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Article

Incidence of the Risk of Malnutrition and Excess Fat Mass, Low Total Lean Tissue, and Gait Speed as Independent Associated Factors in Community-Dwelling Older Adults

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Abstract: Background and aims: Only one cohort study exists on the incidence of the risk of malnutrition (RM) in older adults, though numerous cross-sectional –and that cohort research– have reported several risk factors associated with the prevalence and incidence of this condition. However, alterations of body composition and impaired physical performance as exposition variables of RM have not been explored. This study assessed the incidence of RM and determined its association with excess fat mass, low total lean tissue, gait speed, and handgrip strength as exposition variables for RM in community-dwelling older adults. **Methods:** This is a secondary analysis in older adults (≥ 60 years old) derived from the study “Frailty, dynapenia, and sarcopenia in Mexican adults (FraDySMex)” a prospective cohort project conducted from 2014 to 2019 in Mexico City. At baseline, volunteers underwent body composition analysis and physical performance tests. Several covariates were identified through comprehensive geriatric assessment. At baseline and follow-up, RM was assessed using the long form of the mini nutritional assessment (MNA-LF) scale. Associations between the exposition variables and RM were assessed by multiple logistic regression. **Results:** The cohort included 241 subjects. Average age was 75.6 ± 7.8 years; 83.4% were women. The mean follow-up period was 4.1 years, during which 28.6% of subjects developed RM. This condition was less likely to occur in subjects with excess fat mass index, even after adjusting for several covariates. RM was also more likely to occur in subjects with low total lean tissue index, according to both an unadjusted model and after adjusting for the corresponding variables. Regarding the association between RM and gait speed, RM was also more likely to occur in subjects with low gait speed according to both the unadjusted and adjusted models. Similar results were found for RM in relation to low handgrip strength; however, after adjusting for the associated covariates, models 1 and 2 no longer reached the level of significance. **Conclusion:** The risk of malnutrition diagnosed by MNA-LF was significantly less likely to occur among subjects with excess fat mass, but possibility was significantly higher in those with low total lean tissue and low gait speed after 4.1 years of follow-up in community-dwelling older adults. These results highlight that excess fat mass, low total lean tissue, and low gait speed are first than risk of malnutrition and not vice versa.

Keywords: nutrition disorders; risk factors; body composition; physical functional performance; independent living; and aged; 80 and over

1. Introduction

Older adult populations are more susceptible to developing risk of malnutrition (RM). Currently, pooled analytical data on RM in older subjects highlights the risk of malnutrition as a serious public health problem in aged populations [1]. That analysis reported a prevalence of 46.2% in the geriatric population in different settings on five continents (mostly European countries), and 31.9% among community-dwelling older adults. The latter figure is close to the extreme range of 30.5% reported for community-dwelling older adults [2]. High prevalence has also been reported for other non-Caucasian community-dwelling older adult populations [3–6]. Importantly, the studies included in the pooled data analysis, and other research on the prevalence of RM in the literature, used the long form of the mini nutritional assessment (MNA-LF) scale to assess RM. This scale, generated and validated in older adults [7], has shown a sensitivity and specificity of 96% and 98%, respectively, for diagnosing RM, relative to conventional nutritional assessments and clinic diagnoses [8].

In Mexico, prevalence of RM in community-dwelling older adults ranging from 30.3 to 50%, assessed by the MNA-LF, are reported in cross-sectional studies based on either probabilistic [9] or non-probabilistic sampling [10–14]. A recent study of a national level sample of older adults reported a prevalence of 40.4% using the modified short version of the MNA [15]. All these studies concur in showing high prevalence of RM in community-dwelling older Mexican adults. Regarding to incidence, a cohort study of older Swedish adults found incidence of RM of 8.2, 16.1, 10.8, and 8.2% at 1, 2, 3, and 4-years follow-up, respectively [16]. Overall, RM now appears to constitute a challenging public health issue due to both its current high prevalence and the probability of high incidence in the coming years. Although RM is not listed in the International Disease Classification, it has been related independently to numerous adverse clinical outcomes, such as loss of functionality, impaired physical performance [10,17,18], sarcopenia [19,20], and mortality [18,21]. Some related studies recognize the benefits of nutritional intervention in older adults at risk for RM in distinct health care settings, as it can have a positive effect on energy intake and body weight [22].

Several variables have been associated with RM diagnosed by the short form of the mini nutritional assessment or by the MNA-LF in several cross-sectional studies of community-dwelling older adults [3,4,9–12,15,23,24], one cohort study [16], and a systematic review and meta-analysis of observational research [25]. However, the possible association between the alterations of body composition, particularly excess fat mass and low total lean tissue indices, on the one hand, and impaired physical performance measured by low gait speed and low handgrip strength as exposition variables, on the other, and RM has not yet been explored thoroughly. It is widely believed that these factors can contribute to the early development of RM through diverse mechanisms. In this context, the present study assessed the incidence of the risk of malnutrition and its association with the excess fat mass and low total lean tissue indices, and low gait speed and handgrip strength as exposition variables in community-dwelling older adults after 4.1 years of follow-up.

2. Materials and Methods

2.1. Data sources and study design

This is a secondary analysis based on the primary data generated in the project: “Frailty, dynapenia, and sarcopenia in Mexican adults” (FraDySMex), a prospective cohort study of a non-representative sample of community-dwelling men and women subjects ≥ 50 years old, conducted from 2014 to 2019. The aim of the original project was to assess body composition by dual-energy X-ray absorptiometry (DXA) and physical performance using the short physical performance battery and measures of muscle strength by dynamometry, to diagnose osteoporosis, dynapenia, frailty, and sarcopenia. In addition to the body composition and physical performance measures, volunteers underwent a health and nutritional status evaluation and other functional measurements. Social and demographic characteristics at baseline were also recorded as part of the Comprehensive Geriatric Assessment (CGA). The study was reviewed and approved by the Ethics Committee of the Angeles Mocol General Hospital, and registered at Mexico’s National Institute of Geriatrics (DI-PI-002/2014).

The protocol is in full accordance with the ethical standards established in the 1964 Helsinki Declaration with amendments. All subjects signed an informed consent form.

The methodology for selecting the study population has been published previously [13,26], so here we provide only a brief description. The first round (October-December 2014) consisted in assessing volunteers. In the second round (October-December 2015), new subjects were added and a proportion of the individuals who participated in the first round were reevaluated. The third round (October-December 2019) saw the inclusion of new subjects and reevaluations of some of the people who participated in the first and second rounds. All procedures in all rounds were performed in the Functional Evaluation Research Laboratory at Mexico's National Institute of Geriatrics and the Older Adult Evaluation Center of the Universidad Iberoamericana in Mexico City, where subjects underwent the aforementioned series of objective evaluations, performed by a medical team made up of a physician, a nutritionist, a psychologist, physical therapists, physical rehabilitators, social workers, and geriatricians, all previously, and fully, informed of the study's aims.

