J Nutr Health Aging. 2019;23(10):987-996

HIGH PROTEIN INTAKE IS ASSOCIATED WITH LOWER RISK OF ALL-CAUSE MORTALITY IN COMMUNITY-DWELLING CHINESE OLDER MEN AND WOMEN

R. CHAN[1,2], J. LEUNG[3], J. WOO[1,2,4]

1. Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong; 2. Centre for Nutritional Studies, The Chinese University of Hong Kong, Shatin, Hong Kong; 3. Jockey Club Centre for Osteoporosis Care and Control, The Chinese University of Hong Kong, Shatin, Hong Kong; 4. Jockey Club Institute of Ageing, The Chinese University of Hong Kong, Shatin, Hong Kong. Corresponding author: Dr Ruth Chan, Department of Medicine and Therapeutics, Prince of Wales Hospital, Shatin, N.T., Hong Kong, Tel: 852-3505-2190, Fax: 852-2637-9215, Email: ruthchansm@cuhk.edu.hk

Abstract: *Objectives:* To examine the association of the quantity and the source of protein intake with mortality risk in Chinese older adults. *Design:* Prospective cohort study. *Setting:* Community. *Participants:* Community-dwelling Chinese adults aged >=65 (1,480 men, 1,540 women) in Hong Kong. *Measurements:* Food frequency questionnaire was used to capture baseline dietary intakes including protein, fiber, total grains, vegetables and fruit intakes. Primary outcome measures, identified from the death registry, were death from all causes, cancer and cardiovascular disease (CVD). Other demographic and lifestyle risk factors were also collected. Multivariate Cox proportion hazards regression was used to examine the association of protein intake with mortality risk. *Results:* During a median of 13.8 follow-up years, 963 all-cause deaths, 336 cancer deaths, and 205 CVD deaths were identified. Among men in the highest quintile of total protein intake, all-cause mortality and cancer mortality decreased by 29% [95% confidence interval (CI): 0.55-0.92, p-trend=0.017] and 38% [95% CI: 0.39-0.97, p-trend=0.041] respectively compared with men in the lowest quintile after adjustment for demographics, lifestyle factors and medical conditions. Men in the highest quintile of animal protein intake showed 20% reduced risk of all-cause mortality than men in the lowest quintile (p-trend=0.042). Women in the highest quintile of plant protein intake showed 39% decreased risk of all-cause mortality [95% CI: 0.44-0.85, p-trend=0.019] than those in the lowest quintile. In women, protein intake was not associated with cancer mortality. In both men and women, protein intake was not associated with CVD morality. Further adjustment for other dietary variables attenuated the significant associations. *Conclusions:* Contrary to findings from Caucasian populations of all ages, among Chinese older adults, higher total protein intake was associated with lower all-cause and cancer mortality in Chinese older men. While higher animal protein intake was associated with reduced all-cause mortality in Chinese older men, higher plant protein intake was protective against all-cause mortality in Chinese women. The attenuated associations between protein intake and mortality risk after adjustment for other dietary variables also highlight the role of whole diet approach in mortality risk reduction among older adults.

Key words: Protein, mortality, Chinese.

Introduction

Protein has long been regarded as one of the key nutrients for healthy ageing. Although it is still uncertain what the optimal protein requirement is for older adults, low protein intake has been linked with greater decline of lean mass and higher risk of sarcopenia in older adults (1-3). Other studies also suggest differential effects of animal protein and plant protein on skeletal muscle health (4, 5).

However, the role of the quantity and the source of protein intake in the development of cancer, cardiovascular disease (CVD) as well as mortality has been less investigated, in particular among older population. Observational studies in general show a positive association of the intake of animal protein or animal products with cancer, CVD or all-cause mortality in the general adult population (6-9). Meanwhile, other studies reported metabolic benefits of plant protein and decreased risk of all-cause, cancer and CVD mortality with higher plant protein consumption in general adult population and postmenopausal women (10, 11). These observations echo some recent reviews regarding the promotion of a plant based diet for the prevention and treatment of non-communicable diseases as well as the reduction of morality (12, 13). However, most of the previous studies were conducted among Caucasian general adult population and few included older age group. There is also evidence suggesting that the association of protein intake with risk of mortality was age-dependent. While lower protein intake was associated with lower risk of mortality in the younger age group, higher mortality risk was reported among older adults aged 65 and above with low protein intake regardless of the protein sources (6, 14).

In view of the differences in lifestyle and dietary habits between Caucasian populations and Chinese and the scarcity of evidence on this topic among old age group, we aimed to examine the association of dietary protein intake in terms of both quantity and source with risk of all-cause, cancer and CVD mortality in Chinese community-dwelling older adults aged 65 and over in Hong Kong.

Received June 13, 2019
Accepted for publication June 25, 2019

Published online September 28, 2019, http://dx.doi.org/10.1007/s12603-019-1263-1

Methods

Participants

Subjects were participants of a prospective cohort study examining the risk factors for osteoporosis in Hong Kong (15). Four thousand older adults (2,000 men and 2,000 women) aged 65 years and over living in the community were recruited between 2001 and 2003 by placing recruitment notices in community centres for the older people and housing estates, using a stratified sample so that approximately 33% would be in each of these age groups: 65-69, 70-74, 75+. Participants were volunteers and were able to walk or take public transport to the study site. Compared with the general population of similar age group, participants had higher educational level (9.8% vs. 3.8% with tertiary education), higher proportion of being married (70.7% vs. 59.9%), slightly lower proportion of living alone (10.8% vs. 11.3%) but similar duration of residence in Hong Kong (98.3% vs. 97.1% with duration at or over 15 years) (16). Written informed consent was obtained from all participants. Among 4,000 subjects, 980 subjects were excluded due to either extremely high (5,000 kcal/day), low (500 kcal/day) energy intake or incomplete dietary data (n=6), or prior diagnosis of chronic diseases including cancer, or stroke or heart diseases (n=972), or both (n=2). The final sample included in the analysis was 1,480 men and 1,540 women.

Demographics, general health, and anthropometric measurements

A standardized, structured interview was performed to collect information on age, gender, education level, marital status, smoking habit, alcohol use and medical history. Body weight was measured to the nearest 0.1 kg with participants wearing a light gown, using the Physician Balance Beam Scale (Healthometer, Illinois, USA). Height was measured to the nearest 0.1 cm using the Holtain Harpenden stadiometer (Holtain Ltd, Crosswell, UK). Body mass index (BMI) was calculated as body weight in kg / (height in m)2.

