Lower dietary and circulating vitamin C in middle and older aged men and women are associated with lower estimated skeletal muscle mass.

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Running head: Vitamin C associations with fat free mass

Abbreviations: BIA (Bioelectrical impedance analysis); EPIC (European Prospective Investigation into Cancer and Nutrition); FFM (Fat Free Mass); FFM% (Fat Free mass percentage); FFM_{BMI} (Fat Free Mass standardized by Body Mass Index); FFQ (Food Frequency Questionnaire); HRT (Hormone replacement therapy); ROS (Reactive Oxygen Species).

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ABSTRACT

Background: Age-related loss of skeletal muscle mass contributes to poor outcomes including sarcopenia, physical disability, frailty, type-2 diabetes, and mortality. Vitamin C has physiological relevance to skeletal muscle and may protect it during ageing, but few studies have investigated its importance in older populations.

Objective: Investigate cross-sectional associations between dietary and plasma vitamin C with proxy measures of skeletal muscle mass in a large cohort of middle and older-aged individuals.

Methods: We analysed data from >13,000 men and women in the European Prospective Investigation into Cancer and Nutrition Norfolk cohort, aged 42-82 years. Fat free mass (FFM), as a proxy for skeletal muscle mass, was estimated using bioelectrical impedance analysis and expressed as a percentage of total mass (FFM%) or divided by BMI (FFM_{BMI}). Dietary vitamin C intakes were calculated from 7-day food diary data, and plasma vitamin C was measured in peripheral blood. Multivariable regression models, including relevant lifestyle, dietary and biological covariates, were used to determine associations between FFM measures and quintiles of dietary vitamin C or insufficient versus sufficient plasma vitamin C (<50μmol/L and ≥50μmol/L).

Results: Positive trends were found across quintiles of dietary vitamin C and FFM measures for both sexes, with interquintile differences in FFM% and FFM_{BMI} of 1.0% and 2.3% for men and 1.9 and 2.9% for women (all $p<0.001$). Similarly, FFM% and FFM_{BMI} measures were higher in participants with sufficient versus insufficient plasma vitamin C: 1.6% and 2.0% in men, and 3.4% and 3.9% in women (all $p<0.001$). Associations were also evident in analyses stratified into <65y and ≥65y age groups.

Conclusions: Our findings of positive associations, between both dietary and circulating vitamin C and measures of skeletal muscle mass in middle and older aged men and women, suggests that dietary vitamin C intake may be useful for reducing age-related muscle loss.

Keywords: Sarcopenia, skeletal muscle, frailty, vitamin C, ascorbic acid.

LAY SUMMARY

Sarcopenia is characterized by loss of skeletal muscle mass and function during aging and affects more than 50 million people worldwide over 50 years old. Skeletal muscle is involved in voluntary movement and structural support and so a reduction in amount and strength can lead to frailty, reduced quality of life, and death. It may also cause metabolic disturbances, affecting conversion of food to energy in the body, with implications for obesity and type 2 diabetes. Vitamin C is a nutrient found in fruits and vegetables and can help defend the cells and tissues that make up the body from potentially harmful free radical substances. Unopposed these free radicals can contribute to the destruction of muscle, thus speeding up age-related decline. This study examined the relationship between dietary and blood vitamin C and the estimated mass of muscle in individuals within a large group of older men and women. We found people with the highest amounts of vitamin C in their diet or blood had greater estimated muscle mass, compared to those with the lowest amounts. This study suggests that dietary vitamin C is relevant for muscle health in older people and may be useful for preventing age-related muscle loss.

INTRODUCTION

Sarcopenia is characterized by a progressive and generalized loss of skeletal muscle mass and strength (1-4). Increasing age is a well-recognized risk factor for sarcopenia such that worldwide the condition affects over 50 million people over the age of 50 years (2, 3). Whilst maintenance of strength and function is recognized as important for preventing functional limitations, physical disability, and loss of mobility, less recognized are the metabolic disturbances associated with loss of skeletal muscle mass (4-8). These metabolic disturbances include altered utilization of amino acids, glucose, and fatty acids, as well as contributions to the onset of obesity and type 2 diabetes (4-6, 8, 9). Sarcopenia and age-related skeletal muscle loss are also key contributors to frailty. Despite growing appreciation that reducing loss of skeletal mass and function with age is important, current options for prevention are limited.

The etiology of sarcopenia is multi-factorial with several contributory mechanisms including endocrine causes, age-related changes in circulating cytokines, production of Reactive Oxygen Species (ROS), immobility, and low intakes of protein (1). ROS, which are produced during oxidative metabolism in muscle, and from age-related mitochondrial dysfunction, induce cellular damage in muscle, as does the age-related increase in circulating concentrations of inflammatory cytokines (10-12). Vitamin C, a water-soluble vitamin obtained by consumption of fruits, vegetables, and their products in the diet, has several mechanistic functions relevant to skeletal muscle metabolism and physiology, which could prevent age-related loss of skeletal muscle. The mechanisms for vitamin C in skeletal muscle physiology include synthesis of carnitine and collagen and recent animal studies have further elaborated the role of vitamin C deficiency (13-16). As vitamin C is an electron donor this may reduce oxidative damage to muscle as well as reducing the concentrations of inflammatory cytokines in the circulation (11, 12). Vitamin C deficiency, also known as scurvy, is identified by circulating concentrations of ascorbic acid of <11.4 μmol/L, with concentrations <50 μmol/L considered insufficient. Evidence from validation studies indicates that these circulating concentrations are appropriate biomarkers of dietary vitamin C in epidemiological studies (17-20).

Despite knowledge of the mechanisms by which vitamin C can affect skeletal muscle physiology during aging, the importance of vitamin C in relation to skeletal muscle mass has not been extensively studied. We are unaware of previous epidemiological studies where both dietary and plasma vitamin C have been studied in relation to indices of skeletal muscle mass in both sexes and both middle and older age, although some individual studies have been performed previously (14, 21-26). Given the relevance of vitamin C to skeletal muscle physiology and the lack of previous research on the importance of vitamin C to the sarcopenic risk factor of skeletal muscle mass, the purpose of this study was to investigate the associations between dietary and plasma vitamin C and fat free mass (as a proxy measure of skeletal muscle mass) in a large general population cohort of middle and older aged men and women. We thus investigated the cross-sectional associations between indices of fat free mass and dietary intake, as well as circulating concentrations of vitamin C, in a population of 13,000 free-living men and women in the UK in middle and older age. We also sought to determine the associations in those older and younger than 65 years of age.

SUBJECTS AND METHODS

Study population: The European Prospective Investigation into Cancer and Nutrition (EPIC) study is a prospective cohort involving more than 500,000 study participants from 10 European countries, initially designed to investigate the relationships between diet and cancer. Additional outcomes have been examined within the UK EPIC-Norfolk sub-cohort, consisting of 25,639 men and women aged 40-79 y who attended baseline health checks between 1993 and 1997 (27). A second, follow-up, health check was attended by 17,304 participants aged 42–82 y between 1997 and 2000 in which measures of body composition were made. Our analyses were limited to 6350 men and 7989 women with complete data for dietary vitamin C analyses and 5853 men and 7212 women with complete data for plasma vitamin C analyses (see **Figure 1**).

Dietary assessment: Dietary intake of each participant was assessed using a 7-day food diary. The participants recorded all food and drink consumed within a 7-day period, including details of portion sizes. This method has been found to be more accurate than food-frequency questionnaires in

