1.38)

Notes: n = 1327; OR, Odds Ratio; CI, Confidence Interval; t/w, times per week; ref, reference category. Consumption frequencies of meat and fish: rarely or never; 1-3 t/w; 4-6 t/w; every day or 7-10 t/w. Consumption frequencies of dairy: rarely or never; 1-2 t/w; 3-5 t/w; every day or 6 t/w. Binary logistic regression was the statistic model used. Reference category of the outcome variable was not having functional limitations in walking. The first model was not adjusted for any covariate; socioeconomic model was adjusted for age, sex, geographical location (NUTS II) and education level; lifestyle model was adjusted as for socioeconomic model, plus variables of BMI, smoking status, alcohol intake, physical activity and vegetables consumption; Health model was further adjusted for self-rated health (perceived health status scale), multimorbidity and pharmacological treatment.

In **Table 9** are showed the binary logistic regression models used to assess the association between the prevalence of functional limitation to climb up five steps in relation to high-protein foods consumption, with the lowest frequency as a reference.

No association was found between functional limitation to climb and high-protein foods consumption. As an example, the odds of having functional limitation to climb was similar between participants who eat fish every day compared to rarely or never eating fish (e.g. health model - OR 1.14; 95% CI: 0.50-2.60) (**Table 9**). Same results were found for the other high-protein foods consumption in all models.

Table 9. Association between functional limitation to climb and high-protein foods consumption.

Climb				
	Meat consumption, OR (95% CI)			
	Rarely or never	1-3 t/w or 1-2 t/w	4-6 t/w or 3-5 t/w	Every day or 7-10 t/w or 6 t/w
n	51	492	446	340
Unadjusted model	1.00 (ref)	1.06 (0.59, 1.90)	0.80 (0.44, 1.45)	0.84 (0.46, 1.53)
Socioeconomic model	1.00 (ref)	1.14 (0.62, 2.10)	0.92 (0.49, 1.71)	1.06 (0.57, 1.99)
Lifestyle model	1.00 (ref)	1.20 (0.63, 2.29)	0.90 (0.47, 1.75)	1.12 (0.58, 2.18)
Health model	1.00 (ref)	0.98 (0.48, 1.99)	0.79 (0.39, 1.62)	0.92 (0.44, 1.89)
	Fish consumption, OR (95% CI)			
n	35	443	515	336
Unadjusted model	1.00 (ref)	1.00 (0.50, 2.03)	0.90 (0.44, 1.83)	0.97 (0.48, 1.96)
Socioeconomic model	1.00 (ref)	1.04 (0.50, 2.14)	1.09 (0.52, 2.26)	1.04 (0.50, 2.16)
Lifestyle model	1.00 (ref)	1.15 (0.53, 2.51)	1.25 (0.57, 2.74)	1.23 (0.56, 2.71)
Health model	1.00 (ref)	1.12 (0.50, 2.53)	1.23 (0.54, 2.80)	1.14 (0.50, 2.60)
	Dairy consumption, OR (95% CI)			
n	108	64	117	1040
Unadjusted model	1.00 (ref)	1.10 (0.59, 2.05)	0.93 (0.55, 1.57)	0.86 (0.58, 1.28)
Socioeconomic model	1.00 (ref)	1.15 (0.59, 2.21)	1.01 (0.58, 1.75)	0.89 (0.58, 1.35)
Lifestyle model	1.00 (ref)	1.09 (0.55, 2.16)	0.98 (0.55, 1.74)	0.89 (0.57, 1.37)
Health model	1.00 (ref)	1.09 (0.53, 2.27)	0.88 (0.48, 1.62)	0.94 (0.59, 1.50)

Notes: n = 1329; OR, Odds Ratio; CI, Confidence Interval; t/w, times per week; ref, reference category. Consumption frequencies of meat and fish: rarely or never; 1-3 t/w; 4-6 t/w; every day or 7-10 t/w. Consumption frequencies of dairy: rarely or never; 1-2 t/w; 3-5 t/w; every day or 6 t/w. Binary logistic regression was the statistic model used. Reference category of the outcome variable was not having functional limitations in climbing. The first model was not adjusted for any covariate; socioeconomic model was adjusted for age, sex, geographical location (NUTS II) and education level; lifestyle model was adjusted as for socioeconomic model, plus variables of BMI, smoking status, alcohol intake, physical activity and vegetables consumption; Health model was further adjusted for self-rated health (perceived health status scale), multimorbidity and pharmacological treatment.

Table 10 show the binary logistic regression models used to assess the association between the prevalence of functional limitation to reach and get down a 2.5 kg object from above the head in relation to high-protein foods consumption, with lowest frequency as a reference.