Physical activity assessment

Physical activity level was assessed using the Physical Activity Scale of the Elderly (PASE) (17). This is a 12-item scale measuring the average number of hours per day spent in leisure, household and occupational physical activities over the previous 7 days.

Dietary assessment

Dietary intake was assessed at baseline using a validated food frequency questionnaire (FFQ) developed in a population survey with participants aged between 25 and 74 years, the validity of which has been described elsewhere (18). Mean nutrient quantitation per day was calculated using food composition tables derived from McCance and Widdowson (19) and the Chinese Medical Sciences Institute (20). The FFQ consisted of 280 food items. Each participant was asked to complete the questionnaire – the food item, the size of each portion, the number of times of consumption each day and each week, using the past 12 months prior to the interview as a reference period. Portion size was explained to participants using a catalogue of pictures of individual food portions. For seasonally consumed vegetables and fruits, participants were further asked about the months of food consumption over the past year. The amount of cooking oil was estimated according to the usual cooking methods of preparing standardized portion of different foods and the usual portion of different foods consumed by the participants. In addition to calculations of total protein intake, the amount of animal protein intake and plant protein intake was also determined. Protein intake was expressed as percentage of daily energy consumption for data analysis. Daily intake of total grains, vegetables and fruit was also calculated. Residual method was applied to generate energy-adjusted fiber intake for regression analysis (21).

Mortality ascertainment and follow up period

Mortality data were ascertained from the Hong Kong Government Death Registry. Causes of death were coded according to the 10th revision of the International Classification of Diseases (ICD-10). Data on all-cause mortality, cancer mortality (ICD-10 codes: C00-C97), and CVD mortality (ICD-10 codes: I00-I99) were generated. The follow up period was defined as the time from the baseline examination to the date of death or the date to the latest database update (i.e. 31 March 2017), whichever came first.

Statistical analysis

Statistical analyses were performed separately for men and women using the Statistical Package of the Social Sciences (SPSS) version 24 (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Each of the total, animal and plant protein intake expressed as percentage of total energy intake were stratified into quintiles based on the distribution of each sex. The differences in baseline characteristics across quintiles of each of the total, animal and plant protein intake were examined using chi square test for categorical variables and by one way ANOVA test or non-parametric Krusall-Wallis test for continuous variables, where appropriate.

The Cox proportional hazards model was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for all-cause, cancer or CVD mortality during the follow-up period according to quintiles of each of the total, animal or plant protein intake. Model 1 was age adjusted. Model 2 was further adjusted for baseline BMI (kg/m2), smoking status (never, former, current), alcohol use (never, former, current), PASE, education level (primary or below, secondary or matriculation, tertiary or above), marital status (married, single/divorced/windowed), daily energy intake (kcal), and self-reported history of hypertension (yes, no) and diabetes (yes, no). To address whether the observed associations were independent of other dietary variables, Model 3 was additionally adjusted for daily

Table 1
Baseline characteristics by quintiles of protein intake in men (n=1,480)

Parameters	% energy from total protein					% energy from animal protein					% energy from plant protein				
	Q1 (n=296)		Q5 (n=296)			Q1 (n=296)		Q5 (n=296)			Q1 (n=296)		Q5 (n=296)		
	mean / n	SD / %	mean / n	SD / %	p-value[a]	mean / n	SD / %	mean / n	SD / %	p-value[a]	mean / n	SD / %	mean / n	SD / %	p-value[a]
Age (year)	72.7	4.9	72.1	4.9	0.024	72.7	4.8	72.1	5.0	0.22	72.6	5.4	72.0	4.9	0.21
BMI (kg/m^2)	22.8	3.2	23.5	3.1	0.004	23.1	3.2	23.5	3.1	0.04	23.1	3.2	23.3	3.2	0.71
PASE	92.4	49.3	99.9	57.5	0.17	94.7	48.5	97.0	53.7	0.38	92.4	48.0	100.2	53.2	0.029
Smoking status, n (%)															
Never	97	32.8	106	35.8	0.35	95	32.1	114	38.5	0.25	93	31.4	108	36.5	0.07
Former	156	52.7	154	52.0		153	51.7	143	48.3		153	51.7	138	46.6	
Current	43	14.5	36	12.2		48	16.2	39	13.2		50	16.9	50	16.9	
Drinker, n (%)															
Never	220	74.3	205	69.3	0.10	225	76.0	194b	65.8	0.004	203[b]	68.8	206	69.6	0.31
Former	8	2.7	14	4.7		9	3.1	15	5.1		8	2.7	11	3.7	
Current	68	23.0	77	26.0		62	20.9	86	29.1		84	28.5	79	26.7	
Education level, n (%)															
Primary or below	214	72.3	159	53.7	<0.001	198	66.9	175	59.2	0.06	215	72.6	146	49.3	<0.001
Secondary or matriculation	64	21.6	84	28.4		69	23.3	83	28.0		62	20.9	77	26.0	
Tertiary or above	18	6.1	53	17.9		29	9.8	38	12.8		19	6.4	73	24.7	
Marital status, n (%)															
Married	255	86.1	268	90.5	0.029	260	87.8	268	90.5	0.57	255	86.1	276	93.2	0.002
Single/divorced/windowed	41	13.9	28	9.5		36	12.2	28	9.5		41	13.9	20	6.8	
Self-reported history of hypertension, n (%)	105	35.5	109	36.8	0.52	101	34.1	118	39.9	0.036	101	34.1	91	30.7	0.22
Self-reported history of diabetes, n (%)	36	12.2	52	17.6	0.015	39	13.2	46	15.5	0.20	28	9.5	41	13.9	0.024
Energy intake (kcal/day)	1946.3	514.6	2322.1	638.9	<0.001	1987.2	537.6	2255.1	626.8	<0.001	2071.5	543.0	2237.6	593.6	<0.001
Fiber intake (g/d)[c]	6.8	5.0-9.7	9.9	7.3-13.8	<0.001	8.0	5.5-11.8	8.6	6.5-11.3	0.41	6.6	4.9-8.5	10.8	7.8-14.9	<0.001
Grains intake (g/d)[c]	656.0	512.7-850.9	568.9	447.6-721.0	<0.001	658.7	533.1-873.0	549.0	423.5-692.5	<0.001	515.8	408.1-656.7	597.7	487.7-830.2	<0.001
Vegetable intake (g/d)[c]	153.9	101.4-216.5	263.5	181.1-382.9	<0.001	177.2	114.9-256.7	244.4	171.3-329.0	0.11	161.9	113.9-222.3	248.4	168.6-402.1	0.004
Fruit intake (g/d)[c]	207.6	122.6-316.1	244.7	170.2-356.7	<0.001	211.2	136.0-339.6	238.8	161.2-345.4	<0.001	218.1	129.6-324.8	238.8	170.1-357.2	<0.001

a. p-value for trend for Chi-square test (categorical variables) or one way ANOVA test or p-value difference by non-parametric Krusall-Wallis test (continuous variables) where appropriate; b. One subject did not have data on drinker status; c. median (interquarter range)

