




The association between meat consumption and muscle strength index in young adults: the mediating role of total protein intake and lean mass percentage

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Abstract

Purpose The aim of this study was to analyse the associations between the consumption of different types of meat and the muscle strength index (MSI) and to examine whether this relationship is mediated by total protein intake (TPI) and lean mass percentage (LM%) in young adults.

Methods We conducted a cross-sectional study with first-year university students from Castilla-La Mancha, Spain. Different types of meat consumption (total, red, processed, and white and fish) were separately evaluated using a Food-Frequency Questionnaire. MSI was determined from the handgrip and standing long jump tests. ANCOVA models were used to test the mean differences in MSI by categories of meat consumption. Serial multiple mediation models were used to explore the mediating role of TPI and LM% in the relationship between meat consumption and MSI. All analyses were adjusted for age, sex, and socioeconomic level, identified through a directed acyclic graph. Additional analyses were performed with a small subsample including alcohol intake, tobacco smoking, physical activity, cardiorespiratory fitness, and total energy intake as covariates in the multiple mediation models.

Results A total of 230 students (mean age 21.1 ± 2.1 years, 66.5% women) were included in the analysis. Young adults with higher meat consumption (total, red, and white and fish) had higher MSI adjusted means than their peers with lower meat consumption ($p < 0.05$). These associations did not remain after controlling for TPI and LM%. In adjusted mediation analyses, a significant indirect effect was observed through TPI and LM% in the associations between each of the types of meat consumption and MSI. In the additional analyses, a greater effect of white and fish meat consumption on muscle strength through mediation of TPI and LM% was reported compared to red or processed meat consumption, and no significant effects were observed between processed meat consumption and MSI.

Conclusion Higher consumption of total, red, and white and fish meat was associated with increased MSI in young adults. TPI and LM% mediated this relationship.

Keywords Lean body mass · Meat consumption · Muscle strength · Protein · University students

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Introduction

The consumption of meat has been increasing exponentially worldwide since the 1960s, and projections support a continuous increase in this trend in the coming years [1]. Currently, 92.1% of the adult Spanish population consumes meat daily [2]. Meat represents an important dietary source of protein, in addition to providing a substantial content of saturated and unsaturated fats, minerals, and vitamins [3]. The density of each of these nutrients varies considerably according to the type of meat (i.e. red, processed, white and fish meat) [4]. Differences in protein content occur, especially in terms of density, composition, digestibility, and amino acids provided [5]. Therefore, it is reasonable to consider that the effects of meat consumption on health parameters, such as muscle strength or body composition, vary according to the type of meat consumed [6].

Muscle strength is an important indicator of health status in the general adult population [7]. The intake of high-quality protein, such as that from meat, is essential for lean mass development and maintenance and muscle strength [8]. However, in addition to increased total protein intake, increasing meat consumption may increase total energy intake and, consequently, contribute to body fat storage with adverse effects on body composition and energy regulation [9]. In this context, meat consumption leads to greater muscle strength if the protein and energy provided promote positive changes in lean mass [10, 11]. Therefore, protein intake, lean mass, and muscle strength are closely linked to protein metabolism, energy expenditure, and consequently weight control [12].

Some studies reported that higher total and animal protein intake was positively associated with greater muscle strength in adulthood [10, 13]. However, less consideration has been given to the differential contributions of protein, particularly food sources, to muscle strength [14]. Regarding meat consumption, a meta-analysis of randomized controlled trials (RCTs) showed that beef consumption combined with exercise training represented an effective strategy for increasing lower-limb maximal muscle strength in young adults [15]. Nevertheless, because of the small number of studies and their small sample sizes, the available evidence did not allow for consistent conclusions to be drawn [15].

Considering that eating habits are modifiable aspects of lifestyle and that muscle structure and function in younger adults determine musculoskeletal health and quality of life in adulthood [16], further knowledge of the associations between consumption of different types of meat and muscle strength in young adults is relevant from a public health perspective. In fact, both muscle mass and strength decline progressively after young adulthood [17], so it is

imperative to prevent these age-associated losses to maintain whole-body metabolic homeostasis and locomotory capabilities throughout the life span [18]. Additionally, there is also a need to advance the understanding of the role of total protein intake and percentage of lean mass in the relationship between meat consumption and muscle strength. Therefore, this study aimed to (i) analyse the associations between the consumption of different types of meat and muscle strength in young adults and (ii) examine whether these associations are mediated by total protein intake and lean mass percentage.