No difference was detected between the odds of having functional limitations to reach in participants who reported to eat dairy every day and participants who rarely or never eat dairy (e.g. health model - OR 0.97; 95% CI: 0.61-1.53) (**Table 10**). The same applied to other high-protein foods consumption and respective frequencies in all of the tested models.

Table 10. Association between functional limitation to reach and high-protein foods consumption.

Reach				
	Meat consumption, OR (95% CI)			
	Rarely or never	1-3 t/w or 1-2 t/w	4-6 t/w or 3-5 t/w	Every day or 7-10 t/w or 6 t/w
n	51	490	445	340
Unadjusted model	1.00 (ref)	1.06 (0.59, 1.90)	0.67 (0.37, 1.21)	0.81 (0.45, 1.48)
Socioeconomic model	1.00 (ref)	1.22 (0.66, 2.27)	0.81 (0.43, 1.53)	1.19 (0.63, 2.23)
Lifestyle model	1.00 (ref)	1.33 (0.71, 2.51)	0.87 (0.45, 1.66)	1.35 (0.70, 2.59)
Health model	1.00 (ref)	1.08 (0.54, 2.14)	0.74 (0.37, 1.50)	1.12 (0.55, 2.26)
	Fish consumption, OR (95% CI)			
n	35	443	512	336
Unadjusted model	1.00 (ref)	1.31 (0.63, 2.70)	1.12 (0.54, 2.33)	1.30 (0.63, 2.69)
Socioeconomic model	1.00 (ref)	1.32 (0.62, 2.80)	1.28 (0.60, 2.73)	1.32 (0.62, 2.82)
Lifestyle model	1.00 (ref)	1.39 (0.64, 3.04)	1.43 (0.65, 3.17)	1.57 (0.71, 3.47)
Health model	1.00 (ref)	1.36 (0.60, 3.08)	1.42 (0.62, 3.24)	1.47 (0.65, 3.36)
	Dairy consumption, OR (95% CI)			
n	108	64	116	1038
Unadjusted model	1.00 (ref)	0.76 (0.40, 1.44)	0.89 (0.52, 1.52)	0.93 (0.62, 1.39)
Socioeconomic model	1.00 (ref)	0.72 (0.36, 1.43)	0.91 (0.51, 1.60)	0.89 (0.58, 1.36)
Lifestyle model	1.00 (ref)	0.69 (0.34, 1.38)	0.95 (0.53, 1.69)	0.92 (0.59, 1.42)
Health model	1.00 (ref)	0.66 (0.31, 1.37)	0.89 (0.49, 1.62)	0.97 (0.61, 1.53)

Notes: n = 1326; OR, Odds Ratio; CI, Confidence Interval; t/w, times per week; ref, reference category. Consumption frequencies of meat and fish: rarely or never; 1-3 t/w; 4-6 t/w; every day or 7-10 t/w. Consumption frequencies of dairy: rarely or never; 1-2 t/w; 3-5 t/w; every day or 6 t/w. Binary logistic regression was the statistic model used. Reference category of the outcome variable was not having functional limitations in reaching. The first model was not adjusted for any covariate; socioeconomic model was adjusted for age, sex, geographical location (NUTS II) and education level; lifestyle model was adjusted as for socioeconomic model, plus variables of BMI, smoking status, alcohol intake, physical activity and vegetables consumption; Health model was further adjusted for self-rated health (perceived health status scale), multimorbidity and pharmacological treatment.

Table 11 present the binary logistic regression models used to assess the association between the prevalence of overall functional limitation, defined by a HAQ final score > 0, and high-protein foods consumption.

The odds of having overall functional limitation was similar between participants who eat meat every day and to the ones who rarely or never eat meat (e.g. health model - OR 0.75; 95% CI: 0.32-1.78) (**Table 11**). Same results were detected for the other meat consumption frequencies compared to the lowest one. Similarly, for fish consumption, the odds of having overall functional limitation did not vary between eating fish every day and eating fish rarely or never, in all models (e.g. health model - OR 0.79; 95% CI: 0.28-2.25) (**Table 11**). Concerning milk and dairy, the results were similar to the meat and fish consumption. Nevertheless, there was an exception for consuming dairy 1-2 t/w compared to rarely or never: the odds of having overall functional limitation were 0.41 times lower in the health model (OR 0.41; 95% CI: 0.18-0.91) (**Table 11**).

Table 11. Association between overall functional limitation and high-protein foods consumption.

