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Running head: Modelling bone-related factors in older adults

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ABSTRACT:

Bone mass and quality declines with age, and can culminate in osteoporosis and increased fracture risk. This investigation modelled associations between bone and physical, dietary, and metabolic factors in a group of 200 pre-frail/frail older adults using factor analysis and structural equation modelling (SEM). Exploratory (EFA) and confirmatory factor analysis (CFA) were conducted to compose factors and to assess their robustness. SEM was used to quantify associations between bone and the other factors. Factors arising from EFA and CFA were: Bone (whole body, lumbar and femur bone mineral density and trabecular bone score; good fit), Body composition–lean (lean mass, body mass, vastus lateralis and femoral cross-sectional area; good fit), Body composition–fat (total fat mass, gynoid, android and visceral fat; acceptable fit), Strength (bench and leg press, handgrip and knee extension peak torque; good fit), Dietary intake (kilocalories, carbohydrate, protein and fat; acceptable fit), and metabolic status (cortisol, IGF1, GH and free testosterone; poor fit). SEM using isolated factors showed that body composition (lean) (β=0.66, $p<0.001$), body composition (fat) (β=0.36, $p<0.001$) and strength (β=0.74, $p<0.001$) positively associated with bone. Dietary intake relative to body mass negatively associated with bone (β=-0.28, p=0.001), whereas in absolute terms it showed no association (β=0.01, p=0.911). In a multivariable model, only strength (β=0.38, p=0.023) and body composition (lean) (β=0.34, p=0.045) associated with bone. Resistance training programs that focus on improving lean mass and strength in older individuals may benefit bone in this population.

Keywords: exploratory factor analysis; confirmatory factor analysis; structural equation modelling; lifestyle; bone-health.

New & Noteworthy: We employed factor analysis and structural equation modelling, which are rarely used in nutrition or exercise science, but constitute powerful tools that may overcome limitations of traditional analyses, combining individual related variables into factors or constructs of interest.

Our investigation represents a starting point on this progressive pathway, providing useful insight and a working model for researchers and practitioners who wish to tackle complex problems such as the multi-factorial causes of bone loss in older adults.

INTRODUCTION

Bone mass and quality declines with age (1) and can eventually culminate in osteoporosis, a disease characterized by decreased bone mass, compromised architecture and increased fracture risk (1, 2). Osteoporosis risk is elevated in frail and pre-frail older adults (3), with the term frailty referring to a multi-dimensional geriatric syndrome that affects multiple physiological systems, increasing vulnerability to everyday stressors and risk of adverse events such as falls, incident disability, hospitalization and mortality (4). The development of screening tools, along with targeted preventive or treatment interventions, are essential to protect bone health of high-risk populations. Development of such tools and interventions is, however, fraught with difficulty, given that a wide range of factors contribute to bone metabolism and thus represent potential targets of interest. These include dietary factors (*e.g.,* energy availability, carbohydrate, protein, calcium and vitamin D intake *etc*); physical function (*e.g.,* aerobic capacity, strength, mobility, balance, functional capacity *etc*); body composition (*e.g.,* lean and fat mass and their distribution) and metabolic status (*e.g.,* as determined by levels of circulating hormones such as cortisol, testosterone, IGF-1 *etc*).

Although each of these factors may well influence bone metabolism, their isolated effects are likely to be small, and their interactions are currently unknown. Further, factors such as dietary intake, physical function, body composition and metabolic status are latent constructs, namely complex constructs that cannot be directly measured, but are instead proxied by a range of variables, which may better reflect the construct when assessed collectively rather than in isolation. For example, bone responds to mechanical loads provided by physical activity (5), but these cannot be directly investigated *in vivo*, and so an individual's physical function is proxied by various individual tests (*e.g.,* strength of individual limbs, functional capacity tests, cardiorespiratory tests *etc*). Each of these isolated tests may contribute to an individual's overall physical function, but in isolation, may provide limited insight. In other words, the sum of all parts may be more informative than any one alone. Factor analysis is a statistical tool that combines isolated variables into a single factor that better reflects the broader construct, and is potentially more powerful than analyses that consider single variables in isolation.

