Dietary Total Antioxidant Capacity is Closely Associated with Skeletal Muscle Mass: A Cross-Sectional Study

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Short title: [TAC and Skeletal Muscle Mass]



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10.1017/S0007114524002575

The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

# Abstract

Skeletal muscle is of great importance for human activity and quality of life, as its loss contributes greatly to immobilization, especially for aged individuals. An increased dietary intake of antioxidant vitamins may be beneficial for muscle loss because of aging. However, the quantitative relationship between total antioxidation capacity (TAC) of antioxidant vitamins and muscle mass is undetermined. 4009 participants from the National Health and Nutrition Examination Survey (NHANES) were included. Multivariate linear regression analysis was performed with demographic, lifestyle and dietary intake adjustment factors. The dose saturation effect was also determined by a saturation effect analysis. Subgroup analysis were performed forage and sex. In the fully adjusted model, per unit increase of dietary TAC was associated with an increase of 0.018 g/kg appendicular lean mass (95% CI: 0.007-0.029), 0.014 g/kg trunk lean mass (95% CI: 0.004-0.024) and 0.035 g/kg total lean mass (95% CI: 0.014-0.055). TAC was associated with an decrease of 0.004 kg/kg total percent fat (95% CI: -0.006--0.002), 0.005 kg/kg trunk percent fat (95% CI: -0.007--0.002) and 0.003 kg/m2 BMI (95% CI: -0.006--0.001) at the same time. Subgroup analysis indicated that women and adults <50 years may experience the most significant association between TAC and skeletal muscle mass. We revealed a positive correlation between TAC and lean body mass, a negative association between TAC and body fat and BMI. Saturation values were found among people aged 40–59. Age and sex mediate these associations.

Keywords: Total antioxidation capacity; Skeletal Muscle Mass; Aging; NHANES

# Introduction

Skeletal muscle, one of the most dynamic and plastic tissues in the human body, accounts for approximately 40% of the total body weight in humans and is fundamental to movement, energy homeostasis, and overall quality of life<sup>(1; 2; 3)</sup>. However, skeletal muscle mass begins to decline in middle-aged and older adults, and adults between the ages of 40 and 80 have already lost approximately 20% of their skeletal muscle mass during their lifetime<sup>(4; 5)</sup>. Muscle mass decline makes middle-aged and older adults vulnerable to bone fractures and chronic metabolic diseases, such as type 2 diabetes and obesity, leading to a significant increase in healthcare costs<sup>(6; 7)</sup>. Apart from that, muscle loss has even been reported as an independent risk factor for high mortality in older individuals<sup>(8; 9)</sup>. However, effective and strategic muscle-sparing intervention methods for older adults have not yet been revealed.

In recent years, researchers have found that the level of oxidative stress in skeletal muscle increases with age, and the imbalance between increased reactive oxygen species (ROS) production and overall antioxidant defense is one of the leading causes of muscle damage<sup>(10;</sup> <sup>11)</sup>. At the same time, a series of studies have shown that dietary intake of antioxidant vitamins is associated with lower ROS and better-preserved muscle mass<sup>(12; 13; 14)</sup>; Additionally, exogenous supplementation of appropriate amounts of vitamins can protect against muscle loss during aging<sup>(15; 16)</sup>. Total antioxidant capacity (TAC) is a term that reflects the antioxidant potential of dietary sources, which are mainly a combination of various vitamins<sup>(17; 18; 19; 20; 21)</sup>. Researchers believe that TAC participates in the progression of several diseases, such as hypertension and cancer<sup>(22; 23)</sup>. However, the relationship between TAC and muscle loss has been scarcely studied. In patients with liver cirrhosis, researchers found that TAC was positively correlated with grip strength and arm muscle area<sup>(24)</sup>. Other animal experiments have confirmed that antioxidant supplementation can improve skeletal muscle quality<sup>(25; 26)</sup>. Given the higher risk of muscle mass loss in the middle-aged population than in the younger population, studies targeting TAC and muscle loss in this population are urgent and valuable.