Methods

Experimental design, sample size, and participants

This cross-sectional study is based on data obtained from first-year university students in Castilla-La Mancha, Spain, during the 2017–2018 academic year. The data analysed in this study are part of the database of the investigation “Lifestyle, adiposity and vascular function in university students in Castilla-La Mancha, Spain”, which aimed to estimate the prevalence of obesity in the target population. The sample size was calculated with Epidat software, estimating an obesity prevalence of 23%, an alpha error of 0.05, a statistical power of 80%, and a precision of 5% [19]. Considering a nonresponse rate of 20%, the total sample size was calculated at 300 students. Taking as a sampling frame the list of students enrolled in these university courses, 560 random students aged 18–30 years were invited to participate in the study, of whom 360 (64.3%) agreed and met the following inclusion criteria: not having a learning disability and not having any type of physical or mental disorder. After the exclusion of 130 individuals with missing values for the variables of interest, 230 university students aged 19–29 years were finally included in this analysis. There was no difference in age, sex, or parental socioeconomic status between the invited and finally included students. The study protocol was approved by the Clinical Research Ethics Committee of the “Virgen de la Luz” of Cuenca (REG: 2016jPI1116) and adhered to the principles of the Declaration of Helsinki. Informed consent to participate was obtained from all subjects involved in the study. “A Guideline for Reporting Mediation Analyses” [20] was used for reporting this study (Supplementary material: Table S1).

Study variables

Outcome variable: muscle strength index (MSI)

A muscle strength index (MSI) was calculated as the sum of the standardized z-score of handgrip/body mass and z-score of the standing long jump. Muscle strength variables were assessed after a 4-min warm-up of calisthenic exercises and static stretching. In the handgrip test, a dynamometer

(TKK 5401 Grip-D, Takeya, Tokyo, Japan) was used to measure the maximum isometric handgrip muscle strength. With the elbow in extension, participants had to squeeze the dynamometer gradually and continuously as hard as possible for at least 2 s. The test was performed twice with each hand, and the mean average of the four measurements was reported in kilograms. Additionally, the standing long jump was used to measure the explosive lower body muscular strength. Participants stood behind the jump line, feet shoulder width apart. From this position, they jumped as far as possible, and the test was accepted if they were able to land with both legs in a stable position. The distance jumped was measured in centimetres from the jump line to the back of the heel closest to this line. The best distance of three attempts was recorded and used in the analyses.

Outcome variable–exposure variable: meat consumption

A 137-item Food-Frequency Questionnaire (FFQ) was used to estimate the meat consumption of each meat type [21]. This FFQ contains nine levels of consumption frequencies for each item (never or almost never, 1–3 times per month, once per week, 2–4 times per week, 5–6 times per week, once per day, 2–3 times per day, 4–6 times per day, and more than 6 times per day). In the FFQ questionnaire, all meat servings were specified in grams. Meat consumption was estimated by multiplying the frequency of consumption for each item and the typical portion size specified in the FFQ according to the Spanish food composition tables [22]. For this study, meat consumption was determined in grams per kilogram of body mass (BM) per day (g/kg BM/d). The study sample was classified into three categories of total meat consumption (low, intermediate, and high) based on tertile splits.

Four major groups of meat consumption were created based on the type of meat consumed. Red meat consumption (RMC) was defined as the consumption of beef, veal, lamb, pork, liver (veal, pork), other entrails (brains, heart, sweetbreads), and bacon. Processed meat consumption (PMC) included the consumption of salami, blood sausage, sausage, *sobrassada*, *serrano* and york ham, mortadella, and hamburger. White and fish meat consumption (WFMC) was defined for the consumption of poultry (chicken and turkey), hare, rabbit, white fish (sole, sea bream, hake, whiting, grouper, cod), blue fish (sardine, tuna, bonito, salmon, mackerel), oysters, clams, mussels, squid, octopus, squid, cuttlefish, crustaceans (prawn, shrimp, crayfish), canned fish, and seafood (sardines, anchovies, tuna, bonito). Finally, total meat consumption (TMC) was defined as the sum of RMC, PMC, and WFMC.

Potential mediators: total protein intake (TPI) and lean mass percentage (LM%)

Total protein intake (TPI) was assessed with the 137-item FFQ [21] and determined in g/kg BM/d.

Lean mass (LM) (g) and body fat mass (g) were measured using dual-energy X-ray absorptiometry (DXA) (Lunar iDXA, GE Medical Systems Lunar, Madison, WI 53718, USA). The variable lean mass percentage (LM%) was calculated as follows: $[(LM/\text{total body mass}) * 100]$. All DXA scans were examined using Physician's Viewer, APEX System Software Version 3.1.2. (Bedford, USA). DXA equipment precision was examined daily before each checking session using the GE Lunar calibration phantom, as suggested by the manufacturer. All measurements were performed at high resolution with students in a supine decubital position. Seven trained health researchers performed data collection following standardized procedures to reduce interobserver variability.

