# DO PROTEIN AND ENERGY INTAKES EXPLAIN LONG-TERM CHANGES IN BODY COMPOSITION?

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Abstract: BACKGROUND: Despite evidence that profiles of protein and energy intake can determine short-term (<1y) change in both lean and fat compartments, the role of diet in longer-term, age-related changes in body composition remains unclear. OBJECTIVE: This paper tests for long-term counterparts to the well-established short-term relationships between protein and energy intake and changes in body composition. DESIGN: Using longitudinal data on 608 healthy, non-obese Chinese (50-69y) from the 1993 and 1997 China Health and Nutrition Surveys, sex-specific regression models were created to determine if 3-day mean protein (% of energy) and energy (kJ) intakes at baseline predicted change in midarm muscle area (MAMA) and waist circumference (WC). RESULTS: Although sex-specific U-shaped associations were observed, higher energy intakes were associated with greater gain in WC and less loss of MAMA, and higher protein intakes with less loss of MAMA than lower intakes for both sexes, adjusting for baseline age, height, weight, MAMA, WC, smoking status, activity level, income and urban residence. For males, energy intake below 95% of the Chinese RDA was associated with significantly smaller gains in WC and greater loss of MAMA than energy intake between 95-125% RDA. For both sexes, protein intake below 10.4% of energy was associated with significantly greater loss of MAMA than intake between 10.4-12.1% of energy. For females, energy intake above 125% RDA was associated with significantly greater gains in body fat than intake between 95-125% RDA. CONCLUSION: The results suggest that diet may play an important role in age-related change in body composition.

Key words: Diet, body composition, protein, energy, muscle, sarcopenia, malnutrition, elderly, aging, Chinese.

### Introduction

Research to identify modifiable determinants of age-related changes in body composition has become a major priority in the field of gerontology (1). Age-related changes in body composition, systematic losses of muscle mass with age (known as sarcopenia) and increases in body fat through age seventy, are linked with increased risk of morbidity, functional impairment, and mortality (2, 3).

Potential risk factors for age-related changes in body composition are thought to include catabolic stimuli, such as subclinical inflammation, as well as the withdrawal of anabolic stimuli, decreases with age in physical activity, protein intake, central nervous system input, sex hormones, growth hormone, and insulin action (4). While physical activity and protein intake both stand out as possible targets for lifestyle intervention, the research to date has focused primarily on physical activity. Resistance training has repeatedly been shown to promote muscle maintenance or gain (5-7). Agerelated increases in body fat have been attributed to declines in physical activity (8).

Although dietary intake is recognized as a potentially important risk factor for age-related changes in body composition (9), clear and consistent associations between diet and age-related change in each compartment have not been observed. Despite experimental evidence in support of a direct relationship between protein and/or energy intakes and change

in muscle among older adults (10-15), many observational studies of diet and sarcopenia report null effects (16-20). Since gain of body fat occurs while food intake is decreasing, declines in physical activity are thought to play more of a role than diet (8).

Diet may emerge as a clear determinant of age-related changes in body composition if well-known, short-term (<1y) relationships between diet and acute changes in body composition hold true over the long-term. Experimental and observational results unequivocally link profiles of protein and energy intake with short-term patterns of change in body composition. Results from underfeeding experiments inform us that diets inadequate in both protein and energy lead to loss of both lean and fat mass (e.g. 21-22). Results from refeeding and overfeeding studies demonstrate that diets providing protein and energy intake in excess of requirements lead to gain in both compartments (e.g. 23). Data on protein-sparing modified fasts indicate that diets low in energy but adequate in protein result in loss of body fat with maintenance or gain of lean mass (e.g. 24, 25). Observational research on protein-energy malnutrition (PEM) indicates that a spectrum of diets low in protein, but ranging in energy content, underlie a spectrum of PEM syndromes from nutritional marasmus (loss of both lean and fat mass) to kwashiorkor (loss of lean mass, despite maintenance of body fat) (26). The effect of low protein intake on muscle wasting is also well known to depend on the level of energy intake (26-28). The parallels between PEM-related change in

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body composition and age-related changes in body composition, although previously described in the literature (29, 30), have yet to be explored. If age-related changes in body composition reflect profiles of protein and energy intake, then we might also expect the effects of protein and energy intake to interact.

Despite the accumulated evidence that profiles of protein and energy intake determine patterns of change in both lean and fat compartments over the short-term, and awareness of the interdependence of protein and energy metabolism, research on age-related changes in body composition has focused on one body compartment at a time and/or the effects of single nutrients at a time. This focus has limited previous observational studies to analyses that could not address potential endogeneity between diet and body composition, nor appropriately account for the interrelationships between protein and energy intake and metabolism. These limitations may have obscured dietary effects. Studies that address these limitations may help resolve inconsistencies between the clinical and observational research on the role of diet in age-related change in body composition.

This study tested for long-term counterparts to the well-established short-term relationships between protein and energy intake and changes in body composition. Using models that take into account the endogeneity of diet and body composition as well as interrelationships between protein and energy, we estimated the effects of protein and energy intake on continuous changes in arm muscle and body fat over a 4-year period, simulated the changes in both outcomes associated with particular diet profiles, and tested for interactive effects of protein and energy.

### Methods

### Data

Longitudinal data on 608 healthy adult Chinese, aged 50-69 y, participating in both the 1993 and 1997 China Health and Nutrition Surveys (CHNS), were used for this analysis. This work extends previous descriptive analyses on the same sample of older adults (31).

The CHNS provide unique, detailed data on longitudinal changes in body composition, dietary intake, physical activity, as well as sociodemographic variables. Due to the extremely rapid economic, demographic, and epidemiologic transitions ongoing in China (32-35), these data capture unusually large variation in the lifestyle and anthropometry of free-living individuals. The sampling frame and survey methodology have been described in detail previously (36). The China Health and Nutrition Survey follows human subjects approval procedures that have been approved both by the University of North Carolina School of Public Health and Chinese Academy of Preventive Medicine human subjects protection committees.

To define a sample of healthy older adults, the three inclusion criteria were: 1) Answering 'no' to the question 'over

the past three months have you had any difficulty in carrying out your daily activities and work due to illness?' in both 1993 and 1997 surveys; 2) A weight change of less than 20% from baseline between survey years; and 3) reported energy intake at baseline above 125-138 kJ/kg or 33 kcal/kg body weight. Subjects who experienced a weight change of greater than 20% from baseline or reported an energy intake below the level required for short-term weight maintenance (37, 38) were excluded to avoid confusing acute or illness-related changes in body composition with age-related changes.

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A further inclusion criterion limited the sample to subjects who might be relatively homogeneous with respect to measurement error in the anthropometric measures. Given that the reliability of triceps skinfold measurements may vary greatly between obese and non-obese adults (39, 40), subjects who were obese in either survey year according to the WHO cutoff (BMI\$30) (41) were excluded from the sample.

Of the original 2164 persons, aged 50-69 y, who were interviewed in 1993 and living in provinces that were resurveyed in 1997, 583 were missing both health status and anthropometric information, 116 reported poor health in the three months preceding the survey, and 1465 subjects reported no health-related difficulties. Of these 1465 subjects, 1410 had non-missing anthropometric data and were non-obese. Subjects with missing dietary intake information (n=10) or who reported baseline energy intakes below 125-138kJ/kg or 33 kcal/kg body weight (n=216) were next excluded from the sample.

Between 1993 and 1997, 261 subjects (22% of the sample) were lost to follow-up. Of the remaining 923, 858 reported no health-related difficulties prior to the survey in 1997. Follow-up anthropometric measures were available for 653 of these 858. Further exclusion of subjects who experienced a weight change of more than 20% from baseline (n=40) or had a BMI greater than 30 in 1997 reduced the sample size to 608.

The sample inclusion criterion that subjects be healthy in both 1993 and 1997, prevented a standard evaluation for selection bias from loss-to-follow-up. Although we know that the subjects lost-to-follow-up were significantly older, richer, and had larger waist circumferences at baseline than those not lost-to-follow-up, we know nothing about their health status in 1997. If the missing subjects were unhealthy in 1997, they would have been excluded from the sample, and would not constitute a source of selection bias. If the missing subjects were healthy in 1997, their missing-ness might bias the results towards smaller losses of arm muscle and waist circumference. Previous analyses with this same sample indicate that the older groups at baseline experienced greater loss of arm muscle over the study period, while those with a greater waist circumference at baseline experienced greater loss of body fat (31). Given that older age and ill health are often associated with nonparticipation in surveys, the former scenario is not implausible. An actual Heckman test for selection bias (42) was not possible, however, since this test would have required complete information about the target sample, in this case, information

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on the health status of all subjects in both 1993 and 1997.