Based on the National Health and Nutrition Screening Survey (NHANES) database, the purpose of this study was to investigate the association between dietary TAC of antioxidant vitamins and skeletal muscle mass in middle-aged individuals in the United States after adjusting for potential risk factors.

# Methods

#### **Study population**

NHANES is a representative U.S. population survey that uses complex multilevel probability sampling to provide information on the nutritional status and health status of the general U.S. population. The NHANES research programs were approved by the NCHS Research Ethics Review Committee and received written informed consent from the participants.

This study uses the US NHANES database for the rolling period 2011-2018 (n=39,156). After excluding patients with missing information on demographics, diet, examination, and questionnaires, a total of 4,009 subjects were included in the analysis. Figure 1 shows an example of a selection flow chart.

## **Estimation of TAC from diet**

On the first day of the interview, participants were asked to report in detail all food and beverages consumed in the past 24 hours. Subsequently, after 3-7 days, the researcher collected dietary intake for the past 24 hours again by telephone. The researchers then converted this information into nutrient intakes based on the USDA's Food and Nutritional Database (FNDDS). The antioxidant vitamins recorded in the NHANES dietary interview consisted of vitamin A, vitamin C, vitamin E,  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lycopene, and lutein-zeaxanthin. According to Floegel et al.<sup>(27)</sup>, the individual antioxidant capacity of participants was determined by multiplying the individual amount of antioxidant compounds (antioxidant vitamins) by their antioxidant capacities:

Theoretical TAC= $\Sigma$ (antioxidant content  $\frac{\text{mg}}{100\text{g}}$ \*antioxidant capacity  $\frac{\text{mg VCE}}{100\text{g}}$ )

Antioxidant capacity was measured in the laboratory by chemical combustion and the antioxidant capacity of vitamin C was used as a benchmark to assess the antioxidant capacity

of other vitamins. In our study, we averaged the antioxidant nutrient intakes from the two surveys. TAC was divided into Q1 (0.236 to 22.188 mg VCE/100 g), Q2 (22.188 to 53.255 mg VCE/100 g), Q3 (53.255 to 112.933 mg VCE/100 g), and Q4 (112.933 to 779.247 mg VCE/100 g) according to the survey-weighted quartile.

# Covariates

The demographic factors included age, sex (Men and Women), race (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Other Race), and socioeconomic status (Low, PIR<1.3; Middle,  $1.3 \le$ PIR $\le$ 3.5; High, PIR>3.5). Lifestyle factors consisted of alcohol consumption (yes and no), smoking status (never, former, and now), physical activity (none, moderate, and heavy), and sedentary activity (Table S1). Other factors reported in the study that may influence body mass were obtained from the interview diet data and included protein, dietary fiber, calcium, and phosphorus intake<sup>(28)</sup>.

# **Dependent variables**

There are six dependent variables in this study, including appendicular relative lean mass (relative to body weight, g/kg), trunk relative lean mass (relative to body weight, g/kg), total relative lean mass (relative to body weight, g/kg), total percent fat (percent of body weight, %), trunk percent fat (percent of body weight, %), and body mass index (BMI,  $kg/m^2$ ).

Through dual-energy X-ray absorptiometry (DEXA), the lean body mass (excluding bone mineral content) and fat content of participants' left and right legs, left and right arms, and trunk were measured separately. The appendicular relative lean mass is calculated by summing the lean body mass (excluding bone mineral content) of the left and right legs and arms. In addition, to account for the effects of body weight on these results, all dependent variables are relative to body weight (all lean body mass is per kilogram of body weight g; all fat is per kilogram of body weight kg).