Table 2
Baseline characteristics by quintiles of protein intake in women (n=1,540)

Parameters	% energy from total protein					% energy from animal protein					% energy from plant protein				
	Q1 (n=308)		Q5 (n=308)			Q1 (n=308)		Q5 (n=308)			Q1 (n=308)		Q5 (n=308)		
	mean / n	SD / %	mean / n	SD / %	p-value[a]	mean / n	SD / %	mean / n	SD / %	p-value[a]	mean / n	SD / %	mean / n	SD / %	p-value[a]
Age (year)	73.2	5.6	71.4	4.8	<0.001	72.9	5.6	71.7	4.9	0.031	72.3	5.3	72.1	5.3	0.28
BMI (kg/m^2)	23.7	3.3	24.2	3.8	0.06	23.7	3.4	24.2	3.6	0.07	24.1	5.3	24.0	5.3	0.72
PASE	84.7	31.4	86.7	30.6	0.33	85.7	32.0	84.8	32.8	0.77	83.9	31.8	86.6	30.7	0.17
Smoking status, n (%)															
Never	273	88.6	277	89.9	0.78	275	89.3	286	92.9	0.35	274	89.0	276	89.6	0.96
Former	29	9.4	20	6.5		26	8.4	15	4.9		25	8.1	18	5.8	
Current	6	1.9	11	3.6		7	2.3	7	2.3		9	2.9	14	4.5	
Drinker, n (%)															
Never	297	96.4	297	96.4	0.44	302	98.1	297	96.4	0.27	300	97.4	300	97.4	0.51
Former	0	0	0	0		0	0	0	0		0	0	0	0	
Current	11	3.6	11	3.6		6	1.9	11	3.6		8	2.6	8	2.6	
Education level, n (%)															
Primary or below	279	90.6	240	77.9	<0.001	268	87.0	249	80.8	0.019	277	89.9	246	79.9	0.003
Secondary or matriculation	22	7.1	41	13.3		30	9.7	35	11.4		19	6.2	44	14.3	
Tertiary or above	7	2.3	27	8.8		10	3.3	24	7.8		12	3.9	18	5.8	
Marital status, n (%)															
Married	161	52.3	183	59.4	0.07	151	49.0	191	62.0	0.001	186	60.4	156	50.6	0.005
Single/divorced/windowed	147	47.7	125	40.6		157	51.0	117	38.0		122	39.6	152	49.4	
Self-reported history of hypertension, n (%)	121	39.3	136	44.2	0.42	117	38.0	138	44.8	0.24	118	38.3	126	40.9	1.00
Self-reported history of diabetes, n (%)	25	8.1	42	13.6	0.023	31	10.1	43	14.0	0.06	35	11.4	45	14.6	0.42
Energy intake (kcal/day)	1477.3	441.0	1741.5	524.9	<0.001	1528.6	465.5	1697.5	514.5	<0.001	1589.1	497.2	1673.2	471.3	0.012
Fiber intake (g/d)[b]	6.6	4.7-8.8	9.4	6.8-12.6	<0.001	7.6	5.5-10.4	8.4	6.3-10.7	0.07	6.0	4.3-8.4	10.2	7.1-13.4	<0.001
Grains intake (g/d)[b]	558.8	454.7-675.9	484.7	368.3-589.7	<0.001	572.4	467.0-698.1	477.8	373.5-575.3	<0.001	481.9	391.2-572.8	527.5	429.7-654.3	<0.001
Vegetable intake (g/d)[b]	161.8	113.6-216.8	253.8	185.6-365.5	0.002	184.2	124.9-258.8	231.8	179.0-321.1	0.08	160.6	105.6-228.5	249.4	175.0-376.6	0.07
Fruit intake (g/d)b	194.8	125.2-317.0	228.7	158.3-344.6	<0.001	202.7	128.2-316.3	229.4	157.0-335.9	<0.001	204.6	128.8-311.9	223.6	159.4-330.7	<0.001

a. p-value for trend for Chi-square test (categorical variables) or one way ANOVA test or p-value difference by non-parametric Krusall-Wallis test (continuous variables) where appropriate; b. median (interquarter range)

Table 3
Hazard ratio (HR) (95% CI) of all-cause mortality by quintiles of protein intake in men (n=1,480) and women (n=1,540)