HAQ final score > 0				
	Meat consumption, OR (95% CI)			
	Rarely or never	1-3 t/w or 1-2 t/w	4-6 t/w or 3-5 t/w	Every day or 7-10 t/w or 6 t/w
n	51	491	445	340
Unadjusted model	1.00 (ref)	0.94 (0.45, 1.95)	0.63 (0.30, 1.31)	0.75 (0.36, 1.59)
Socioeconomic model	1.00 (ref)	1.09 (0.51, 2.33)	0.79 (0.37, 1.71)	1.11 (0.51, 2.42)
Lifestyle model	1.00 (ref)	1.07 (0.49, 2.34)	0.76 (0.34, 1.66)	1.10 (0.50, 2.46)
Health model	1.00 (ref)	0.73 (0.32, 1.71)	0.56 (0.24, 1.31)	0.75 (0.32, 1.78)
	Fish consumption, OR (95% CI)			
n	35	443	513	336
Unadjusted model	1.00 (ref)	0.64 (0.26, 1.61)	0.69 (0.27, 1.74)	0.76 (0.30, 1.93)
Socioeconomic model	1.00 (ref)	0.66 (0.26, 1.70)	0.81 (0.31, 2.10)	0.81 (0.31, 2.11)
Lifestyle model	1.00 (ref)	0.68 (0.26, 1.83)	0.82 (0.30, 2.22)	0.85 (0.31, 2.30)
Health model	1.00 (ref)	0.63 (0.22, 1.76)	0.79 (0.28, 2.26)	0.79 (0.28, 2.25)
	Dairy consumption, OR (95% CI)			
n	108	64	116	1039
Unadjusted model	1.00 (ref)	0.52 (0.26, 1.04)	1.18 (0.62, 2.26)	0.94 (0.58, 1.51)
Socioeconomic model	1.00 (ref)	0.53 (0.25, 1.09)	1.26 (0.64, 2.49)	0.94 (0.57, 1.56)
Lifestyle model	1.00 (ref)	0.48 (0.23, 1.03)	1.20 (0.60, 2.40)	0.92 (0.55, 1.55)
Health model	1.00 (ref)	0.41 (0.18, 0.91)	0.99 (0.48, 2.07)	0.93 (0.53, 1.61)

Notes: n = 1327; OR, Odds Ratio; CI, Confidence Interval; t/w, times per week; ref, reference category. Consumption frequencies of meat and fish: rarely or never; 1-3 t/w; 4-6 t/w; every day or 7-10 t/w. Consumption frequencies of dairy: rarely or never; 1-2 t/w; 3-5 t/w; every day or 6 t/w. Binary logistic regression was the statistic model used. Reference category of the outcome variable was not having functional limitations (HAQ final score = 0). The first model was not adjusted for any covariate; socioeconomic model was adjusted for age, sex, geographical location (NUTS II) and education level; lifestyle model was adjusted as for socioeconomic model, plus variables of BMI, smoking status, alcohol intake, physical activity and vegetables consumption; Health model was further adjusted for self-rated health (perceived health status scale), multimorbidity and pharmacological treatment.

In summary, there was no association between high-protein foods consumption (meat, fish and dairy) and functional limitation outcomes (**Table 7**, **Table 8**, **Table 9**, **Table 10** and **Table 11**). The absence of association was clear in the unadjusted models and also in the more complex models (socioeconomic, lifestyle and health models), adjusted for potential confounders. Adjustment for regular physical activity in the lifestyle model did not modify the findings.

shows the detailed results of the binary logistic regression of the health model, used to assess the association between the prevalence of overall functional limitation, defined by a HAQ score > 0, and high-protein foods consumption.

Although there was not found an association between high-protein foods and functional limitations outcomes, variables like age, sex, BMI, multimorbidity, use of medication and self-perceived health status were significant associated ($p < 0.05$) with overall functional limitation (HAQ final score > 0) (**Table 12**), as previously reported in scientific literature (21, 114-122). The prevalence of functional limitations was associated with age: each additional year was associated with 1.07 (95% CI, 1.04–1.10) times the odds of having functional limitations. Being a woman (OR 1.84; 95% CI 1.28–2.65) and having a higher BMI were also associated with functional limitation. Each unit increase in the BMI scale increased in 1.08 (1.03–1.12) the odds of having functional limitation. Perceived health status (OR 0.96; 95% CI 0.96–0.97), absence of multimorbidity (OR 0.67; 95% CI 0.49–0.92) and no use of medication (OR 0.43; 95% CI 0.26–0.69) were inversely associated with functional limitation prevalence.