This approach attempts to describe variability across observed measures by assuming the existence of a lesser number of unobservable variables known as factors, latent variables, constructs or dimensions (6). Structural equation modelling (SEM) combines factor analysis and multiple regression, and can be used to analyse structural relationships between factors. These techniques have substantial potential to explore relationships between complex factors and to better address common issues in observational research, but despite this potential, they are infrequently used. Accordingly, the aim of the current study is to use factor analysis and SEM to explore potential associations between bone, and body composition, physical function, dietary intake and metabolic status in a group of frail and pre-frail older adults.

MATERIALS AND METHODS

Experimental Design

This study comprises an observational, cross-sectional evaluation of baseline data collected during the Pro-Elderly study, which was an RCT that investigated the influence of protein and protein derivative supplementation in combination with resistance training on health-related parameters in pre-frail and frail older adults. The protocol and main results of that study are described in detail elsewhere (7). Within the current analysis, outcomes were considered in relation to five factors, namely bone, physical function, dietary intake, body composition and metabolic status, with bone considered to be the dependent variable of interest, and all others as potentially explanatory factors. An overview of all factors and included outcome variables is described in Figure 1 and all outcome variables were selected based on availability within the original dataset. Exploratory factor analysis (EFA) was initially conducted to develop the factors and robustness of the resultant factors was then assessed using confirmatory factor analysis (CFA). Finally, SEM was used to quantify potential associations between each of the explanatory factors (body composition, dietary intake, physical function and metabolic status) and bone.

Participants

Baseline data from men and women who participated in the original study (7) were considered for inclusion. All participants were aged 65 years or more, and classified as frail or pre-frail according to the criteria proposed by Fried et al. (8). Exclusion criteria included: use of exogenous insulin or steroid based drugs; use of protein and/or amine based dietary supplements; consuming a calorie or food-group restricted diet; currently engaging in resistance training and having any uncontrolled chronic condition that precluded exercise training. The study design was approved by the local ethical review board (CAAE: 37499314.0.0000.5391) and all participants provided written informed consent prior to participation.

Factors (bone, body composition, dietary intake, physical function, metabolic status)

All factors were developed based upon variables available within the original dataset. The full protocol for each outcome is described in detail elsewhere (7) and the most pertinent details summarised herein.

The dependent factor (*bone*) included bone mineral density of the whole body, lumbar spine and total femur and femoral neck using dual-energy X-ray absorptiometry (DXA; Hologic QDR 4500, Hologic, Inc., Bedford, MA, USA). Trabecular micro-architecture was assessed through calculation of the trabecular bone score (TBS), using available software in the Hologic DXA scanner (9). All scans were performed by the same experienced technician. *Body composition* comprised a range of measurements including height; body mass; lean mass; total, android, gynoid and visceral fat mass and cross-sectional area (CSA) of the vastus lateralis and rectus femoris muscles. Body fat and lean mass, along with their distribution, were assessed using the total body DXA scan, and CSA was determined using an ultrasound B-mode with a 7.5 MHz linear-array probe (SonoAce R3, Samsung-Medison, Gangwon-do, South Korea), 5 MHz linear-array probe (Philips, VMI, industry and commerce