Protein intake (% energy)	Q1	Q2		Q3		Q4		Q5		p-trend (linear)
Men (n=1,480)										
% energy from total protein										
Mean (SD) % energy from total protein	11.5 (1.3)	14.2 (0.5)		16.1 (0.6)		18.1 (0.6)		21.9 (2.3)		
N	296	296		296		296		296		---
No. of deaths	146	116		112		109		111		---
Person-years	3,382	3,628		3,665		3,763		3,717		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.67	0.52-0.86	0.64	0.50-0.82	0.67	0.52-0.86	0.67	0.53-0.86	0.004
Model 2*, HR (95%CI)	1 (Ref)	0.72	0.57-0.93	0.68	0.53-0.87	0.73	0.57-0.94	0.71	0.55-0.92	0.017
Model 3**, HR (95%CI)	1 (Ref)	0.75	0.58-0.96	0.71	0.55-0.92	0.79	0.6-1.03	0.77	0.58-1.01	0.11
% energy from animal protein										
Mean (SD) % energy from animal protein	4.6 (1.1)	7.1 (0.6)		8.9 (0.5)		10.9 (0.6)		14.7 (2.5)		
N	296	296		296		296		296		---
No. of deaths	138	120		109		114		113		---
Person-years	3,471	3,574		3,716		3,686		3,708		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.84	0.66-1.07	0.73	0.57-0.94	0.75	0.58-0.96	0.77	0.60-0.99	0.026
Model 2, HR (95%CI)	1 (Ref)	0.89	0.69-1.13	0.74	0.57-0.95	0.78	0.61-1.01	0.80	0.62-1.03	0.042
Model 3, HR (95%CI)	1 (Ref)	0.87	0.68-1.11	0.75	0.58-0.97	0.80	0.62-1.04	0.79	0.60-1.03	0.07
% energy from plant protein										
Mean (SD) % energy from plant protein	4.5 (0.6)	5.7 (0.2)		6.5 (0.2)		7.6 (0.4)		11.0 (2.5)		
N	296	296		296		296		296		---
No. of deaths	128	138		102		111		115		---
Person-years	3,546	3,415		3,807		3,682		3,705		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	1.19	0.93-1.51	0.76	0.59-0.99	0.79	0.61-1.02	0.88	0.68-1.13	0.020
Model 2, HR (95%CI)	1 (Ref)	1.24	0.97-1.58	0.84	0.64-1.09	0.87	0.67-1.13	0.97	0.75-1.26	0.16
Model 3, HR (95%CI)	1 (Ref)	1.26	0.98-1.63	0.89	0.67-1.18	0.91	0.68-1.22	1.08	0.82-1.43	0.64
Women (n=1,540)										
% energy from total protein										
Mean (SD) % energy from total protein	11.5 (1.3)	14.2 (0.6)		16.0 (0.5)		17.9 (0.6)		21.7 (3.0)		
N	308	308		308		308		308		---
No. of deaths	94	74		73		69		59		---
Person-years	3,846	3,920		3,877		3,931		4,046		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.77	0.57-1.05	0.81	0.60-1.10	0.75	0.55-1.03	0.72	0.52-1.00	0.05
Model 2, HR (95%CI)	1 (Ref)	0.74	0.55-1.01	0.78	0.58-1.07	0.75	0.55-1.03	0.70	0.50-0.98	0.05
Model 3, HR (95%CI)	1 (Ref)	0.74	0.54-1.01	0.81	0.59-1.11	0.74	0.53-1.03	0.67	0.46-0.96	0.044
% energy from animal protein										
Mean (SD) % energy from animal protein	4.1 (1.8)	6.5 (0.5)		8.2 (0.5)		10.0 (0.6)		13.8 (3.4)		
N	308	308		308		308		308		---
No. of deaths	80	78		76		70		65		---
Person-years	3,860	3,909		3,900		3,939		4,015		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	1.04	0.76-1.42	0.91	0.66-1.24	0.90	0.65-1.24	0.89	0.64-1.23	0.30
Model 2, HR (95%CI)	1 (Ref)	1.01	0.74-1.38	0.89	0.65-1.22	0.91	0.66-1.26	0.89	0.64-1.24	0.37
Model 3, HR (95%CI)	1 (Ref)	0.99	0.72-1.36	0.87	0.63-1.20	0.88	0.63-1.23	0.84	0.58-1.20	0.24
% energy from plant protein										
Mean (SD) % energy from plant protein	5.2 (0.7)	6.4 (0.2)		7.2 (0.2)		8.3 (0.4)		11.5 (2.6)		
N	308	308		308		308		308		---
No. of deaths	92	71		72		74		60		---
Person-years	3,848	3,988		3,898		3,936		3,949		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.68	0.5-0.93	0.69	0.51-0.94	0.75	0.55-1.02	0.63	0.46-0.88	0.024
Model 2, HR (95%CI)	1 (Ref)	0.65	0.48-0.89	0.73	0.53-0.99	0.73	0.54-1.00	0.61	0.44-0.85	0.019
Model 3, HR (95%CI)	1 (Ref)	0.67	0.49-0.92	0.79	0.57-1.09	0.79	0.56-1.10	0.65	0.45-0.92	0.08

*Model 2: Further adjusted for BMI, smoking, alcohol, education, PASE, marital status, daily energy intake, and self-reported history of hypertension and diabetes; **Model 3: Further adjusted for energy adjusted fiber intake, sex-specific quintiles of daily total grains intake, daily fruit intake, and daily vegetable intake

Table 4
Hazard ratio (HR) (95% CI) of cancer mortality by quintiles of protein intake in men (n=1,102) and women (n=1,291)