Covariates

Information on potential confounders of the study associations was also assessed. Self-reported information was obtained for age (years), sex (female, male), socioeconomic level based on the level of education of the participants' parents (high, intermediate, low), alcohol intake (alcoholic beverages drinker, nondrinker), and tobacco smoking (current smoker, nonsmoker). Moreover, BMI was calculated as mass divided by the square of the height (kg/m^2), both objectively measured under standardized conditions. Total energy intake was estimated based on the answers to the 137-item FFQ [21] and determined in total calories/day [22]. Likewise, data on carbohydrate intake and lipid intake were also obtained. Cardiorespiratory fitness was assessed with the Course Navette test, and the maximal oxygen consumption ($\text{VO}_2 \text{ max}$) was estimated using the Leger 20-m shuttle-run formula $(31.025 + (3.238 \times \text{velocity}) - (3.248 \times \text{age}) + (0.1536 \times \text{age} \times \text{velocity}))$ [23]. Finally, information on objectively measured physical activity was obtained for a small subsample ($n = 118$) and used specifically in the additional analyses. Participants wore GENEActive accelerometers (ActivInsights) on their wrists for seven consecutive days. The devices were set at a fixed frequency of 30.0 Hz for collecting raw acceleration data measured in milli-g ($1000 \text{ mg} = 1 \text{ g} = 9.81 \text{ m/s}^2$) for each movement axis (x , y , and z) to estimate the young adult's physical activity. We considered valid measurements those available for at least five consecutive days, including one weekend day. For this study, the mean minutes per day of moderate and vigorous physical activity were estimated according to previous studies [24].

Body mass (BM) is associated with meat consumption, muscle strength, and the two mediators analysed regardless of sex and age [12, 25]. For this reason, instead of considering BM as another possible adjustment covariate, we considered that incorporating BM in the definition of the exposure, outcome, and mediator variables would be more appropriate. Therefore, meat consumption and TPI were calculated in terms of daily intake in grams per kg of BM. LM%, being a proportion of BM, did not require any specific procedure in this regard. Likewise, BM was considered for the calculation of MSI (the sum of the standardized *z*-score of handgrip/BM and *z*-score of the standing long jump). In summary, BM was not included as a possible adjustment variable because its potential biasing effect was intrinsically controlled in the definition of the main study variables.

To identify the minimum sufficient adjustment set (MSAS) for the total effect of meat consumption on muscle strength, we built a theoretical causal diagram based on previous knowledge available in the scientific literature. We used the online tool DAGitty [26] to construct a directed acyclic graph (DAG) [27]. The covariates sex, age, and socioeconomic level were identified as the MSAS.

Statistical analysis

Initially, statistical (Kolmogorov–Smirnov) and graphical (normal probability plots) methods were used to evaluate the normal distribution of continuous variables. One-way analysis of variance and the Kruskal–Wallis test were used to analyse the associations between characteristics of the study sample by the TMC categories (low, intermediate, and high). Moreover, the associations between categorical variables and TMC categories were tested through the Chi-square test (Table 1).

Bivariate correlation coefficients were calculated to examine the relationship between MSI, TPI, LM%, and each group of meat consumption (Table 2).

To test the mean differences in MSI by categories of all groups of meat consumption, analysis of covariance models controlled for age, sex, and socioeconomic level (model 1), and additionally for TPI and LM% (model 2) were used. Post hoc pairwise multiple comparisons using the Bonferroni test to identify significant differences between means of MSI by the categories of meat consumption were used (Fig. 1).

Mediation analyses were conducted to examine whether TPI and LM% mediate the association between meat consumption and MSI using PROCESS SPSS Macro, version 3.5 [28]. This macro used bootstrapping methods for testing mediation hypotheses [28]. For these analyses, we selected a serial multiple mediation model using 5000 bootstrap samples to calculate confidence intervals (CI 95%) with TPI as the first mediator, controlling for age, sex, and socioeconomic level (i.e. the MSAS covariates identified through

the DAG). We separately analysed TMC, RMC, PMC, and WFMC as the main independent variables. The mediation model used (Model 6 with 2 mediators) [28] explores the total (*c*) and direct effects (a_1 , a_2 , b_1 , b_2 , d , and c') that indicate the unstandardized regression coefficient and significance between meat consumption and MSI. Additionally, this model examines three indirect effects (IE₁, IE₂, and IE₃) that indicate the change in MSI for each unit change in meat consumption that is mediated by TPI and LM%. The IEs were considered significant when the 95% CI did not contain zero. Pairwise contrasts calculate all possible comparisons between specific IEs. Following the Hayes recommendation [28], the complete and partial mediation concepts were not used in this study. As shown in Supplementary material: Fig. S1, when controlling the analyses for the MSAS covariates, the bias pathways were completely closed, and only the causal pathways (both direct and indirect, i.e. through mediators) remained open. Therefore, age, sex and socioeconomic level were added as covariates in these analyses. Additional mediation analyses were performed with the adjustment for other covariates identified through the DAG (Fig. S1) as relevant confounding factors in the diet-muscle strength relationship available for a subsample ($n = 118$) in our dataset, such as alcohol intake, tobacco smoking, physical activity, cardiorespiratory fitness, and total energy intake. The mediation analyses in this study are exploratory.

All statistical analyses were performed using IBM SPSS Statistics software (Version 24.0; IBM Corp., Armonk, NY, USA), and $p < 0.05$ was considered to indicate significance.

Results

Of the 360 students who agreed to participate in the study, a total of 230 young adults (mean age 21.1 ± 2.1 , 66.5% female) were finally included in this analysis. Table 1 presents the descriptive characteristics of the study participants. Compared with the low category of TMC, those with high TMC presented lower BMI values, higher intake of all macronutrients, and higher values of total energy intake and MSI.