2.2. Study population

All participants were community-dwelling adults and older adults from two municipalities (of a total of 16) in southeast Mexico City (Magdalena Contreras and Álvaro Obregón), characterized by high poverty levels and older populations. Subjects were invited regardless of their health, mental, nutritional, and functional status via home visits by a psychologist or social worker, or through flyers left in churches, seniors' community centers, social security centers, and health clinics in the aforementioned places.

No institutionalized individuals or potential subjects with an acute or chronic disease that could affect the measurements required by the study protocol were not invited during recruitment. Potential volunteers had to reach the installations or laboratories on their own. Upon arriving, they underwent a cognitive assessment, and the mini-mental state examination (MMSE) was applied. Subjects had to be able to answer the study questionnaires by themselves. If they had MMSE scores of 10 points or less [27], they were assisted by the caregiver. All subjects included at baseline with complete data were contacted by phone and home visits at follow-up and invited to participate in the second assessment.

Baseline data were collected from October 2014 to December 2015, while follow-up data were gathered from October-December 2019. At baseline, 540 potential men and women subjects were included. The inclusion criteria for the present analysis were men and women subjects ≥ 60 years old. Subjects at baseline and follow-up had to have the measurements required to test the hypothesis variables. Furthermore, the RM assessed by the MNA-LF, and all data collected in the CGA at baseline such as the cognitive function assessments, record of depressive symptoms, comorbidity, smoking and alcohol consumption, and the number of medications taken. Functional status was assessed by the basic activities of daily living (BADL) and instrumental activities of daily living (IADL) scales. Evaluations of physical performance were based on handgrip strength and gait speed, and the social and demographic characteristics were also assessed, and body composition by DXA. The exclusion criteria before follow-up were potential subjects with incomplete files, individuals < 60 years of age, and all those diagnosed with RM at baseline by the MNA-LF.

2.3. Risk of malnutrition as the main response variable

At both baseline and follow-up, RM was assessed by the MNA-LF scale [7] and applied by trained personnel. During face-to-face interviews, all volunteers were asked about their lifestyle, medications, mobility, signs of depression or dementia, the number of meals taken per day, food and fluid intake, autonomy of feeding, and self-perceptions of their health and nutrition. They then underwent an anthropometric assessment because the MNA-LF requires data on body weight, height, and arm and calf circumference measurements. Participants' nutritional status was classified according to their MNA-LF scores: < 17 , malnutrition or undernutrition; 17-23.5, risk of malnutrition; and ≥ 24 , normal nutritional status [7]. RM, as the main response variable or outcome, was coded as categorical variable. For this analysis, the MNA-LF was dichotomized into well-nourished (24-30

points) and at risk for malnutrition (<17 points), since only nine individuals had mal- or undernutrition.

2.4. Anthropometry

The anthropometric variables of body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using a SECA mBCA 514 scale (MFBIA; SECA®, Hamburg, Germany) and a SECA 264 Free-Standing Wireless 360 Stadiometer (SECA®, Hamburg, Germany). BMI was calculated based on these measurements. Arm and calf circumferences were measured according to ISAK technique. The BMI and arm and calf circumferences were incorporated into the MNA-LF. The BMI was also used to diagnose overweight and obesity [28]. All variables were measured by trained personnel as part of the anthropometry protocol.

2.5. Body composition measured by DXA

At baseline, whole and regional body composition was measured in fasting conditions by DXA (Hologic Discovery-WI, Hologic Inc, Bedford, MA). Volunteers wore a disposable gown. All metallic objects were removed. Measurements and calibrations of the DXA system were carried out following the manufacturer's instructions. Finally, DXA scans were edited to estimate appendicular lean tissue following published recommendations [29]. For the present analysis only total lean tissue (TLT), bone mineral content (BMC), and fat mass (FM) were considered. The TLT and FM results were then divided by height in meters squared to obtain the TLT and FM indices, respectively (TLTI, kg/m² and FMI, kg/m², respectively), which were then used to derive the exposition variables. In addition, the published FMI cut-off points were used to diagnose excess fat mass in the whole sample [30].

2.6. Physical performance assessment

Gait speed and handgrip strength were the main components of the physical performance assessment at baseline. Results were used to derive the exposition variables. Both were elements of the CGA. After receiving instructions, volunteers performed the gait speed test, recorded as a regular 6-meter walk on the GAIT Ritel instrumented mat (platinum 20,204 × 35.5 × 0.25 inches, 100 Hz sampling rate). Results were recorded in meters/second (m/s). Isometric grip strength was measured in both hands with an adjustable handgrip strength dynamometer (JAMAR Hydraulic Hand Dynamometer, Lafayette, IN), following the standardized, approved protocol [31,32]. Results were recorded in kilograms (kg). The mean of three trials for each hand was recorded, and only the highest value was used for the later analyses.

2.7. Association between the exposition variables and the risk of malnutrition

The total lean tissue and fat mass indices are the main body composition compartments associated with RM. Together with other factors, these two indices were recently associated with RM in older people. Results showed that RM was less likely to occur in those subjects under 85 years old, those with normal swallowing and normal gait speed, and higher TLTI, fat mass, and BMI values, while in those over 85 years old increased likely of suffering RM according to the adjusted and unadjusted models [24]. One cohort study showed that RM was less likely to occur in people with higher triceps skinfold thickness and handgrip strength, and better overall physical health, while risk increased with greater age and low serum albumin at baseline (16). For the present analysis, alterations of body composition compartments, particularly TLT and excess fat mass divided by height in meters squared, were considered separately as exposition variables. The former was categorized as normal or low based on sex-specific cut-off points. The dividing point was a value <20th percentile of the data distribution of the study population, while excess fat mass was determined by the sex-specific FMI cut-off points reported by Kelly et al., (2009) [30].

Regarding the components of impaired physical performance as exposition variables of RM, we considered gait speed (m/s) and handgrip strength (kg), two aspects recently associated with RM, among other variables. RM was less likely to occur in older adults with normal gait speed values

(≥ 8 m/s) [24], and in those with greater handgrip strength at baseline [16]. For our purposes, normal gait speed for older men and women subjects was considered ≥ 0.8 and ≥ 0.7 m/s, respectively, so low gait speed for men was ≤ 0.8 m/s, while for women it was ≤ 0.7 m/s. Handgrip strength was categorized as normal at scores of >18 kg and >10 kg for men and women, respectively, so low handgrip strength was ≤ 18 kg in men and ≤ 10 kg in women. These cut-off points were derived from the distribution of the variables in the same study population, and correspond to values $<20^{\text{th}}$ percentile as recommended by EWGSOP2 [33]. These exposition variables were coded as categorical (Table 1).