Table 12. Association between functional limitation prevalence and high-protein foods consumption (detailed health model of the binary logistic regression).

		p	OR	95% CI for OR
Meat consumption	*Rarely or never*	0.265		
	1-3 t/w	0.474	0.73	(0.32, 1.71)
	4-6 t/w	0.180	0.56	(0.24, 1.31)
	Every day or 7-10 t/w	0.514	0.75	(0.32, 1.78)
Fish consumption	*Rarely or never*	0.463		
	1-3 t/w	0.373	0.63	(0.22, 1.76)
	4-6 t/w	0.664	0.79	(0.28, 2.26)
	Every day or 7-10 t/w	0.657	0.79	(0.28, 2.25)
Dairy consumption	*Rarely or never*	0.074		
	1-2 t/w	0.029	0.41	(0.18, 0.91)
	3-5 t/w	0.985	0.99	(0.48, 2.07)
	Every day or 6 t/w	0.790	0.93	(0.53, 1.61)
Age		0.000	1.07	(1.04, 1.10)

		p	OR	95% CI for OR
Sex	Female	0.001	1.84	(1.28, 2.65)
NUTS II	*Norte*	0.833		
	Centro	0.240	1.27	(0.85, 1.89)
	Lisboa	0.961	1.01	(0.68, 1.50)
	Alentejo	0.423	1.33	(0.66, 2.67)
	Algarve	0.864	0.93	(0.41, 2.11)
	Azores	0.353	1.38	(0.70, 2.71)
	Madeira	0.981	1.01	(0.54, 1.88)
Education level	*Primary education*	0.251		
	Secondary education	0.290	0.75	(0.44, 1.28)
	Tertiary education	0.158	0.69	(0.41, 1.16)
BMI		0.000	1.08	(1.03, 1.12)
Smoking status	*Never smoked*	0.874		
	Smoked in the past	0.657	0.92	(0.64, 1.32)
	Smokes daily or occasionally	0.909	1.04	(0.56, 1.90)
Alcohol intake	*Never consumed*	0.142		
	Daily intake	0.062	0.71	(0.50, 1.02)
	Occasional intake	0.766	0.94	(0.62, 1.42)
Regular physical activity		0.076	0.76	(0.57, 1.03)
Vegetables consumption	*Rarely or never*	0.300		
	1-2 t/w	0.585	1.38	(0.44, 4.34)
	3-5 t/w	0.538	0.72	(0.26, 2.02)
	Every day or 6 t/w	0.774	0.86	(0.32, 2.34)
Perceived health status scale		0.000	0.96	(0.96, 0.97)
Absence of multimorbidity		0.013	0.67	(0.49, 0.92)
Takes no medication		0.001	0.43	(0.26, 0.69)
Constant		0.200	0.14	

Notes: n = 1327; OR, Odds Ratio; CI, Confidence Interval; *p*, p-value; BMI, body mass index; t/w, times per week; reference categories are in *italic*. Binary logistic regression was the statistic model used. Functional limitation was considered when HAQ final score > 0. Reference category of the outcome variable was not having functional limitations (HAQ final score = 0). The health model was adjusted for age, sex, geographical location (NUTS II), education level, BMI, smoking status, alcohol intake, physical activity, vegetables consumption, self-rated health (perceived health status scale), multimorbidity and pharmacological treatment.

All the models were also tested using the highest consumption category ("Every day or 6 times/week" or the "Every day or 7-10 times/week") as a reference, but no association was established between high-protein food consumption and functional limitations.

A linear regression was also conducted using the HAQ final score as a continuous outcome variable, but again no association was found between high-protein food consumption and functional limitations (**Table 13**).

Table 13. Association between functional limitation prevalence and high-protein foods consumption (detailed health model of the linear regression).

		Unstandardized Coefficients B	Unstandardized Coefficients SE	Standardized Coefficients β	*t*	*p*	95% CI for B
Meat consumption	Rarely or never	0.05	0.07	0.01	0.62	0.537	(-0.99, 0.19)
	1-3 t/w*						
	4-6 t/w	-0.07	0.04	-0.04	-1.76	0.079	(-0.14, 0.01)
	Every day or 7-10 t/w	-0.08	0.04	-0.04	-1.83	0.068	(-0.16, 0.01)
Fish consumption	Rarely or never	0.04	0.09	0.01	0.45	0.654	(-0.13, 0.21)
	1-3 t/w	-0.06	0.04	-0.04	-1.56	0.119	(-0.13, 0.02)
	4-6 t/w*						
	Every day or 7-10 t/w	0.08	0.04	0.04	1.81	0.071	(-0.01, 0.16)
Dairy consumption	Rarely or never	0.02	0.05	0.01	0.32	0.752	(-0.09, 0.12)
	1-2 t/w	0.06	0.07	0.02	0.89	0.371	(-0.07, 0.20)
	3-5 t/w	-0.07	0.06	-0.03	-1.30	0.194	(-0.18, 0.04)
	Every day or 6 t/w*						
Age		0.02	0.00	0.16	7.34	0.000	(0.01, 0.02)
Sex	Female	0.23	0.04	0.15	5.33	0.000	(0.14, 0.31)
NUTS II	Norte*						
	Centro	-0.09	0.04	-0.05	-1.99	0.047	(-0.17, -0.00)
	Lisboa	-0.09	0.05	-0.05	-1.95	0.051	(-0.18, 0.00)
	Alentejo	-0.00	0.06	-0.00	-0.03	0.975	(-0.13, 0.12)
	Algarve	-0.09	0.08	-0.02	-1.08	0.282	(-0.24, 0.07)
	Azores	0.00	0.06	0.00	0.02	0.984	(-0.12, 0.12)
	Madeira	-0.08	0.06	-0.03	-1.42	0.156	(-0.19, 0.03)
Education level	Primary education*						
	Secondary education	-0.11	0.07	-0.03	-1.57	0.116	(-0.25, 0.03)
	Tertiary education	-0.12	0.07	-0.04	-1.79	0.074	(-0.25, 0.01)
BMI		0.01	0.00	0.06	2.97	0.003	(0.00, 0.02)
Smoking status	Never smoked*						
	Smoked in the past	0.04	0.05	0.02	0.82	0.415	(-0.05, 0.13)
	Smokes daily or occasionally	-0.05	0.08	-0.01	-0.58	0.561	(-0.20, 0.11)
Alcohol intake	Never consumed*						
	Daily intake	-0.16	0.04	-0.10	-3.95	0.000	(-0.24, -0.08)
	Occasional intake	-0.08	0.04	-0.04	-1.90	0.057	(-0.16, 0.00)