Bivariate correlations among meat consumption, MSI, TPI and LM% are presented in Table 2. TMC, RMC, and WFMC were significantly associated with MSI, TPI, and LM%.

Figure 1 depicts the mean differences in MSI by categories of meat consumption (TMC, RMC, PMC, and WFMC). When adjusted for age, sex, and socioeconomic level (model 1), participants with high TMC, RMC, and WFMC showed significantly higher values in MSI than those with low categories of meat consumption. When TPI and LM% were added to model 1 as covariates (model 2), the aforementioned associations lost statistical significance. No association between PMC and LM% was observed (Fig. 1).

Table 1 Descriptive characteristics of the study sample by total meat consumption

Characteristic	Total ^a (<i>n</i> = 230)	Total meat consumption (g/kg BM/d)			<i>p</i> -value ^b
		Low (<i>n</i> = 77)	Intermediate (<i>n</i> = 77)	High (<i>n</i> = 76)	
Sociodemographic					
Age (years)	21.1 ± 2.1	21.0 ± 1.7	21.2 ± 2.4	21.1 ± 2.2	0.740
Female (%)	66.5	58.4	68.8	72.4	0.165
Socioeconomic level (level of parental education, %)					
University studies (high)	22.5	21.3	24.0	22.1	0.712
Secondary studies (intermediate)	60.4	58.7	64.0	58.4	
Primary studies or no education (low)	17.1	20.0	12.0	19.5	
Lifestyle habits					
Alcohol intake (%)	87.3	81.8	89.6	90.7	0.199
Tobacco smoking (%)	14.7	10.8	18.4	14.9	0.421
Moderate and vigorous physical activity (min/d)	145.51 ± 249.51	225.99 ± 48.62	222.62 ± 82.08	224.32 ± 78.12	0.608
Cardiorespiratory fitness (VO ₂ max estimate, mL/kg/min)	38.65 ± 8.18	38.05 ± 8.74	38.12 ± 7.94	39.61 ± 7.94	0.455
Anthropometric					
Lean mass (%)	70.1 ± 9.4	69.3 ± 9.4	69.1 ± 10.3	72.0 ± 8.1	0.108
Fat mass (%)	29.3 ± 9.0	30.3 ± 9.4	30.0 ± 9.4	27.5 ± 7.9	0.115
BMI (kg/m ²)	23.1 ± 3.5	24.5 ± 4.4	23.1 ± 2.9	21.8 ± 2.5	< 0.001
Dietary intake					
Meat consumption (g/kg BM/d)					
Total meat	4.1 ± 2.4	2.2 ± 0.7	3.7 ± 0.5	6.6 ± 2.5	< 0.001
Red meat	1.0 ± 0.7	0.5 ± 0.3	0.9 ± 0.4	1.6 ± 0.8	< 0.001
Processed meat	0.7 ± 0.6	0.4 ± 0.3	0.7 ± 0.4	1.1 ± 0.7	< 0.001
White and fish meat	2.4 ± 1.7	1.2 ± 0.5	2.1 ± 0.6	3.9 ± 2.1	< 0.001
Macronutrient intake (g/kg BM/d)					
Protein	1.8 ± 0.8	1.2 ± 0.5	1.7 ± 0.4	2.6 ± 0.9	< 0.001
Carbohydrate	4.7 ± 2.5	3.5 ± 2.0	4.5 ± 2.2	6.1 ± 2.7	< 0.001
Lipids	1.9 ± 1.0	1.3 ± 0.7	1.7 ± 0.6	2.7 ± 1.2	< 0.001
Total energy intake (kcal)	2703 ± 1223	2060 ± 956	2514 ± 841	3545 ± 1321	< 0.001
Muscle strength					
Muscle strength index ^c	-0.07 ± 1.8	-0.33 ± 1.9	-0.03 ± 1.7	0.15 ± 1.6	0.042
Handgrip strength (kg)	29.4 ± 8.6	28.1 ± 7.2	29.4 ± 8.4	30.8 ± 10.0	0.528
Standing long jump (cm)	160.0 ± 47.3	154.9 ± 52.5	161.4 ± 48.2	163.5 ± 41.4	0.632

BM body mass

^aFor the following variables, the total number of participants was lower due to missing data: alcohol intake and BMI (*n* = 229), tobacco smoking (*n* = 224), and physical activity (*n* = 118)

^bObtained for continuous variables with one-way analysis of variance (normally distributed: moderate and vigorous physical activity; lean mass; fat mass; muscle strength index; and standing long jump) or Kruskal–Wallis test (nonnormally distributed: age; BMI; cardiorespiratory fitness; total, red, processed, and white and fish meat consumption; protein, carbohydrate, and lipids intake; total energy intake; and handgrip strength) and for categorical variables with the Chi-square test

^cSum of the BM-standardized *z*-score of dynamometry and standing long jump test. *P* values marked with bold indicate statistically significant differences between the tertiles of meat consumption

The results of the multiple serial mediation models are presented in Fig. 2. Total effects were found, indicating that the higher the TMC, RMC, PMC, and WFMC were, the higher the MSI (paths c). Furthermore, similarly for each type of meat studied, TPI and LM% mediated the associations between meat consumption and MSI. Indirect effect 3 (IE₃) was the only statistically significant pathway for

these mediation analyses. The IE₃ means that the higher the consumption of all types of meat was, the higher the TPI, which would be associated with improvements in LM% and, therefore, with gains in MSI (Fig. 2).