Ltda, Lagoa Santa, Brazil), according to the protocol described by Lixandrão et al. (10) and using image software (Image J, National Institutes of Health, Bethesda, MD, USA). *Physical function* was assessed using one-repetition maximal testing of the upper- and lower-limbs (bench press and leg press); isometric strength tests (handgrip and knee extension peak torque); and the timed-up-and-go and timed-stands tests. Knee extension peak torque of the dominant leg was determined during ballistic isometric voluntary contractions using an isokinetic dynamometer (Biodex Medical, Inc., Shirley, NY, USA). Handgrip strength was assessed using a hand-held dynamometer (TKK 5101; Takei, Tokyo, Japan). Dynamic balance and the risk of falls was evaluated using the Biodex Balance System, with overall stability indexes, sagittal and transverse axis determined by the equipment's software. The timed-stands test (11) consisted of the number of stands the participant was able to perform from a standard-height armless chair (i.e., 45 cm) within 30 seconds, while the timed up-and-go test (12) was done by registering the time (in seconds) that each participant required to rise from a chair, walk three meters, turn around, and sit back down in the same chair. *Dietary intake* was assessed using self-reported food diaries, undertaken on 3 non-consecutive days (2 weekdays and 1 weekend day). Oral and written instructions were provided by a certified dietitian on how to register food consumption and portion sizes correctly using household measures. Food portions were also confirmed by a dietitian, alongside the patient, using a visual album with real photos of foods. Protein, carbohydrate, fat and overall energy intake, were calculated using dietary software (Avanutri – online version, Rio de Janeiro, Brazil). *Metabolic status* was assessed based on circulating hormone levels within blood and urine samples collected in the morning after an overnight fast and included cortisol, growth hormone (GH), insulin-like growth factor 1 (IGF-1), free testosterone and 25-hydroxy vitamin D (25(OH)D). All hormones were measured by the Central Laboratory Division at the Faculty of Medicine Clinical Hospital (HCFMUSP, São Paulo, Brazil), where the original trial took place. Coefficient of variability for all of these analyses were reported by the Laboratory Division as being between 1.7 and 3.6% (13).

Statistical Analysis

Separate EFA models were conducted on all outcomes within each of the considered factors (see Figure 1) to identify which outcomes contributed to each construct. CFA and SEM models were implemented and assessed using the *lavaan* package (14) in the statistical program R (version 3.3.1 R Core Team, 2016) (15). To fit CFA models, factor means were set to zero and factor variances were estimated using the marker variable method where a single absolute loading for each factor was set to the value 1 as a means of scaling the factor variance (16). To account for potential departures from normality a robust maximum likelihood estimator was used adjusting standard errors of parameter estimates (17). The robust method selected was the Satorra-Bentler correction which corrected for non-normality based on the extent of the average multivariate kurtosis that existed in the data (16). Each of the practices selected conform with standard CFA guidelines (17).

The absolute fit of individual CFA models was assessed using the standardized root mean square residual (SRMR) derived from a residual analysis of the sample correlation matrix and the model implied correlation matrix. Relative fit was assessed using the comparative fit index (CFI) comparing the independence model and the target model tested. The fit indices were selected prior to conducting the analyses and provide quantitative evaluations of model fit on a continuous scale. In practice, however, researchers are generally more interested in qualitative assessments of model fit using terms such as 'poor', 'fair', or 'good' (18, 19), with CFI's of ≥ 0.95; 0.90 – 0.95 and ≤ 0.90, described as good, acceptable and poor fits; whereas SRMR thresholds for good, acceptable and poor were considered to be ≤ 0.05, 0.05 – 0.08 and ≥ 0.8, respectively (19). SEMs were then used to model relationships between factors with at least appropriate fit, with bone mass considered the dependent outcome. Strength of associations between exploratory factors and bone mass were quantified with standardized betas (β) and associated null hypothesis (β = 0) tests. Initially, SEM models with bone mass and a single explanatory factor were conducted, with a final full model

179 including all factors with appropriate fit. Additionally, and due to the potential of sex to confound
180 potential associations, sensitivity analyses were also completed with female participants only (77% of
181 sample).

182

183 **RESULTS**

184 *Participant Characteristics*

185 Data were available for 200 participants, and their characteristics are described in Table 1. Participants
186 were aged 72 ± 6 years, with BMI of 28 ± 5 kg/m^2. Most were female (77%) and characterized as pre-
187 frail (89%). Lumbar spine t-scores for women and men were -1.44 ± 1.31 and 0.19 ± 1.98, while total
188 femur t-scores were -1.14 ± 1.03 and -0.52 ± 0.98, respectively.