		Unstandardized Coefficients B	Unstandardized Coefficients SE	Standardized Coefficients β	*t*	*p*	95% CI for B
Regular physical activity		-0.19	0.03	-0.13	-5,78	0.000	(-0.25, -0.13)
Vegetables consumption	Rarely or never	0.18	0.09	0.05	2.13	0.033	(0.02, 0.35)
	1-2 t/w	0.14	0.06	0.05	2.41	0.016	(0.03, 0.25)
	3-5 t/w	0.01	0.04	0.01	0.32	0.752	(-0.06, 0.09)
	Every day or 6 t/w*						
Perceived health status scale		-0.02	0.00	-0.39	-18.08	0.000	(-0.02, -0.01)
Absence of multimorbidity		0.17	0.04	0.10	4.55	0.000	(0.10, 0.24)
Takes no medication		0.11	0.06	0.04	1.89	0.059	(-0.00, 0.23)
Constant		-0.01	0.25		-0.05	0.957	(-0.50, 0.48)

Notes: n = 1731-2393; B, unstandardized coefficient; β, Beta estimate (standardized coefficient); SE, Standard Error; *t*, t-value; *p*, p-value; CI, Confidence Interval; BMI, body mass index; t/w, times per week. Linear regression was the statistic model used. The outcome was analysed as a continuous variable (HAQ final score). The health model was adjusted for age, sex, geographical location (NUTS II), education level, BMI, smoking status, alcohol intake, physical activity, vegetables consumption, self-rated health (perceived health status scale), multimorbidity and pharmacological treatment. *These variables were excluded from the model due to impossible tolerances.

5. DISCUSSION

This study determined the prevalence of functional limitations among community-dwelling Portuguese older adults and analysed the association between high-protein foods and functional limitation outcomes, using data from a national cohort representative sample of the Portuguese population.

5.1 Prevalence of functional limitations

Overall functional limitations' prevalence among community-dwelling Portuguese older adults (n = 2236) was 81.0%. The prevalence increased with age, with the highest level in the + 85 years group (93.1%). Participants between 65 to 75 years old had a 76.9% prevalence of functional limitations, lower than the ones aged 76-85 (86.8%). Women presented a higher functional limitations' prevalence (87.1%) when compared to men (70.0%). When stratified by educational level, low educational attainment level was related to a higher prevalence (82.6%) of functional limitations.

Regarding the ability to perform ADLs without any difficulty, 60.1 - 60.2% reported being able to stand up from a straight chair; 60.8 - 61.1% were able to walk outdoors on flat ground; 53.0 - 53.1% had the ability to climb up five steps, and 56.4 - 56.7% could reach and get down a 2.5 kg object from above their head.

Despite methodological differences, these results seem to be in line with previously national and international published results.

According to the 2011 Census, about 19% of the resident population in Portugal were older adults of which 50% had great difficulty or were unable to perform at least one of the six ADLs (seeing, listening, walking, memory/concentration, bathing/dressing, understanding others/making themselves understood) (19). The proportion of the population with difficulty in carrying out at least one ADL increased with age. In older adults aged 65-69 years, the incidence rate of at least one functional disability was 30% (19). For the 75-79 age group, the proportion of people unable or that find very difficult to carry out at least one activity is over 50% (19). The proportion of women who are unable or find it very difficult to carry out at least one of the day-to-day activities is higher than the proportion of men, in all age groups. On average, the proportion of women with at least one limitation was 10% above men. Only 4.4% of the older population (88 269) had great difficulty or could not perform any of the six activities. Of the people aged 65 and over who had at least one difficulty, 70% could not or did have much difficulty walking or climbing stairs (19).