Additional mediation analyses in a subsample adjusted for alcohol intake, tobacco smoking, physical activity, cardiorespiratory fitness, and total energy intake showed overall

Table 2 Bivariate correlations among muscle strength, protein intake, lean mass, and each group of meat consumption

Meat consumption (g/kg BM/d)	MSI	TPI (g/kg BM/d)	LM%
Total meat	0.174**	0.872**	0.177*
Red meat	0.171**	0.641**	0.138*
Processed meat	0.112**	0.613**	0.164*
White and fish meat	0.165**	0.735**	0.132*

BM body mass, LM% lean mass percentage, MSI muscle strength index, TPI total protein intake

Values are the correlation coefficients. * $p < 0.05$, ** $p < 0.01$

similar results (Supplementary material: Fig. S2). However, the following changes in the mediation effects should be noted: the IE₃ between PMC and MSI did not remain

statistically significant, and the IE₃ among WFMC and MSI was higher compared to the IE₃ between RMC and MSI.

Discussion

This study analysed the associations of total meat consumption (TMC), red meat consumption (RMC), processed meat consumption (PMC), and white and fish meat consumption (WFMC) with muscle strength index (MSI) in Spanish young adults and explored the mediating roles of total protein intake (TPI) and lean mass percentage (LM%) in these relationships. The main findings were as follows: (i) university students with high meat consumption (TMC, RMC and WFMC) had higher MSI than their peers with lower meat consumption, regardless of age, sex, and socioeconomic level; and (ii) TPI

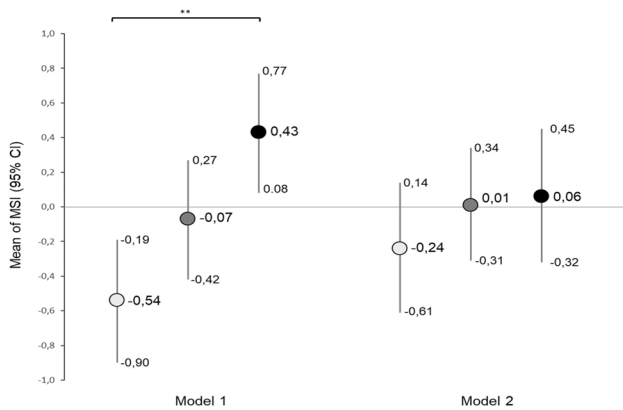
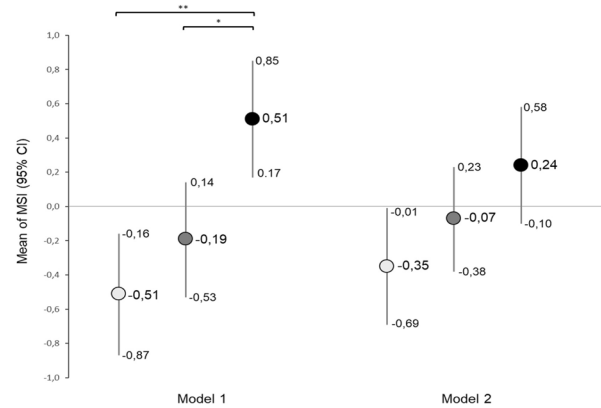
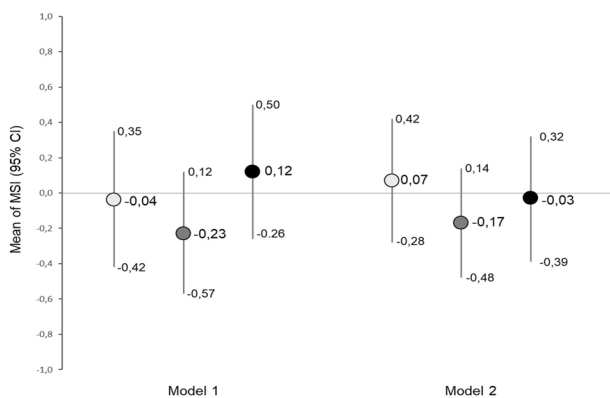
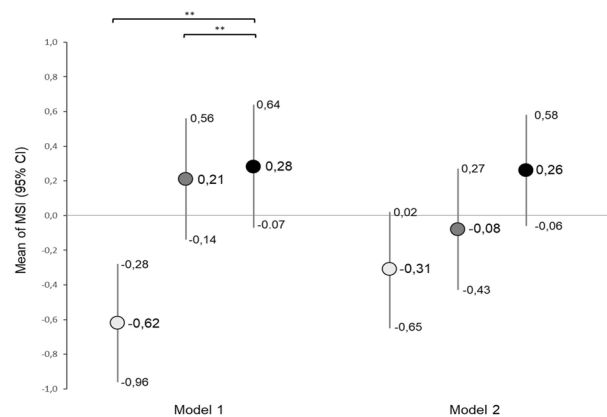
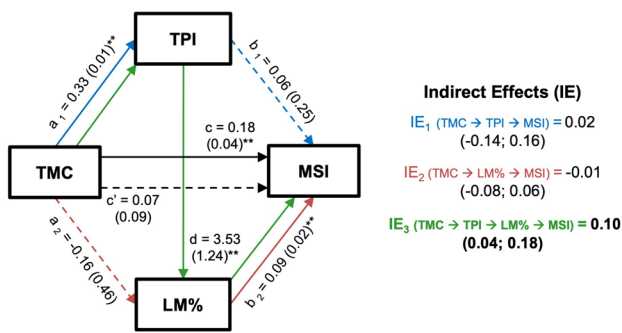
(a) Total Meat Consumption (TMC)**(b) Red Meat Consumption (RMC)****(c) Processed Meat Consumption (PMC)****(d) White and Fish Meat Consumption (WFMC)**