97 estimating dietary nutrient intake when compared with weighted records (18, 28). The Data Into
98 Nutrients for Epidemiologic Research (DINER) software used to document and convert the dietary
99 information provided by the 7-day food diaries into nutrient quantities has been described previously
100 (29). Non-dietary data was collected through health and lifestyle questionnaires at each health check
101 that included questions on smoking, alcohol consumption, social class, occupational history, past
102 history of disease, short family history of main disease end-points, a short section on exercise, and
103 reproductive history for women (27).
104
105 *Body composition:* Each participant had their height and weight measured using standardized
106 methodology (27) at both health checks: Height was measured to the nearest millimetre without shoes,
107 using a freestanding stadiometer; weight was measured to the nearest 0.2kg without shoes and in light
108 clothing, using digital scales. Body fat was measured at the 2nd health check using a bioelectrical
109 impedance analysis (BIA) machine (TANITA Body Fat Monitor/Scale TBF-531), with individuals in
110 the standing position, from which Fat Free Mass (kg) (FFM) was calculated as a proxy measure of
111 skeletal muscle mass. The percentage Fat Free Mass (FFM%; FFM ÷ total mass × 100) and FFM_{BMI}
112 (FFM/BMI) were calculated according to previously established and recommended indices for scaling
113 for the effects of body size on the proportion of FFM (2).
114
115 *Plasma vitamin C:* To obtain vitamin C plasma concentration, non-fasting blood samples were taken
116 from participants using venipuncture. Blood samples were protected from light and without delay the
117 plasma fraction was isolated and immediately stabilized with metaphosphoric acid. Stabilized samples
118 were stored at -70°C and within a week the plasma vitamin C concentration was estimated by
119 fluorometric assay (30, 31).
120
121 *Statistical methods*: STATA statistical software (version 15; Stata Corp.) was used to examine the
122 relationship between Vitamin C and FFM. Sex differences for variables used in our analysis models
123 were tested by independent sample t-test for continuous or chi-square for categorical variables. Our
124 analyses combined dietary data from health check 1, with covariate, body composition data, and

plasma vitamin C data from health check 2. Univariate regression was first used to investigate the differences in FFM across sex-specific quintiles (Q) of dietary vitamin C intake. Due to the established sex differences in body composition all the analyses were stratified by sex. A multivariable model was then tested, incorporating biological (age; menopausal status; HRT status; corticosteroid use; and statin use), lifestyle (smoking status classified as current, former, or never; social class classified by 6 categories; and physical activity status classified as inactive, moderately inactive, moderately active and active), and dietary covariates (total energy; protein intake as a percentage of total energy; number of days participant filled in diary; and the energy intake to estimated energy requirement ratio (EI:EER)). In order to test for trends we used the median values for quintiles as a continuous variable. We also calculated the adjusted values for FFM and used these to determine the percentage differences in FFM between specific vitamin C quintiles. ANCOVA was used to determine whether these differences were statistically significant with a p value less than <0.05. The European Food Safety Authority consider a serum ascorbic acid concentration of less than 11.4 μmol/L as deficient, less than 50μmol/L as insufficient, and 50 μmol/L or more as sufficient (17, 32). As the number classified as deficient in this cohort was small we performed the plasma vitamin C analyses using the broader categorization of insufficiency (plasma vitamin C <50 μmol/L; n=2035 in men and n=1212 in women) versus sufficiency (plasma vitamin C ≥ 50 μmol/L; n=3818 in men and n=6000 in women). Similarly, to the diet analyses, we first tested an unadjusted regression model to identify any differences in indices of FFM according to categories of plasma vitamin C intake. We then tested a model adjusted for age, menopausal status, HRT status, statin use and corticosteroid use, smoking, physical activity status, and social class. All models were repeated stratifying by age group (<65y or ≥ 65y). We performed additional analyses to compare the main models relating dietary vitamin C to FFM indices, calculated with vitamin C contributions from food and drinks only, with models which also included vitamin C contributions from supplements, calculated using data from the vitamin and mineral supplement database (ViMiS) developed for EPIC-Norfolk (33). Univariate regression was used to test the association between dietary and plasma vitamin C. Exclusions were made where participants had missing values for any variables included in the multivariable model (see **Figure 1**). Those who had extreme BIA values (>1000 or <300 ohms) and those who had FFM <25 kg or a BMI

153 ≥36 kg/m^2, were excluded because estimating FFM from BIA including these values would be
154 inaccurate (34). Women who were missing menopausal status, had their status recoded to
155 postmenopausal if > 55y and ever users of HRT, or premenopausal if < 50 and never users of HRT.
156 Any participants missing data for smoking status were recoded as former smokers since there was
157 higher prevalence of smoking in this cohort than in the UK population as a whole. All models were
158 defined a priori using evidence from previous research, and thus P values <0.05 have been considered
159 statistically significant in individual analyses.
160
161 **RESULTS**
162 Participant characteristics are summarized in **Table 1** and **Supplemental Table 1,** for men and
163 women, as mean and SD for continuous variables or percentage for categorical variables. There were
164 6350 men and 7989 women with complete data for dietary vitamin C analyses; for plasma vitamin C
165 analyses, there were 5853 men and 7212 women. Intakes of vitamin C ranged from 36.6 (9.33) (mean,
166 SD) for men in Q1 to 170 (44.8) in Q5 and from 38.9 (9.41) in Q1 to 171 (43.4) in women, and mean
167 interquintile differences were 133 and 132 mg/day, respectively. In this study 0.9% men and 0.2% of
168 women were classified as deficient according to plasma concentrations of <11.4 μmol/L and 34.7% of
169 men and 16.8% of women were classified as insufficient (plasma concentrations <50 μmol/L).
170
171 Positive associations were found between dietary vitamin C and FFM% in both men and women
172 ($p<0.001$, n=6350 men; and $p<0.001$, n=7989 women) after adjustment for covariates, with significant
173 interquintile differences (Q5 vs Q1) in FFM% of +1.0% ($p<0.001$, n=6350) in men, and +1.9%
174 ($p<0.001$, n=7989) in women (see **Table 2).** Similar associations were also found between vitamin C
175 and FFM_{BMI} ($p<0.001$ for both men and women) after adjustment for covariates; and interquintile
176 differences were significant (**see Figure 2)**, with Q5 vs Q1 differences in FFM_{BMI} of +2.3% in men,
177 and +2.9% in women (all $p<0.01$). Results of additional analyses including the contribution of vitamin
178 C from supplements showed positive associations between total vitamin C intakes and FFM measures
179 and no substantial differences to the results of the non-supplement models presented here in full.
180

Significant differences in FFM% were found between individuals with sufficient vs insufficient plasma vitamin C concentrations ($p<0.001$, n=5853 men; and $p<0.001$, n=7212 women) after adjustment for covariates (see **Table 3**). In the adjusted model, men who had sufficient plasma vitamin C concentrations (n=3818) had a mean FFM% 1.6% higher than men with insufficient concentrations (n=2035); in women the difference was 3.4% (n=3818 sufficient versus, n=1212 insufficient). Similarly, significant differences in FFM_{BMI} were found between individuals with sufficient or insufficient plasma vitamin C concentration ($p<0.001$ for both men and women) after adjustment for covariates. In men, the difference between sufficient vs insufficient individuals was +2.0% for FFM_{BMI} in men and +3.9% in women. In age-stratified analyses (<65y and ≥65y) (Figure 2**, Figure 3**, and **Supplementary Tables** 1**, 2, and 3**), although the baselines for FFM measures differed between the age groups, similar significant trends to those found in all-age analyses were evident for dietary or plasma vitamin C and measurements of FFM.

Results from the univariate regression between dietary and plasma vitamin C in the whole cohort population showed that for every 1mg increase in vitamin C intake per day there was an increase in plasma vitamin C concentration of 0.478 μmol/L ($p<0.001$, n=13033). The rate was greater in men than women: 0.647 μmol/L per mg increase in men ($p<0.001$, n=5832), and 0.392 μmol/L per mg increase in women ($p<0.001$, n=7201).

The greatest percentage (%) contributions of different food groups to the daily vitamin C intake of the population were from fruits, vegetables, potatoes and fruit juices (84.4% of the total intake in men and 87.1% in women) (**Figure 4**). Overall, the greatest contribution to vitamin C intake was from fruit intake (26.5% in men and 32% in women), followed by vegetables (25.2% for both sexes).

DISCUSSION

To our knowledge, this is the first study assessing the relationship between dietary and circulating vitamin C and the sarcopenic risk factor of loss of skeletal muscle mass in a large UK cohort of both men and women of middle and older age. Our results show significant positive associations between

dietary vitamin C intake and measures of FFM (as proxies for skeletal muscle mass) using multivariable regression models, adjusted for known lifestyle and biological covariates. The magnitude of differences seen with the indices of FFM between individuals with intakes of vitamin C in the lowest and highest quintiles was greater for FFM_{BMI} than FFM%, with the largest difference of 2.9% seen in FFM_{BMI} of women. Importantly, our findings with dietary vitamin C intake values derived from self-reported food diary data are reinforced and validated by our analyses using biomarker data. These showed similar trends and strong associations of circulating vitamin C and indices of FFM, with statistically significant differences between sufficient and insufficient concentrations with the largest difference of 3.9% seen in FFM_{BMI} of women. While associations were significant for both sexes, the scale of the associations was greater in women than in men. Similar associations were seen in age-stratified analyses for those above and below 65 years of age, although the magnitude of differences in FFM measures varied by age group. Previous studies have shown that people over the age of 50 experience a 0.5-1% loss of muscle mass a year (35), thus suggesting, by comparison, that the magnitude of the differences seen in our analyses could be of clinical importance.