Anthropometric measurements were taken by trained health workers in both 1993 and 1997. Approximately 55% of the interviewers were the same for 1993 and 1997. Testing for inter-observer reliability was undertaken. Body weight was measured to the nearest tenth of a kilogram with a beam balance scale. Height was measured without shoes to the nearest tenth of a centimeter using a portable stadiometer. Triceps skinfold thickness was measured to the nearest millimeter using Halpenden calipers. Mid-upper arm circumference (MAC) was measured to the nearest tenth of a centimeter using graduated tape. Specific training on anthropometric measurement techniques was provided at the beginning of each survey over a 2-week period that included interobserver reliability training and testing and followed NCHS training and collection protocol. The health workers also asked all subjects if they were current or past smokers. As indicated above, subjects with missing anthropometric data for either survey year were excluded from the sample.

Specially trained interviewers obtained detailed individual-level diet data via 24-hour recall for three consecutive days as well as household-level changes in food inventory over the same 3-day period. The dietary information was recorded in terms of raw ingredients/foods. At the time of the last (3rd) diet recall, the food inventory information was used to cross-check the diet recall data. Later, using an algorithm developed by Guo et al (43), we also used household-level information on the disappearance of cooking oil to estimate each individual's intake of cooking oil. In China, where a small amount of oil is commonly used to cook for all members of the household simultaneously, it is difficult for each subject to accurately report his/her own oil intake. We included individual intake of cooking oil in each subject's estimated total energy intake.

The 1991 Food Composition Table for China (44) was used to calculate 3-day mean daily protein and energy intakes from the raw food consumption data for each individual. According to Willett (45), 3-day mean energy and protein intakes adequately represent usual intakes for these nutrients. Although the energy intakes observed in the CHNS studies have not been validated using a gold standard technique, they have been shown to predict BMI in CHNS adults (34, 36). A validation study of CHNS dietary intake is currently underway. As indicated above, subjects with missing dietary data at baseline (n=10) were excluded from the sample.

Although absolute intakes are described in Tables 1 and 3, protein and energy intakes were expressed in relative units to help control for differences in individual dietary requirements. With energy intake expressed as a percent of the Chinese agesex-and activity specific Recommended Daily Allowance (RDA) in sex-specific models that include proxies for dietary requirements (age, height, weight, smoking, and activity level), the present analysis evaluates the effects of the adequacy of energy intake. Protein intake expressed as a percent of energy effectively reduces variation in absolute protein intake due to

differences in body size, activity level, and metabolic efficiency (45).

Protein intakes were expressed relative to total energy intake instead of relative to body weight or the protein RDA for several additional reasons. Aside from the fact that the protein RDA for older adults remains a source of controversy (46), in observational studies, neither protein relative to body weight nor protein relative to the RDA reflects the metabolic interrelationships between protein and energy for each individual. In clinical studies where protein intakes (g/kg or %RDA) are assigned to each individual with an explicitly defined level of energy intake (and confounding by energy intake controlled by randomization or matching), the metabolic profile of each subject can be gauged. In observational studies, however, where the protein-energy combination is not defined a priori by the researcher, and protein intakes (g/kg or %RDA) can occur with a range of different energy intakes, the metabolic response to a given protein intake is unknown.

A further limitation of protein intake expressed in g/kg or % of RDA units is that in observational studies involving energyadjusted models, these variables may yield misleading results (47). Because protein is an energy-yielding nutrient, an absolute increase in protein intake necessarily implies a concomitant increase in total energy intake or, if total energy is held constant, shifts in the proportion of energy from other nutrients. The addition of one gram of protein, for example, to a plate of food increases the energy content of the food by 16.7 kJ (4 kcal). Alternatively, if we hold constant the overall energy content, then the addition of one gram of protein necessarily means providing less energy from fat or carbohydrate. This covariance of protein with total energy intake and other macronutrients interferes with the interpretation of independent effects of absolute protein intake. In models that control for energy intake, g/kg or %RDA protein effects actually represent the effects of a shift in dietary composition, not just effects of changes in absolute protein intake.

The expression of protein as a percent of energy intake in this study avoids the use of controversial RDA values for protein, allows protein-energy combinations to be gauged, and facilitates the estimation of protein-specific effects (as independent from the effects of total energy intake). In multivariable models that include energy intake, the coefficient for protein (% of energy) represents the effect of a unit increase in the protein composition of the diet, holding total energy intake constant (47).

Physical activity was recorded as a categorical variable. Very light or light activity was characterized by work as an office worker or work in a sitting or standing position; moderate or heavy activity as work carried out by drivers, electricians, or farmers. The activity variable was designed by the Chinese Nutrition Society to reflect total energy expenditure, and intended for use in calculating the Chinese Recommended Daily Allowances. The variable significantly predicts energy intake and weight status among Chinese adults

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(36). Missing values for the activity variable (n=13) were imputed using age, sex, urban/rural residence, occupation, and self-reported information on the present condition of the upper and lower extremities (functioning normally, having some problems but not affecting daily activities and work, slightly affecting daily activities, affecting daily activities - help is required).

In addition to age (years) and gender, the CHNS collects information on place of residence (urban/rural) and income. Given the rapid urbanization-related changes occurring in China, these are key indicators for the present sample. Urban/rural residence was defined according to the Chinese census definition, which considers small towns and city neighborhoods as urban, and villages and suburbs as rural. Questions on income probed for any income-producing activity each person might have engaged in during the previous year. Income from market and non-market activities, and nonmonetary government subsidies (food subsidies in the form of ration coupons) were included in the income estimate. Per capita household income was deflated by the relative Retail Price Index which was 100 at base year of 1985 (48). While there were no missing values for the age, gender, and urban/rural variables, 2 missing income values were imputed using the age, gender, and residence information.

### Body composition indices

Midarm muscle area (MAMA) was chosen as the indicator of arm muscle mass for the present analyses. Estimates of MAMA were calculated from midarm circumference and triceps skinfold measures for each individual using the equation recommended by WHO (41, 49). Waist circumference was chosen as the indicator of body fat for this analysis. In validation studies in adults over age 18 and healthy elderly, waist circumference has consistently appeared to be a good measure (0.70<r<0.95) of total body fat as well as abdominal fat for both sexes (50-53).

### Analysis

All procedures were carried out using the STATA statistical package (54). To avoid interpretive problems related to the endogeneity of diet and body composition, the following analyses evaluated the effects of baseline diet on changes in body composition, instead of the effects of changes in diet on changes in body composition. After crude (unadjusted) stratified analysis, two multivariable modeling approaches were considered for estimating the effects of protein and energy intake adjusted for various covariates: a more conventional model, which estimates separate effects of protein and energy; and a second model, where particular protein-energy profiles are studied instead of independent nutrient-specific effects. The first modeling approach requires an interaction term between the main effects of protein and energy intake to test for interactive effects. The alternative approach, by contrast, involves indicator variables that each represent the overall

interactive effect of a particular protein-energy combination. Given interest in the independent effects of each nutrient, and to allow comparison of our results with those from previous observational studies, we used the first modeling approach to estimate the main effects of protein and energy intake, respectively, on each outcome of interest controlling for covariates. Next, we combined the respective main effects to determine if profiles of protein and energy intake predict the expected patterns of change in body composition. Lastly, we tested for interactive effects of protein and energy intake on change in MAMA using both modeling approaches.

# Respective main effects of protein and energy on each outcome

Sex-specific multivariable ordinary least squares regression (OLS) models were fit to test for independent main effects of protein and energy intake on change in MAMA and change in waist circumference, respectively, adjusting for various covariates. The models were specified with dietary protein content (protein as a percent of energy) and energy adequacy (%RDA) as main exposures. In contrast to the nutrient density method of energy adjustment described by Willett & Stampfer (47), energy adequacy was entered into the models instead of absolute energy intake (kJ). Although the models allow between-person differences in absolute energy intake, they control for the etiologically relevant variable vis-a-vis change in body composition, energy adequacy.