# Statistical analysis

The statistical analysis was conducted by using the statistical computing and graphics software R (version 4.2.1) and EmpowerStats (version 5.0). Continuous variables were compared for between-group differences using t-tests or one-way ANOVA, expressed as mean  $\pm$  standard error (SE), and categorical variables were compared for between-group differences using nonparametric tests, as well as expressed as frequencies (percentages). After satisfying the linear regression assumptions, we determined the beta and 95% confidence interval (CI) by analyzing a multivariate linear regression between the TAC and all outcomes. The multivariate linear regression was built using three models: Model 1: not adjusted; Model 2: adjusted for sex, age, race, and socioeconomic status; Model 3: adjusted for all covariates. In addition, considering the non-normality of the TAC distribution, we again performed multivariate linear regression analyses by log-transforming the TAC. Smoothed curve fits were carried out concurrently with the variable adjustments. We used a threshold effects analysis model to examine the relationship and saturation effect between TAC and body mass. Finally, subgroup analysis was used to determine the population who experienced the most benefit. We used dietary day one sample weight to analyze all the results, and P <0.05 was considered statistically significant.

#### Results

#### **3.1 Descriptions of participants**

The characteristics of weighted demographics, dietary data, and lifestyle of the participants are shown in Table 1. A total of 4,009 participants were included in this study. Of these participants, the average age was 49.69, and 50.18% were man. Among different groups of TAC (quartiles, Q1-Q4), age, sex, race, socioeconomic status, smoking, physical activity, appendicular relative lean mass, trunk relative lean mass, total relative lean mass, total percent fat, trunk percent fat, protein, dietary fiber, calcium, and phosphorus were all significantly different (P < 0.05). The relationships between the dependent variables and the covariates can also be seen in Table S2.

### 3.2 Relationship between TAC and skeletal muscle mass

There was a significant positive association between dietary TAC and lean body mass in three weighted univariate and multivariate linear regression models (Table 2). In the fully adjusted model, each 1-unit increase in dietary TAC was associated with an increase of 0.018 g/kg appendicular lean mass (95% CI, 0.007, 0.029), 0.014 g/kg trunk lean mass (95% CI, 0.004, 0.024) and 0.035 g/kg total lean mass (95% CI, 0.014, 0.055).

Dietary TAC also showed a significant negative association with total percent fat, trunk percent fat, and BMI (Table 3). Assuming linearity, each 1-unit increase in dietary TAC was associated with -0.004 kg/kg total percent fat (95% CI: -0.006, -0.002), -0.005 kg/kg trunk percent fat (95% CI: -0.007, -0.002) and -0.003 kg/m<sup>2</sup> BMI (95% CI: -0.006, -0.001). Furthermore, after log-transforming TAC, a significant association between TAC and skeletal muscle mass was still found (Table S3).

# 3.3 Dose-response relationships and their saturation effect

Figure 2 shows the dose-response relationship between dietary intake and total antioxidant capacity for all outcomes. Combining the smoothing curve and TAC quartile, a saturation effect was found between TAC and all outcomes. Then, a saturation effect analysis explored these turning points and the saturation effect value was 67.433 mg VCE/100 g in the appendicular relative lean mass, 64.072 mg VCE/100 g in the trunk relative lean mass, 64.809 mg VCE/100 g in the total relative lean mass, 67.433 mg VCE/100 g in the total percent fat, 65.955 mg VCE/100 g in the trunk percent fat and 71.167 mg VCE/100 g in BMI (Table 4).

#### 3.4 Subgroup analysis of the association between dietary TAC and skeletal muscle mass

Our study population contained participants aged 40 to 59 years with a mix of both men and women participants, so we also explored how age and sex influenced the aforementioned associations (Table 5, and Figure S1-S2). When stratifying by age, the associations were significant in patients aged 40-50 years rather than in those aged 50-59 years. In the subgroup analysis of sex, women participants had significant associations between dietary TAC and skeletal muscle mass. Therefore, women younger than 50 years may experience the best benefits from dietary TAC.

# Discussion

The present analysis was conducted to determine the relationship between dietary TAC intake and body mass components in adults over 40 years old. The US population data were extracted from the NHANES database. The results showed that for adults who had an increased risk of skeletal muscle mass loss, higher dietary TAC is related to greater preservation of appendicular lean mass, trunk lean mass, and total lean mass. Also, higher dietary TAC intake is associated with lower total percent fat, trunk percent fat, and BMI.