Protein intake (% energy)	Q1	Q2		Q3		Q4		Q5		p-trend (linear)
Men (n=1,102)										
% energy from total protein										
Mean (SD) % energy from total protein	11.5 (1.2)	14.2 (0.5)		16.1 (0.6)		18.1 (0.6)		21.9 (2.2)		
N	199	227		226		231		219		---
No. of deaths	49	47		42		44		34		---
Person-years	2,530	3,011		3,078		3,139		2,989		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.73	0.49-1.09	0.64	0.43-0.97	0.70	0.46-1.05	0.58	0.37-0.90	0.022
Model 2*, HR (95%CI)	1 (Ref)	0.76	0.51-1.15	0.67	0.44-1.01	0.71	0.47-1.08	0.62	0.39-0.97	0.041
Model 3**, HR (95%CI)	1 (Ref)	0.82	0.54-1.25	0.72	0.47-1.11	0.80	0.51-1.24	0.66	0.41-1.07	0.12
% energy from animal protein										
Mean (SD) % energy from animal protein	4.7 (1.0)	7.1 (0.6)		8.9 (0.6)		10.9 (0.6)		14.6 (2.4)		
N	204	226		223		228		221		---
No. of deaths	46	50		36		46		38		---
Person-years	2,626	2,964		3,059		3,090		3,008		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.99	0.66-1.48	0.65	0.42-1.01	0.83	0.55-1.24	0.75	0.49-1.15	0.11
Model 2, HR (95%CI)	1 (Ref)	1.09	0.72-1.63	0.67	0.43-1.04	0.81	0.54-1.22	0.76	0.49-1.18	0.08
Model 3, HR (95%CI)	1 (Ref)	1.01	0.67-1.52	0.68	0.43-1.06	0.80	0.52-1.23	0.70	0.44-1.12	0.07
% energy from plant protein										
Mean (SD) % energy from plant protein	4.5 (0.6)	5.7 (0.2)		6.5 (0.2)		7.6 (0.4)		11.1 (2.5)		
N	213	212		231		223		223		---
No. of deaths	45	54		37		38		42		---
Person-years	2,828	2,771		3,161		3,009		2,978		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	1.24	0.83-1.84	0.75	0.49-1.16	0.74	0.48-1.14	0.91	0.59-1.38	0.13
Model 2, HR (95%CI)	1 (Ref)	1.35	0.91-2.01	0.85	0.54-1.31	0.89	0.57-1.39	1.06	0.69-1.63	0.52
Model 3, HR (95%CI)	1 (Ref)	1.44	0.94-2.20	0.94	0.59-1.52	0.95	0.58-1.55	1.27	0.80-2.03	0.88
Women (n=1,291)										
% energy from total protein										
Mean (SD) % energy from total protein	11.6 (1.3)	14.2 (0.6)		16.1 (0.5)		17.9 (0.6)		21.7 (3.0)		
N	240	258		262		263		268		---
No. of deaths	26	24		27		24		19		---
Person-years	3,216	3,446		3,479		3,497		3,642		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.85	0.49-1.48	0.95	0.55-1.63	0.84	0.49-1.47	0.68	0.38-1.23	0.26
Model 2, HR (95%CI)	1 (Ref)	0.83	0.47-1.45	0.94	0.55-1.63	0.81	0.46-1.43	0.62	0.34-1.13	0.16
Model 3, HR (95%CI)	1 (Ref)	0.84	0.48-1.48	1.00	0.57-1.76	0.81	0.45-1.45	0.59	0.31-1.13	0.15
% energy from animal protein										
Mean (SD) % energy from animal protein	4.1 (1.2)	6.5 (0.5)		8.2 (0.5)		10.0 (0.6)		13.8 (3.5)		
N	253	256		255		262		265		---
No. of deaths	25	26		23		24		22		---
Person-years	3,362	3,425		3,414		3,488		3,591		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	1.05	0.61-1.81	0.90	0.51-1.58	0.93	0.53-1.63	0.86	0.49-1.53	0.52
Model 2, HR (95%CI)	1 (Ref)	1.02	0.59-1.78	0.88	0.5-1.56	0.92	0.52-1.62	0.82	0.46-1.46	0.43
Model 3, HR (95%CI)	1 (Ref)	1.00	0.57-1.75	0.89	0.50-1.58	0.94	0.53-1.68	0.76	0.41-1.42	0.39
% energy from plant protein										
Mean (SD) % energy from plant protein	5.2 (0.7)	6.4 (0.2)		7.2 (0.2)		8.3 (0.4)		11.5 (2.6)		
N	249	252		255		266		269		---
No. of deaths	33	15		19		32		21		---
Person-years	3,302	3,451		3,414		3,508		3,605		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.42	0.23-0.77	0.54	0.31-0.95	0.88	0.54-1.43	0.57	0.33-0.99	0.43
Model 2, HR (95%CI)	1 (Ref)	0.44	0.24-0.82	0.58	0.33-1.03	0.98	0.6-1.62	0.55	0.32-0.96	0.41
Model 3, HR (95%CI)	1 (Ref)	0.44	0.24-0.83	0.62	0.34-1.12	1.04	0.61-1.78	0.57	0.31-1.02	0.56

*Model 2: Further adjusted for BMI, smoking, alcohol, education, PASE, marital status, daily energy intake, and self-reported history of hypertension and diabetes; **Model 3: Further adjusted for energy adjusted fiber intake, sex-specific quintiles of daily total grains intake, daily fruit intake, and daily vegetable intake

Table 5
Hazard ratio (HR) (95% CI) of CVD mortality by quintiles of protein intake in men (n=1,003) and women (n=1,259)

Protein intake (% energy)	Q1	Q2		Q3		Q4		Q5		p-trend (linear)
Men (n=1,003)										
% energy from total protein										
Mean (SD) % energy from total protein	11.6 (1.3)	14.2 (0.5)		16.1 (0.6)		18.1 (0.6)		21.9 (2.2)		
N	178	201		206		210		208		---
No. of deaths	28	21		22		23		23		---
Person-years	2,408	2,856		2,865		2,971		2,912		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.59	0.33-1.03	0.59	0.34-1.03	0.66	0.38-1.15	0.72	0.41-1.25	0.38
Model 2*, HR (95%CI)	1 (Ref)	0.56	0.32-1.00	0.57	0.32-1.00	0.69	0.39-1.22	0.73	0.41-1.29	0.48
Model 3**, HR (95%CI)	1 (Ref)	0.58	0.32-1.04	0.58	0.32-1.06	0.70	0.38-1.26	0.75	0.41-1.39	0.58
% energy from animal protein										
Mean (SD) % energy from animal protein	4.7 (1.0)	7.1 (0.6)		8.9 (0.6)		10.9 (0.6)		14.7 (2.4)		
N	181	204		208		202		208		---
No. of deaths	23	28		21		20		25		---
Person-years	2,494	2,791		2,957		2,842		2,926		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	1.17	0.68-2.04	0.78	0.43-1.41	0.75	0.41-1.36	1.05	0.60-1.85	0.58
Model 2, HR (95%CI)	1 (Ref)	1.18	0.68-2.06	0.72	0.39-1.31	0.76	0.41-1.39	1.06	0.59-1.88	0.60
Model 3, HR (95%CI)	1 (Ref)	0.58	0.32-1.04	0.58	0.32-1.06	0.70	0.38-1.26	0.75	0.41-1.39	0.49
% energy from plant protein										
Mean (SD) % energy from plant protein	4.5 (0.6)	5.6 (0.2)		6.5 (0.2)		7.6 (0.4)		11.0 (2.6)		
N	192	189		210		210		202		---
No. of deaths	24	31		16		25		21		---
Person-years	2,685	2,563		3,014		2,928		2,821		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	1.27	0.74-2.16	0.58	0.31-1.09	0.81	0.46-1.43	0.82	0.45-1.47	0.17
Model 2, HR (95%CI)	1 (Ref)	1.25	0.73-2.15	0.57	0.3-1.08	0.81	0.46-1.45	0.83	0.45-1.50	0.18
Model 3, HR (95%CI)	1 (Ref)	1.31	0.74-2.34	0.63	0.32-1.25	0.94	0.49-1.79	0.92	0.47-1.77	0.47
Women (n=1,259)										
% energy from total protein										
Mean (SD) % energy from total protein	11.5 (1.3)	14.2 (0.6)		16.1 (0.5)		17.9 (0.6)		21.7 (3.1)		
N	236	252		249		257		265		---
No. of deaths	22	18		14		18		16		---
Person-years	3,213	3,438		3,387		3,498		3,631		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.73	0.39-1.37	0.61	0.31-1.19	0.73	0.39-1.37	0.76	0.40-1.46	0.42
Model 2, HR (95%CI)	1 (Ref)	0.62	0.33-1.17	0.55	0.28-1.08	0.68	0.36-1.29	0.76	0.39-1.47	0.51
Model 3, HR (95%CI)	1 (Ref)	0.66	0.34-1.26	0.58	0.28-1.17	0.72	0.37-1.41	0.78	0.38-1.62	0.60
% energy from animal protein										
Mean (SD) % energy from animal protein	4.1 (1.2)	6.5 (0.5)		8.2 (0.5)		10.0 (0.6)		13.8 (3.5)		
N	244	248		251		258		258		---
No. of deaths	16	18		19		20		15		---
Person-years	3,313	3,373		3,415		3,524		3,542		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	1.18	0.6-2.31	1.09	0.56-2.12	1.20	0.62-2.31	0.96	0.48-1.95	0.96
Model 2, HR (95%CI)	1 (Ref)	1.09	0.56-2.16	1.03	0.53-2.02	1.22	0.63-2.36	0.98	0.48-1.99	0.92
Model 3, HR (95%CI)	1 (Ref)	1.07	0.54-2.15	1.08	0.54-2.15	1.19	0.60-2.35	0.93	0.42-2.03	0.99
% energy from plant protein										
Mean (SD) % energy from plant protein	5.2 (0.7)	6.4 (0.2)		7.2 (0.2)		8.3 (0.4)		11.5 (2.6)		
N	239	256		250		249		265		---
No. of deaths	23	19		14		15		17		---
Person-years	3,271	3,495		3,414		3,404		3,583		---
Model 1 (Age-adjusted), HR (95%CI)	1 (Ref)	0.68	0.37-1.25	0.53	0.27-1.03	0.61	0.32-1.17	0.67	0.36-1.26	0.20
Model 2, HR (95%CI)	1 (Ref)	0.60	0.32-1.12	0.55	0.28-1.07	0.55	0.28-1.07	0.63	0.33-1.19	0.17
Model 3, HR (95%CI)	1 (Ref)	0.62	0.33-1.18	0.63	0.31-1.27	0.62	0.30-1.26	0.71	0.35-1.45	0.41