Since the respective relationships between dietary protein content and energy adequacy and each outcome appeared nonlinear in the crude stratified analysis (see Table 2), protein and energy intakes were each expressed as categorical dummy variables in all models. To simplify the presentation of results, we chose the same cutoffs to categorize subjects for both sexes (<95% and \$125% of the Chinese age-,sex- and activityspecific RDA for energy, and <10.4 and \$12.1% of energy for protein). The cutoff values not only allow the same meaning relative to the RDA or total energy intake for both sexes, but also approximate the tertile values for protein (10.4, 12.2% of energy for females; 10.4, 12.0 % of energy for males) and energy intake (102.7, 121.5%RDA for females, 96.0, 118.5%RDA for males) for both sexes in this sample. Among females, the 95-125% of RDA cutpoints for energy intake also more clearly distinguish between intakes below or above the RDA for energy than the actual tertile values. We did not adopt the more conventional 66.7 and 133.3 percentile values for categorizing energy intake, as these seemed too low and too high to represent sub-optimal or slightly excessive energy intakes which might have adverse effects over the long-term.

In addition to the protein and energy intake dummy variables, the multivariable OLS models included baseline age, height, weight, arm muscle, waist circumference, smoking status (current/non-smoker), activity level, income, and urban/rural residence. The baseline anthropometric measures, smoking and activity variables were considered proxies for

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dietary requirements and potential confounders of dietary effects. Whether attributable to biological differences in dietary requirements or to statistical regression to the mean, body size at baseline was positively and significantly associated with loss of MAMA and waist circumference (31). Since loss of stature (kyphosis) has been shown to inflate estimates of change in waist circumference, the change in height between survey years was also controlled in all of the models (55). To account for the potential non-independence of data for subjects from the same household, the standard errors for the estimated coefficients were adjusted using the robust variance estimator available with the cluster command in STATA. Differences with a probability level below 0.05 from any of the models were considered statistically significant.

To illustrate the respective effects of protein and energy intake on each outcome, we used the results from the sexspecific OLS models to predict the change in MAMA and change in waist circumference associated with each level of protein and energy, respectively. These predictions assumed the mean value for all other covariates in the model - i.e. the predictions assumed that all subjects were of average age, height, weight, waist circumference, MAMA, smoking status, activity level, income, and urban/rural residence at baseline.

## Simultaneous effects of both protein and energy intake on both outcomes

Given the goal of the present analysis to determine if profiles of protein and energy intake determine patterns of change in both MAMA and body fat, the next step in our analysis was to model how both protein and energy intakes simultaneously determined change in both outcomes. We combined the outcome-specific OLS models described above into sexspecific seemingly unrelated regression models (SUREG) (54, 56) . The four categorical dummy variables representing approximate tertiles of protein and energy intake, respectively, were included in all of the SUREG models with baseline age, height, weight, arm muscle, waist circumference, smoking status (current/non-smoker), activity level, income, urban/rural residence and the change in height between survey years as independent variables. The joint estimates from the SUREG models were used to test whether energy intake simultaneously influenced change in both MAMA and waist circumference, and, similarly, whether protein intake simultaneously determined both outcomes. To illustrate the simultaneous effects of protein and energy intake on both outcomes, the results from the SUREG models were used to predict mean changes in MAMA and waist circumference associated with particular baseline diets. Again, in these simulations all other covariates were set at their mean value.

### Interactive effects of protein and energy intake

We next tested for interactive effects of protein and energy intake on change in MAMA. All possible interaction terms between the tertile dummy variables representing dietary protein content and energy adequacy were added to the sexspecific main effects models predicting change in MAMA. As an alternative check for effect modification between protein and energy intake, we also fit sex-specific models with eight indicator variables representing nine possible protein-energy intake combinations: LPLE, LPME, LPHE, MPLE, MPME, MPHE, HPLE, HPME, HPHE (where L, M, H refer to the lowest, medium, and highest intake categories (approximate tertiles), and P and E refer to protein and energy, respectively). While the first modeling approach involved three parameters to describe each interaction, the effect of each nutrient plus their interaction, the second approach treated each protein-energy intake combination as a block and involved only one comparison. While both approaches allowed for the nonlinearity of the relationships under study, the second approach provided a less fragmented measure of the protein-energy combination and maximized the available statistical power. All multivariable models adjusted for the same covariates.

Table 1
Characteristics of 608 healthy, non-obese adults aged 50 to 69y, participating in the 1993
and 1997 China Health and Nutrition Surveys

	Male	Female
	(n=298)	(n=310)
	Mean (SD)	Mean (SD)
Sociodemographic variables		
Age (y)	57.6 (5.6)	57.5 (5.3)
Income (Yuan/y)	1303.0 (1270.8)	1167.2 (1178.7)
Moderate or heavy activity	70.0	69.7
(% of sample)		
Urban residents (% of sample)	24.8	25.5
Current smokers (% of sample)	67.8	9.4
Anthropometry		
Baseline height (m)	162.7 (6.0)	151.6 (5.9)
Baseline weight (kg)	56.8 (8.2)	49.5 (7.6)
Δ Weight (kg)	0.2 (4.0)	0.03 (3.6)
Baseline BMI (kg/m2)	21.4 (2.6)	21.5 (2.8)
Δ BMI (kg/m2)	0.1 (1.5)	0.1 (1.6)
Baseline MAMA (cm2)	40.4 (7.8)	33.6 (7.8)
Δ MAMA (cm2)	-2.0 (7.3)	-1.8 (7.5)
Baseline waist circumference	76.7 (8.2)	76.2 (8.8)
(cm)		
Δ Waist circumference (cm)	1.0 (6.5)	0.7 (7.1)
Dietary intake variables	*	
Protein (g)	80.9 (21.0)	70.9 (20.2)
Protein (g/kg Body weight)	1.4 (0.4)	1.4 (0.4)
Protein (% of energy)	11.3 (1.9)	11.5 (2.2)
Energy (kJ)	11979.2 (2537.0)	
Energy (kJ/kg Body weight)	213.8 (49.0)	211.3 (46.4)
Energy (% of Chinese RDA)	110.2 (26.2)	114.0 (26.4)

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#### Results

Selected characteristics of the sample are shown in Table 1. A majority of subjects lived in rural areas, and reported moderate or heavy activity levels. At baseline in 1993, the absolute mean protein intakes for males (80.9g, SD=21.0g) and females (70.9 g, SD=20.2g) were within the ranges recommended by the Chinese Nutrition Society (70-80 g/d for males; 60-70g/d for females) (44). The mean energy intakes at baseline also met the age-sex- and activity specific Chinese RDA for energy. The protein and energy intakes reported for this sample may appear greater than normal intakes for other healthy samples, since the estimates for this sample were calculated from raw foods. Between 1993 and 1997, the subjects experienced an average decrease in MAMA of 1.9 (7.4) cm2 and an average increase in waist circumference of 0.9 (6.8) cm.

Table 2 presents the unadjusted changes in MAMA and waist circumference experienced by this sample of older adults, stratified by gender, protein and energy intake. Stratified in this way, the respective relationships between protein and energy intake and each outcome appeared non-linear. Among males, U-shaped patterns were observed, such that subjects with the lowest and highest protein intakes lost more MAMA than subjects with medium protein intakes. Those with the lowest and highest energy intakes gained less in waist circumference than those with the mid-range energy intakes. Among females,

dose-response relationships between protein intake and change in either compartment were not apparent. Smaller losses of MAMA were noted only for protein intakes above 12.1 percent of energy among females. Although energy intake appeared linearly associated with change in MAMA, the relationship with change in waist circumference appeared more J-shaped.

Figures 1-3 illustrate the adjusted effects of protein and energy intake on changes in MAMA and waist circumference, respectively. These estimates were predicted from multivariable models that controlled for baseline age, height, weight, arm muscle, waist circumference, income, urban/rural residence, smoking status, activity level, and change in height between survey years. To avoid violating the linearity assumption inherent in OLS models, protein and energy intakes were expressed as categorical dummy variables, rather than continuous variables, in these models.

# Baseline protein and energy intakes and change in waist circumference

Higher energy intakes were associated with greater gain in body fat for both sexes, although the shape of results differed by sex (see Figure 1). Among males, mean daily energy intake below 95% of the RDA was associated with significantly smaller gains in body fat than energy intakes between 95-125% of the RDA. Among females, mean daily energy intakes above 125% of the RDA were associated with significantly greater gains in body fat than energy intakes between 95-125% RDA.