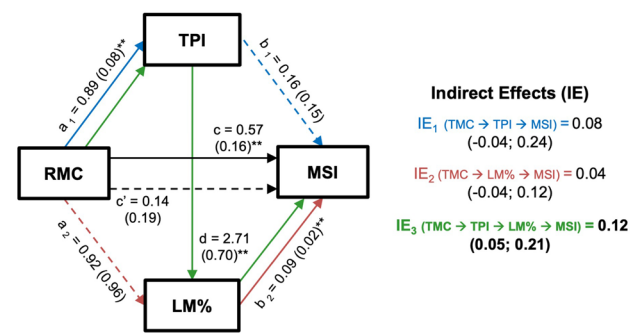
Fig. 1 Mean differences (95% confidence intervals) in muscle strength index by categories of consumption (g/kg BM/d) of different types of meat. Model 1: adjusted for age (y), sex (female, male), and socioeconomic level (level of parental education); Model 2: Model 1 adjusted for total protein intake (g/kg BM/d) and lean mass (%). The Bonferroni post hoc test was used to identify statistical significance in

pairwise mean comparisons of muscle strength index by the categories (low, intermediate, and high) of total (A), red (B), processed (C), and white and fish (D) meat consumption. The colour of the figures indicates low (white), intermediate (grey), and high (black) meat consumption. * $p < 0.05$, ** $p < 0.01$. MSI muscle strength index

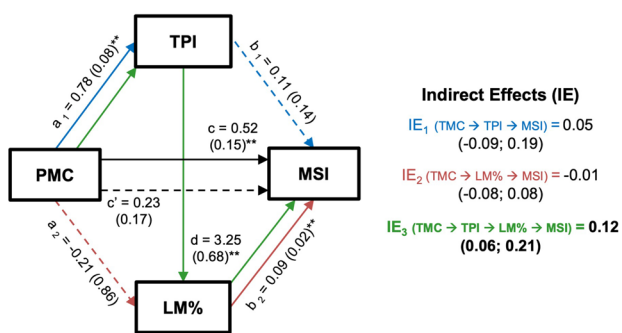
(a) Total Meat Consumption (TMC)



(b) Red Meat Consumption (RMC)



(c) Processed Meat Consumption (PMC)



(d) White and Fish Meat Consumption (WFMC)

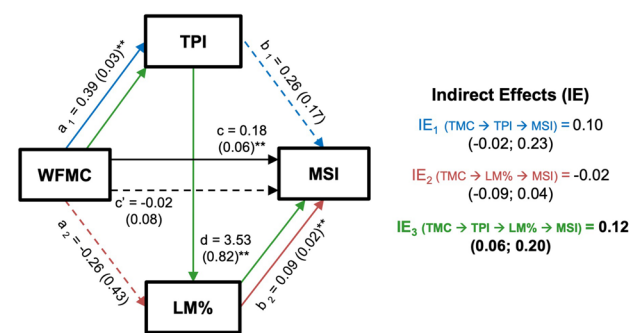


Fig. 2 Mediation analysis of the association between meat consumption (g/kg BM/d) and muscle strength index. Serial multiple mediation models were used with total protein intake (g/kg BM/d) and lean mass (%) as mediators, controlled for age (y), sex (female, male), and socioeconomic level (level of parental education). Values for the a_1 , a_2 , b_1 , b_2 , d , c , and c' paths are expressed as the unstandardized regression coefficient (standard error). IE_1 , IE_2 , and IE_3 are expressed

as unstandardized regression coefficients (95% confidence intervals). Continuous lines (pathways) and bold values (IEs) indicate a statistically significant effect. * $p < 0.05$, ** $p < 0.01$. IE indirect effect, $LM\%$ lean mass percentage, MSI muscle strength index, PMC processed meat consumption, RMC red meat consumption, TMC total meat consumption, TPI total protein intake, $WFMC$ white and fish meat consumption

and $LM\%$ mediated the association between meat consumption (TMC, RMC, PMC and WFMC) and MSI .