SHARE (Survey of Health, Ageing and Retirement in Europe) and sibling studies like HRS (Health and Retirement Study; USA) and ELSA (English Longitudinal Study of Ageing; England) comprise measures of disability which include questions regarding functional limitations, ADLs and IADLs (123). Respondents in HRS (51+ years; n = 18469) reported considerably more difficulty with climbing stairs (18.8%), getting up from a chair (39.7%), walking (14.8%) and extending arms above shoulders (15.8%) compared to SHARE (50+ years; n = 32442) and ELSA participants (50+ years; n = 9718) (123). SHARE participants had the lowest prevalence of functional limitations: 11.8% reported difficulty in walking 100 meters, 17.3% had difficulty getting up from a chair, 10.7% climbed one flight of stairs with difficulty and 9.7% reported to have difficulty extending arms above shoulders (123). Additionally, the highest level of disability (one or more functional limitation, %) was found in participants of HRS (69.3%), compared to ELSA (55.8%) and SHARE participants (51.6%) (123).

Data for individuals aged 65 and older from the HRS (n = 10905), ELSA (n = 5437), and SHARE (n = 13408) revealed that difficulty in dressing and bathing had the highest prevalence and eating the lowest, overall (12.7% and 11.9% vs. 3.9%, respectively) and in each survey (124). Overall difficulty in walking was 6.6% and varied between surveys (HRS: 10.9%; ELSA: 4.7%; SHARE: 3.8%) (124).

In a study conducted with Portuguese (n = 2080), Italian (n = 3583) and Spanish (n = 3570) data from SHARE of 2011 (n = 9233), 78.8% of participants (50+ years) were considered to be non-limited and 21.2% were limited (moderately and severely limited) (125). Regarding Portuguese data, 75.5% did not have functional limitation, compared to 13.7% who were moderately limited and 10.9% severely limited (125). Additionally, women were 2.3% more likely to experience severe functional limitation than men, and overcame a 10% probability threshold of suffering from severe limitation around 5 years earlier (125). Having a university degree reduced the probability of severe functional limitation by 3.5% as compared to none educational level. Differences were wider for the oldest old: women aged 65-79 years old were 3.3% more likely to suffer severe limitations, the excess risk increasing to 15.5% among those older than 80 (125).

In the second wave of the European health interview survey, conducted between 2013 and 2015, individuals aged 15 and over were evaluated about their health state, which among others, referred to the main physical and sensory functional limitations (for example, related to vision, hearing or walking). Specifically, older adults (aged 65 and over) were asked whether they had difficulty in doing certain types of activities, the latter grouped into personal care activities (for example, getting in and out of a bed or a chair) or household activities (for example, preparing meals or doing housework). More than two thirds of participants aged 65 or over reported physical and sensory functional limitations (126). This study concluded that age is another important factor that may explain differences in functioning. In the oldest age group, at EU-28 level, around two thirds of people aged 65 and over reported such type of limitations, a share that was at 69.2% in Portugal (126). In relation to personal care activities, on average, about 24% of the European population aged 65 and over reported (moderate or severe) limitations, with Portuguese older adults situating at approximately 21.5%. Within the older population, limitations with respect to personal care or household activities were more prevalent among women and among older adults with low educational attainment (the share of people reporting limitations in personal care activities was higher for those with at most lower secondary education, as compared to those having completed upper secondary education or post-secondary non-tertiary education or higher) (126).

The functional autonomy of the Portuguese population with 65 years and over was assessed between 1985 and 2005, using data from four National Health Surveys undertaken in Portugal (1987, 1995, 1998, 2005). The study found a decreasing tendency in the proportion of individuals with at least one disability over the years (74.5% in 1987; 60% in 1995; 65.8% in 1998 and 62.1% in 2005) (122). Variables that explained disability were age, sex, education, income, chronic diseases and self-perception of health status (122).

In this study we found a higher prevalence of functional limitation (81.0%) in a sample of community-dwelling Portuguese older adults (n = 2236). Although methodological differences can partly explain the difference in values found, some hypotheses can be drawn. Possible explanations to this reverse trend may be the population ageing and the increasing prevalence of obesity and other chronic diseases (4), combined with rising physical inactivity among Portuguese population (127). Population ageing is the 21st century's dominant demographic phenomenon (128) and obesity has markedly increased during recent decades (129). The risk for functional limitations is elevated by the growing prevalence of obesity, which in turn increases the prevalence of simultaneously occurring debilitating diseases such as arthritis (129).