Table 2
Unadjusted mean (SD) change in midarm muscle area (MAMA) and waist circumference between 1993 and 1997 by gender, protein and energy intake

	Males					Females				
		MAMA		Waist c	Waist circumference		MAMA		Waist circumference	
		Baseline		Baseline	seline Δ m) (cm)		Baseline (cm2)	Δ (cm2)	Baseline (cm)	. Δ
		(cm2)		(cm)						(cm)
	n		Mean (	SD)		n		Mean (	SD)	
Whole sample	298	40.4	-2.0	76.7	1.0	310	33.6	-1.8	76.2	0.7
, 11010 om-1-		(7.8)	(7.3)	(8.2)	(6.5)		(7.8)	(7.5)	(8.8)	(7.1)
Protein intake			. ,							
Lowest	100	39.6*	-2.6	74.4*	1.0	100	32.7	-2.2	74.5*	1.3
(<10.4% of Energy)		(7.0)	(5.9)	(6.7)	(6.1)		(6.4)	(7.2)	(8.1)	(6.7)
Mid-range	108	39.7+	-0.4 *	76.5*	1.4	101	33.8	-2.1	75.5*	0.3
(10.4 to 12.1% of Energy)		(0.8)	(7.4)	(8.9)	(6.5)		(6.8)	(6.5)	(7.4)	(7.4)
Highest	90	42.0	-3.4	79.5	0.5	109	34.4	-1.0	78.4 🦫	0.5
(≥12.1% of Energy)		(8.3)	(8.3)	(8.0)	(7.2)		(9.5)	(8.6)	(10.0)	(7.2)
Energy intake										
Lowest	92	38.6*	-2.4	74.4*	0.7	79	30.4*	-0.8*	71.3*	0.6
(<95 % of RDA)		(6.7)	(6.1)	(7.1)	(6.0)		(6.0)	(6.4)	(7.1)	(7.9)
Mid-range	125	40.5	-1.6	76.1*	1.7	140	34.3	-1.2*	77.2	0.2+
(95 to 125% of RDA)		(8.6)	(7.4)	(7.7)	(6.5)		(7.0)	(7.4)	(8.9)	(7.3)
Highest	81	42.1	-2.4	80.2	0.3	91	35.5	-3.5	78.9	1.6
(≥125 %RDA)		(7.4)	(8.5)	(9.0)	(7.1)		(9.3)	(8.3)	(8.3)	(5.8)

Δ: Change in arm muscle area between 1993 and 1997; \* p-value<0.05 for comparison with the corresponding value for the highest protein or highest energy intake category; + p-value<0.10 for comparison with the corresponding value for the highest protein or highest energy intake category

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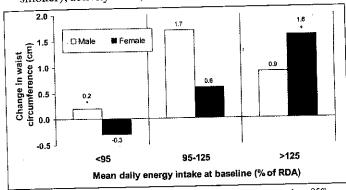
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Figure 1

Baseline energy intake and change in waist circumference among older adults (50-70 y) participating in the 1993 and 1997 China Health and Nutrition Surveys Predicted mean change in waist circumference from ordinary least squares regression models adjusting for baseline age, height, weight, arm muscle, waist circumference, smoking status (current/non-smoker), activity level, income, and urban/rural residence.



\* p-value <0.05 compared to 95-125% RDA value; + p-value<0.1 compared to <95% RDA value in the multivariable models

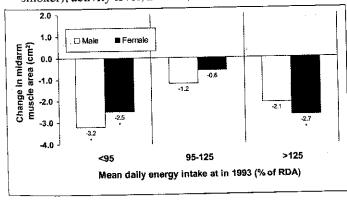
No significant associations were observed between protein intake at baseline and change in waist circumference

### Baseline protein and energy intakes and change in MAMA

Lower energy intakes were associated with greater loss of arm muscle for both sexes (see Figure 2). Energy intakes below 95%RDA were associated with significantly greater loss of arm muscle than intakes between 95-125% RDA. Unexpectedly, significantly greater losses of arm muscle were observed among the females with energy intakes above 125% of the RDA. A similar trend was apparent among males.

Figure 2

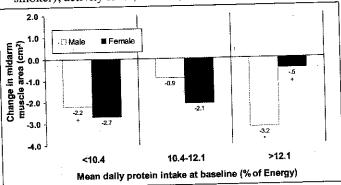
Baseline energy intake and change in mid-arm muscle area§ among older adults (50-70 y) participating in the 1993 and 1997 China Health and Nutrition Surveys; §Predicted mean change in mid-arm muscle area from ordinary least squares regression models adjusting for baseline age, height, weight, arm muscle, waist circumference, smoking status (current/non-smoker), activity level, income, and urban/rural residence.



\* p-value <0.05 compared to 95-125% RDA value in the multivariable models

Figure 3

Baseline protein intake and change in mid-arm muscle area§ among older adults (50-70 y) participating in the 1993 and 1997 China Health and Nutrition Surveys; §Predicted mean change in mid-arm muscle area from ordinary least squares regression models adjusting for baseline age, height, weight, arm muscle, waist circumference, smoking status (current/non-smoker), activity level, income, and urban/rural residence.



\* p-value <0.05 compared to 10.4-12.1% of Energy value; + p-value <0.1 compared to 10.4-12.1% of Energy value in the multivariable models

Lower protein intakes were associated with greater loss of arm muscle (see Figure 3). Among males, protein intake below 10.4% of energy was associated with significantly greater losses of arm muscle than protein intake between 10.4-12.1% of energy. Among females, protein intake greater than 12.1% of energy was associated with significantly smaller losses of arm muscle than protein intakes between 10.4 and 12.1% of energy. Again, we observed an unexpected U-shape, this time for the effect of protein intake on change in MAMA. Among males protein intakes above 12.1% were associated with significantly greater losses of arm muscle instead of gains.

# Baseline protein and energy intakes and change in both MAMA and waist circumference

Next, we modeled the simultaneous effects of protein and energy intake on change in both body compartments. The OLS models described above were combined into sex-specific seemingly unrelated regression models. For both males and females, energy intake below 95% RDA was simultaneously, significantly associated with loss of both MAMA and waist circumference (p-value for the test of jointly zero outcomes =0.01 for both sexes) compared with higher intakes as reference group. Protein intake retained the significant effects on change in MAMA described above.

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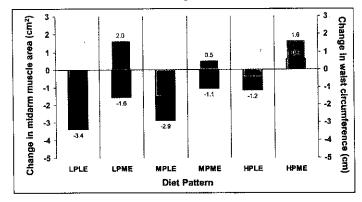
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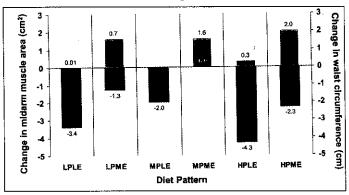
### Figure 4

Predicted¶ change in both mid-arm muscle area and waist circumference associated with specific protein and energy intakes

4a: among females







¶Predicted means from seemingly unrelated regression models with four dummy variables representing the tertiles of protein and energy intake, respectively. The models adjusted for baseline age, height, weight, arm muscle, waist circumference, smoking status (current/non-smoker), activity level, income, and urban/rural residence.

Figures 4a and 4b illustrate the changes in arm muscle and body fat associated with diets lowest, medium, or highest in protein (LP, MP, or HP) and lowest or medium in energy intake (LE or ME) at baseline, for males and females, respectively. To simplify these figures, the adverse effects of the highest energy groups (LPHE, MPHE, and HPHE), described above, are not shown. The relationships presented in the figures mirror the shorter-term relationships reported in the literature. Among females (Figure 4a), the diets lowest in both protein and energy intake were associated with comparable loss of arm muscle, but less gains in body fat than diets comparable in protein intake but medium in energy. The medium and higher protein diets were associated with less loss of arm muscle than the lower protein diets. Medium protein diets that were higher in energy were associated with greater gains in body fat than diets comparable in protein, but lower in energy. Similarly, greater gains in body fat were observed with the highest- protein, higher energy diets than the highest protein diets that were lower in energy.

Among males, protein and energy intakes were associated

with similar patterns of change in arm muscle and body fat (see Figure 4b). The diets lowest in both protein and energy were associated with comparable loss of arm muscle, but less gains in body fat than the diets low in protein but higher in energy. The medium protein diets were associated with less loss of arm muscle than the lower protein diets. The medium protein diets that were higher in energy were associated with greater gains in body fat than the medium protein diet lowest in energy. The results for the males differed from those for the females only with respect to the large losses of arm muscle associated with the highest protein intakes.