Only a small number of previous studies have investigated associations between either dietary or circulating vitamin C concentrations and indices of skeletal muscle mass or function (21-26). Of the three previous cohort studies investigating indices of skeletal muscle mass and vitamin C, two found significant differences in skeletal muscle mass measures ranging from 1% to 3.2% between extreme quintiles of vitamin C intake in women, and up to 3.5% across quartiles of vitamin C intake in men and women over a follow up period of 2.6 years (24, 26); the third found no associations between skeletal muscle mass measures and plasma vitamin C concentrations (21). Three other studies found no association between intake of vitamin C and prevalence of sarcopenia (36-38). All these previous studies investigating either dietary or circulating vitamin C and FFM, muscle function, or sarcopenia, were in smaller populations than in our study and none investigated associations in a mixed population stratified by sex (21-26, 36-38).

Mechanisms: The mechanistic roles for vitamin C in skeletal muscle physiology include the synthesis of carnitine and collagen. These are important as collagen is a key structural component of skeletal muscle cells and tendons, and carnitine is essential for metabolism of long chain fatty acids during physical activity (15, 16). Animal studies have further elucidated the mechanisms relating to skeletal muscle atrophy, and the morphological changes caused by deficiency of dietary vitamin C. The main drivers appear to be upregulation of the ubiquitin ligases atrogin1/muscle atrophy F-box (MAFbx) and muscle RING-finger protein 1 (MuRF1), and a reduction in production of ROS (13-16). Moreover, muscle atrophy was reversed by re-introduction of vitamin C into the diet in one of these studies (13).

Deficiency and low intakes: In general terms, the prevalence of vitamin C deficiency is greater in men than women and is high in low income and vulnerable populations in care (39-42). Whilst the prevalence of very low plasma concentrations of vitamin C, indicative of scurvy, in our cohort population was 0.9% men and 0.2% of women, more than a third of men (35%) and a sixth of women (17%) were vitamin C insufficient. Within the US 14% of men and 8% of women of a similar age to our study are deficient (43), and the prevalence of insufficiency in the UK population, as a whole, is 57% in men and 39% in women (42). In terms of the dietary guidelines for vitamin C, these range from the older UK guidelines with a recommended UK estimated average requirement (EAR) of 40 mg/d to the European Food Safety Authority (EFSA) guidelines of 90 mg/d in men and 80 mg/d in women (17, 44, 45). Within our cohort 11% of men and 10% of women had intakes of vitamin C below the UK EAR, with the equivalent for the EFSA guidelines, of 59% in men and 47% in women. In our study, only those people in Q4 and Q5 of vitamin C intake consumed amounts at or above the EFSA guidelines, indicating that 60% of the population were consuming insufficient vitamin C.

Given that we found potentially clinically significant effects of insufficient dietary and circulating vitamin C on FFM, strategies to reduce the proportion of individuals with insufficient vitamin C status by increasing vitamin C intake may be beneficial for skeletal muscle health at a population level. This suggestion is also supported by a previous dietary supplementation intervention study where increased

intake of vitamin C caused an increase in concentration of vitamin C in plasma, but also in the vastus lateralis (the largest of the quadriceps leg muscles) (46).

Analysis of dietary data for our cohort showed that for both men and women the greatest contributors to vitamin C intake were fruits, vegetables, potatoes, and fruit juices (Figure 4). Although such foods are typically readily available and easy to prepare and small increases in daily consumption should be achievable, limitations in income, access, and availability, exist in at risk populations. In our cohort the mean interquintile differences in vitamin C intakes for men and women were 133 and 132 mg/day, respectively, more than a fourfold difference between Q1 and Q5. The individuals in Q1, consuming a mean of approximately 40 mg/day, would need to eat the equivalent of one citrus fruit (e.g. an orange), a glass of apple juice and a vegetable accompaniment with a meal (e.g. cabbage or broccoli) to achieve the same vitamin C intake as individuals in Q5.

Strengths and limitations: We believe our study is a significant advance on previous research in this area and has a number of particular strengths. This is the largest study to examine concurrent associations between dietary and plasma vitamin C and measures of skeletal muscle (FFM% and FFM_{BMI}) in both men and women and both middle and older age (21-26). Dietary intakes were calculated using 7-day food diaries. This method provides more accurate estimates of vitamin C than FFQs, which have been shown to systematically estimate intakes of vitamin C by approximately 50% more than the time dependent methods of 7-day diaries or 24 hour recalls (18, 47). The use of plasma vitamin C in this study is advantageous as this measurement accounts for factors that affect the absorption and metabolism of dietary vitamin C (e.g. current smoking habit). This helps to validate our findings since plasma vitamin C is a good biomarker of vegetable and fruit consumption and avoids the potential reporting bias in using dietary intake records (18, 19). However, blood samples were taken from non-fasted participants and thus steady state vitamin concentrations may have been overestimated. We adjusted for recognized risk factors and lifestyle variables, including protein intake, that are known to affect skeletal muscle mass. To acknowledge any potential contribution of vitamin C supplementation, in parallel analyses, we tested models with and without supplementation data and

found that supplementation did not materially alter our findings. A general limitation of our study is the cross-sectional design, which means we cannot infer a causative link between vitamin C intake and skeletal muscle mass measures, and we cannot assess temporality of associations. Dietary data was derived from food intake only, and thus excluded supplements, however when we tested the quantitative contribution of vitamin C supplementation there were no significant changes to our findings. BIA was used to measure body composition, which is regarded to be less precise than DXA measurements. However in healthy individuals, BIA is considered as an accurate, practical non-invasive data collection method, for measurements of body composition when compared with the more precise DXA method (48, 49). For sarcopenia diagnosis both low muscle mass and low muscle function are expected, but presarcopenia (itself a major risk factor for sarcopenia) is characterized by low muscle mass without overt effects on muscle function (50). The metabolic consequences of loss of skeletal muscle are also potentially important for sarcopenia (4). Thus, although muscle function data were not available for our analyses, our study investigating FFM as an estimate of skeletal muscle mass is still valuable for understanding risk of sarcopenia. Our analyses have been conducted on a generally health population cohort and we did not adjust our analyses for chronic disease status which may influence sarcopenia outcomes. Lastly, the EPIC-Norfolk cohort is a population from a geographically defined area, with little outward migration, and consisting mainly of Caucasian participants, thus our results may be less applicable to different ethnic groups.

Overall, our findings suggest that consuming a diet high in vitamin C has potential for protection of skeletal muscle health during aging and provides reinforcement to the benefits of following healthy eating guidelines by consuming sufficient fruits and vegetables. Further studies are required that include longitudinal analyses and intervention trials to investigate the long-term effects of increasing dietary or supplemental vitamin C on skeletal muscle health during ageing.

Conclusion

This study has shown significant positive associations between both dietary and circulating vitamin C and measures of skeletal muscle in a large cohort of free-living middle and older aged men and

women. These results suggest that ensuring sufficient dietary vitamin C intake, by promoting a diet rich in fruits and vegetables, may help to reduce age-related loss of skeletal muscle and thus have wide-reaching public health benefit.

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TABLES

Table 1 Participant characteristics of the EPIC-Norfolk cohort population stratified by sex for the dietary vitamin C (n=14339) and the plasma vitamin C group (n=13065).[1]