The association of high meat consumption with higher MSI is consistent with a prospective study in which the consumption of different types of meat (red meat, chicken, and fish) had a similar positive association with muscle strength in the general adult population [10]. Concerning the consumption of different types of meat in young adults, consumption of lean beef (~ 113 g/d) increases muscle protein synthesis after ingestion in healthy young adults [29]. However, compared with no meat consumption, lean beef consumption (~ 135 g/d) after exercise sessions (3 times per week) for 9 weeks showed no significant difference in maximum leg and chest strength [30]. Consistently, the findings from another RCT indicated that no muscle adaptations were found in maximum leg and chest strength with consumption (~ 46 g/d) of different types of meat (beef and

chicken) during 8 weeks of periodized exercise training in resistance-trained university students [31]. Nevertheless, because these studies did not analyse the mediating effects of TPI and $LM\%$, their role in MSI changes cannot be ruled out. Furthermore, it should be considered that in this study, the increase in MSI according to higher meat consumption was regardless of socioeconomic level (and age and sex), and this association may be due to the mediating effect of TPI and $LM\%$. Therefore, it seems premature to state that meat consumption positively or negatively influences muscle strength without considering TPI and $LM\%$.

Three major differences between our study and other existing studies should be noted. First, studies associating meat consumption with muscle strength typically refer to changes in the maximum muscle strength for the upper or lower body regions separately. Second, other studies have analysed meat consumption, especially beef consumption, along with a

resistance training component. Third, our definition of meat consumption (g/kg BM/d) is different from others more frequently used (i.e. g/d). Therefore, it is possible that the characteristics of the methodology adopted to collect and analyse dietary data were behind some differences observed between studies and difficult the comparison across studies.

The serial multiple mediation model revealed one probable pathway to understand the association between meat consumption and MSI (IE_3). The IE_3 is a specific pathway from increasing meat consumption groups to higher MSI by increasing TPI and subsequently increasing LM%. First, the association of high meat consumption with higher TPI and LM% is consistent with a prospective study that found that protein intake (~4.3% of total energy intake) from meat consumption (red, processed, and white) during puberty was related to greater levels of fat-free mass index in young adults [32]. Our data indicated that the increase in LM% following higher meat consumption could be attributed to the high protein content of meat consumed, although we cannot be certain that other dietary protein sources (e.g. eggs, dairy, and vegetables) influence this association. Additionally, it is necessary to highlight that although PMC was associated with higher MSI through the IE_3 pathway in the main mediation analysis, additional mediation models with further adjustment including physical activity reported no significant effects (total, direct or indirect) between PMC and MSI. Moreover, no significant differences were found in the means of MSI according to the different categories of PMC (ANCOVA results). It should be considered that this is partially because in our sample, the PMC was the lowest proportion among all types of meat, so differences in MSI exclusively related to PMC could be expected to be difficult to detect. Therefore, according to our findings, the association between PMC and MSI remains inconclusive, and future research is certainly warranted in this regard.

Finally, the IE_3 indicated that meat consumption, through the mediation of TPI and LM%, increased MSI. Meat consumption exhibits rapid protein digestion and amino acid absorption, resulting in greater postprandial plasma amino acid availability and peak plasma leucine concentrations [33]. Both muscle strength and lean mass improve with increased essential amino acid availability, especially leucine, which has been established as a key factor in muscle protein synthesis [5]. Lean mass, which is predominantly represented by skeletal muscle, has been positively associated with muscle strength because it influences the development of muscle fibres, noncontractile tissue (i.e. collagen, fat), muscle metabolism, oxidative stress, and neuromuscular junctions [34]. Regarding our results, LM% appears to be a key factor in understanding the improvement of MSI through meat consumption and TPI. However, a direct causal pathway cannot be assumed because TPI also influences other characteristics

(i.e. insulin resistance, serum metabolites) that affect muscle function [35]. Therefore, meat consumption is important for building and maintaining muscle strength in young adults [25], always with a positive net protein balance that is influenced by TPI and physical activity [12]. Specifically, it has been suggested that moderate to vigorous physical activity is the most important intensity for developing muscle strength in young adults [36].

It is important to consider a holistic approach (e.g. environment, lifestyle habits, dietary pattern, protein food, food matrix, and constituent of protein) for the overall recommendation of protein and meat intake [37]. In this sense, in our study sample, those who reported higher TMC showed higher total energy intake and better body composition levels (i.e. BMI, fat mass, lean mass). According to previous studies, high meat consumption has been associated with higher nutrient and overall dietary quality compared to low meat consumption [38], which is consistent with better body composition levels [32, 39]. Furthermore, although the higher TMC group did not report a greater amount of moderate to vigorous physical activity, it is important to consider that the type of activity performed (e.g. high-intensity intervals, aerobic, strength) [40] and light physical activities [41] can lead to considerable differences in energy expenditure and muscle function. Therefore, the interactions between food protein quality, diet quality, type of physical activity and energy expenditure are important factors in analysing the associations of meat consumption with body composition and muscle strength levels.