### Interactive effects of protein and energy on change in MAMA

The predicted estimates shown in figures 4a and 4b suggested potential interactions between protein and energy intake. The level of energy intake appeared to modify the effect of protein intake on change in MAMA, and vice versa. For both sexes, the diets lowest in protein and energy intake appeared associated with greater loss of MAMA than diets similar in protein, but higher in energy intake. These apparent differences were not statistically significant, however. When interaction terms were added to the models that generated figures 4a and 4b, no significant interactions were observed (data not shown).

Considering that the multiple dummy variables and associated interaction terms might limit our power to detect significant interactive effects, we tested for effect modification using an alternative specification. We replaced the dummy variables and interaction terms with eight indicator variables representing nine particular protein-energy combinations in the models predicting change in MAMA. Table 3 describes the actual protein and energy intakes, as well as unadjusted changes in MAMA of subjects in each of these categories. With the alternative specification, the effect of protein intake on change in MAMA varied significantly by energy level (see Figure 5). Females who consumed diets lowest in both protein and energy (LPLE) lost significantly more MAMA than those with comparably low protein intake but energy intake between 95-125% of the RDA (LPME). Males who consumed diets mid-range in protein and energy gained significantly more MAMA than males with comparable protein intake but lower energy intake (MPLE).

The effect of energy intake on change in MAMA also appeared to vary significantly by level of protein intake. Females with the lowest energy and protein intakes lost significantly more MAMA than females with comparable energy intakes, but higher protein intakes (LPLE vs. HPLE). The effect of mid-range energy intake appeared more beneficial among females with the highest protein intake than with medium protein intake (MPME vs HPME). Among males, significantly greater gains in MAMA were observed for the MPME group than the LPME group.

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Table 3

Baseline protein and energy intakes of subjects in each of the nine protein-energy intake groups

Protein									
	n	g	% of Energy	g/kg BWT	k.J Mear	% of RDA 1 (SD)	kJ/kg BWT	Baseline cm <sup>2</sup>	cm <sup>2</sup>
Male									
Lowest protein,	36	55.9	9.5	1.1	9821.5	82.7	187.0	38.6	-2.1
Lowest energy		(9.4)	(0.6)	(0.2)	(1270.3)	(7.4)	(30.5)	(6.9)	(6.3)
Lowest protein,	38	66.7	9.5	1.3	11786.7	107.6	226.4	39.2	-2.0
Mid-range energy		(10.6)	(0.8)	(0.2)	(1751.8)	(7.6)	(40.6)	(6.8)	(5.4)
Lowest protein,	26	78.0	8.8	1.4	14943.2	143.1	269.0	41.6	-4.2
Highest energy		(13.3)	(1.1)	(0.3)	(2100.0)	(16.3)	(51.0)	(7.3)	(6.2)
Mid-range protein,	35	65.9	11.3	1.3	9765.9	82.0	185.8	37.4*	-1.4+
Lowest energy		(7.9)	(0.6)	(0.2)	(1122.1)	(8.3)	(27.6)	(6.5)	(5.3)
Mid-range protein,	42	83.0	11.3	1.5	12261.6		222.2	39.2	0.7*
Mid-range energy	.2	(12.8)	(0.5)	(0.3)	(1659.0)		(38.1)	(8.5)	(7.7)
Mid-range protein,	31	101.8	11.3	1.6	14965.7		241.8	43.1	-0.6+
Highest energy	JI	(20.6)	(0.5)	(0.5)	(2633.0)		(61.5)	(8.0)	(9.0)
Highest protein,	21	80.5	14.0	1.4	9637.0	83.7	171.1	40.7	-4.4
Lowest energy	21	(11.1)	(1.4)	(0.2)	(1156.5)		(25.9)	(6.4)	(6.7)
Highest protein,	45	94.2	13.5	1.6	11671.3		198.7	42.9	-3.3
Mid-range energy	43	(13.7)	(1.2)	(0.3)	(1406.2)		(33.9)	(9.7)	(8.2)
Highest protein,	24	110.9	13.5	1.9	13814.3		230.1	41.4	-2.6
Highest energy	24	(16.2)	(0.8)	(0.4)	(2140.1)		(52.3)	(7.0)	(9.9)
Female									
Lowest protein,	32	45.2	9.2	1.0	8192.7	83.6	183.3	30.9	-2.8
Lowest energy		(6.7)	(0.8)	(0.1)	(982.8)		(27.6)	(6.1)	(6.4)
Lowest protein,	40	57.3	9.4	1.2	10246.6		210.0	33.6	-0.9
Mid-range energy		(8.7)	(0.7)	(0.2)	(1292.9)		(38.5)	(6.0)	(7.5)
Lowest protein,	28	68.2	9.1	1.4	12551.6		261.9	33.4	-3.3
Highest energy		(11.1)	(0.9)	(0.3)	(1799.1)		(53.6)	(7.0)	(7.5)
Mid-range protein,	24	54.8	11.4	1.2	8059.6		178.7	30.3+	-0.1+
Lowest energy		(6.4)	(0.5)	(0.2)	(916.7)		(28.0)	(6.2)	(6.7)
Mid-range protein,	51	66.2	11.1	1.4	9898.5		202.5	34.4	-2.2
Mid-range energy	51	(8.1)	(0.5)	(0.2)	(1114.6)		(28.5)	(5.7)	(6.1)
Mid-range protein,	26	83.8	11.2	1.6	12495.5		241.8	35.7	-3.9
Highest energy	20	(13.7)	(0.5)	(0.3)	(2025.9)		(48.1)	(8.2)	(6.9)
Highest protein,	23	69.5	14.0	1.5	8297.3		184.1	29.8*	1.2*
Lowest energy	23	(13.7)	(1.9)	(0.4)	(1115.5)		(29.3)	(5.8)	(5.5)
Highest protein,	49	83.5	13.8	1.7	10168.4		206.3	34.7	-0.4
Mid-range energy	77	(13.6)	(2.0)	(0.4)	(1156.0		(41.0)	(8.8)	(8.6)
Highest protein.	37	102.3	13.7	1.9	12544.5		234.7	36.9	-3.3
Highest energy	31	(18.1)	(1.3)	(0.4)	(2152.2		(52.7)	(11.3)	(9.8)

RDA: Age-sex, and activity-specific recommended daily allowance for Chinese adults (44); BWT: Body weight; MAMA: Mid-arm muscle area;  $\Delta$ : Change in arm muscle area between 1993 and 1997; Protein intake cutoffs: Lowest, <10.4% of energy; Mid-range 10.4- 12.1% of energy, Highest, \$12.1% of energy; Energy intake cutoffs: Lowest, <95% of RDA; Mid-range 95-125% of RDA, Highest, \$125% of RDA; \* p-value<0.05 for comparison with the corresponding value for the lowest protein, highest energy group; + p-value<0.10 for comparison with the corresponding value for the lowest protein, highest energy group

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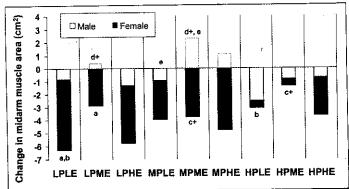
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### DIET AND LONG-TERM CHANGE IN BODY COMPOSITION

Figure 5
Predicted† change in mid-arm muscle area associated with specific patterns of protein and energy intake



†Predicted means from sex-specific seemingly unrelated regression models with eight indicator variables representing particular protein-energy intake combinations: LPLE, LPME, LPHE, MPLE, MPME, MPHE, HPLE, HPME, HPHE (where L, M, H refer to the lowest, medium, and highest intake categories), and P and E refer to protein and energy, respectively). The models controlled for baseline age, height, weight, arm muscle, wast circumference, smoking status (current/non-smoker), activity level, income, and urban/rural residence. The predicted means with the same letter were significantly different in the multivariable models (p-value < 0.05). Letters with a plus sign represent differences with a p-value<0.10.

#### Discussion

The results of this observational study suggest that diet may play an important role in age-related changes in body composition. The results provide evidence of long-term counterparts to well-established short-term relationships between diet and changes in body composition. Although slightly different by sex, the observed associations were consistent with the known positive associations between dietary intake and short-term changes in body composition. The lowest protein intakes were associated with greater loss of arm muscle than higher protein intakes. The lowest energy intakes were associated with greater loss of arm muscle than higher energy intakes. Higher energy intakes were associated with more gain in waist circumference than lower energy intakes. Profiles of protein and energy intake predicted the expected patterns of change in body composition over a four-year period. The effect of protein on change in arm muscle also appeared to depend on the level of energy adequacy.