Characteristics	**Dietary Vitamin C**		**Plasma vitamin C**	
	Men	**Women**	**Men**	**Women**
	(n=6350)	**(n=7989)**	**(n=5853)**	**(n=7212)**
Age, *years*	62.9 (9.03)	61.5 (9.04)	62.9 (8.98)	61.5 (9.01)
BMI, kg/m^2	26.7 (3.05)	26.1 (3.73)	26.7 (3.02)	26.1 (3.70)
Weight, *kg*	80.9 (10.8)	67.7 (10.4)	80.9 (10.7)	67.6 (10.4)
Height, *cm*	174 (6.61)	161 (6.12)	174 (6.60)	161 (6.13)
FFM%, *%*	76.7 (5.78)	60.9 (8.25)	76.7 (5.74)	61.0 (8.20)
FFM_{BMI}, kg/m^2	2.33 (0.257)	1.58 (0.259)	2.33 (0.256)	1.59 (0.258)
Vitamin C intake, *mg/day*	89.8 (50.7)	93.7 (50.1)	89.9 (50.5)	94.0 (50.3)
Plasma Vitamin C, *µmol/L*	--	--	56.9. (21.3)	68.9 (24.5)
Protein, *g/day*	83.4 (17.6)	66.2 (13.7)	83.5 (17.6)	66.3 (13.7)
Protein *% energy*	14.8 (2.40)	15.5 (2.77)	14.8 (2.39)	15.5 (2.78)
Energy intake, *kcal/day*	2286 (500)	1735 (378)	2289 (501)	1736 (379)
Smoking, *% [n]*				
Current	8.54 [542]	8.71 [696]	8.46 [495]	8.72 [629]
Former	55.5 [3524]	31.9 [2551]	55.6 [3254]	32.1 [2312]
Never	34.0 [2284]	59.4 [4742]	35.9 [2104]	59.2 [4271]
Physical activity, *% [n]*				
Inactive	27.3 [1736]	25.9 [2070]	26.8 [1566]	25.4 [1829]
Moderately inactive	25.1 [1595]	32.5 [2600]	24.9 [1458]	32.9 [2374]
Moderately Active	25.0 [1590]	34.2 [1933]	25.4 [1485]	24.1 [1737]
Active	22.5 [1429]	17.3 [1386]	23.0 [1344]	17.6 [1272]
Corticosteroid use, *% [n]*				
Current	4.16 [264]	5.09 [407]	4.05 [237]	5.06 [365]
Menopausal status, *% [n]*				
Premenopausal	--	5.95 [475]	--	5.92 [427]
Perimenopausal <1y	--	3.33 [266]	--	3.30 [238]
Perimenopausal 1-5y	--	17.5 [1399]	--	17.4 [1256]
Postmenopausal >5y	--	73.2 [5849]	--	73.4 [5291]
HRT use, *% [n]*				
Current	--	21.3 [1704]	--	21.4 [1543]
Former	--	17.9 [1431]	--	17.9 [1288]
Never	--	60.8 [4854]	--	60.8 [4381]
Statins use, *% [n]*				
Yes	5.46 [347]	3.63 [290]	5.43 [318]	3.56 [257]

Number of days participant filled in diary	6.75 (1.16)	6.81 (1.01)	6.75 (1.15)	6.81 (1.01)
Vitamin C supplementation, *% [n]*				
Yes	34.5 [695]	39.8 [1425]	34.1 [633]	39.8 [1281]
Social class, *% [n]*				
Professional	8.24 [523]	6.83 [546]	8.18 [479]	6.74 [486]
Managerial	40.7 [2587]	36.9 [2950]	40.9 [2395]	37.0 [2668]
Skilled Non manual	12.6 [797]	19.4 [1554]	12.4 [723]	19.6 [1413]
Skilled manual	22.4 [1422]	19.7 [1577]	22.4 [1312]	19.8 [1426]
Semi skilled	12.5 [781]	11.9 [950]	12.4 [727]	11.9 [855]
Nonskilled	2.35 [149]	3.34 [267]	2.27 [133]	3.24 [234]

[1] Values are mean (SD) or % [n]. Differences between men and women for all characteristics had p values <0.01, according to t-test for continuous or chi-square for categorical variables. Fat Free Mass Percentage (FFM%), Fat Free Mass standardized by BMI (FFM_{BMI}).

Table 2 Associations between quintiles of dietary vitamin C and skeletal muscle mass in men and women aged 42-82 years.[1]

Men (n=6350)	**Vitamin C**		**FFM%**		**FFM_{BMI}**	
Quintile	Mean± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (n=1270)	36.6 ± 9.33	38.5	76.2 ± 0.169	76.3 ± 0.128	2.27 ± 0.007	2.30 ± 0.007
2 (n=1270)	57.9 ± 5.38	57.7	76.9 ± 0.161**	76.7 ± 0.124*	2.32 ± 0.007***	2.32 ± 0.007*
3 (n=1270)	78.4 ± 6.64	78.3	76.8 ± 0.159*	76.7 ± 0.124*	2.33 ± 0.007***	2.33 ± 0.007**
4 (n=1270)	107 ± 10.1	106	76.8 ± 0.166**	76.8 ± 0.125**	2.34 ± 0.007***	2.34 ± 0.007***
5 (n=1270)	170 ± 44.8	157	76.9 ± 0.156***	77.1 ± 0.125***	2.36 ± 0.007***	2.35 ± 0.007***
Q5-Q1 diff [1]	--	--	0.694 (0.244, 1.14)	0.763 (0.404, 1.121)	0.088 (0.068, 0.108)	0.052 (0.032, 0.071)
% difference [2]	--	--	0.910	1.00	3.88	2.25
P trend	--	--	0.024	<0.001	<0.001	<0.001
Women (n=7989)	**Vitamin C**		**FFM%**		**FFM_{BMI}**	
Quintile	Mean± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (n=1598)	38.9 ± 9.41	40.6	60.1 ± 0.217	60.3 ± 0.166	1.55 ± 0.007	1.56 ± 0.006
2 (n=1598)	62.6 ± 5.93	62.5	61.0 ± 0.208**	60.9 ± 0.162*	1.58 ± 0.007***	1.58 ± 0.006*
3 (n=1598)	83.9 ± 6.75	83.6	61.0 ± 0.207**	60.9 ± 0.162*	1.59 ± 0.006***	1.59 ± 0.006**
4 (n=1598)	112 ± 9.88	111	61.0 ± 0.198**	60.9 ± 0.163*	1.59 ± 0.006***	1.58 ± 0.006**
5 (n=1597)	171 ± 43.4	159	61.5 ± 0.201***	61.5 ± 0.164***	1.61 ± 0.006***	1.61 ± 0.006***
Q5-Q1 diff	--	--	1.40 (0.83, 1.97)	1.14 (0.680, 1.61)	0.064 (0.046, 0.082)	0.05 (0.028, 0.062)
% difference	--	--	2.33	1.90	4.17	2.89
P trend	--	--	<0.001	<0.001	<0.001	<0.001

[1] Values are presented as means ± SEM. The p-trend was calculated using ANCOVA.

[2] Q5-Q1 calculates the absolute difference between the means of quintile(Q) 5 and Q1, with 95% confidence intervals.

[3] % difference calculates the percentage difference between the means of Q5 and Q1.

*p < 0.05; **p < 0.01; ***p < 0.001 versus quintile 1, according to ANCOVA.

Adjusted model includes age, total energy, protein intake as a percentage of total energy, Estimated Energy Requirement, smoking status, physical activity, corticosteroid use, menopausal status, HRT use, statins use, number of days participant filled out in the diary, social class.

Table 3 Associations between plasma vitamin C concentration (<50μmol/L and ≥50μmol/L) and skeletal muscle mass in men and women aged 42-82 years.[1]

Men (n=5853)	**Dietary Vitamin C**		**Plasma Vitamin C**		**FFM%**		**FFM_{BMI}**	
Vitamin C categories	Mean ± SD	Median	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
Insufficient n=2035	72.9 ± 39.7	63.4	35.9 ± 10.8	39.0	75.9 ± 0.131	75.9 ± 0.126	2.29 ± 0.006	2.30 ± 0.006
Sufficient n=3818	99.0 ± 53.3	88.0	68.1 ± 16.5	64.0	77.2 ± 0.090	77.2 ± 0.092	2.35 ± 0.004	2.34 ± 0.004
Absolute diff [2]	--	--	--	--	1.29 (0.980, 1.59)	1.25 (0.943, 1.56)	0.060 (0.046, 0.073)	0.047 (0.033, 0.061)
% difference [3]	--	--	--	--	1.70	1.65	2.61	2.05
P value	--	--	--	--	<0.001	<0.001	<0.001	<0.001
Women (n=7212)	**Dietary Vitamin C**		**Plasma Vitamin C**		**FFM%**		**FFM_{BMI}**	
Vitamin C categories	Mean± SD	Median	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
Insufficient n=1212	73.1 ± 40.6	63.8	37.1 ± 10.1	40.0	59.3 ± 0.260	59.3 ± 0.235	1.53 ± 0.008	1.54 ± 0.007
Sufficient n=6000	98.2 ± 51.0	88.4	75.4 ± 21.4	72.0	61.3 ± 0.103	61.3 ± 0.104	1.60 ± 0.003	1.60 ± 0.003
Absolute diff	--	--	--	--	2.03 (1.53, 2.54)	2.03 (1.53, 2.54)	0.068 (0.052, 0.084)	0.060 (0.044, 0.076)
% difference	--	--	--	--	3.43	3.43	4.43	3.91
P value	--	--	--	--	<0.001	<0.001	<0.001	<0.001

[1] Values are presented as means ± SEM. P values were calculated using ANCOVA comparing the two categories.