2.8. Assessment of covariates

The variables used as covariates were evaluated as part of the CGA. A questionnaire was applied to obtain sociodemographic information on subjects' age, occupation, marital status, years of schooling, and medical services. Based on the occupational data gathered, the main source of income was classified as having a pension. Information on living arrangements was coded as living alone or not. Age, gender, educational level, living alone, being single, widowed, or divorced, not having a pension, and health insurance status are factors that have been associated with RM in older people in earlier studies [4,9,15,25].

Cognitive status was assessed and classified using the MMSE instrument, validated for a Spanish-speaking population [27]. Cognitive decline or impairment was considered at MMSE scores ≤ 23 points for individuals with 5 years of schooling, ≤ 19 points for those with 1-4 years of education, and ≤ 16 for those with no schooling or <1 year. Depressive symptoms were assessed by the Center for Epidemiologic Studies Depression Scale, Brief Version (CESD-7), validated for older Mexican adults [34]. The presence of these symptoms was considered when subjects scored 5 or more points on the scale. Cognitive decline, depression, or symptoms of depression have been associated with RM in older people (3, 9, 12, 16, 23).

Comorbidity was assessed by the Charlson comorbidity scale (Charlson ME, 1987) [35], which was applied by trained personnel following the published instructions and recommendations [36]. Comorbidity was coded as no (≤ 2 diseases) or yes (≥ 3 diseases) [13]. Polypharmacy was assessed by asking participants to give the names of their medications, their pharmaceutical form, dosage frequency, duration of use, and whether or not they were prescribed medically. Polypharmacy was defined as yes, if 5 or more medications were being taken, and no, if the number was ≤ 4 drugs/day [37].

Data on current or previous smoking habits, frequency of alcohol consumption, and use of drugs were also assessed and coded as follows: alcohol consumption if subjects drank ≥ 2 glasses/day [38], and current smoking as yes or no [39]. Both comorbidities and smoking have been associated with RM in community-dwelling older adults [4], while polypharmacy has been signaled in cases of home-care individuals [40]. In addition, oral health (number of dental pieces, dry mouth, xerostomia) has been related to RM [23,41], as have poor self-perceptions of health [15,16].

Functional status was assessed by the Barthel scale for BADLs [42] and Lawton and Brody scale for IADLs [43]. Functional dependence was present if volunteers had scores of ≤ 90 or ≥ 1 on the BADL and IADL instruments, respectively. Loss of functionality has been associated with RM in older people [9,10,23], whereas both handgrip strength and healthy gait speed as continuous variables are deemed to be protector factors from RM in terms of the physical performance components of community-dwelling older adults [16,24].

2.9. Statistical analysis

The student's t-test for continuous variables and chi-squared test for categorical variables were used to detect significant between-group differences with and without risk of malnutrition (p -value ≤ 0.05). Results are reported as mean values with standard deviation (SD) or percentages (%), respectively. A simple logistic regression analysis was used to test for potential associations between several of the independent variables and risk of malnutrition as the response variable (Table 2). Two criteria were established to determine a potential association: a p -value ≤ 0.2 and a biological plausibility of the odds ratio (OR). Multiple logistic regression using automated stepwise analysis

(beginning with forward using a p-value ≤ 0.05) was applied to test the association between each exposed variable and the occurrence or development of risk of malnutrition. It is important to note that due to the collinearity between the exposition variables and several covariates the regression models were generated, separately. Once obtained, the preliminary models were assessed for interaction at a p-value ≤ 0.1 . Collinearity was assessed using a correlation matrix ($r \leq 0.85$). The linearity assumption of the model was not assessed because it included only categorical variables. The OR and 95% confidence interval (CI) for the occurrence of risk of malnutrition were calculated using the variables of body composition alterations and impaired physical performance after adjusting for several covariates in the final models. All analyses were performed with the statistical package STATA 16.0 for Windows (Stata Corp, College Station).

3. Results

3.1. Baseline characteristics

At baseline, 540 men and women completed all the required data. According to their MNA-LF scores, 22.9% had RM, with women having a higher proportion as compared with men (84 vs. 16%). None of the subjects diagnosed with RM by the MNA-LF at baseline were included in the follow-up study ($n=100$), so the potential sample at follow-up consisted of 440 men and women. Some of those volunteers; however, had incomplete MNA-LF data ($n = 55$), and 40 were below 60 years of age. Other losses occurred during follow-up due to the following main recorded causes: excluded candidates ($n=39$), unable to contact due to change of address ($n=16$), failed to attend the appointment ($n=6$), and death ($n=43$). This left a total sample for analysis at baseline and follow-up of 241 men and women. Figure 1 shows the flow chart of volunteers throughout the cohort study.