Considerable evidence from clinical studies of obesity (overfeeding studies), starvation and the treatment of obesity (underfeeding studies), as well as the treatment of malnourished individuals (refeeding studies) demonstrate that energy and protein intakes, relative to dietary requirements, predict simultaneous change in both the lean and fat compartments (e.g. 21-25). Among females with energy intakes below 125% of the age-sex-and activity specific RDA, and males with protein intakes below 12.1 % of energy, the simultaneous effects of protein and energy intakes on change in arm muscle and body fat paralleled the short-term associations between PEM and changes in body composition (26). While diets lower

in both protein and energy were associated with loss of both arm muscle and body fat, diets comparably low in protein but higher in energy were associated with loss of arm muscle, but gain of body fat.

The interactions between protein and energy intake observed in this study were consistent with what we might expect from the literature on protein and energy metabolism. In keeping with the comments of Morais et al (28), the diets lowest in both protein and energy intake were associated with greater losses or smaller gains of MAMA than diets higher in one or both nutrients.

# Inconsistent results from previous research on diet and age-related change in body composition

In addition to providing evidence of long-term counterparts to well-established short-term associations, this study may help resolve inconsistent findings in the clinical and observational literature on diet and age-related changes in body composition. Although experimental studies report significant or non-significant positive associations between protein intake and change in skeletal muscle mass or area after 9-12 weeks among older adults (10-15), the existing observational studies involving older adults do not report positive effects of protein or energy intake on muscle outcomes (16-20).

Previous work on this same sample of older adults in China suggested that the role of diet might become clearer if we considered both outcomes simultaneously (31). We estimated the prevalence of different patterns of long-term changes in body composition and described these patterns in terms of baseline body size, mean dietary intake, activity level, income and urban residence. Despite reports of no association between protein or energy and muscle (16-20), the previous analysis showed that when changes in arm muscle were classified as gains or losses of arm muscle concurrent with gains or losses of body fat, females who lost arm muscle, but gained fat appeared to have a significantly lower protein intake than those who gained both arm muscle and fat, and a significantly greater energy intake than those who lost both arm muscle and body fat (31). The emergence of these significant differences in protein and energy intake suggested that dietary effects might have been obscured in earlier studies by complex interrelationships between body compartments and/or protein-energy metabolism. Although our descriptive analysis suggested that different patterns of change in body composition differ with respect to mean dietary intake, it did not estimate the magnitude or characterize the shape of underlying associations. To fill these gaps, the present longitudinal study estimated the magnitude and direction of effects of both protein and energy intake on changes in both arm muscle and body fat. Unlike previous studies, the present analysis considered the endogeneity of diet and body composition, potential confounding of protein effects by the level of energy adequacy, as well as potentially interactive effects of protein and energy.

The significant effects of protein and energy intake observed

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in this study indicate that null effects in previous observational studies may indeed be attributable to complexities involved in multivariable modeling of observational data, U-shaped associations, confounding by energy intake, and/or interactions between protein and energy intake. Underlying U-shaped associations could have produced null results in previous observational studies that relied on correlation analyses and ordinary regression models which assume a linear association between variables (e.g. 19, 20). Confounding by energy intake and/or misleading results from multivariable models where protein intake was entered in g/kg units (17, 20) may also have contributed to apparent null results. The multivariable models in previous studies also did not include or test interaction terms between protein and energy intakes. Without interaction terms or sub-group specific analyses, the reference group implicit in models with continuous protein and energy intakes as independent variables would have been the subjects with the lowest protein intakes and the lowest energy intakes. In the present study, the effect of protein appeared weaker among subjects with the lowest energy intakes than among subjects with energy intakes in the mid-range. Finally, poor representation of etiologically relevant variables and pathways may have led to null results in previous observational analyses. The fact that interactive effects of protein and energy only became apparent in this study when diet profiles were treated as the main exposures instead of specific nutrients, illustrates how null results in previous observational studies may be attributable to complexities of model specification.

The present study may help resolve confusion about the role of diet in age-related changes in body composition, because it contributes observational data that corroborate clinical findings, at the same time as offering possible explanations for previously observed null effects.

### Non-linear associations

Despite the protective effects of higher protein and energy intakes compared to the lowest intakes, the relationships observed in this study were non-linear. Among females, the highest energy intakes (>125% of the Chinese RDA) were unexpectedly associated with loss of muscle, rather than gain. Among males, protein intakes above 12.1% of energy were associated with loss of muscle, rather than gain. While it is always possible that the U-shaped associations observed in this study reflect measurement error, confounding by unobserved variables (such as illness or medication use), or statistical artefacts, they agree with current nutrition theory that both under- and overconsumption of protein and energy can result in negative outcomes. Findings from a 10-year longitudinal study of nutritional intake and physical health in older adults (57) suggest that the present results among women may reflect true relationships. Vellas et al (57) report that healthy elderly women with baseline energy intakes below or above the current RDA were more likely to become frail, sick or die than those with energy intakes in the midrange. Also, women with protein

intakes greater than the midrange of 0.8-1.2 g/kg of body weight tended to have fewer health problems. Although speculative, we wonder whether insulin resistance might mediate U-shaped relationships between protein and energy intake and long-term change in muscle. While we have no data to pursue this hypothesis, insulin resistance has been implicated as a determinant of sarcopenia (4).

Despite control for the adequacy of energy intake, the observed U-shaped effects may be attributable to residual confounding by total energy intake. Because a given absolute amount of protein may be relatively great for persons with low energy intake, it is possible that adverse effects of the highest level of protein intake reflect effects of low energy intake. Similarly, the adverse effects of high energy intake among females may reflect effects of low protein intake. Results from the models involving nine particular diet profiles instead of nutrient specific main effects argue against this explanation, however. The males who consumed HPLE, HPME, and HPHE diets all lost significantly more MAMA than males consuming diets lower in protein (MPME) content. Also, females who consumed the LPHE diets lost a similar amount of MAMA than females who consumed diets comparably high in energy but higher in protein content (MPHE or HPHE diets).

### Sex differences

The results of this study suggest that males and females may respond differently to given levels of dietary protein content. Among males, the smallest losses of MAMA were associated with protein intake between 10.4 and 12.1% of energy. Among females, the smallest losses were associated with protein intake above 12.1% of energy. Although elderly men have previously been found to be more efficient than elderly women in the use of egg protein to maintain nitrogen balance (58), this sexspecific result may reflect sex differences in body size and energy requirements, particularly those related to physical activity. Larger individuals (males) require more energy than smaller individuals (females) to perform the same tasks, because they have to move their greater mass (59-63). The division of absolute protein intake by total energy intake to express protein as a percent of energy could, therefore, have produced apparently different effects of dietary protein content, even though the effects or levels of absolute protein intake might be similar. Sex differences in dietary requirements might also be attributable to differences in sex hormones, underlying illness and/or smoking status. While 68% of males reported current smoking, smoking was comparatively rare among women (9%). Smoking status was not associated with protein intake above 12.1% of energy among males, however.

### Potential limitations

We cannot rule out possible confounding of the present results by changes in dietary intake. Baseline protein and energy intakes were studied instead of changes over time to avoid interpretive problems related to the endogeneity of diet

### DIET AND LONG-TERM CHANGE IN BODY COMPOSITION

and body composition. Working with levels of intake instead of changes over time, the present study design attempted to unravel the feedback loop between diet and body composition. The work assumed a biological mechanism where a given level of dietary intake over a long period results in gradual changes in body composition, which in turn determine new dietary requirements and new levels of dietary intake at later points in time. We assumed that three-day mean protein and energy intakes at baseline adequately reflect the habitual or 'usual' intake of these nutrients over an extended period of time. Between 1993 and 1997, however, the absolute energy and protein intakes decreased significantly for this sample by an average 543.9 kJ/d and 3.5 g/d, respectively. We do not know exactly when between 1993 and 1997 the actual dietary changes occurred. Since the body composition measurements were taken at the same time that dietary data were collected in 1993 and 1997, we cannot know whether the decreases in energy intake happened before or after the observed changes in body composition. If energy intakes decreased before any changes in MAMA and/or waist circumference, then the observed changes in body composition may be attributable to changes in intake instead of baseline levels of intake. The effect of low energy on change in MAMA may be underestimated, since subjects with low energy intake at baseline were less likely to decrease their energy intakes than subjects with higher energy intake at baseline. Even if all of the dietary changes happened before the observed changes in body composition, the associated confounding would not explain away the reported relationships. Multivariable models that controlled for the change in protein (% of energy) and change in energy intake between 1993 and 1997 produced a similar pattern of results to those presented here (data not shown). Lastly, in the case where dietary changes happened subsequent to changes in body composition, it would be inappropriate to include dietary change as a covariate in models predicting change in body composition - tantamount to having the same variable on both sides of the regression equation. Data from additional points in time and more complex statistical models (such as structural equations models) would be necessary to better model the endogenous relationships between diet and body composition.