[2] Absolute diff calculates the absolute difference between means of the two categories, with 95% confidence intervals.

[3] % difference calculates the percentage difference between means of the two categories.

Adjusted model includes age, smoking status, physical activity, corticosteroid use, menopausal status, HRT use, statins use, and social class.

FIGURE LEGENDS

Figure 1: Flow chart of participants through the study.

Figure 2: Adjusted fat free mass measures for individuals of the EPIC-Norfolk cohort stratified by sex (column A, men; column B, women), age group, and quintiles of dietary vitamin intake (n = 14,339).

*p < 0.05; **p < 0.01; ***p < 0.001 versus quintile 1, according to ANCOVA.
Adjusted model includes age, total energy, protein intake as a percentage of total energy, Estimated Energy Requirement, smoking status, physical activity, corticosteroid use, menopausal status, HRT use, statins use, number of days participant filled out in the diary, social class. Values are presented as mean ± SEM.

Figure 3: Adjusted fat free mass measures for individuals of the EPIC-Norfolk cohort stratified by sex (column A, men; column B, women), age group, and plasma categories of vitamin C (<50 μmol/L and ≥50 μmol/L) (n = 13,065).

Values are presented as mean ± SEM
*p < 0.05; **p < 0.01; ***p < 0.001 versus insufficient, according to ANCOVA.
Adjusted model includes age, smoking status, physical activity, corticosteroid use, menopausal status, HRT use, statins use, and social class.
Men<65 years: Insufficient n=1038; Sufficient n=2171. *Men≥65 years:* Insufficient n=997; Sufficient n=1647. *Women<65 years*: Insufficient n=668; Sufficient n=3745. *Women≥65 years:* Insufficient n=544; Sufficient n=2255.

Figure 4: Percentage composition of food groups for vitamin C intake of individuals of the EPIC-Norfolk cohort.

Men: Fruit (26.5%); Vegetables (25.2%); Juice (18.6%); Potatoes (14.1%); Meat/Protein (3.0%); Dairy (3.0%); Grains (1.6%); Fats/sugar (3.9%); Non-alcoholic beverages (3.2%); Other (0.7%). Women: Fruit (32.0%); Vegetables (25.2%); Juices (19.5%); Potatoes (10.3%); Meat/protein (2.0%); Dairy (2.7%); Grains (1.7%); Fats/sugars (2.7%); Non-alcoholic beverages (2.8%); Other (0.7%). Fruit includes apples, apricots, avocado, bananas, berries, blueberries, citrus, fig/dates, grapes, melon, mixed fruits, other, peach/nectarines, pears and plums. Vegetables include herbs, brassica (cabbage), carrots, cauliflower, cucumber, green beans, other, peas, salad, and tomato. Juices include juices and citrus juice. Meat/protein includes eggs, game, meat products, poultry, red meat, red meat dish, red meat products, and offal. Dairy includes cheeses, cream, milk (semi skimmed, skimmed, full), and yogurts. Grains include bread, oat cereal, cereals, pasta, and rice. Fat/sugars includes savoury biscuits, sweet biscuits, cake, confectionary, fats, jam, syrups, pastry and batter products, cereal puddings, milk-based puddings, savoury sauces, sweet sauces, savoury foods, and sugars. Non-alcoholic beverages include coffee, tea, and squash. Other includes beans, bean dish, dressings, diet meals, meals, nuts, seeds, pickles, and soups.