To the best of our knowledge, the association between dietary TAC and skeletal muscle mass has not yet been investigated in a cohort with this size and  $scope^{(29; 30)}$ . Consistent with a previous cross-sectional study in cirrhotic outpatients, dietary TAC was positively associated with arm muscle area<sup>(6)</sup>. In a three-year-long cohort study, higher dietary antioxidant intake had positive effects on BMI and abdominal fat [10]. Another study of children and adolescents showed that dietary antioxidant intake had an inverse association with total body fat in obese subjects<sup>(11)</sup>. Above all, dietary TAC intake has an inspiring effect on lean body mass, fat, and BMI<sup>(31; 32)</sup>.

Although some studies have been deployed to detect the association between antioxidant intake and body components in particular populations, including children and adolescents, women, and healthy young adults, they not only primarily focused on the effects of single antioxidant intake, which might not fully explain the synergistic effects of all antioxidant vitamins in the diet<sup>(12)</sup>, but also provide less knowledge of the middle-aged population who suffer a higher risk of skeletal muscle mass loss<sup>(33)</sup>. In this study, we paid attention to the comprehensive TAC values rather than considering the effects of single compounds, and we focused on the people who may experience greater benefits from the above results.

Although the underlying mechanisms between TAC and body composition were not elaborated in our study, by reviewing previously reported studies, we hypothesized that oxidative stress plays an integral role. Oxidative stress levels in skeletal muscle increase with age, which may lead to impaired muscle protein synthesis and muscle fiber damage<sup>(34; 35)</sup>. Whereas, increased dietary TAC may protect muscles from damage by neutralizing free radicals and reducing oxidative stress. In addition, antioxidants have anti-inflammatory effects and can reduce inflammatory responses in muscle tissue<sup>(36)</sup>. Inflammation is a known

contributor to muscle atrophy; therefore, by reducing inflammation, a high TAC diet may help maintain muscle mass<sup>(37)</sup>.

Dose-response curves suggest that all outcomes displayed a close correlation with dietary TAC. However, there also displayed a saturation effect of correlation between dietary TAC and skeletal muscle mass. All these results indicated that higher dietary TAC would likely improve lean body mass and decrease body fat and BMI. The saturation effect revealed that there was a threshold effect between dietary TAC and all outcomes. A subsequent subgroup analysis indicated that women and individuals aged 40-50 years will experience maximum benefits from higher dietary TAC on skeletal muscle mass. Our findings not only provide possible nutritional interventions for slowing or preventing the decline of muscle mass and function in middle-aged and older adults but also provide detailed recommendations for dietary intake in relation to the challenges of aging regarding muscle loss and fat gain.

However, there are still some limitations in our study. First, this study was a cross-sectional design, which means that the causal relationship between dietary TAC and skeletal muscle mass could not be clearly determined owing to its original survey. Second, vitamin supplementation, such as vitamin C supplementation, is not taken into consideration while only focusing on dietary TAC intake in this design<sup>(38)</sup>. Finally, the bioavailability of dietary vitamins in participants was not included in this study because of the defect value in the NHANES dataset<sup>(39)</sup>. Furthermore, more work should be done to investigate the relationship between serum TAC levels and skeletal muscle mass both clinically and experimentally in the future to figure out their causal effect and potential mechanism.

In summary, our results not only found a simple linear positive association between TAC and lean body mass and a negative association between body fat but also a saturation threshold. This result is encouraging for enhancing health management of muscle loss and fat gain in middle-aged populations.

#### Acknowledgements

We gratefully acknowledge the contributions of all staffs who work on the National Health and Nutrition Examination Surveys.

#### **Conflict of Interest**

The authors declare no conflicts of interest.

#### **Financial Support**

None.

# **Authors' Contributions**

Conceptualization, L.W. and S.C.; Methodology, J.Z. and W.F.; Software, J.Z. and W.F;

Formal Analysis, J.Z. and W.F.; Writing - Original Draft Preparation, J.Z. and W.F.;

Writing – Review & Editing, L.W., J.Z. and W.F.; Supervision, L.W.. All authors have read and approved the final manuscript.