In this context, the impact of both total protein and meat intake on health outcomes should be considered a U-shaped curve; that is, only in moderate and balanced quantities are they potentially beneficial for health [42]. However, the complexity and diversity of these associations, marked by different elements such as the type of meat consumed or the physiological characteristics/requirements of individuals, make it difficult to establish healthy limits for meat consumption [5, 43]. To meet functional needs and promote skeletal muscle protein synthesis and muscle strength, the intake of good-quality protein between 0.8 and 3.5 g/kg BM/d is recommended for healthy adults [5, 44]. Regarding the recommendations for meat consumption ranges, the evidence is mostly related to red meat [29, 30, 45]. An adequate (i.e. without health risks) RMC has been reported between 100 and 200 g/d in interventional studies [29, 30, 45], although the evidence is still not conclusive and may vary according to individual anthropometric and metabolic characteristics. Previous evidence recommended limiting [46] or reducing RMC to an average of 70 g/d [43] or less than 500 g/week [1]. Considering that a typical serving of meat (i.e. 100–160 g) contains between 15 and 45 g of protein depending on the type-quality-preparation of meat [21, 45], the

abovementioned range of TPI and RMC coincides with the average consumption observed in our study sample.

Although the health effects of high RMC and PMC are still inconclusive, most evidence points out an adverse effect for colorectal cancer [46]. Regarding muscle function, processed meat contains a considerable amount of sodium, nitrite, and saturated and trans fats [47], which may yield increased inflammation and endothelial dysfunction [48] and can consequently reduce physical functioning [49]. It should be considered that, in this study, the increase in MSI according to higher meat consumption was for all types of meat, except for processed meat, according to the results of both ANCOVA and additional mediation analyses. Meanwhile, global evidence of WFMC is more consistent to support beneficial associations with different health outcomes, such as a lower risk of total mortality [50] or a reduced risk of incident cardiovascular diseases [51]. The nutritional composition of white and fish meat has greater cardioprotective properties than red or processed meat because of its lower total fat content and higher polyunsaturated and monounsaturated fat content and because it is a less plentiful source of heme iron [47]. In turn, both proteins and fatty acids from fish meat have shown better digestibility, which improves absorption and, consequently, the bioavailability of their end products (i.e. amino acids and unsaturated fatty acids) [52]. Moreover, dietary effects have been proven to be more related to the food matrix than to specific foods [53]. In this context, the Mediterranean diet is a healthy dietary pattern associated with higher WFMC rather than RMC or PMC. Finally, the production of white and fish meat has a lower environmental impact than that of red or processed meat [54]. Considering that in this study the potential benefits of WFMC for MSI are comparable to those of other types of meat (and with a greater effect in the results of the additional mediation analyses), our findings reinforce the body of evidence supporting the recommendation for increased white and fish meat consumption as a healthier substitute for red and processed meat.

Our study has some limitations that should be acknowledged. First, the cross-sectional design limits us from stating causal associations, and the results must be read with caution. Specifically, longitudinal studies are needed to determine whether our highest meat consumption category has prospective beneficial effects for MSI and, importantly, is safe in terms of cardiovascular and metabolic health parameters. Second, dietary variables were collected through a questionnaire, which might have some degree of measurement error due to recall and information biases. Third, the dietary behaviour, lifestyle and body composition of university students are specific to the early stage of adult life, so the generalization of our results to middle-aged and older adult populations will require additional testing. Fourth, although we analysed an MSI normalized by BM, other anthropometric parameters

related to muscle performance could alternatively be used in this normalization procedure, such as height or hand size. Finally, although serial mediation analyses were performed, they were exploratory and, thus, we cannot exclude other dietetic components or mediators that could have influenced the association between meat consumption and MSI. Likewise, although we adjusted the analyses for some major potential confounders identified through the DAG method (i.e. age, sex, and socioeconomic level), residual confounding cannot be eliminated. This limitation was partially overcome because additional analyses in a smaller subsample also included the adjustment of the covariate's alcohol intake, smoking, physical activity, cardiorespiratory fitness, and total energy intake.

In conclusion, the relationship between regular meat consumption and muscle strength, although still lacking confirmation from prospective observational and experimental studies, reinforces the importance of this food group as an essential item in a healthy diet, especially in young adults. More importantly, with respect to the benefits for muscle function and body composition, our data suggest that the consumption of white and fish meat contributes in the same way as red or processed meat through the mediation of total protein intake and lean mass percentage. In fact, the additional mediation analyses through this same pathway indicate a greater effect of white and fish meat consumption on muscle strength than red or processed meat consumption. Thus, our findings agree with dietary guidelines of healthy diets such as the Mediterranean diet, which recommends the preferential consumption of white and fish meat over the consumption of red or processed meat.

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Availability of data and materials The datasets generated and analysed in this study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate The study protocol was approved by the Clinical Research Ethics Committee of the “Virgen de la Luz” of Cuenca (REG: 2016jPI1116) and adhered to the principles of the Declaration of Helsinki. Informed consent to participate was obtained from all subjects involved in the study.

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