Figure 1

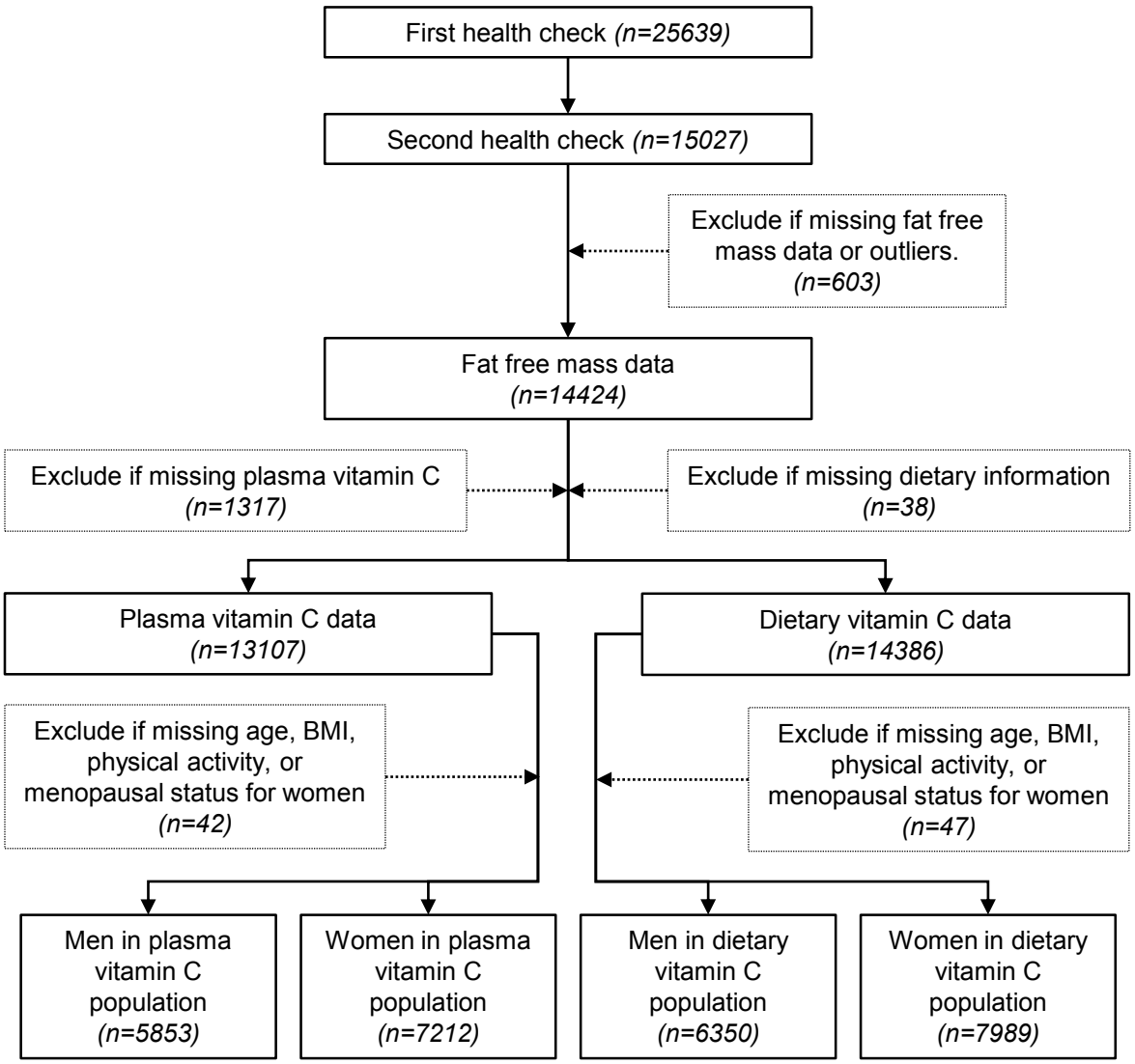

Figure 2

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Figure 2

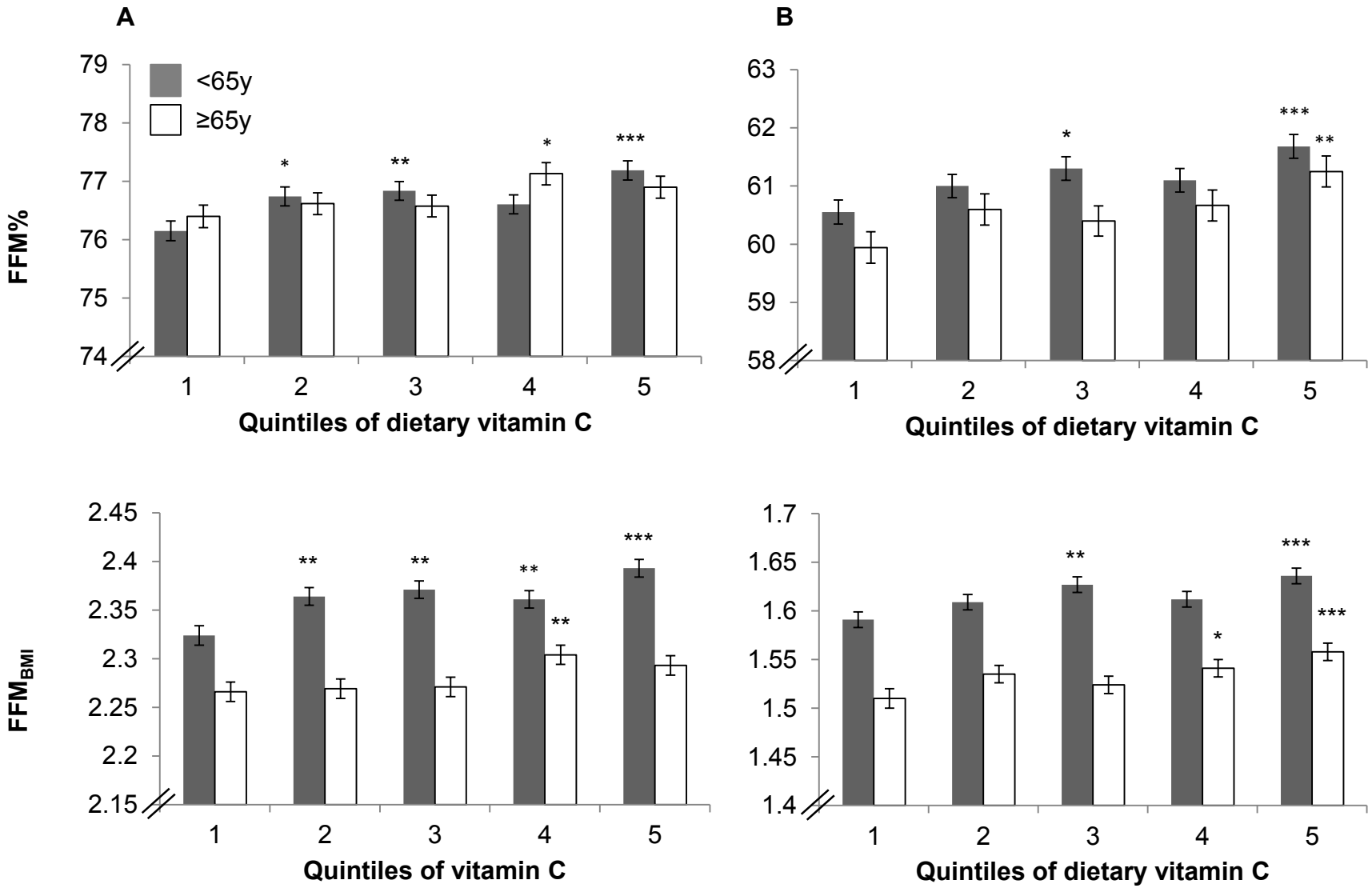


Figure 3

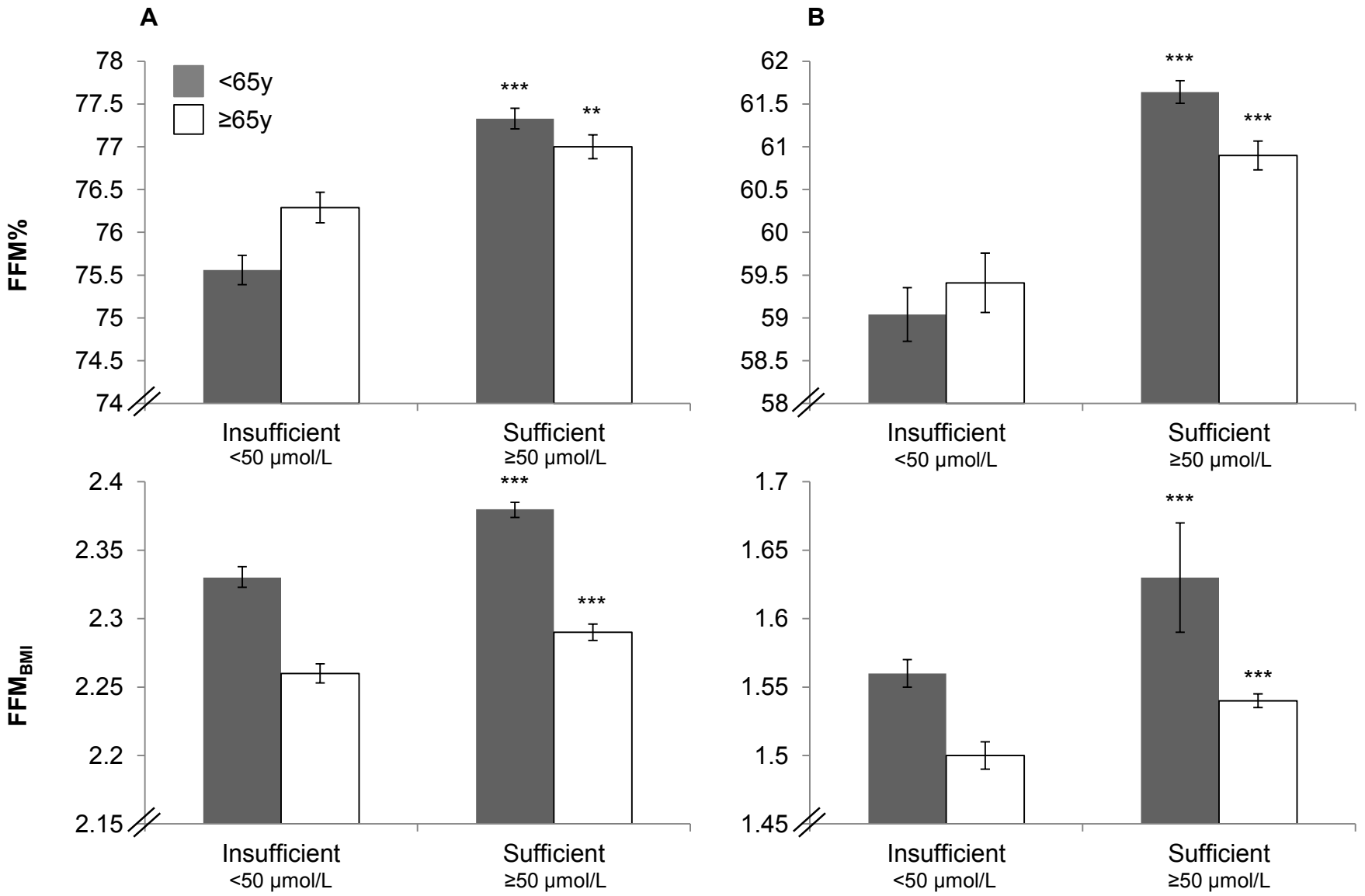
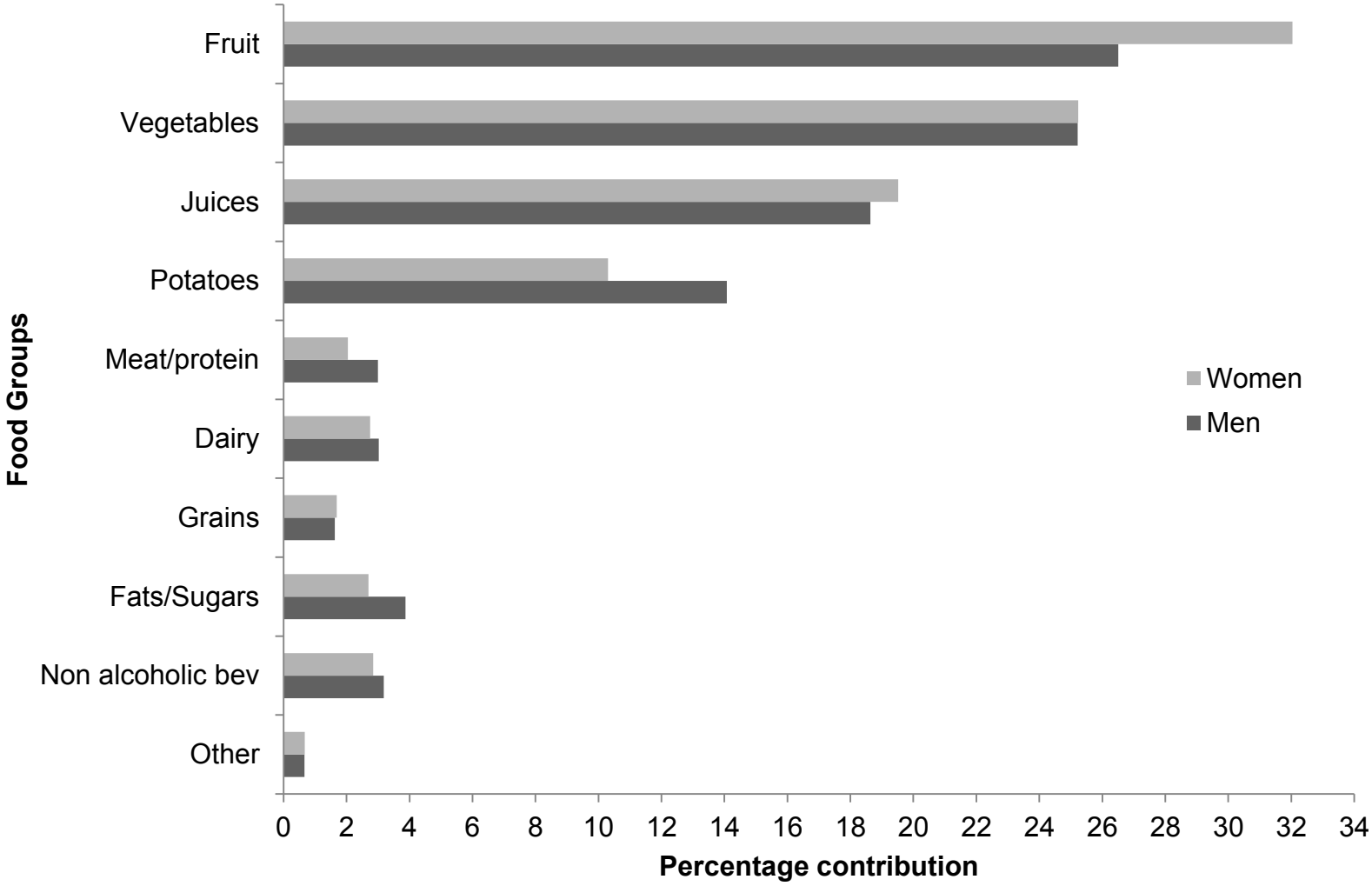