5.2 Dietary intake

Regarding dietary intake, we found that frequent consumption of vegetables, soup and fresh fruit was more prevalent among individuals who consumed fish every day or 7-10 times/week. It seems that participants who have higher fish consumption tend to eat a more-healthy diet.

5.3 High-protein foods and functional limitations

There was no association between high-protein foods (meat, fish and dairy) and functional limitations among Portuguese older adults. Explanations about these results may rely on the self-reported nature of the main data used in the analysis. Additionally, as some authors have been pointing out, people's diets consist of a variety of foods with complex combinations of nutrients and the examination of only single foods could result in identification of erroneous associations between dietary factors and functional decline in older adults (86). In future studies, the dietary pattern approach using factor and cluster analysis could provide more information regarding the risk of potential functional decline in older individuals (86). Beyond the potential reasons mentioned to the lack of association, protein content of meals consumed by older adults was not quantified. There can be a great variation between meals composed by high-protein foods, and consequently protein content may be very different. Moreover, it would have been useful to include other protein-source foods (soy, nuts, seeds and legumes) and to assess the prevalence of inadequate protein intake among study participants in order to know if protein needs were supplied. It should be noted that few participants reported to have a low frequency consumption (rarely or never) of high-protein foods, which may have influenced the representativeness of this low consumption level.

In fact, several studies have associated low protein consumption with functionality (63, 66, 83, 84, 86-89, 96, 98) in older adults, among other outcomes such as muscle mass (79, 80). But, as pointed out by Gaytán-González et al., "this may be a vicious circle" (96), as the lack of functionality is a risk factor for low protein intake (130). As suggested this authors, "there should be more research in other ways to assess daily living functionality, like physically active and sedentary time, along with protein intake assessment to expand knowledge about their possible interaction" (96).

As an example, Bradlee et al. found that higher intake of animal-protein foods, alone, but especially in combination with a physically active lifestyle, was associated with preservation of muscle mass and functional performance in older adults (85). The study showed that higher intakes of protein-source foods (red meat, poultry, fish, dairy, and soy, nuts, seeds and legumes) were associated with higher % skeletal muscle mass over 9 years, particularly among women (85). Men and women with higher intakes of foods from animal sources had a higher % skeletal muscle mass regardless of activity; beneficial effects of plant-based protein foods were only evident in physically active adults (85). Active participants with higher intakes of animal or plant protein-source foods had 35% lowest risks of functional decline and among less active individuals, only those consuming more animal protein-source foods had reduced risks of functional decline (Hazards Ratio: 0.71; 95% CI: 0.50-1.01) (85). Comparing with the results of the present study, participants with higher intakes of meat had similar odds of having overall functional limitation than participants who rarely or never eaten meat (e.g. health model - OR 0.75; 95% CI: 0.32-1.78). Regarding fish and dairy consumption, parallel results were found (e.g. health model: fish - OR 0.79; 95% CI: 0.28-2.25; dairy - OR 0.93; 95% CI: 0.53-1.61), in all the models.

By contrast, other studies did not found association between protein intake and function outcomes or muscle mass and strength in older adults (81, 97, 131). Results from Sahni et al. show no association between animal protein intake and lean mass or muscle strength (81). Their findings suggest that maintaining adequate protein intake with age may help preserve muscle mass and strength in adult men and women and that dietary protein types may differentially affect muscle mass and strength. The authors mention that whether plant protein is a marker of dietary quality or has a direct effect on muscle strength (independent of lean mass) needs to be further clarified (81). In a Chinese study that did not found association between total protein intake and functional decline, the mentioned reasons to the absence of association were: 1) the relative total protein intake of the study sample was comparable or even high, compared with other published studies among Caucasians; 2) the participants were able to walk or take public transport and the ceiling effect of higher functional status may account for the absence of associations between protein intake and physical performance measures (98). Unexpectedly, this study found that higher vegetable protein intake was associated with reduced muscle loss in the sample and although the underlining reasons are unknown, some suggestions are cited: 1) other nutrients, such as vitamin D, folic acid, and antioxidant intakes may also affect muscle mass, strength and performance in older adults. Therefore, whether reduced muscle loss is attributed exclusively to high vegetable protein intake or to the antioxidant property of plant-based foods deserves additional research; 2) Chinese population generally has higher intake of soy foods and soy proteins than Western populations. Soy proteins contains all amino acids essential to human nutrition and their quality is almost equivalent to animal sources (98). Additionally, there is evidence to suggest that increases in muscle strength and size were not influenced by the predominant source of protein consumed by older men with adequate total protein intake (131).