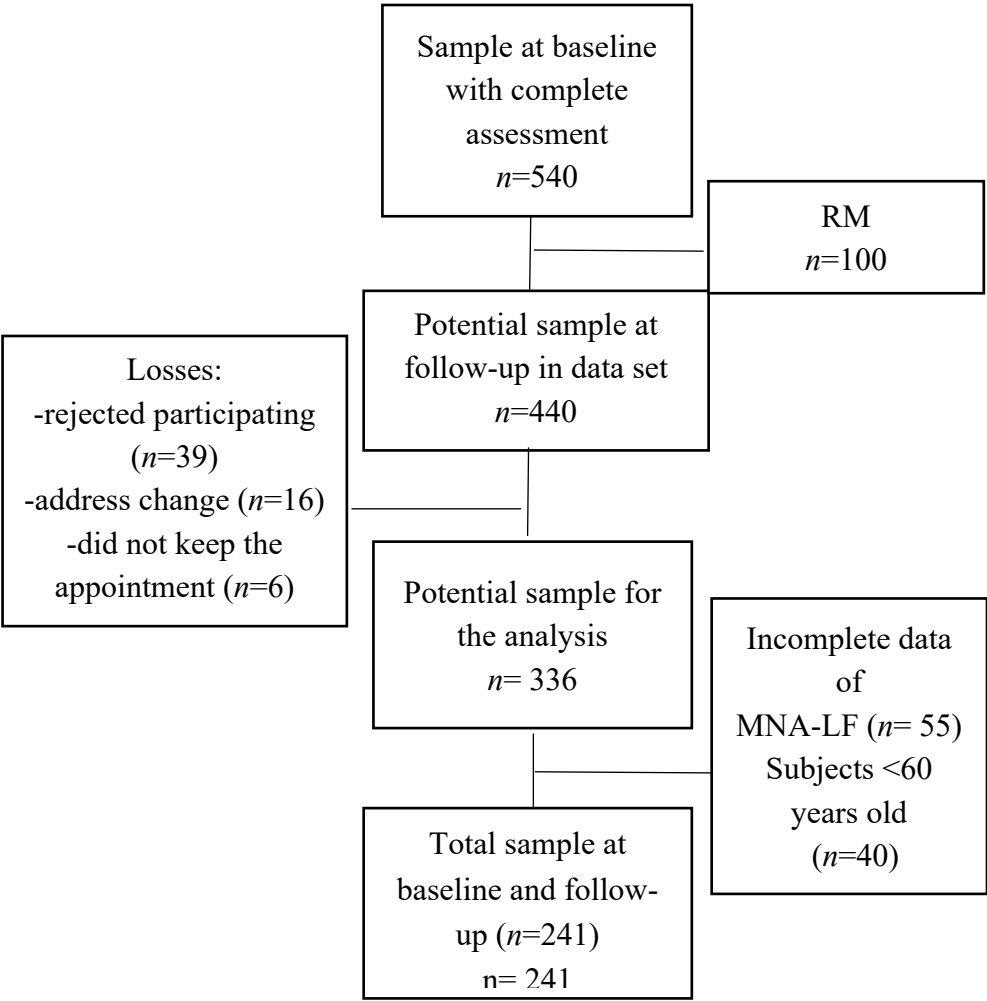


Figure 1. Flow chart of the volunteers during the cohort study. Abbreviations: RM= risk of malnutrition, MNA-LF= long form of the mini nutritional assessment.

At baseline, the sample of 241 men and women (83.4% women) subjects over 60 years old had an average of 75.6 years and well-nourished status according to the MNA-LF. In addition, 85.4% of the sample had good health by self-perception, with only 14.5% reporting poor health. Regarding fat mass measured by DXA, 85.4% of the sample had excess fat mass according to the FMI classification, while 21% had low TLTI, 36.2% had comorbidities, and 85.4% had obesity estimated by BMI ≥ 30 kg/m². Regarding physical performance and functional status, 33.2% had low gait speed, 37.7% low handgrip strength, while 14.1% and 32.3% had dependency according to the BADL and IADL scales, respectively.

Table 1 shows the behavior of several variables according to the four exposition variables measured at baseline. Regarding the behavior of these variables according to the fat mass index taken as the dichotomic variable, subjects with excess FMI were younger than the normal group. Mean values for body weight, BMI, fat mass, TLT, and BMC were all higher than those for the normal FMI group ($p \leq 0.05$). In line with these findings, the MNA score was significantly higher compared to the result for the normal FMI group. The low TLTI group was also younger; nevertheless, they had significantly lower mean values of body weight, BMI, fat mass, TLT, and BMC. In addition, the mean value of the MNA score was significantly lower than in the normal TLTI group. In contrast, the low gait speed group was older, and the proportion of subjects with depression symptoms and dependency, measured by IADL, was higher ($p \leq 0.05$). Concurring with these results, they also had significantly lower mean values of HGS compared to the normal gait speed group. As well, the MNA score was lower than in the normal gait speed group. Finally, the low handgrip strength group was also older than the normal group, and there was a higher proportion of subjects with dependency by IADL in the low HGS group, accompanied by a lower mean value of the MNA score compared to the normal HGS group.

Table 1. Baseline anthropometric and body composition, comprehensive geriatric assessment, and sociodemographic data according to the exposition variables.

Variables	Body composition					Physical performance		
	Fat mass index					Gait speed		
	Total lean tissue index					Handgrip strength		
	Excess ^a <i>n</i> = 206	Normal <i>n</i> =35	Low ^b <i>n</i> = 51	Normal <i>n</i> =190	Low ^c <i>n</i> =80	Normal <i>n</i> =161	Low ^d <i>n</i> =91	Normal <i>n</i> =150
<i>Gender</i>								
Women	171 (83.0)	30 (85.7)	39 (76.5)	162 (85.3)	64 (80.0)	137 (85.1)	75 (82.4)	126 (84.0)
Man	35 (16.9)	5 (14.3)	12 (23.5)	28 (14.7)	16 (20.0)	24 (14.9)	16 (17.6)	24 (16.1)
Age, years	75.1 \pm 7.9	79.1 \pm 7.2*	77.1 \pm 8.2	79.1 \pm 7.2*	79.1 \pm 7.8	73.9 \pm 7.6*	78.7 \pm 7.6	73.8 \pm 7.7*
<i>Anthropometry</i>								
Height, m	1.5 \pm 0.0	1.4 \pm 0.0	1.5 \pm 0.0	1.5 \pm 0.0	1.49 \pm 0.0	1.50 \pm 0.0	1.49 \pm 0.0	1.51 \pm 0.0
Body weight, kg	60.6 \pm 12.1	51.4 \pm 7.6*	57.7 \pm 10.4	67.3 \pm 12.8*	65.4 \pm 14.2	65.2 \pm 12.3	64.1 \pm 14.9	65.9 \pm 11.5
Body mass index, kg/m ²	29.7 \pm 4.7	23.1 \pm 2.8*	24.8 \pm 3.8	29.8 \pm 4.8*	29.1 \pm 5.6	28.6 \pm 4.8	28.5 \pm 5.7	28.9 \pm 4.6
<i>Body composition</i>								
Total mass by DXA, kg	65.6 \pm 12.4	45.7 \pm 13.2	51.1 \pm 14.4	65.9 \pm 12.6*	62.7 \pm 15.7	62.8 \pm 13.6	61.7 \pm 16.5	63.4 \pm 12.8
Fat mass, kg	28.1 \pm 7.2	16.2 \pm 3.2*	22.2 \pm 6.2	27.6 \pm 7.9*	26.8 \pm 8.8	26.4 \pm 7.5	26.4 \pm 9.6	26.6 \pm 6.7
Fat mass index, kg/m ²	12.4 \pm 3.2	7.2 \pm 1.3*	9.5 \pm 2.6	12.3 \pm 3.5*	11.9 \pm 3.9	11.6 \pm 3.3	11.8 \pm 4.1	11.7 \pm 3.1
Total lean tissue, kg	35.7 \pm 7.6	31.3 \pm 4.7*	29.8 \pm 6.9	36.5 \pm 7.0*	35.1 \pm 7.6	35.1 \pm 7.4	34.5 \pm 7.5	35.5 \pm 7.5
Bone mineral content, kg	1.7 \pm 0.8	1.3 \pm 0.5*	1.5 \pm 0.5	1.7 \pm 0.9*	1.6 \pm 0.5	1.7 \pm 0.9	1.7 \pm 1.2	1.7 \pm 1.6
<i>Marital status</i>								
Married	60 (29.1)	14 (40.0)	15 (29.4)	59 (31.1)	18 (22.5)	56 (34.8)	29 (31.9)	45 (30.0)
Single, divorce or separate	58 (28.2)	9 (25.7)	15 (29.4)	52 (27.4)	19 (23.8)	48 (29.8)	22 (24.2)	45 (30.0)
Widower	88 (42.7)	12 (34.3)	21 (41.2)	79 (41.6)	43 (53.8)	57 (35.4)	40 (43.9)	60 (40.0)