Figure 4



Supplementary Table 1. Participant characteristics of the EPIC-Norfolk cohort population stratified by sex for the dietary vitamin C (n=14339) and the plasma vitamin C groups (n=13065) and also stratified by age group.[1]

	Dietary vitamin C group				Plasma vitamin C group			
	Men		Women		Men		Women	
Characteristic	**<65y (n=3477)**	**≥65y (n=2873)**	**<65y (n=4887)**	**≥65y (n=3102)**	**<65y (n=3209)**	**≥65y (n=2644)**	**<65y (n=4413)**	**≥65y (n=2799)**
Age, *years*	56.0 (5.4)	71.3 (4.2)	55.5 (5.3)	71.1 (4.2)	56.0 (5.3)	71.2 (4.2)	55.5 (5.3)	71.0 (4.2)
BMI, kg/m^2	26.6 (3.1)	26.9 (3.0)	25.8 (3.7)	26.5 (3.7)	26.6 (3.0)	26.8 (3.0)	25.8 (3.7)	26.5 (3.7)
Weight, *kg*	81.9 (10.9)	79.7 (10.5)	68.1 (10.5)	67.1 (10.3)	81.9 (10.9)	79.8 (10.4)	68.1 (10.5)	67.0 (10.2)
Height, *cm*	175.3 (6.5)	172.3 (6.3)	162.3 (5.9)	159.0 (5.8)	175.4 (6.5)	172.3 (6.3)	162.4 (5.9)	159.0 (5.9)
FFM percentage, *%*	76.7 (5.8)	76.7 (5.7)	61.1 (8.3)	60.6 (8.1)	76.8 (5.8)	76.7 (5.7)	61.3 (8.3)	60.6 (8.1)
FFM_{BMI}, kg/m^2	2.4 (0.3)	2.3 (0.2)	1.6 (0.3)	1.5 (0.2)	2.4 (0.3)	2.3 (0.2)	1.6 (0.3)	1.5 (0.2)
Vitamin C intake, *mg/day*	90.4 (51.7)	89.0 (49.6)	93.7 (51.0)	93.6 (48.7)	90.7 (51.5)	89.0 (49.3)	94.0 (51.2)	93.9 (48.9)
Plasma Vitamin C, *µmol/L*					57.2 (19.8)	56.5 (23.0)	69.6 (23.6)	67.8 (25.8)
Protein, *g/day*	86.3 (18.3)	79.9 (15.9)	67.1 (13.9)	64.7 (13.2)	86.4 (18.3)	80.0 (16.0)	67.2 (14.0)	64.8 (13.2)
Protein *% energy*	14.7 (2.4)	15.0 (2.4)	15.4 (2.7)	15.8 (2.8)	14.7 (2.3)	15.0 (2.4)	15.4 (2.7)	15.8 (2.8)
Energy intake, *kcal/day*	2382 (509)	2873 (464)	1776 (386)	1670 (355)	2383 (509)	2173 (466)	1777 (388)	1672 (354)
Smoking, *n (%)*								
Current	333 (9.6)	209 (7)	496 (10)	200 (6)	301 (9)	194 (7)	451 (10)	178 (6)
Former	1716 (49)	1808 (63)	1505 (31)	1046 (34)	1584 (49)	1670 (63)	1363 (31)	949 (34)
Never	1428 (41)	856 (30)	2886 (59)	1856 (60)	1324 (41)	780 (30)	2599 (59)	1672 (60)
Physical activity, *n (%)*								
Inactive	719 (21)	1017 (35)	941 (19)	1129 (36)	646 (20)	920 (35)	827 (19)	1002 (36)
Moderately inactive	843 (24)	752 (26)	1563 (32)	1037 (33)	767 (24)	691 (26)	1430 (32)	944 (34)
Moderately Active	979 (28)	611 (21)	1333 (27)	600 (19)	912 (28)	573 (22)	1190 (27)	547 (20)
Active	936 (27)	493 (17)	1050 (21)	336 (11)	884 (28)	460 (17)	966 (22)	306 (11)
Corticosteroid use, *n (%)*	106 (3)	158 (5)	204 (4)	203 (7)	96 (3)	141 (5)	185 (4)	180 (6)

Menopausal status, *n (%)*								
Premenopausal			473 (10)	2 (0.0006)			425 (10)	2 (0.07)
Perimenopausal<1yr			266 (5)				238 (5)	
Perimenopausal1-5yrs			1381 (28)	18 (0.006)			1239 (28)	17 (6)
Postmenopausal>5yrs			2767 (57)	3082 (99)			2511 (57)	2780 (99)
HRT use, *n %*								
Current			1456 (30)	248 (8)			1323 (30)	220 (8)
Former			1036 (21)	395 (13)			926 (21)	362 (13)
Never			2395 (49)	2459 (79)			2164 (49)	2217 (79)
Statins use								
Yes	153 (4)	194 (7)	103 (2)	187 (6)	141 (4)	177 (22)	90 (2)	167 (6)
Days diary completed	6.6 (1.4)	6.9 (0.9)	6.8 (1.1)	6.9 (0.8)	6.6 (1.3)	6.9 (0.8)	6.8 (1.1)	6.9 (0.8)
Vitamin C supplements	354 (38)	341 (32)	934 (43)	491 (35)	315 (36)	318 (32)	850 (43)	
Social class, *n (%)*								
Professional	283 (8)	240 (8)	348 (7)	198 (6)	261 (8)	218 (8)	310 (7)	176 (6)
Managerial	1433 (41)	1154 (40)	1859 (38)	1091 (35)	1327 (41)	1068 (40)	1689 (38)	979 (35)
Skilled non-manual	407 (12)	390 (14)	843 (17)	711 (23)	363 (11)	360 (14)	761 (17)	652 (23)
Skilled manual	818 (24)	604 (21)	1043 (21)	534 (17)	758 (24)	554 (21)	941 (21)	485 (17)
Semi-skilled	419 (12)	362 (13)	591 (12)	359 (12)	393 (12)	334 (13)	538 (12)	317 (11)
Non-skilled	78 (2)	71 (2)	143 (3)	124 (4)	70 (2)	63 (2)	55 (1)	115 (4)

[1] Differences between men and women. Values are mean (SD) or n (%). Vitamin C group characteristics at first health check. Plasma vitamin C characteristic at second health check (time of BIA measures). Fat Free Mass Percentage (FFM%), Fat Free Mass standardised by BMI (FFM_{BMI})

Supplementary Table 2. Associations between quintiles of dietary vitamin C and fat free mass, adjusted mean values, in men and women aged 42-82 years[1]