Furthermore, age, sex, BMI, multimorbidity, medication use and perceived health status were significantly associated ($p < 0.05$) with functional limitations. The prevalence of functional limitations was directly associated with age (OR 1.07; 95% CI, 1.04–1.10), being a woman (OR 1.84; 95% CI 1.28–2.65), BMI (OR 1.08; 95% CI, 1.03–1.12). Perceived health status (OR 0.96; 95% CI 0.96–0.97), absence of multimorbidity (OR 0.67; 95% CI 0.49–0.92) and no use of medication (OR 0.43; 95% CI 0.26–0.69) were inversely associated with functional limitation prevalence.

These results appear to be consistent with findings from studies previously reported in scientific literature (21, 114-122, 132). For example, in a cross-sectional study of the survey on Health, Ageing, and Retirement in Europe (n = 13974 adults aged 50+), functional limitation was associated with females, age, self-rated health, and an increased number of chronic conditions, disease symptoms and depressive symptoms (21). In the WHO Study on Global Ageing and Adult Health (SAGE), involving representative cohorts of respondents (≥ 50 years) in six countries (China, Ghana, India, Mexico, Russia, and South Africa), followed as they age, found that health status score declined with age, as expected. At each age in each country, the score for males was higher than for females; women lived longer than men on average, but had poorer health status (3). In a study with older adults (≥ 75 years) from the Centro region of Portugal, participants (n = 1153) were classified in respect to ADLs performance. 31.4% presented excellent or good performance, 21.9% had small limitation, 23.2% had moderate limitation, 7.1% had severe limitation and 16.5% had total limitation. Differences were observed between age groups ($p < 0.001$), with a worse classification for the elderly, and between genders ($p < 0.001$), with greater limitations in women (133).

5.4 Study limitations and strengths

Some limitations of this study should be considered. Given this study's nature, reverse causality may be present in the cross-sectional data. Selection bias may also have occurred from the first to the second wave of EpiDoC study, when participants were lost, which could have led to systematic errors in the association. Consequential loss of power in data due to attrition may have affected associations, and the presence of very healthy older 'survivors' might have posed additional source of bias. The sample used in this study did not include institutionalized older adults, so our results may not apply to this particularly susceptible group. Moreover, dietary intake was assessed with food frequency questions in EpiDoC 2, as at the moment of data collection there was no validated questionnaire for telephone use regarding dietary intake. The food frequency questionnaire used was qualitative and partially assessed the dietary habits of participants, therefore reflecting recent exposure instead of long-term exposure. Additionally, data regarding dietary habits was self-reported. Other studies found that women are more likely to under-report than men, and under-reporting is more common among overweight and obese individuals (134). Overall meat/poultry/fish intake tend to be overestimated in national dietary surveys, when disaggregation of mixed dishes is not taken into account (135). In the present study, eggs' consumption was not assessed and therefore was not considered as a high-protein food source. Additionally, data from chronic diseases were based on self-reported data. Physical activity was also self-reported, which may imply the existence of substantial measurement error and thus contribute to the possibility of residual confounding by physical activity. The outcomes of functional limitation were based on self-reported measures as for high-protein foods consumption, which are subject to misreporting. Despite being important, self-report measures should be utilized cautiously and serve only as one component of the assessment of function, according to some authors (42). Indeed, self-report measures are valuable in defining the patient's perspective but have been shown to differ substantially from physical performance measures that involve quantification of output, and are dramatically influenced by changes in pain (42). However, previous studies support that self-reports have a physiologic basis and can accurately predict preclinical disability and related impairment (136). Concerning the regression models, although they were adjusted for confounding variables, it is likely there are residual confounders, which may influence the relationship we intended to study. As an observational study it can be more subject to potential confounding than randomized clinical trials. Finally, in the future it would be interesting to assess functional limitations through repeated standardized measures of functional status from sequential exams.

The strengths of this study are the fact it was performed in a national representative sample, with a robust study design and a high number of participants, and the careful adjustment made for potential confounders in the regression models.

6. CONCLUSION

Community-dwelling Portuguese older adults have a high prevalence of functional limitations, emphasizing the need to address this situation with a dedicated intervention. The characterization of the functional limitations of older population is of particular importance, given the demographic profile of the population residing in Portugal.

This study's approach relied on function-related questions, such the ability to stand up, walk, and reach, and their relationship with self-reported high-protein foods consumption. Unlike other authors, an association between high-protein food consumption and functional limitations was not found. However, results imply there is a need to keep investigating this relationship in older adults. These findings need to be replicated in other studies in different settings.

There was no association between self-reported low consumption of high-protein foods and functional limitation. However, age, being a woman, overweight, multimorbidity and low quality of life (low perceived health status) were significantly associated ($p < 0.05$) with functional limitations in Portuguese older adults.

Further investigations may strengthen the current evidence about this subject and contribute to food and nutritional recommendations update, aiming the promotion of an active and healthy ageing.

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