*Model 2: Further adjusted for BMI, smoking, alcohol, education, PASE, marital status, daily energy intake, and self-reported history of hypertension and diabetes; **Model 3: Further adjusted for energy adjusted fiber intake, sex-specific quintiles of daily total grains intake, daily fruit intake, and daily vegetable intake

energy adjusted fiber intake (g/d), and sex-specific quintiles of daily total grains intake (g/d), daily fruit intake (g/d), and daily vegetable intake (g/d). Test for trend was examined by entering quintiles of each of the total, animal or plant protein intake as a continuous variable in all models. Since intake of total fat, saturated fat, polyunsaturated fat and monounsaturated fat showed high degree of collinearity with intake of total, animal and plant protein, they were not included in the multivariate models. An α level of 5%, 2 sided was considered as statistically significant.

Results

Participants' characteristics

There were significant differences in baseline characteristics between participants who were included (n=3,020) and those who were excluded (n=980) for analysis. Participants excluded for analysis were older, less physically active, had higher BMI and higher proportion of self-reported history of hypertension and diabetes, and more likely to be male, former smokers as well as former alcohol users ($p<0.05$) than participants included for analysis (data not shown). During a median of 13.8 years of follow-up, 963 all-cause deaths (594 men, 369 women), 336 cancer deaths (216 men, 120 women), and 205 CVD deaths (117 men, 88 women) were identified.

Mean daily protein intake was 87.1 g for men and 65.3 g for women, and the corresponding protein intake per kilogram body weight was 1.4 for men and 1.2 for women. Animal protein contributed to 55.5% for men and 51% for women of the total protein intake. Baseline characteristics of men and women in the final sample across quintiles of percentage of energy from total protein intake, animal protein intake and plant protein intake are shown in Tables 1 and 2. Men in the highest quintile of total protein intake were younger, had higher BMI, higher education level, higher proportion of being married, and higher proportion of history of diabetes than those in the lowest quintile. They also showed different dietary intake profiles than those in in the lowest quintile. Men in the highest quintile of animal protein intake had higher BMI, higher proportion of current smoker, and higher proportion of history of hypertension than those in the lowest quintile. Their dietary intake of energy, fruit, and grains also differed from those in in the lowest quintile. Men in the highest quintile of plant protein intake were more physically active, had higher education attainment, and had higher proportion of being married and having history of diabetes than those in the lowest quintile. They also showed higher intake of daily energy, fiber, total grains, vegetables and fruit than those in in the lowest quintile (Table 1).

Women in the highest quintile of total protein intake were younger, had higher education level and higher proportion of having history of diabetes than those in the lowest quintile. They also showed different dietary intakes than those in in the lowest quintile of total protein intake. Women in the highest quintile of animal protein intake were younger, had higher education attainment and higher proportion of being married than those in the lowest quintile. They also showed higher intake of daily energy and fruit, but lower grains consumption than those in in the lowest quintile. Women in the highest quintile of plant protein intake were more educated and had lower proportion of being married than those in the lowest quintile. Their dietary intakes differed from those in in the lowest quintile of plant protein intake (Table 2).

Protein intake and mortality

Compared with men in the lowest quintile, men in the highest quintile of total protein intake showed a significantly reduced risk of all-cause mortality in the age adjusted model and the model adjusted for BMI, smoking status, alcohol use, physical activity, daily energy intake, and other demographic and medical conditions. However, the significant results were attenuated when other dietary variables were further adjusted. Similar results were observed for animal protein intake. In contrast, while plant protein intake showed a significantly inverse trend with all-cause mortality in men in the age adjusted model, the trend was no longer significant in all the multivariate adjusted models (Table 3).