Men (n=6350)	**FFM%**				**FFM$_{BMI}$**			
	<65y (n=3477)		**≥65y (n=2873)**		**<65y (n=3477)**		**≥65y (n=2873)**	
Vitamin C Quintile	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
1	75.9±0.23	76.2±0.17	76.6±0.25	76.4±0.19	2.30±0.01	2.32±0.01	2.24±0.01	2.27±0.01
2	76.8±0.22	76.7±0.16	77.0±0.24	76.6±0.19	2.36±0.01	2.36±0.01	2.27±0.01	2.27±0.01
3	76.9±0.22	76.8±0.16	76.5±0.23	76.6±0.19	2.38±0.01	2.37±0.01	2.28±0.01	2.27±0.01
4	76.6±0.23	76.6±0.16	77.1±0.24	77.1±0.19	2.37±0.01	2.36±0.01	2.31±0.01	2.30±0.01
5	77.3±0.21	77.2±0.16	76.5±0.23	76.9±0.19	2.41±0.01	2.39±0.01	2.30±0.01	2.29±0.01
Q5-Q1 difference	1.34 (0.72, 1.95)	1.04 (0.57, 1.51)	-0.08 (-0.74, 0.58)	0.43 (-0.11, 0.97)	0.11 (0.08, 0.14)	0.07 (0.04, 0.10)	0.06 (0.03, 0.09)	0.03 (-0.001, 0.06)
% difference	1.76	1.36	-0.10	0.57	4.80	2.98	2.63	1.20
P trend	0.001	<0.001	0.663	0.05	<0.001	<0.001	<0.001	0.01
Women (n=7989)	**FFM%**				**FFM$_{BMI}$**			
	<65y (n=4887)		**≥65y (n=3102)**		**<65y (n=4887)**		**≥65y (n=3102)**	
Vitamin C Quintile	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
1	60.2±0.28	60.6±0.21	59.9±0.34	59.9±0.27	1.58±0.01	1.59±0.01	1.50±0.01	1.51±0.01
2	61.3±0.27	61.0±0.20	60.5±0.32	60.6±0.27	1.61±0.01	1.61±0.01	1.54±0.01	1.54±0.01
3	61.2±0.26	61.3±0.20	60.7±0.33	60.4±0.26	1.63±0.01	1.63±0.01	1.53±0.01	1.52±0.01
4	61.2±0.25	61.1±0.20	60.6±0.32	60.7±0.27	1.62±0.01	1.61±0.01	1.54±0.01	1.54±0.01
5	61.8±0.26	61.7±0.20	61.1±0.31	61.3±0.27	1.64±0.01	1.64±0.01	1.56±0.01	1.56±0.01
Q5-Q1 difference	1.52 (0.78, 2.26)	1.13 (0.55, 1.71)	1.22 (0.32, 2.12)	1.31 (0.55, 2.06)	0.07 (0.04, 0.09)	0.04 (0.02, 0.07)	0.06 (0.04, 0.09)	0.05 (0.02, 0.08)
% difference	2.52	1.86	2.04	2.18	4.17	2.79	4.14	3.32
P trend	0.001	<0.001	0.015	0.001	<0.001	<0.001	<0.001	0.001

[1]Values are presented as means ± SEM. The P-trend was calculated using ANCOVA. % difference calculates the difference between the mean values of quintile 5 and quintile 1. Adjusted model includes age, total energy, protein intake as a percentage of total energy, Estimated Energy Requirement, smoking status, physical

activity, corticosteroid use, menopausal status, HRT use, statins use, number of days participant filled out in the diary, social class. **Dietary Vitamin C intake (mean ± SD; mg/day, median) by vitamin C quintiles (Q).** *Men<65*years: Q1 (n=685) 36.4±9.6, 38.5;Q2(n=693) 57.8±5.3, 57.7; Q3 (n=699) 78.4±6.6, 78.3; Q4 (n=699) 106.8±10.2, 105.8; Q5 (n=701) 171.0±46.7, 156.7. *Women<65years:* Q1(n=975) 38.7±9.5, 40.6; Q2(n=995) 62.7±5.9, 62.6; Q3(n=961) 83.9±6.8,83.6; Q4(n=979) 111.5±9.9, 111.2; Q5(n=977) 172.0±47.2, 159.2. *Men≥65 years:* Q1(n=585) 36.8±9.0, 38.5; Q2(n=577) 57.9±5.5, 57.7; Q3(n=571) 78.4±6.7, 78.3; Q4 (n=571) 106.1±10.0, 105.8; Q5(n=569) 167.7±42.2, 156.7. *Women≥65 years*: Q1(n=623) 39.2±9.3, 40.6; Q2(n=603) 62.6±5.9, 62.6; Q3 (n=637) 83.8±6.7, 83.6; Q4 (n=619) 112.0±9.8, 111.2; Q5(n=620) 170.3±36.8, 159.2.

Supplementary Table 3. Associations between plasma vitamin C categories and fat free mass, adjusted mean values, in men and women aged between 42-82years stratified by age group[1]

Men (n=5853)	**FFM%**				**FFM_{BMI}**			
	<65y (n=3209)		**≥65y (n=2644)**		**<65y (n=3209)**		**≥65y (n=2644)**	
Vitamin C category	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Insufficient <50µmol/L	75.5±0.18	75.6±0.18	76.3±0.19	76.3±0.18	2.32±0.01	2.33±0.01	2.26±0.01	2.26±0.01
Sufficient ≥50µmol/L	77.4±0.12	77.3±0.12	77.0±0.14	77.0±0.14	2.39±0.01	2.38±0.01	2.30±0.01	2.29±0.01
Q5-Q1 difference	1.87 (1.44, 2.29)	1.77 (1.34, 2.19)	0.64 (0.19, 1.08)	0.71 (0.27, 1.16)	0.07 (0.05, 0.09)	0.06 (0.04, 0.08)	0.04 (0.02, 0.06)	0.03 (0.02, 0.05)
% difference	2.47	2.34	0.83	0.94	3.02	2.49	1.69	1.52
P value	<0.001	<0.001	0.005	0.002	<0.001	<0.001	<0.001	<0.001
Women (n=7212)	**FFM%**				**FFM_{BMI}**			
	<65y (n=4413)		**≥65y (n=2799)**		**<65y (n=4413)**		**≥65y (n=2799)**	
Vitamin C category	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Insufficient <50µmol/L	59.1±0.35	59.0±0.32	59.6±0.26	59.4+0.35	1.56±0.01	1.56±0.01	1.50±0.01	1.50±0.01
Sufficient ≥50µmol/L	61.6±0.13	61.6±0.13	60.9±0.10	60.9±0.17	1.63±0.004	1.63±0.004	1.54±0.003	1.54±0.01
Q5-Q1 difference	2.55 (1.87, 3.22)	2.61 (1.93, 3.28)	1.27 (0.52, 2.03)	1.49 (0.73, 2.25)	0.07 (0.05, 0.10)	0.07 (0.05, 0.09)	0.05 (0.02, 0.07)	0.05 (0.02, 0.07)
% difference	4.31	4.40	2.14	2.51	4.75	4.68	3.07	3.11
P value	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Values are presented as means ± SEM. P values were calculated using ANCOVA comparing the two vitamin C categories. Adjusted model adjusted for age, smoking status, physical activity, corticosteroid use, menopausal status, HRT use, statins use, and social class. **Dietary Vitamin C intake (mean ± SD; mg/day, median) by vitamin C categories.** *Men<65 years*: Insufficient (n=1038) 72.7±40.7, 62.8; Sufficient (n=2171), 99.2±53.9, 87.8. *Men≥65 years:* Insufficient (n=997) 73.1±38.6, 64.0; Sufficient (n=1647) 98.7±54.4, 88.0. *Women<65 years*: Insufficient (n=668) 73.5±40.5, 64.0; Sufficient (n=3745), 97.7±52.0, 87.5. *Women≥65 years:* Insufficient (n=544) 72.6±40.8, 63.7; Sufficient (n=2255) 99.1±49.3, 89.9. **Plasma Vitamin C (mean ± SD; µmol/L, median) by vitamin C**

categories *Men<65 years*: Insufficient (n=1038) 36.5±10.5, 39; Sufficient (n=2171), 67.1±15.0, 64. *Men≥65 years:* Insufficient (n=997) 35.2±11.1, 38; Sufficient (n=1647) 69.4±18.2, 65. *Women<65 years*: Insufficient (n=668) 38.5±9.6, 41; Sufficient (n=3745), 75.2±20.9, 72. *Women≥65 years:* Insufficient (n=544) 35.5±10.4, 38; Sufficient (n=2255) 75.6.1±22.1, 72.