189

190 **Table 1:** Descriptive characteristics of study population

	Overall (N = 200)	**Female (N = 154)**	**Male (N = 46)**
Main characteristics			
Age	72.2 ± 6.1	72.2 ± 5.9	72.3 ± 6.7
Height (m)	1.58 ± 0.08	1.55 ± 0.06	1.66 ± 0.06
Weight (kg)	70 ± 15	68 ± 14	77 ± 15
BMI	28.0 ± 5.1	28.1 ± 5.2	27.7 ± 4.6
Frailty level			
Pre-frail	180 (90%)	136 (88%)	44 (96%)
Frail	20 (10%)	18 (12%)	2 (4%)
Bone mass			
Lumbar spine BMD (g/cm^2)	0.936 ± 0.179	0.898 ± 0.147	1.069 ± 0.219
Lumbar spine t-score	-1.16 ± 1.56	-1.44 ± 1.31	-0.19 ± 1.98
Femoral neck BMD (g/cm^2)	0.702 ± 0.119	0.685 ± 0.118	0.761 ± 0.103
Femoral neck t-score	-1.47 ± 0.97	-1.54 ± 1.02	-1.24 ± 0.754
Total femur BMD (left) (g/cm^2)	0.842 ± 0.148	0.810 ± 0.133	0.952 ± 0.149
Total femur t-score	-1.00 ± 1.05	-1.14 ± 1.03	-0.52 ± 0.98
Whole body BMD (g/cm^2)	1.014 ± 0.119	0.986 ± 0.106	1.113 ± 0.107
Whole body t-score	-1.51 ± 1.35	-1.69 ± 1.36	-0.90 ± 1.13
Whole body TBS	1.25 ± 0.10	1.23 ± 0.09	1.30 ± 0.13
TBS z-score	-0.17 ± 1.07	-0.23 ± 1.05	0.03 ± 1.12
Body composition			
Total lean mass (kg)	42.9 ± 8.	39.4 ± 5.8	54.8 ± 7.0
Total fat mass (kg)	25.1 ± 9.3	26.3 ± 9.1	21.2 ± 8.8

	Overall (N = 200)	Female (N = 154)	Male (N = 46)
Fat mass percentage (%)	35 ± 8	38 ± 6	27 ± 6
Visceral fat (g)	722 ± 267	694 ± 249	818 ± 303
Android fat (g)	2,174 ± 899	2,175 ± 890	2,172 ± 939
Android fat percentage (%)	38 ± 8	39 ± 8	34 ± 8
Gynoid fat (g)	4,162 ± 1,551	4,499 ± 1,536	3,015 ± 943
Gynoid fat percentage (%)	38 ± 8	42 ± 5	28 ± 5
Femoris CSA (cm^2)	4.35 ± 1.37	3.88 ± 0.88	5.80 ± 1.61
Vastus lateralis CSA (cm^2)	20.1 ± 4.5	18.8 ± 3.7	24.1 ± 4.5
Physical Function			
Hand grip (kg)	26 ± 8	23 ± 5	36 ± 7
Up and go (s)	7.06 ± 1.31	7.17 ± 1.26	6.68 ± 1.41
Timed Stand (repetitions)	13.27 ± 2.00	13.21 ± 2.08	13.44 ± 1.71
Falls Risk	1.62 ± 0.73	1.58 ± 0.73	1.74 ± 0.73
Instability, low	0.79 ± 0.30	0.76 ± 0.28	0.92 ± 0.33
Instability, moderate	1.45 ± 0.58	1.38 ± 0.50	1.68 ± 0.76
Instability, high	2.78 ± 1.25	2.66 ± 1.20	3.19 ± 1.38
Leg press (kg)	53 ± 28	45 ± 22	80 ± 28
Bench press (kg)	29 ± 12	24 ± 8	43 ± 13
Peak torque (Nm)	99 ± 43	84 ± 27	149 ± 46
Dietary Intake			
Energy intake (Kcal)	1,471 ± 412	1,399 ± 336	1,703 ± 536
Energy intake (Kcal/RMR*)	1.14 ± 0.32	1.13 ± 0.30	1.18 ± 0.37
Carbohydrate (g)	60 ± 19	56 ± 15	71 ± 25
Protein (g)	200 ± 60	190 ± 48	232 ± 80
Protein (g/kg/day)	0.89 ± 0.29	0.87 ± 0.28	0.95 ± 0.30
Fat (g)	47 ± 17	46 ± 16	54 ± 20
Metabolic status			
25-hydroxy vitamin D (ng/mL)	27 ± 9	26 ± 10	28 ± 9
Cortisol (µg/dL)	11.1 ± 3.9	10.9 ± 3.9	11.7 ± 4.1
GH (ng/mL)	0.65 ± 1.04	0.76 ± 1.16	0.28 ± 0.31
IGF-1 (ng/mL)	121 ± 49	117 ± 43	135 ± 65
Free testosterone (pmol/L)	59 ± 102	7 ± 8	227 ± 80
Total testosterone (nmol/L)	176 ± 262	24 ± 19	508 ± 238
SHBG (nmol/L)	76 ± 40	78 ± 40	69 ± 38