<i>Living arrangements/Living alone</i>									
No	157 (76.2)	27 (77.1)	62 (77.5)	122 (77.5)	62 (77.5)	122 (75.8)	66 (72.5)	118 (78.7)	
Yes	49 (23.8)	8 (22.9)	18 (22.5)	39 (24.2)	18 (22.5)	39 (24.2)	25 (27.5)	32 (21.3)	
<i>Education/Years of schooling</i>									
<10 years	122 (58.2)	22 (62.9)	27 (52.9)	117 (61.6)	56 (70.0)	88 (54.7)	56 (61.5)	88 (58.7)	
≥10 years	13 (37.4)	84 (40.8)	24 (47.1)	73 (38.4)	24 (30.0)	73 (45.3)*	35 (38.5)	62 (41.3)	
<i>Income source</i>									
<i>Pension</i>									
No	65 (31.5)	9 (25.7)	23 (45.1)	51 (26.8)	23 (28.8)	51(31.7)	29 (31.9)	45 (30.0)	
Yes	141 (68.5)	26 (74.3)	28 (54.9)	139 (73.2)*	57 (71.3)	110 (68.3)	62 (68.1)	105 (70.0)	
<i>Medical services</i>									
None	8 (3.9)	1 (2.9)	1 (1.9)	8 (4.2)	2 (2.5)	7 (4.3)	1 (1.1)	8 (5.3)	
Government	187 (90.8)	32 (91.5)	172 (90.5)	47 (92.2)	73 (91.3)	146 (90.7)	85 (93.4)	134 (89.3)	
Private or others	11 (5.3)	2 (5.7)	10 (5.3)	3 (5.9)	5 (6.3)	8 (4.9)	5 (5.5)	8 (5.3)	
<i>Self-perception of health</i>									
Bad	27 (13.1)	8 (22.9)	11 (21.6)	24 (12.6)	19 (23.8)	16 (9.9)	19 (20.9)	16 (10.7)	
Good	179 (86.9)	27 (77.1)	49 (78.4)	166 (87.4)	61 (76.3)	145 (90.1)*	72 (79.1)	134 (89.3)*	
<i>Depression symptoms/CESD-7^e</i>									
No, ≤4 CESD-7 scale	120 (58.3)	19 (54.3)	31 (60.8)	108 (56.8)	36 (45.0)	103 (63.9)	54 (59.3)	85 (56.7)	
Yes, ≥5 CESD-7 scale	86 (41.8)	16 (45.7)	20 (39.2)	82 (43.2)	44 (55.0)	58 (36.0)*	37 (40.7)	56 (43.3)	
<i>Comorbidity</i>									
No, ≤2 diseases	153 (74.3)	23 (65.7)	32 (62.7)	144 (75.8)	57 (71.3)	119 (73.9)	65 (71.4)	111 (74.9)	
Yes, ≥3 diseases	53 (25.7)	12 (34.3)	19 (37.3)	46 (24.2)	23 (28.8)	42 (26.1)	26 (28.6)	39 (26.0)	
<i>Cognitive impairment/MMSE^f</i>									
No	181 (87.9)	31 (88.6)	45 (88.2)	167 (87.9)	59 (73.8)	153 (95.0)	77 (84.6)	135 (90.0)	
Yes	25 (12.1)	4 (11.4)	6 (11.8)	23 (12.1)	21 (26.3)	8 (4.9)*	14 (15.4)	15 (10.0)	
<i>Other diseases</i>									
<i>Osteoarthritis</i>									
No	148 (72.2)	22 (64.7)	38 (76.0)	132 (69.8)	54 (69.2)	116 (72.1)	62 (68.9)	109 (72.5)	
Yes	57 (27.8)	12 (35.3)	12 (24.0)	57 (30.2)	24 (30.8)	45 (27.9)	28 (31.1)	41 (27.5)	
<i>Alcohol consumption</i>									
<2 glasses/day	110 (53.4)	17 (50.0)	26 (50.9)	101 (53.3)	49 (61.3)	78 (48.8)	47 (51.7)	80 (53.7)	
>2 glasses/day	96 (46.6)	17 (50.0)	25 (49.0)	88 (46.6)	31 (38.8)	82 (51.3)	44 (48.4)	69 (46.3)	
<i>Smoking</i>									
No	187 (90.8)	33 (94.3)	49 (96.1)	171 (90.0)	76 (95.0)	144 (89.4)	87 (95.6)	133 (88.7)	
Yes	19 (9.2)	2 (5.7)	2 (3.9)	19 (10.0)	4 (5.0)	17 (10.6)	4 (4.4)	17 (11.3)*	
<i>Polypharmacy</i>									
No, ≤4 drugs/day	102 (49.5)	15 (42.9)	25 (49.0)	92 (48.4)	34 (42.5)	83 (51.6)	40 (43.9)	77 (51.3)	
Yes, ≥5 drugs/day	104 (50.5)	20 (57.1)	26 (50.9)	98 (51.6)	46 (57.5)	78 (48.5)	51 (56.0)	73 (48.7)	
<i>Oral health/dry mouth</i>									
No	179 (86.8)	26 (74.3)	41 (80.4)	163 (86.2)	64 (79.8)	141 (87.6)	78 (85.7)	126 (84.6)	
Yes	27 (13.2)	9 (25.7)*	10 (19.6)	26 (13.8)	16 (20.3)	20 (12.4)	13 (14.3)	23 (15.4)	
<i>Nutritional status</i>									
MNA score ^g	25.3±2.7	23.8±3.4*	24.1±2.9	25.3±2.7*	24.2±2.9	25.5±2.7*	24.4±2.78	25.5±2.8*	
<i>Dependency/BADL^h</i>									
No	177 (85.9)	30 (85.7)	42 (82.4)	165 (86.8)	60 (75.0)	147 (91.3)	75 (82.4)	132 (88.0)	
Yes	29 (14.1)	5 (14.3)	9 (17.7)	25 (13.2)	20 (25.0)	14 (8.7)*	16 (17.6)	18 (12.0)	
<i>Dependency IADLⁱ</i>									
No	141 (68.5)	22 (62.9)	34 (66.7)	129 (67.9)	35 (43.8)	128 (79.5)	52 (57.1)	111 (74.0)	
Yes	65 (31.6)	13 (37.1)	17 (33.3)	61 (32.1)	45 (56.3)	33 (20.5)*	39 (42.9)	39 (26.9)*	
HGS, kg	14.4±6.4	13.1±6.2	13.4±6.0	14.4±6.4	12.8±6.2	14.8±6.3*	8.9±3.8	17.4±5.2	
GS, m/s	0.88±0.0	0.88±0.0	0.88±0.0	0.89±0.0	0.87±0.0	0.89±0.0*	0.88±0.0	0.89±0.0	