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Figure 1. Flowchart for participant inclusion and exclusion.



Figure 2. Dose-response relationship between dietary TAC and skeletal muscle mass and body fat. (A) Appendicular relative lean mass (g/kg), (B) Trunk relative lean mass (g/kg), (C) Total relative lean mass (g/kg), (D) Total percent fat (kg/kg), (E) Trunk percent fat (kg/kg), and (F) BMI (kg/m<sup>2</sup>).

	All	01	02	02	04	P-val
	(N=4,009)	QI	Q2	Q3	Q4	ue
Age (year)	49.69±0.17	48.91±0.29	49.83±0.21	49.81±0.32	50.22±0.32	0.025
Sex						0.044
Mag	50.18	46.63	52.84	47.08	54.58	
Men	(1.21)	(2.35)	(2.23)	(2.55)	(2.54)	
Women	40.91(1.21)	53.37	47.16	52.92	45.42	
	49.81(1.21)	(2.35)	(2.23)	(2.55)	(2.54)	
Race						0.027
Mexican	9 20 (1 01)	(7)(102)	9.1373	7.02(1.16)	0.97(1.67)	
American	8.39 (1.01)	0.70(1.03)	(1.39)	7.93(1.16)	9.87 (1.67)	
Other	5.96 (0.79)	4.25 (0.97)	5.02 (0.02)	(0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,	7.26(1.08)	
Hispanic	5.80 (0.78)	4.25 (0.87)	5.92 (0.93)	0.08 (1.04)	7.20 (1.08)	
Non-Hisp	66.19	70.50	66.23	66.87	(0, 0, 4/2, 0, 0)	
anic White	(2.23)	(3.10)	(2.66)	(2.76)	60.84(2.98)	
Non-Hisp	10.18	10.25	10.01	9.3991	11.12	
anic Black	(1.02)	(1.52)	(1.30)	(1.13)	(1.14)	
Other	0.29 (0.77)	9.05 (1.05)	9.70(1.10)	0.72(1.00)	10.92	
Race	9.38 (0.77)	8.25 (1.25)	8.70(1.10)	9.72 (1.09)	(1.48)	
Socio-econo						< 0.00
mic status						1
T	18.58	24.07(2.68)	19.27	14.48	16.41	
Low	(1.46)	24.07(2.68)	(1.47)	(1.68)	(1.86)	
N.C. 1.11	31.60	35.94	33.36	30.99	25.85	
Middle	(1.49)	(2.53)	(2.80)	(2.30)	(2.17)	
TT' 1		39.99	47.37	54.53	57.74	
нıgn	49.82(2.04)	(3.07)	(3.07)	(2.68)	(2.81)	
BMI (kg/m <sup>2</sup> )						0.054

# Table 1. Baseline characteristics of the participants grouped by TAC quartiles

Thin		0.76(0.10)	0.83 (0.34)	0.89(0.42)	0.05 (0.53)	0.3663	
	1 11111	0.70 (0.19)	0.85 (0.54)	0.89 (0.42)	0.95 (0.55)	(0.19)	
	Normal	24.81	21.18	21.23	27.99	28.90	
	Normai	(1.03)	(1.73)	(1.65)	(2.27)	(2.04)	
	Overweig	36.59	37.77	37.67	34.76	36.18	
	ht	(1.24)	(2.14)	(2.03)	(2.67)	(2.20)	
Obese	Ohaga	37.84	40.22	40.21	36.29	34.55	
	Obese	(1.32)	(1.93)	(2.09)	(2.45)	(2.43)	
A	Alcohol						0.613
	No	22.63	23.90	20.33	22.62	23.65	
	NO	(1.10)	(2.19)	(1.68)	(2.23)	(2.30)	
<b>N</b> 7	77.37	76.10	79.67	77.38	76.35		
	1 85	(1.10)	(2.19)	(1.68)	(2.23)	(2.30)	
S	moking						< 0.00
		50 56	45.06	50.25	57 11	5676	1
Never	(1, 42)	43.90	(2, 21)	(2.50)	(2, 67)		
		(1.43)	(2.32)	(2.21)	(2.50)	(2.07)	
	Former	24.03	(2.06)	(2,20)	(2.04)	(2.54)	
		(1.02)	(2.00)	(2.20)	(2.04)	(2.34)	
	Now	(1.16)	32.85	(2.06)	(1.01)	(1.80)	
г	N'1	(1.10)	(2.25)	(2.06)	(1.91)	(1.89)	-0.00
r							<0.00
F	Activity		52.09	11 75	41.02	25.90	1
	No	44.25 (1.3)	(2.(2))	44.75	41.93	33.89	
		21.45	(2.03)	(2.12)	(2.74)	(2.55)	
	Moderate	31.43 (1.20)	28.89	34.04 (2.20)	33.21	29.55 (2.22)	
		(1.39)	(2.58)	(2.29)	(2.62)	(2.33)	
	Heavy	24.52	17.13	21.21	24.86	34.56	
-	(1.35)	(2.07)	(2.01)	(2.56)	(2.65)		