Data presented as mean ± standard deviation (SD) for continuous variables or absolute number and proportion (%) for categorical variables. N = total number of participants; BMI = body mass index; BMD = bone mineral density; TBS = trabecular bone score; CSA = cross-sectional area; RMR = resting metabolic rate, estimated using the Harris-Benedict equation; GH = growth hormone; IGF-1 = insulin-like growth factor 1; SHBG = Sex hormone-binding globulin.

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192 *Factor selection and fit*

193 The outcomes representing each factor, along with their overall fit quantified using CFA are presented
194 in Table 2. Based on clear positive and negative EFA loading patterns, changes were made to the
195 factors that were theoretically defined prior to analysis. These were: 1) body composition was split

into two distinct factors (lean and fat), as loading patterns suggested that these represented different constructs; and 3) physical function comprised only strength related outcomes, as loading patterns suggested that other physical function tests, such as timed-up-and-go and timed-stands, did not further contribute to explaining variance within the physical function factor. Based on these updates, the factors carried forward to the confirmatory analysis were: bone; body composition (fat); body composition (lean); strength; dietary intake and metabolic status. Results of the confirmatory analysis indicated that the bone, body composition (lean) and strength measures had a good fit, and that body composition (fat) and dietary intake had acceptable fit. Poor fit was obtained for metabolic status and so this factor was not included within the subsequent SEM analysis.

Table 2: Factor weightings and fit indices for Confirmatory Factor Analysis models

Factor	Components	Factor weighting	Fit indices
Bone	Whole body BMD	0.91	
N = 164	Lumbar BMD	0.86	CFI: 0.972 – Good fit
	Femur BMD	0.78	SRMR: 0.035 – Good fit
	Whole body TBS	0.45	
Body composition (lean)	Lean mass	0.97	
	Body mass	0.82	CFI: 0.957 – Good fit
N = 179	Vastus lateralis CSA	0.75	SRMR: 0.047 – Good fit
	Femoral CSA	0.70	
Body composition (fat)	Fat mass	0.99	
N = 173	Gynoid fat	0.89	CFI: 0.900 – Acceptable fit
	Android fat	0.83	SRMR: 0.079 – Acceptable fit
	Visceral fat	0.55	
Strength	Supine press	0.93	
N = 185	Leg press	0.91	CFI: 0.973 – Good fit
	Peak torque	0.87	SRMR: 0.021 – Good fit
	Hand grip	0.82	
Dietary Intake (absolute values)	Kilocalories	0.97	
	Protein	0.84	CFI: 0.939 – Acceptable fit
N = 187	Fat	0.76	SRMR: 0.055 – Acceptable fit
	Carbohydrate	0.70	
Dietary Intake (relative values)	Kilocalories/kg	0.99	
	Protein/kg	0.86	CFI: 0.940 – Acceptable fit
N = 187	Fat/kg	0.73	SRMR: 0.051 – Acceptable fit
	Carbohydrate/kg	0.71	
Metabolic status	Cortisol	0.80	CFI: 0.638 – Poor fit
N = 184	IGF1	0.20	SRMR: 0.099 – Poor fit

	GH	0.18
	Free Testosterone	0.09

Factor weightings for individual components and fit indices for bone, body composition, strength, dietary intake and metabolic status factors. Fit indices were evaluated as such: CFI ≥ 0.95 = good fit, 0.95 – 0.90 = acceptable fit, and ≤ 0.90 poor fit; SRMR ≤ 0.05 = good fit, 0.05 – 0.08 = acceptable fit; and ≥ 0.8 poor fit. CFI = comparative fit index; SRMR = standardized root mean squared error; BMD = bone mineral density; TBS = trabecular bone score; CSA = cross-sectional area; IGF-1 = insulin-like growth factor 1; GH = growth hormone.