Results are reported as mean values ± SD, and number and percentages. ^aExcess fat mass index, ≥6.0 kg/m² in men and ≥9.0 kg/m² in women. ^bLow total lean tissue index, ≤15.6 kg/m² in men and ≤13.5 kg/m² in women. ^cLow gait speed ≤0.8 m/s in men, ≤0.7 m/s in women. ^dLow handgrip strength, ≤18 kg in men, ≤10 kg in women. ^eCESD-7, Center for Epidemiologic Studies Depression Scale. ^fMMSE, Mini Mental State Examination ≤23 points were

obtained with 5 years of school education, ≤ 19 points with between 1 and 4 years of education, ≤ 16 without education or with < 1 year of education. ^aMNA, mini nutritional assessment ^bDependency based on the BADL, basic activities of daily living, ≤ 90 points on the Barthel index. ⁱDependency based on IADL, instrumental activities of daily living < 8 points on the Lawton & Brody scale. * $p \leq 0.05$.

The mean follow-up period was 4.1 years. In that time, 28.6% of the sample developed RM. The cumulative incidence was more pronounced in women than in men (84.1 *vs.* 15.9%). Regarding the factor of incidence according to the exposure variables, Table 2 shows that a lower proportion of older adults (25.2%) with excess FMI developed a risk of malnutrition compared to the normal group (48.5%) at 4.1 years of follow-up. In another result, 41.1% of the subjects with low TLTI had developed RM at follow-up, compared to just 24.7% of the normal TLTI group. Similar findings emerged when the incidence of the risk of malnutrition was compared between the low and normal gait speed and handgrip strength groups. Overall, these results suggest that older subjects with excess FMI presented lower incidences of RM, while those with low TLTI, low gait speed, and low HGS had higher incidences of RM than subjects without these conditions at 4.1 years of follow-up.

Table 2. Cumulative incidence of the risk of malnutrition in community-dwelling older adults at 4.1 years of follow-up.

Variables	Incidence of risk of malnutrition		p-value
	Yes (n= 69)	No (n= 172)	
Fat mass index			
Excess (n= 206)	52 (25.2)	154 (74.8)	0.004
Normal (n= 35)	17 (48.5)	18 (51.5)	
Total lean tissue index			
Low (n= 51)	22 (41.1)	29 (58.9)	0.009
Normal (n= 190)	47 (24.7)	143 (75.3)	
Gait speed			
Low (n= 80)	33 (41.2)	47 (58.8)	0.002
Normal (n= 161)	36 (23.8)	125 (76.2)	
Handgrip strength			
Low (n= 91)	33 (36.2)	52 (63.8)	0.041
Normal (n= 150)	36 (24.0)	114 (76)	

Excess FMI, fat mass index, ≥ 6.0 kg/m² in men, ≥ 9.0 kg/m² in women. Low TLTI, total lean tissue index, ≤ 15.6 kg/m² in men, ≤ 13.5 kg/m² in women. Low gait speed ≤ 0.8 m/s in men, ≤ 0.7 m/s in women. Low handgrip strength, ≤ 18 kg in men, ≤ 10 kg in women.

Table 3 shows the potential associations among our hypothesis, and several independent variables, and RM as the response variable. Results of the simple logistic regression applied show that excess FMI, low TLTI, low gait speed, low HGS, marital status, education, good self-perceptions of health, depression symptoms, cognitive impairment, comorbidity, polypharmacy, dry mouth, and dependency by the BADL and IADL, were all associated with the risk of malnutrition, estimated by the MNA-LF.

Table 3. Association between the hypothesis variables and several independent variables with RM by simple logistic regression analysis in community-dwelling older adults.

Variables	OR	p-value
Excess FMI, kg/m ²	0.35	0.006
Low TLTI, kg/m ²	2.30	0.011
Gender, women	1.06	0.862
Civil status		
Single, divorced, or separated	0.55	0.125
Widowed	0.93	0.841
Living alone	0.67	0.267
Education, < 10 years of schooling	1.98	0.025
Without a pension	1.23	0.500

Without medical services	1.35	0.674
Good self-perception of health	0.15	0.000
Depression symptoms, ≥ 5 CESD-7 scale	1.48	0.168
Cognitive impairment	1.91	0.110
Comorbidity, ≥ 3 diseases	1.87	0.042
Osteoarthritis	0.93	0.842
Polypharmacy, ≥ 5 drugs/day	1.57	0.118
Dry mouth	3.95	0.000
Dependency by the BADL	6.24	0.000
Dependency by the IADL	2.82	0.000
Low gait speed, m/s	1.96	0.020
Low handgrip strength, kg	1.80	0.042
Alcohol consumption, > 2 glasses/day	1.41	0.547
Smoking	0.76	0.610

Excess FMI, fat mass index, ≥ 6.0 kg/m² in men, ≥ 9.0 kg/m² in women. Low TLTI, total lean tissue index, ≤ 15.6 kg/m² in men, ≤ 13.5 kg/m² in women. Low gait speed ≤ 0.8 m/s in men, ≤ 0.7 m/s in women. Low handgrip strength, ≤ 18 kg in men, ≤ 10 kg in women. CESD-7 Center for Epidemiologic Studies Depression Scale. Cognitive impairment by MMSE, mini mental state examination ≤ 23 points were obtained with 5 years of school education, ≤ 19 points with 1-4 years of education, ≤ 16 without education or with < 1 year. Dependency based on the BADL, basic activities of daily living, ≤ 90 points on the Barthel index. Dependency based on the IADL, instrumental activities of daily living, < 8 points on the Lawton & Brody scale. Low gait speed ≤ 0.8 m/s in men, ≤ 0.7 m/s in women. Low handgrip strength, ≤ 18 kg in men, ≤ 10 kg in women.

3.2. Final models of the association between the exposition variables and the risk of malnutrition at 4.1 years of follow-up

Regarding the association between alterations of body composition and RM, the condition was less likely to occur in subjects with excess fat mass index according to the unadjusted model (OR: 0.35 [95% CI: 0.17-0.74]). This result remained significant after adjusting for BADL dependency, low gait speed, and good self-perceptions of health (model 1; OR: 0.24 [95% CI: 0.09-0.64]), and even for IADL dependency, low gait speed, and good self-perceptions of health (model 2; OR: 0.35 [95% CI: 0.16-0.82]) compared to the group without excess fat mass index (Table 4). In contrast to its relation to the FMI, RM was more likely to occur in men and women with a low TLTI, according to the unadjusted model (OR: 2.30 [95% CI: 1.21-4.39]). This finding also remained significant after adjusting for BADL dependency, dry mouth, and self-perceptions of health (model 1; OR: 2.01 [95% CI: 1.06-3.83]), and even for IADL dependency, dry mouth, gait speed, and self-perceptions of health (model 2; OR: 1.96 [95% CI: 1.03-3.72]).