'Protein or energy intakes' in the above discussion refer to energy intakes relative to the RDA and dietary protein content, not to absolute levels of protein or energy intake. The specification of protein intake as a percentage of energy intake constitutes a limitation of this study in so far as it complicates the interpretation of the results in terms of protein adequacy or protein recommendations - which are conventionally expressed in absolute terms (g or g/kg BWT). Explicitly, in this study some subjects with the same absolute protein intakes were classified as having different (lowest, mid-range, or highest) relative protein intakes if their total energy intakes differed.

The activity variable used in this analysis leaves open the potential for incomplete control of relevant aspects of physical activity. Given that the CHNS measure of activity level was

designed to reflect total energy expenditure and intended for use in calculating the Chinese RDA, it may be a better index of aerobic activity than weight-bearing exercise. Since resistance training, but not aerobic exercise, is associated with increases in muscle mass, we may have incompletely controlled for heterogeneity related to weight-bearing exercise.

The present sample of non-obese individuals, aged 50 to 69 y, was chosen to minimize bias related to differentially distributed differences in measurement errors in the MAMA measure. Errors in this measure are known to increase with age and obesity (64, 65). Although MAMA has been shown to correlate reasonably well with criterion measures in healthy younger adults (66-68), in older adults, MAMA correlates less well with arm muscle and poorly with total body muscle (64). In addition to errors in the measurement of triceps skinfold and midarm circumference, invalid geometric assumptions may contribute to overestimation of the true arm muscle area. Among elderly subjects in the US, bone-free MAMA has been shown to overestimate true muscle and bone area by as much as 30% in men and 50% in women (65). If geometric deviations from circularity increase over time, the overestimation in MAMA may be worse at follow-up than at baseline, leading to apparent increases in muscle mass over time.

The changes in arm muscle and waist circumference reported here should also be interpreted with caution. Anthropometric measures are not precise enough to detect subtle changes in muscle or body fat (49), and may not reflect changes in whole body compartments. While limited information suggests that waist circumference and MAMA are meaningful among Chinese groups (69, 70), validation studies of change in either measure are not available in the literature. Given the limitations of the MAMA measure, we interpret MAMA as an index of arm muscle mass rather than total body muscle and recognize that changes in this variable may not reflect sarcopenia in the whole body.

### Conclusion

In this longitudinal study of older adults in China, profiles of protein and energy intake were associated with particular patterns of change in arm muscle and fat mass. The effects of protein and energy interacted to determine change in arm muscle. These relationships, observed over a four-year period, paralleled the well-established short-term associations between protein and energy intake and change in body composition. The results suggest that diet may play an important role in agerelated change in body composition.

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- Rosenberg IH. Sarcopenia: origins and clinical relevance. J Nutr 1997;127:990S-991S. Muller DC, Elahi D, Sorkin JD, Andres R. Muscle mass: it's measurement and influence on aging. The Baltimore Longitudinal study of aging. In Rosenberg IH (Ed). Nutritional Assessment of Elderly Populations Measure and Function. Raven Press, New York: 1995, p50-62

References

- Borkan GA, Norris AH. Fat redistribution and the changing body dimensions of the adult male. Human Biol 1977; 49(3): 495-513.

  Roubenoff R, Hughes VA. Sarcopenia: Current concepts. J Gerontol Med Sci 2000;
- 55(12):M716-M724.
- ith CN, Frontera WR, O'Reilly KP, Evans WI. Body composition in elderly men: effect of dietary modification during strength training. J Am Geriatr Soc 1992;40: 155-162.
- Finatarone MA, O'Neil EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB, Kehayias JJ, Lipsitz LA, Evans WJ. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Eng J Med 1994;330:
- Finatrone MA, Marks EC, Ryan NC. High-intensity strength training in nonage Effects on skeletal muscle. JAMA 1990; 263: 3029-34.
- Morley JE. Anorexia of aging: physiologic and pathologic. Am J Clin Nutr 1997;66: 760-773. 8.
- Dutta C, Hadley EC. The significance of sarcopenia in old age. J Gerontol Series A 1995; 50A 1-4.

  Meredith CN, Frontera WR, O'Reilly KP, Evans WJ. Body composition in elderly men:
- effect of dietary modification during strength training. J Am Geriatr Soc 1992;40: 155-
- Fiatarone MA, O'Neil EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB. Kehayias JJ, Lipsitz LA, Evans WJ. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Eng J Med 1994;330: 1769-1775.
- Gray-Donald K, Payette H, Boutier V. Randomized clinical trial of nutritional supplementation shows little effect on functional status among free-living frail elderly. J supplementation shows litt Nutr 1995;125: 2965-2971
- Castaneda C, Charnley JM, Evans WJ, Crim MC. Elderly women accommodate to low protein diet with losses of body cell mass, muscle function, and immune response. Am J Campbell WW, Crim MC, Young VR, Joseph LJ, Evans WJ. Effects of resistance
- training and dietary protein intake on protein metabolism in older adults. Am J Physiol 1995; 268; E1143-1153.
- Castaneda C, Gordon PL, Fielding RA, Evans WJ, Crim MC. Marginal protein intake results in reduced plasma IGF-I levels and skeletal muscle fiber atrophy in elderly

- 19.

- Castaneda C, Gordon PL, Fielding RA, Evans WJ, Crim MC. Marginal protein intake results in reduced plasma IGF-1 levels and skeletal muscle fiber atrophy in elderly women. J Nutr Health Aging 2000;4(2):85-90.

  Munro HN, McGandy RB, Hartz SC, Rusell RM, Jacob RA, Otradovec CL. Protein nutriture of a group of free-living elderly. Am J Clin Nutr 1987; 46: 586-92.

  Neiman DC, Sherman KM, Arabatzisk K, Underwood BC, Barbosa JC, Johnson M, Shultz TD, Lee J. Hematological, anthropometric, and metabolic comparisons between vegetarian and non-vegetarian elderly women. Int J Sports Med 1989; 10: 243-50.

  Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol 1998;147(8):755-63.

  Baumgartner RN, Waters DL, Gallagher D, Morley JE, Garry PJ. Predictors of skeletal muscle mass in elderly men and women. Mech Ageing Dev 1999;107: 123-136.

  Starling RD, Ades PA, Poehlman ET. Physical activity, protein intake, and appendicular skeletal muscle mass in older men. Am J Clin Nutr 1999; 70: 91-96.

  Keys A, Brozek J, Henschel A, Michelson O, Taylor HL. The Biology of Human Starvation. University of Minnesota Press, Minneapolis: 1950.

  Vansant G, Van Gaal L, Van acker K, DeLeeuw I. Short and long term effects of a very low calorie diet on RMR and body composition. Int J Obesity 1989;13 (Suppl.): 87-89.

  Orphanidou CI. McCargar LJ, Birmingham CL, Belzberg AS. Changes in body composition and fat distribution after short-term weight gain in patients with anorexia nervosa. Am J Clin Nutr 1997; 65: 1034-41.
- nervosa. Am J Clm Nutr 1997; 65: 1034-41.

  Apfelbaum F, Bostsarron J, Lacatis D. Effect of caloric restriction and excessive caloric intake on energy expenditure. Am J Clin Nutr 1971; 24: 1405-1409.

  Van Gaal LF, Snyders D, De Leeuw IH, Bekaert JL. Anthropometric and calorimetric van Caloric expenses of the page of t Van Gaal LF. Snyders D, De Leeuw IH, Bekaert JL. Anthropometric and calorimetric evidence for the protein sparing effects of a new protein supplemented low calorie preparation. Am J Clin Nutr 1985; 41: 540-44.

  Waterlow JC. Protein energy malnutrition. Edward Arnold, London: 1993.