Structural Equation Model

Due to missing data, not all analyses presented with 200 observations. 156 to 161 observations were available for the single explanatory factor models, and 143 for the multi explanatory factor model (Table 3). Initial SEM models of bone and each individual factor indicated significant relationships between bone and strength (β = 0.74; p = <0.001; good fit); body composition – lean (β = 0.66; p = <0.001; acceptable fit); body composition – fat (β = 0.36; p = <0.001; poor fit) and dietary intake with outcomes expressed relative to body mass (β = -0.28; p = 0.001, acceptable fit). The latter finding of a negative relationship between dietary intake and bone was deemed unusual, given that many aspects of dietary intake (*e.g.,* protein intake or total calories) were hypothesised to positively influence bone in this population. A sensitivity analysis was conducted based on dietary intake expressed in absolute terms, and showed no relationship with bone (β = 0.01; p = 0.911; good fit). Final SEM models, including all factors, indicated that only strength (β = 0.74; p = 0.037) and body composition – lean (β = 0.66; p = 0.045) significantly associated with bone. However, overall fit of the SEM model with all factors included indicated poor fit (CFI = 0.767 and SRMR = 0.138). Further details of the full SEM models are provided in Table 3. Sensitivity analyses conducted on females showed no substantial differences compared to results found in the whole sample, with lean mass and strength factors also being associated with bone mass (Supplementary Table 1, available online in https://doi.org/10.17605/OSF.IO/D67HP).

Table 3: Structural Equation Model

Model	B	p values	Fit indices
Single explanatory factor models			
Bone ~ Lean N = 156	0.66	<0.001	CFI: 0.883 – Acceptable fit SRMR: 0.077 – Acceptable fit
Bone ~ Adipose N = 167	0.36	<0.001	CFI: 0.717 – Poor fit SRMR: 0.150 – Poor fit
Bone ~ Strength N = 158	0.74	<0.001	CFI: 0.950 – Good fit SRMR: 0.049 – Good fit
Bone ~ Dietary intake (Absolute) N = 161	0.01	0.911	CFI: 0.952 – Good fit SRMR: 0.050 – Good fit
Bone ~ Dietary intake (Relative) N = 161	-0.28	0.001	CFI: 0.934 – Acceptable fit SRMR: 0.077 – Acceptable fit
SEM with absolute dietary intake			
Multi explanatory factor model			
Bone ~ **Lean +** **Adipose +** **Strength +** **Dietary intake** N = 143	 0.34 0.02 0.37 -0.08	 0.045 0.767 0.037 0.140	CFI: 0.767 – Poor fit SRMR: 0.138 – Poor fit
SEM with relative dietary intake			
Multi explanatory factor model			
Bone ~ Lean + **Adipose +** **Strength +** **Dietary intake** N = 143	0.32 -0.01 0.38 -0.02	0.049 0.924 0.023 0.125	CFI: 0.796 – Poor fit SRMR: 0.126 – Poor fit

β = standardized beta coefficient; N = number of participants included in each model; CFI = comparative fit index; SRMR = standardized root mean squared error; SEM = structural equation modelling.

DISCUSSION

The aim of this investigation was to explore the use of factor analysis and SEM to model relationships between bone and other factors related to physical function, body composition, dietary intake and

metabolic status in a group of frail and pre-frail older adults. Multi-factor structural equation models indicated that only strength and lean mass associated with bone. Accordingly, strength-based tests, or resistance-based interventions may represent the most viable targets when considering risk assessment screening tools and bone-promoting interventions in this population.