For the association between RM and aspects of impaired physical performance, such as low gait speed, Table 4 shows that the condition was more likely to occur in men and women with low gait speed, according to the unadjusted model (OR: 2.43 [95% CI: 1.36-4.32]) after adjusting for dry mouth, the FMI, and self-perceptions of health (model 1; OR: 2.01 [95% CI: 1.06-3.83]), and even for dry mouth, the TLTI, and self-perceptions of health (model 2; OR: 1.96 [95% CI: 1.03-3.72]). Similar results were found for low handgrip strength, though in this case after adjusting for the associated covariates, both models 1 and 2 lost significance.

Table 4. Final models of the association between alterations of body composition, and impaired physical performance and risk of malnutrition in community-dwelling older people.

	Model 1		Model 2	
	OR Unadjusted (CI 95%)	p-value	OR Adjusted (CI 95%)	p-value
<i>Fat mass index, kg/m²</i>				
Normal	1.00		1.00	
Excess	0.35 (0.17-0.74)	0.006	0.24 (0.09-0.64)	0.005
<i>Total lean tissue index, kg/m²</i>				
Normal	1.00		1.00	
Low	2.30(1.21-4.39)	0.011	2.06(0.98-4.32)	0.054
<i>Gait speed, m/s</i>				
Normal	1.00		1.00	
Low	2.43 (1.36-4.32)	0.003	2.01 (1.06-3.83)	0.032

Normal	1.00		1.00		1.00	
Low	2.43(1.36-4.32)	0.003	2.01(1.06-3.83)	0.032	1.96(1.03-3.72)	0.038
Handgrip strength, kg						
Normal	1.00		1.00		1.00	
Low	1.80 (1.02-3.18)	0.042	1.68 (0.90-3.16)	0.061	1.63(0.87-3.05)	0.125

Fat mass index, kg/m²; Model 1 adjusted for BADL, gait speed, and self-perception of health. Model 2 adjusted for IADL, gait speed, and self-perception of health. Total lean tissue index, kg/m²; model 1 adjusted for BADL, dry mouth, and self-perception of health; model 2 adjusted for IADL, dry mouth, gait speed, and self-perception of health. Gait speed, m/s; model 1 adjusted for dry mouth, fat mass index, and self-perception of health; model 2 adjusted for dry mouth, total lean tissue index, and self-perception of health. Handgrip strength, kg; model 1 adjusted for dry mouth, fat mass index, and BADL; model 2 adjusted for dry mouth, total lean tissue index, and self-perception of health. Excess fat mass by FMI ≥6.0 kg/m² in men, ≥9.0 kg/m² in women. Low TLT by TLTI, ≤15.6 kg/m² in men, ≤13.5 kg/m² in women. CESD-7 Center for Epidemiologic Studies Depression Scale. Low handgrip strength, ≤18 kg in men, ≤10 kg in women. Low gait speed ≤0.8 m/s in men, ≤0.7 m/s in women. Low handgrip strength, ≤18 kg in men, ≤10 kg in women.

4. Discussion

At baseline, all subjects ≥60 were free of RM according to their MNA-LF scores. However, a significant proportion of the sample had excess fat mass measured by the FMI classification [30], and overweight and obesity by the BMI categories [28]. Around 29% of the sample –mostly women– developed RM at follow-up. These data on the incidence of RM enhance our understanding of the etiology of this condition in community-dwelling older adults by providing a basis for estimating the risk of developing RM, and other diseases, for that matter. Moreover, the unadjusted and adjusted models that incorporated several covariates showed that RM was less likely to occur in subjects with excess fat mass assessed by the FMI classification, but more likely to occur in subjects with low TLTI and low gait speed at follow-up. None of these results have been reported in groups of community-dwelling older people worldwide.

Based on the clinical consequences reported, the volunteers diagnosed with RM are probably at a greater risk of functional dependency [10,17], sarcopenia [18,19], and mortality [20,21], compared to well-nourished older subjects. From a public health perspective, the diagnosis and early confirmation of low TLTI, low gait speed, and low handgrip strength should be warning signals of the need to take action to treat the people so affected and prevent further progress or evolution of RM and its numerous adverse clinical consequences in community-dwelling older adults, especially those in low- and middle-income countries. It seems mandatory to assess body composition and impairment physical performance, and other factors to early detect risk of malnutrition in the older adult populations (Tables 1 and 4).

4.1. Previous studies related to the causality of risk of malnutrition

According to our search, only one cohort study on the association between several risk factors and RM was found. That published work reported that high age, low self-perceptions of health, and depressive symptoms were factors strongly associated with RM at follow-up [16]. The results of several published cross-sectional studies on the association between risk factors and RM in older populations [3,4,9–12,15,23,24] led us to hypothesize that excess fat mass, low TLT, low gait speed, and low handgrip strength, separately, could be associated with RM at follow-up in community-dwelling older people. Results of the present study also found that RM is less likely to occur in subjects with excess fat mass (measured by DXA and FMI classification) as categorical variable, assessed. These results are in line with those published in a cross-sectional study on the association between fat mass by DXA and RM [24] and triceps skinfold measurements, on the one hand, and RM at baseline, on the other, in a cohort of older adults [16]. It seems that RM is less likely to occur, as well, in subjects with excess fat mass, but also, increments in fat mass index and highest values of fat mass increase the risk of impaired physical performance [44] and physical dependency [45], respectively, in older adults. Finally, we cannot neglect the fact that high fat mass is related to several

cardiovascular disease outcomes [46], and mortality [47]. At this time, more cohort studies are necessary to support or reject the results reported herein.

Regarding the association between the TLTI and RM, results also confirm the findings, published previously, on community-dwelling older New Zealanders [24], though our findings are related to low TLTI as the categorical variable, while the researchers in the New Zealand study [24] reported that RM was less likely to occur in subjects with high fat-free mass index values as the continuous variable. In contrast, we found that RM was more likely to occur in subjects with low TLTI than in the normal group. In fact, our older subjects who developed RM had 2 fewer kilograms of TLT than the normal group at baseline, and 31.8% of the subjects who developed RM had low TLTI, compared to just 16.8% in the normal group. Overall, these results are clearly important.

Unfortunately, the approach used in our work is impractical for daily clinical practice in the study region due to the limited availability of methods and equipment for assessing body composition. However, many published BIA equations are available to accurately estimate fat-free mass in older adults, and DXA could be another method for assessing total lean tissue in older populations, so it may be possible to overcome this limitation. The early measurement of fat-free mass using ethnic- and gender-specific BIA or anthropometric equations [48–50], or TLT by DXA, will certainly help researchers and clinicians identify individuals with low TLTI, before this is reflected in MNA-LF scores.