Sedentary	423 16+13	457 07+44	405 20+12	122 80+24	202 05+10	
Activity	423.10±13.	437.07±44.	40 <i>3</i> .30±13.	433.80±24.	595.95±10.	0.288
(min/day)	/8	39	81	24	93	
Appendicula						
r relative	274.69±1.1	268.91±1.8	273.86±2.0	275.11±2.4	281.22±1.9	< 0.00
lean mass	0	8	3	4	7	1
(g/kg)						
Trunk relative lean mass (g/kg)	321.92±0.9 7	318.37±1.8 3	322.33±1.3 4	322.17±1.9 1	324.99±1.8 3	0.036
Total relative lean mass (g/kg)	635.72±1.9 5	625.74±3.5 7	635.19±3.1 4	636.75±4.2 3	645.74±3.5 9	0.001
Total percent fat	33.88±0.20	34.91±0.36	33.95±0.32	33.74±0.44	32.86±0.37	<0.00 1
Trunk percent fat	33.45±0.21	34.47±0.32	33.79±0.32	33.02±0.44	32.46±0.39	<0.00 1
Protein (g/day)	84.90±0.91	68.47±1.31	84.33±1.72	92.21±1.77	95.10±2.00	<0.00 1
Dietary fibre(g/day)	17.63±0.30	11.25±0.31	16.03±0.36	20.17±0.61	23.30±0.54	<0.00 1
Calcium	978.90±15.	768.33±23.	953.71±23.	1051.35±31	1150.83±34	< 0.00
(mg/day)	96	79	92	.77	.66	1
Phosphorus	1435.37±17	1159.40±25	1409.23±26	1558.90±33	1622.60±32	< 0.00
(mg/day)	.07	.29	.57	.21	.79	1

Data are presented by % (SE) for categorical variables or mean  $\pm$  SE for continuous variables.

	Append	icular	relative	Trunk	relative	lean mass	Total re	lative lea	an mass
	lean ma	ss (g/kg)		(g/kg)			(g/kg)		
	Model	Model	Mode	Mode	Model	Madal 2	Model	Model	Model
	1	2	13	11	2	Wodel 3	1	2	3
TAC TAC quartil	0.052 (0.036 , 0.068) <0.00 1	0.029 (0.018 , 0.039) <0.00 1	0.018 (0.00 7, 0.029 ) <0.00 1	0.026 (0.01 3, 0.039 ) <0.00 1	0.017 (0.007 , 0.026) <0.00 1	0.014 (0.004, 0.024) 0.008	0.082 (0.053, 0.111) <0.001	0.048 (0.028 , 0.067) <0.001	0.035 (0.014 , 0.055) <0.00 1
es									
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2	4.948 (1.098 , 8.797) 0.012	1.059 (-1.32 7, 3.444) 0.384	-0.03 6 (-2.37 , 2.266 ) 0.976	<ul> <li>3.962</li> <li>(0.92</li> <li>4,</li> <li>7.000</li> <li>)</li> <li>0.011</li> </ul>	1.602 (-0.63 5, 3.839) 0.161	1.247 (-0.948, 3.442) 0.266	9.452 (2.681, 16.223 ) 0.006	3.046 (-1.52 1, 7.612) 0.191	1.752 (-2.67 3, 6.177) 0.438
Q3	6.201 (2.402 , 10.00 1) 0.001	5.778 (3.413 , 8.143) <0.00 1	4.114 (1.73 6, 6.492 ) <0.00	3.806 (0.80 8, 6.805 ) 0.013	4.237 (2.018 , 6.455) <0.00 1	3.931 (1.664,6.19 9) <0.001	11.008 (4.324, 17.691 ) 0.001	10.841 (6.313 , 15.369 ) <0.00	9.153 (4.581 , 13.724 ) 0.002