The positive association shown between strength and lean mass with bone is unsurprising, given that the muscle and bone are considered to act as a unit (20), and that the predominant factor to influence bone metabolism is mechanical strain (21), which is directly mediated by muscle mass. For this reason, prevention of age-related muscle loss is a key strategy for the prevention of osteoporosis (22). It is important to highlight that both factors were maintained in the final model, indicating that the combination of information related both to muscle mass and function is more informative than either one alone. As such, risk screening strategies for low bone mass in frail and pre-frail older adults may benefit from the inclusion of batteries of strength-based tests, in addition to more commonly used assessments of muscle mass. The available evidence indicates that resistance training does not influence BMD in older adults (23), although it is worth highlighting that only few studies of this kind have been conducted, and those available generally had small samples and relatively short intervention periods, rendering the possibility of Type 2 error plausible. In contrast, resistance-based training programs have proven efficacy in improving muscle mass (24, 25), strength (25–29) and function (29–31) in elderly and frail individuals (for a comprehensive review, see Lopez et al (32)). As such, and given the independent relationship between bone and both muscle mass and strength reported in this investigation, resistance training programs may bring about indirect benefits for bone in this population, although it is recommended that they are implemented as early as possible, in an attempt to prevent or attenuate age-related bone loss, given that recovery of bone once lost in older adults is difficult.

Of equal interest to the factors identified as associating with bone within the current investigation are those that did not. All of the factors included within this model have the theoretical potential to

influence bone, however although single factor models showed a significant association between bone and fat mass and dietary intake, these were not maintained within the final multi-factor SEM. This may be due to typical challenges when modelling related variables, such as collinearity (*e.g.,* those who have more muscle may also have more fat), unaccounted confounding factors, or to noise in the measurement leading to either spurious, or undetected, associations. Differentiating between these potential explanations is beyond the scope of the current investigation, however in the case of dietary intake, the latter explanation does seem most likely. Single factor models showed a negative association with dietary intake when scaled to body mass, however the same factors expressed in absolute terms showed no association with bone. This change from no association (*i.e.,* a horizontal regression line) to a negative association (downward sloping regression line) is likely to represent a scaling artefact, reflecting the lower relative values assigned to heavier individuals when simply dividing dietary intake by mass in comparison to an important clinical outcome, namely bone. As such, our interpretation of these data is that there was no association between dietary intake and bone within this dataset. Whether this truly represents a lack of association, or Type 2 error, is unknown, but it is important to acknowledge that the food diaries used within the current study provide only a snapshot estimation of an individual's usual dietary intake. Considering that bone is slow to respond to stimuli, longitudinal evaluations of an individual's longer-term habitual dietary intake may be warranted to more accurately model these relationships.

The current investigation has both strengths and weaknesses that should be considered when interpreting results. Although factor analysis and SEM are rarely used in nutrition or exercise science, they constitute powerful tools that may overcome certain limitations of more traditional analysis approaches, including the combination of a larger number of individual related variables, into a smaller number of factors or constructs of interest (33). This is important as our interest in nutrition and exercise science is at least implicitly focussed on these constructs of interest and ultimately their interactions. For example, strength may be proxied by an exercise such as a leg press, but interest is not placed in this specific exercise and its associated biomechanical constraints. Instead, researchers

are interested in the more general construct of strength and how that is manifested across many movements and tests. Factor analysis and SEM when built progressively can create valid and reliable measures and factors, with the potential to address more foundational questions regarding interrelationships and ultimately causal pathways. This approach is not without its limitations, however. It is important to highlight that the factors used herein were developed based on the variables available within an existing dataset, but that this dataset was not collected with this specific purpose in mind. Our intention with this investigation, was to explore the potential of this approach to answer a topical and important question within our field, namely what factors associate with bone in a group of frail and pre-frail older adults. Development and validation of factors is a large undertaking, requiring strong theoretical justifications, large, purpose-built datasets and extensive validation. Our analysis provides a first step in employing this approach to model these very complex relationships, but further research is required to build on these findings, and formally develop and validate factors that associate with bone in this, and other, populations.

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Figure captions

Figure 1. Schematic illustrating all variables included within the initial exploratory factor analysis (EFA)