In women, those in the highest quintile of total protein intake tended to show a significantly reduced risk of all-cause mortality than those in the lowest quintile. While no significant trend between animal protein intake and all-cause mortality was noted in women, women with the highest quintile of plant protein intake showed a lower risk of all-cause mortality than those in the lowest quintile in the age adjusted model and the model adjusted for BMI, smoking status, alcohol use, physical activity, daily energy intake, and other demographic and medical conditions. However, the significant association was attenuated when other dietary variables were further adjusted (Table 3).

Compared with men in the lowest quintile, men in the highest quintile of total protein intake showed a significantly reduced risk of cancer mortality in the age adjusted model and the model adjusted for BMI, smoking status, alcohol use, physical activity, daily energy intake, and other demographic and medical conditions. However, the significant association was attenuated when other dietary variables were further adjusted. No significant association of cancer morality with animal protein intake or plant protein intake was observed in men (Table 4). In women, total protein intake or types of protein intake were not associated with cancer mortality (Table 4). In both men and women, no significant association of CVD morality with total protein intake, animal protein intake or plant protein intake was detected (Table 5).

Discussion

Our study is one of the few studies evaluating the association of protein intake and mortality risk in older adults. Our study showed that higher total protein intake was associated with lower risk of all-cause mortality in both men and women. Higher animal protein intake was associated with reduced risk of all-cause mortality in men whereas higher plant protein intake was linked with decreased risk of all-cause mortality in women. Such associations were attenuated when other dietary variables were further adjusted. There was also no significant association of CVD morality with total protein intake, animal protein intake or plant protein intake in both men and women.

The gender difference in the results may be explained by several reasons. Men in general showed less healthy dietary patterns and lifestyle than women, thus data from men may be more heterogeneous compared with women, in which significant associations between protein intake and mortality was more easily detected in this study. Another reason may be explained by fewer deaths among women compared with men.

Few studies have examined the separate association of animal protein intake and plant protein intake with mortality risk, and the results were inconclusive. In the Iowa Women's Health Study, while plant protein intake was inversely associated with CVD mortality but not associated with cancer or total mortality in the postmenopausal women, no significant association of total protein intake or animal protein intake with CVD, cancer or total mortality was observed (10). In contrast, results from two prospective US cohort studies suggested that increasing animal protein intake was associated with higher CVD mortality risk and higher plant protein intake was associated with lower all-cause and CVD mortality in men aged 40 to 75 years and women aged 30 to 55 years (22). The results were partly consistent with those from a recent study among 2,641 Finnish men aged 42 to 60 years. During the average follow-up of 22.3 years, higher total protein intake and animal protein intake was associated with higher risk of total mortality. Although no significant association between plant protein intake and total mortality risk was found, a higher animal-to-plant protein ratio was linked with lower mortality risk (9).

Differences in the covariates being adjusted in the multivariate models, the protein intake level, as well as the age of the studied population may partly explain the differential findings. The protein intake of our studied population was comparable or even high, compared with other published studies among Caucasian population. The mean relative total protein intake of our participants was 1.4 and 1.2 g/kg body weight respectively for men and women whereas that of the aforementioned Finland study was 1.2 g/kg body weight (9). Moreover, although the mean percentage of energy from protein intake was similar between our study and other studies (i.e. ~16%) (6, 9, 10), animal protein and plant protein each contributed to about 50% of the total protein intake in our study. In contrast, most previous studies showed that animal protein and plant protein contributed to about 70% and 30% of the total protein intake respectively (6, 9). The relatively higher proportion of total protein intake from plant sources in our participants possibly reflect the cultural difference in dietary practice, for example, Chinese population generally has higher intake of soy foods and soy proteins than Caucasian populations (23). In our participants with replete protein intake, a lower ratio of animal protein intake to plant protein intake may be beneficial for health (24, 25).

The age-dependent association between protein intake and mortality may also help explain the inverse association of protein intake and mortality in our study. In some previous studies, higher protein intake was linked with higher mortality in participants aged at or below 65 years but lower mortality in those aged over 65 (6, 26). High protein intake was found to increase the activity of pro-anabolic signalling pathways that drive the ageing process and promote cancer development in rodent studies and among middle-aged humans (27, 28). However, with ageing there is protein anabolic resistance (29), therefore increased amino acid intake, such as leucine intake and cysteine intake resulted from increased dietary protein intake may provide beneficial anabolic effects in older adults and higher protein may not be harmful but necessary. For example, leucine helps promote anabolism by stimulating mTORC1 activity in older adults and cysteine could counteract the age-related decline in glutathione synthesis, which helps to combat the increased oxidative status during ageing (14). In contrast to the recent advocacy by the EAT-Lancet commission regarding the promotion of a universal healthy reference diet characterized by plenty consumption of plant-based foods and limited intake of animal foods that is beneficial for human health in the general adult population (30), our findings imply that one cannot simply apply such recommendations to older people. This 'paradoxical' finding is similar to the observations that while higher BMI is associated with increased risk of mortality in young adults in general, higher BMI is protective for older people (31, 32).

There are strengths and limitations in this study. The strengths included reliable mortality data based on an official database, inclusion of several non-dietary variables and adjustment for their confounding effect in the analyses. However, dietary assessment by FFQ is subject to recall bias, thus the measurement error may attenuate associations between diet and outcome measures. Furthermore, our sample as a whole showed different demographic characteristics compared with the general older population in Hong Kong. Since participants were volunteers and were able to walk or take public transport to the study site, our sample may also be more health conscious and have good baseline health status. Besides, there were differences in demographic and lifestyle characteristics between those included and those excluded for the analysis. Therefore, the results may not be generalized to the general population.

In conclusion, higher total protein intake was associated with lower all-cause and cancer mortality in Chinese older men. While higher animal protein intake was associated with reduced all-cause mortality in Chinese older men, higher plant protein intake was protective against all-cause mortality in Chinese women. These 'paradoxical' findings suggest that while lowering protein intake, in particular animal protein intake is beneficial for reducing mortality and other adverse health outcomes in the general adult population, such dietary recommendations cannot be simply applied to the older population with different physiological and protein anabolism status. Dietary recommendations for older people should take into account such age-related physiological changes and culture-specific dietary habits. The attenuated associations between protein intake and mortality risk after adjustment for other dietary variables also highlight the role of whole diet approach in mortality risk reduction among older adults.

Funding: The work was supported by grants from the Research Grants Council of Hong Kong (CUHK 4101/02M); the Hong Kong Jockey Club Charities Trust; and the Centre for Nutritional Studies, The Chinese University of Hong Kong.