  Munro HN. General aspects of the regulation of protein metabolism by diet and by hormones. In Munro HN, Allison JB (Eds.). Mammalian Protein Metabolism, Vol. 1.

  New York Academy Press, New York: 1964, 381-481.

  Morais JA, Gouggeon R, Pencharz PB. Jones PJH. Ross R, Marliss EB. Whole-body protein purpoyer in healthy elderly. Am J Clin Nutr 1997;66:880-9

- Morais JA, Gouggeon K, Pencharz PB, Jones PJH, Ross K, Mariiss EB. Whoie-body protein turnover in healthy elderly. Am J Clin Nutr 1997;66:880-9. Winick M. Hunger Disease, Studies by the Jewish Physicians in the Warsaw Ghetto. New York: John Wiley & Sons: 1979. Roubenoff R, Kehayias JJ. The meaning and measurement of lean body mass. Nutr Rev 1001; 40:64:162-76. 29
- 1991; 49 (6): 163-75
- Stookey JD, Adair L, Stevens J, Popkin BM. Patterns of long-term change in body composition are associated with diet, activity, income and urban residence among older adults in China. J Nutr 2001; 131: 2433S-2440S.

  Popkin BM, Keyou G, Zhai F, Guo X, Ma H, Zohoori N. The nutrition transition in China: a cross-sectional analysis. Eur J Clin Nutr 1993;47(5): 333-46.

  Popkin BM. The nutrition transition in low-income countries: an emerging crisis. Nutr

- Popkin BM, Pacratakul S, Zhai F, Ge K, Dictary and environmental correlates of obesity in a population study in China. Obes Res 1995; 3(Suppl.): 135s-143s.

- 35. Popkin BM. The nutrition transition and its health implications in lower income countries Pub Health Nutr 1998: 1: 5-21
- Pacratakul S, Popkin BM, Ge K, Adair LS, Stevens J. Changes in diet and physical activity affect the body mass index of Chinese adults. Intl J Obesity 1998; 22: 424-32.
- World Health Organization (WHO). Energy and protein requirements: report of joint FAO-WHO-UNU expert consultation. World Health Organization, Geneva: 1985. Roberts SB. Effects of aging on energy requirements and the control of food intake in men. J Gerontol. Series A 1995; 50A (Special issue): 101-106.
- Reid JR, Evans MC, Ames R. Relationships between upper-arm anthropometry and soft-tissue composition in postmenopausal women. Am J Clin Nutr 1992; 56:463-6. Kuczmarski RJ, Fanelli MT, Koch GG. Ultrasonic assessment of body composition in obese adults: overcoming the limitations of the skinfold caliper. Am J Clin Nutr 1987; 45: 717-24.
- WHO Expert Committee. Physical status: The use and interpretation of anthropometry WHO Technical Report Series 854. World Health Organization, Geneva, Switzerland:
- Heckman J. The common structure of statistical models of truncation, sample sele and limited dependent variables and a simple estimator for such models. Annals Econ Soc Measurement 1976; 5: 475-492.
- Soc Measurement 1976; 5: 475-492.

  Guo X, Popkin BM, Mroz TA, Zhai F. Food price policy can significantly reduce the negative effects of the nutrition transition in China. J Nutr 1999;129: 994-1001.

  Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine (INFH-CAPM) The Food Consumption Tables. People's Medical Publication House, China: 1001
- 46
- China: 1991.

  Willett W, Stampfer M. Nature of variation in diet. In: Willett W (Ed.). Nutritional Epidemiology, 2nd Edition. Oxford University Press, New York: 1998, p.33-49.

  Campbell WW, Evans WJ. Protein requirements of elderly people. Eur J Clin Nutr 1996; 50 Suppl 1:S180-3.

  Willett W, Stampfer M. Implications of total energy intake for epidemiologic analyses. In: Willett W (Ed.). Nutritional Epidemiology, 2nd Edition. Oxford University Press, New York: 1998 p. 273-301. New York: 1998, p.273-301.
  China State Statistical Bureau (SSB). China's Statistical Yearbook. China Statistical
- Press, Beijing (in Chinese): 1992.
- Wright RA, Heymsfield S. Nutritional Assessment. Blackwell Scientific Publications, Boston: 1984. 49.
- Seidell JC, Bjorntorp P, Sjostrom L, Sannerstedt R, Krotkiewski M, Kvist H. Regional distribution of muscle and fat mass in men-new insight into the risk of abdominal obesity using computed tomography. Int J Obesity 1989; 13: 289-303.

  Seidell JC, Oosterlee A, Deurenberg P, Hautvast JGAJ, Ruijs JHJ. Abdominal fat depots
- measured with computed tomography: effects of degree of obesity, sex, and agc. Eur. J. Clin Nutr 1988: 42: 805-15
- Clin Nutr 1988; 42: 805-15.

  Ross R, Leger L, Morris D, De Guise J, Guardo R. Quantification of adipose tissue by MRI: relationship with anthropometric variables. J Appl Physiol 1992; 72(2): 787-95.

  Ross R, Shaw KD, Martel Y, de Guise J, Avruch L. Adipose tissue distribution measured by magnetic resonance imaging in obese women. Am J Clin Nutr 1993; 57: 53.
- StataCorp. Stata Statistical Software: Release 6.0. Stata Corporation, Texas: 1999. Chumlea WC, Roche AF, Webb P. Body size, subcutaneous fatness and total body fat in older adults. Int J Obesity 1984; 8: 311-17.
- Zellner A. An efficient method of estimating seemingly unrelated regressions and tests for aggregation bias. J Am Stat Assoc 1962: 57;348-368.
- Vellas BJ, Hunt WC, Romero LJ, Koehler KM, Baumgartner RN, Garry PJ. Changes in nutritional status and patterns of morbidity among free-living elderly persons: a 10-yr longitudinal study. Nutr 1997; 13(6): 515-9.
- Bidlack WR, Wang W. Nutrition requirements of the elderly. In Morley JE, Glick Z, Rubenstein LZ (Eds.) Geriatric Nutrition, A Comprehensive Review, 2nd Edition. Raven Press, New York: 1995, 25-49. 58.
- Curtis DE, Bradfield RB. Long-term energy intake and expenditure of obese housewives. Am J Clin Nutr 1971; 24:1410-1417.

  Haagarty P, Valencia ME, McNeil G, Gonzales NL, Moja SY, Pinelli A, Quihui L, Savado MS, Esparza J, Ashton J, Milne E, James WPT. Energy expenditure during heavy work and its interaction with body weight. Br J Nutr 1997;77:359-373.

  Heshka S, Yang MU, Wang J, Burt P, Pi-Sunyer FX. Weight loss and change in resting metabolic rate. Am J Clin Nutr 1990; 52:981-6.
- Dulloo AG, Jacquet J. Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signals from fat stores. Am J Clin Nutr 1998;
- department in management of the second of th

- wateriow JC: Inc nature and significance of nutritional adaptation. Eur J Clin Nutr 1999: 53 Suppl 1, S2-S5
  Reid JR, Evans MC, Ames R. Relationships between upper-arm anthropometry and soft-tissue composition in postmenopausal women. Am J Clin Nutr 1992: 56: 463-6.
  Baumgartner RN, Rhyne RL, Garry PJ, Heymsfield SB. Imaging techniques and anatomical body composition in aging. J Nutr 1993; 123: 444-48.
  Heymsfield SB, McManus C, Smith J, Stevens V, Nixon DW. Anthropometric measurement of muscle mass: project equations for calculation bears for a provided and proposed to the control of th measurement of muscle mass: revised equations for calculating bone-free arm muscle area. Am J Clin Nutr 1982; 36: 680-90.
- 67
- area. Am J Clin Nutr 1982; 36: 680-90. Heymsfield SB, Olafson RP, Kutner MH, Nixon DW. A radiographic method of quantifying protein-calorie undemutrition. Am J Clin Nutr 1979; 32: 693-702. Heymsfield SB, Smith R. Aulet M, Bensen B, Lichtman S, Wang J, Pierson RN. Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry. Am J
- Eston RG, Fu F, Fung L. Validity of conventional anthropometric techniques for
- predicting body composition in healthy Chinese adults. Br J Sp Med 1995; 29(1): 52-56. Kwok T. Woo J, Chan HHL, Lau E. The reliability of upper limb anthropometry in older Chinese people. Int J Obesity 1997; 21: 542-47