# Table 2. Multivariate linear regression analysis of TAC and lean mass

	Accepted manuscript								
			1					1	
Q4	12.30 4 (8.431 , 16.17 7) <0.00 1	6.807 (4.374 , 9.239) <0.00 1	3.576 (1.03 9, 6.113 ) 0.006	6.619 (3.56 3, 9.675 ) <0.00 1	4.108 (1.826 , 6.390) <0.00 1	3.087 (0.667, 5.506) 0.012	19.999 (13.18 7, 26.811 ) <0.001	11.655 (6.998 , 16.313 ) <0.00 1	7.609 (2.732 , 12.487 ) 0.002
P for	< 0.00	< 0.00	< 0.00	< 0.00	< 0.00	<0.001	<0.001	< 0.00	< 0.00
trend	1	1	1	1	1	<0.001	<0.001	1	1

Model 1: without adjustment.

Model 2: age, sex, race, and socio-economic status were adjusted.

Model 3: Model 2 plus smoking, alcohol, physical activity, sedentary activity, protein, dietary fiber, calcium, and phosphorus were adjusted.

 $\beta$ , 95% confidence intervals (CIs), and P value are presented.

	Total relevant fat (kg/kg)			Trunk rele	evant fat (kg	/kg)	BMI $(kg/m^2)$		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
	-0.008	-0.005	-0.004	-0.009	-0.007	-0.005	-0.004	-0.003	-0.003
TAC	(-0.011,	(-0.007,	(-0.006,	(-0.012,	(-0.009,	(-0.007,	(-0.006,	(-0.005,	(-0.006,
	-0.005)	-0.003)	-0.002)	-0.006)	-0.004)	-0.002)	-0.001)	-0.001)	-0.001)
	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003	0.008	0.006
TAC									
quartiles									
Q1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	-0.957	-0.316	-0.202	-0.686	-0.309	-0.152	-0.068	-0.008	-0.114
02	(-1.652,	(-0.795,	(-0.666,	(-1.363,	(-0.872,	(-0.697,	(-0.613,	(-0.534,	(-0.650,
Q2	-0.262)	0.162)	0.261)	-0.010)	0.255)	0.393)	0.478)	0.551)	0.422)
	0.007	0.195	0.392	0.047	0.283	0.585	0.808	0.976	0.677
	-1.168	-1.152	-1.011	-1.454	-1.525	-1.304	-0.654	-0.502	-0.786
- <b>-</b>	(-1.854,-	(-1.626,	(-1.490,	(-2.122,	(-2.083,	(-1.867,	(-1.192,	(-1.040,	(-1.340,
Q3	0.482)	-0.677)	-0.532)	-0.786)	-0.966)	-0.741)	-0.115)	0.036)	-0.233)
	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.017	0.068	0.005
	-2.048	-1.209	-0.832	-2.017	-1.545	-1.004	-0.989	-0.817	-0.924
04	(-2.747,	(-1.697,	(-1.343,	(-2.697,	(-2.120,	(-1.605,	(-1.507,	(-1.370,	(-1.514,
Q4	-1.349)	-0.721)	-0.320)	-1.336)	-0.971)	-0.403)	-0.410)	-0.263)	-0.333)
	< 0.001	< 0.001	0.001	< 0.001	< 0.001	0.001	< 0.001	0.004	0.002
P for trend	<0.001	< 0.001	< 0.001	<0.001	< 0.001	<0.001	< 0.001	<0.001	< 0.001

Table 3. Multivariate linear regression analysis of TAC and fat/BMI.