Acknowledgement: We wish to thank all participants for their participation.

Conflict of interest: The authors declare no conflict of interest associated with this manuscript.

Ethical standard: This study followed the guidelines laid down in the Declaration of Helsinki, and was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

References

1. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr. 2008;87(1):150-5.
2. Scott D, Blizzard L, Fell J, Giles G, Jones G. Associations between dietary nutrient intake and muscle mass and strength in community-dwelling older adults: the Tasmanian Older Adult Cohort Study. J Am Geriatr Soc. 2010;58(11):2129-34.
3. Huang RY, Yang KC, Chang HH, Lee LT, Lu CW, Huang KC. The Association between Total Protein and Vegetable Protein Intake and Low Muscle Mass among the Community-Dwelling Elderly Population in Northern Taiwan. Nutrients. 2016;8(6):pii: E373.
4. Chan R, Leung J, Woo J, Kwok T. Associations of dietary protein intake on subsequent decline in muscle mass and physical functions over four years in ambulant older Chinese people. J Nutr Health Aging. 2014;18(2):171-7.
5. Lord C, Chaput JP, Aubertin-Leheudre M, Labonte M, Dionne IJ. Dietary animal protein intake: association with muscle mass index in older women. J Nutr Health Aging. 2007;11(5):383-7.
6. Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, et al. Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. Cell Metab. 2014;19(3):407-17.
7. Orlich MJ, Singh PN, Sabate J, Jaceldo-Siegl K, Fan J, Knutsen S, et al. Vegetarian dietary patterns and mortality in Adventist Health Study 2. JAMA Intern Med. 2013;173(13):1230-8.
8. Pan A, Sun Q, Bernstein AM, Schulze MB, Manson JE, Stampfer MJ, et al. Red meat consumption and mortality: results from 2 prospective cohort studies. Arch Intern Med. 2012;172(7):555-63.
9. Virtanen HEK, Voutilainen S, Koskinen TT, Mursu J, Kokko P, Ylilauri MPT, et al. Dietary proteins and protein sources and risk of death: the Kuopio Ischaemic Heart Disease Risk Factor Study. Am J Clin Nutr. 2019;109(5):1462-71.
10. Kelemen LE, Kushi LH, Jacobs DR, Jr., Cerhan JR. Associations of dietary protein with disease and mortality in a prospective study of postmenopausal women. Am J Epidemiol. 2005;161(3):239-49.
11. Kahleova H, Fleeman R, Hlozkova A, Holubkov R, Barnard ND. A plant-based diet in overweight individuals in a 16-week randomized clinical trial: metabolic benefits of plant protein. Nutr Diabetes. 2018;8(1):58.
12. Kahleova H, Levin S, Barnard N. Cardio-Metabolic Benefits of Plant-Based Diets. Nutrients. 2017;9(8):pii: E848.
13. Campbell TC. A plant-based diet and animal protein: questioning dietary fat and considering animal protein as the main cause of heart disease. J Geriatr Cardiol. 2017;14(5):331-7.
14. McCarty MF, DiNicolantonio JJ. An increased need for dietary cysteine in support of glutathione synthesis may underlie the increased risk for mortality associated with low protein intake in the elderly. Age. 2015;37(5):96.
15. Wong SY, Kwok T, Woo J, Lynn H, Griffith JF, Leung J, et al. Bone mineral density and the risk of peripheral arterial disease in men and women: results from Mr. and Ms Os, Hong Kong. Osteoporos Int. 2005;16(12):1933-8.
16. Census and Statistics Department. Hong Kong 2006 Population Bycensus Thematic Report: Older Persons. Hong Kong: Census and Statistics Department. 2006.
17. Washburn RA, Smith KW, Jette AM, Janney CA. The Physical Activity Scale for the Elderly (PASE): development and evaluation. J Clin Epidemiol. 1993;46(2):153-62.
18. Woo J, Leung SSF, Ho SC, Lam TH, Janus ED. A food frequency questionnaire for use in the Chinese population in Hong Kong: Description and examination of validity. Nutr Res. 1997;17:1633-41.
19. Paul AA, Southgate DAT. McCance & Widdowson's: The Composition of Foods. 4th ed. London: HMSO. 1978.
20. Yang Y, Wang G, Pan X. China Food Composition 2002. Peking: University Medical Press. 2002.
21. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997;65(4 Suppl):1220S-8S.
22. Song M, Fung TT, Hu FB, Willett WC, Longo VD, Chan AT, et al. Association of animal and plant protein intake with all-cause and cause-specific mortality. JAMA Intern Med. 2016;176(10):1453-63.
23. Messina M, Messina V. The role of soy in vegetarian diets. Nutrients. 2010;2(8):855-88.
24. Chan R, Leung J, Woo J. Association between estimated net endogenous acid production and subsequent decline in muscle mass over four years in ambulatory older Chinese people in Hong Kong: a prospective cohort study. J Gerontol A Biol Sci Med Sci. 2015;70(7):905-11.
25. Chan R, Leung J, Woo J. Estimated net endogenous acid production and risk of prevalent and incident hypertension in community-dwelling older people. World J Hypertens. 2015;5(4):129-36.
26. Bates CJ, Mansoor MA, Pentieva KD, Hamer M, Mishra GD. Biochemical risk indices, including plasma homocysteine, that prospectively predict mortality in older British people: the National Diet and Nutrition Survey of people aged 65 years and over. Br J Nutr. 2010;104(6):893-9.
27. Bartke A. Impact of reduced insulin-like growth factor-1/insulin signaling on aging in mammals: novel findings. Aging Cell. 2008;7(3):285-90.
28. Fontana L, Weiss EP, Villareal DT, Klein S, Holloszy JO. Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans. Aging Cell. 2008;7(5):681-7.
29. Burd NA, Gorissen SH, van Loon LJ. Anabolic resistance of muscle protein synthesis with aging. Exerc Sport Sci Rev. 2013;41(3):169-73.
30. Willett W, Rockstrom J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. Lancet. 2019;393(10170):447-92.
31. Winter JE, MacInnis RJ, Wattanapenpaiboon N, Nowson CA. BMI and all-cause mortality in older adults: a meta-analysis. Am J Clin Nutr. 2014;99(4):875-90.
32. Woo J. Body mass index and mortality. Age Ageing. 2016;45(3):331-3.