Gait speed as a categorical variable has also been associated with RM, showing that this was less likely to occur in community-dwelling older adult subjects with healthy values ($\geq 0.8\text{m/s}$) [24]. The present study also confirmed this association, but using low gait speed as the hypothesis variable. Results showed that RM was more likely to occur in subjects with low gait speed even after adjusting for their corresponding variables (see models 1 and 2, and Table 4). Note that few studies have explored this association. It is well known that the gait speed test is normally used to diagnose severe sarcopenia [33], assess physical functioning [51,52], and identify people at high risk of adverse health outcomes [53] and/or disability [54]. But gait speed is also a simple, reproducible measure of physical performance, so future studies on the risk factors for RM should explore this as an exposition variable to confirm or reject the findings of our work.

Finally, only one cohort study to date has analyzed the association between several risk factors and RM at baseline. Those researchers found that RM was less likely to occur in subjects with high handgrip strength as a continuous variable in community-dwelling older people in Sweden. However, in the model built after follow-up, handgrip strength did not appear among the associated risk factors for the incidence of RM [16]. The researchers in a cross-sectional study did not find any association between these two variables in older New Zealander subjects [24]. In the present study; however, there was an association between low handgrip strength as a hypothesis variable and the incidence of RM according to the unadjusted OR, though it lost significance after adjusting for several covariates. Results are shown in models 1 and 2 (Table 4). Upon examining the p-values found (model 1), it is likely that the small sample size influenced the significance of this association. As mentioned above, 37.7% ($n=15$) of men and women had low handgrip strength. Future studies should recruit a more adequate sample size to determine if this association reaches statistical significance. Clearly, additional work is required to clarify this possible association.

4.2. Potential biological mechanisms

The association found may be supported by the following mechanisms. Triacylglycerols in adipose tissue or fat mass represent a major energy reserve in the body. Under conditions of high energy demand, such as low energy intake or caloric deficit, stored triacylglycerols are hydrolyzed by lipase and released into the bloodstream as free fatty acids to satisfy constant energy needs. In fact, it has been reported that MNA scores of 17–23.5 (risk of malnutrition) detected elderly patients with poor nutritional intake and, at the same time, normal albumin levels or no loss of body weight [7]. In addition, eating only one or two meals a day [9], or consuming fewer than three snacks daily [55], increased the risk of malnutrition compared to subjects who ate 3 or more meals a day, or more than three snacks daily. Thus, it is to be expected that RM is less likely to occur in subjects with excess

fat mass, subjects with RM probably satisfy their energy needs, as the results of our study confirm. On the other hand, total lean tissue, or fat-free mass, is associated with energy intake, an association mediated by the resting metabolic rate. Fat-free mass and the resting metabolic rate, together, explain 62% of the variance in energy intake [56]. Our results suggest that low TLT could be the main determinant of the resting metabolic rate, hence, low energy requirements, and this condition could foster a low energy intake, which could contribute to the development of RM.

Finally, the relation between nutritional status and physical performance in older adult populations is well known. Well-nourished subjects walk significantly faster than those with RM or malnourishment [57]. Impaired physical performance –low gait speed, for example– can have an early effect on some of the activities involved in buying and preparing food, thus impacting dietary caloric intake. Gait speed correlates positively with all body mass measures, TLT, percentage of body fat, and the BMI [24]. Therefore, a synergistic effect among low TLT, low fat mass, and low gait speed can be sustained. In addition, the older people in this study who developed RM at 4.1 years follow-up had less fat mass and lean tissue at baseline, compared to the men and women in the group without RM.

4.3. Strengths and limitations

One of the strengths of this study is its cohort design, which allowed us to explore temporal associations and causality. Significantly, unlike the earlier cross-sectional studies and the one cohort study found, we used excess fat mass, low TLT, low handgrip strength, and low gait speed as the exposition variables, then adjusted the models for several covariates reported in the literature and based on their tested association with RM using simple regression analysis. In addition, the exposition variables were assessed using such reference methods as DXA for body composition and the GAIT Rite instrumented mat for gait speed. Two other strengths of this study are that robust statistical analyses were carried out to determine associations, and the follow-up period was sufficiently long to allow us to determine associations between several exposition variables and RM.

The limitations of cohort studies associated with losses at follow-up are well-known. However, the percentage of loss in this study was relatively low (just 12%) and was taken into account in our calculations of the sample size. This limitation could also be overcome because there were no significant differences in several of the demographic, body composition, nutritional status, and clinical characteristics between the subjects who were lost and those who remained for the follow-up study (see Annex I).

Another limitation is a possible selection bias, since our sample was made up of volunteers who came to the centers where the evaluations were carried out on their own, so older adults with greater impairment of their physical or functional performance may have been excluded, possibly resulting in an underestimate of the incidence of RM and the number of people with excess fat mass, low TLT, and low gait speed and handgrip strength. Finally, this study tested the association between alterations of body composition and impaired physical performance by adjusting for several covariates, but did not consider such biochemical and nutritional parameters as hematocrit, glucose, albumin, erythroid sedimentation rate, and triglycerides, all of which have been associated with RM in clinical settings.

5. Conclusions

The accumulated incidence of the risk of malnutrition was relatively high, around 29% in the sample of community-dwelling older adults. RM was less likely to occur in older subjects with excess fat mass by the FMI classification, but more likely to occur in older men and women subjects with a low total lean tissue index and low gait speed at 4.1 years of follow-up in community-dwelling older people, even after adjusting for several covariates. Also, our findings highlight that excess fat mass, low total lean tissue, and low gait speed are first than risk of malnutrition and not vice versa. Future prospective cohort studies are required to confirm or reject our hypotheses for older adult populations. Due to the high incidence of RM in community-dwelling older people, and its possible short-term clinical consequences, early assessment of body composition and physical performance to

detect excess fat mass, low total lean tissue indices, and low gait speed, respectively, is recommended to detect RM in older adult populations before the onset of this condition.

Author Contributions: ML-T contributed to study design, data collection, statistical data analysis, and manuscript writing. OR-C was responsible for the study design (FraDySMex) and data collection, and critically reviewed the paper. HJV-E, she participated in the statistical analyses and interpretation, and reviewed the paper. JE-R contributed to the statistical analyses and interpretation, and critically reviewed the models generated and assessed by multiple logistic regression analysis, as well as the entire document. AL-L contributed to the study design of and data collection, and the final review of the manuscript. HA-M is the corresponding author, derived the new hypotheses tested in this secondary analysis, participated in interpreting the statistical analyses and manuscript writing and critically reviewed the entire document. All authors read and approved the final manuscript.

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Conflicts of Interest: All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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