Model: without adjustment.

Model 2: age, sex, race, and socio-economic status were adjusted.

Model 3: Model 2 plus smoking, alcohol, physical activity, sedentary activity, protein, dietary fiber, calcium, and phosphorus were adjusted.

 $\beta$ , 95% confidence intervals (CIs), and P value are presented.

	TAC turning point (K), mg VCE/100 g	< K	> K
Appendicular		0.077	0.006
relative lean mass	67.433	(0.035, 0.118)	(-0.008, 0.019)
(g/kg)		< 0.001	0.396
		0.071	0.003
Irunk relative lean	64.072	(0.029, 0.112)	(-0.009, 0.016)
mass (g/kg)		0.001	0.610
<b>T 1 1 1</b>	64.809	0.171	0.009
Total relative lean		(0.087, 0.254)	(-0.017, 0.034)
mass (g/kg)		<0.001	0.498
<b>T</b> . 1		-0.018	-0.001
Total percent fat	67.433	(-0.027, -0.010)	(-0.003, 0.002)
(kg/kg)		<0.001	0.640
		-0.025	-0.001
Trunk percent fat	65.955	(-0.035, -0.015)	(-0.004, 0.002)
(kg/kg)		<0.001	0.600
		-0.016	0.001
BMI (kg/m <sup>2</sup> )	71.167	(-0.025, -0.006)	(-0.004, 0.002)
		0.001	0.651

Table 4. Saturation effect analysis of TAC on all outcomes

Age, sex, race, socio-economic status, smoking, alcohol, physical activity, sedentary activity, protein, dietary fiber, calcium, and phosphorus were adjusted.

 $\beta$ , 95% confidence intervals (CIs), and P value are presented.

		T1.	T-4-1	T-4-1	T1.	
	Appendicular	Trunk	Total	Total	Trunk	
	relative lean mass	relative	relative	percent	percent	BMI
		lean mass	lean mass	fat	fat	$(kg/m^2)$
	(g/kg)	(g/kg)	(g/kg)	(kg/kg)	(kg/kg)	
Age (year)						
	0.022	0.013	0.039	-0.004	-0.006	-0.004
~50	0.023	(-0.000,	(0.012,	(-0.007,	(-0.009,	(-0.008,
≤30	(0.008, 0.037)	0.027)	0.066)	-0.001)	-0.002)	-0.001)
	0.002	0.055	0.005	0.006	0.001	0.009
	0.014 (-0.000, 0.029) 0.052	0.014	0.031	-0.003	-0.004	-0.003
>50		(-0.000,	(0.003,	(-0.006,	(-0.007,	(-0.006,
		0.027)	0.058)	-0.000)	-0.001)	0.001)
		0.053	0.032	0.025	0.022	0.134
Sex						
	0.007	0.006	0.014	-0.002	-0.003	-0.002
	0.007	(-0.007,	(-0.013,	(-0.004,	(-0.006,	(-0.005,
men	(-0.007, 0.021)	0.019)	0.040)	0.001)	0.000)	0.001)
	0.322	0.350	0.308	0.281	0.067	0.202
	0.022	0.023	0.061	-0.006	-0.007	-0.005
	0.032	(0.008,	(0.032,	(-0.009,	(-0.011,	(-0.009,
women	(0.01/, 0.04/)	0.037)	0.090)	-0.003)	-0.003)	-0.002)
	<0.001	0.002	< 0.001	< 0.001	< 0.001	0.003

# Table 5. Association of dietary TAC with all outcomes stratified by age and sex

Race, socioeconomic status, smoking, alcohol, physical activity, sedentary activity, protein, dietary fiber, calcium, and phosphorus were adjusted.

 $\beta,\,95\%$  confidence intervals (CIs), and P value are presented.