Associations of dietary protein intake with bone mineral density: an observational study in 70,215 UK Biobank participants

Steell L[1,2*], Sillars A[1*], Welsh P[1], Iliodromiti S[4], Wong SC[2], Pell JP[3], Sattar N[1], Gill JMR[1], Celis-Morales CA[1*] and Gray SR[1*],

[1] Institute of Cardiovascular and Medical Sciences, [2] Developmental Endocrinology Research Group, School of Medicine, University of Glasgow, Glasgow, UK [3] Institute of Health and Wellbeing University of Glasgow, Glasgow, UK and [4] Department of Obstetrics and Gynaecology, School of Medicine, University of Glasgow

* denotes joint first and senior authors

Corresponding author. Dr Stuart Gray, Institute of Cardiovascular and Medical Sciences, University of Glasgow, BHF Glasgow Cardiovascular Research Centre, Glasgow, G12 8TA, UK. E-mail: stuart.gray@glasgow.ac.uk

Abstract

Purpose

Adequate dietary protein intake is important for the maintenance of bone health; however, data in this area is ambiguous with some suggestion that high protein intake can have deleterious effects on bone health. The aim of the current study was to explore the associations of protein intake with bone mineral density (BMD).

Methods

We used baseline data from the UK Biobank (participants aged 40-69 years) to examine the association of protein intake with BMD (measured by ultrasound). These associations were examined, in women (n= 39,066) and men (31,149), after adjustment for socio-demographic and lifestyle confounders and co-morbidities.

Results

Protein intake was positively and linearly associated with BMD in women (β-coefficient 0.010 [95% CI 0.005; 0.015, p<0.0001] and men (β-coefficient 0.008 [95% CI 0.000; 0.015, p=0.044]); per 1.0 g/kg/day increment in protein intake, independently of socio-demographics, dietary factors and physical activity.

Conclusions

The current data have demonstrated that higher protein intakes are positively associated with BMD in both men and women. This indicates that higher protein intakes may be beneficial for both men and women.

Keywords

Bone, diet, protein, bone mineral density

Introduction

The current dietary recommendation for protein intake is for all adults to consume 0.8 grams of protein per kg body mass per day (g/kg/day) [1,2]. There is a wealth of research which has suggested that higher protein intakes may be beneficial for the maintenance of muscle mass [3,4], due to protein's anabolic properties [5]. Furthermore, higher protein intakes have been suggested for weight loss due to proteins ability to reduce hunger and promote fullness [6], mitigate reductions in fat-free mass and resting energy expenditure, and augment reductions in plasma triglycerides and blood pressure [7,8]. There have, however, been some concerns that diets high in protein could result in bone mineral depletion – the acid-ash hypothesis which has contributed to the popularity of the so-called "alkaline diet". According to this hypothesis, diets which result in the metabolic production of acid, such as high protein diets, result in demineralisation of the skeleton and an increase in urinary calcium excretion [9]. The data in support of this hypothesis are limited.

Indeed, systematic review of randomized trials and observational studies found no support for the acid-ash hypothesis [10]. On top of this a meta-analysis of randomised trials and prospective cohort studies did not indicate any differences between high and low protein diets in and their associations with BMD and/or bone mineral content loss over time [11]. It is worth noting that in this meta-analysis the conclusions were limited by the clear heterogeneity in the studies in terms of study design, doses, durations and outcomes, as acknowledged by the authors. A more recent meta-analysis concluded that data tentatively supports the hypothesis that protein intakes above the current recommendations may be beneficial for BMD [12], although again limitations in the data were noted including a high level of heterogeneity. Interestingly some data from a small cohort (n=2,919) has indicated that the relationship between protein and bone may not be the same between sexes, with higher protein intakes

being of benefit to women but detrimental to men [13]. Further work, in a larger cohort, is required to determine if the associations between protein intake and BMD differ by sex. The UK Biobank data allows us to test the association between protein intake and BMD in the largest cohort studied which also allows us to investigate potential sex differences in associations.

The aim of the current study, therefore, was to explore the associations of reported protein intake with BMD in UK Biobank, a large general population cohort study of participants aged 40-69 years.

Methods

Study design

UK Biobank is a large, population cohort study. Between 2007 and 2010, 502,628 participants, aged 40–69 years, were recruited and participated in baseline assessments at 22 centres across England, Scotland and Wales. Detailed information was obtained via a self-completed, touch-screen questionnaire and face-to-face interview, and trained staff undertook a series of measurements using standard operating procedures. The main outcome measured in this study was BMD. The independent predictor variable of interest was daily protein intake (g/kg/day). We chose to express protein intake in g/kg/day as these are the units in which current recommendations are given. Socio-demographic factors (age, ethnicity and area-based socioeconomic deprivation index), smoking status, body weight, physical activity, grip strength, sedentary behaviour, total energy intake, and dietary intake (total energy, alcohol, fruit and vegetable, calcium and potassium) were treated as potential confounders.

Study procedures

Dietary information was collected via the Oxford WebQ; a web-based 24 h recall questionnaire which was developed specifically for use in large population studies and has been validated against an interviewer-administered 24 h recall questionnaire [14]. The Oxford WebQ derives energy intake (total and from specific macronutrients) from the information recorded in McCance and Widdowson's, The Composition of Food, 5th edition [15]. For participants who completed more than one online dietary questionnaire, mean values were calculated from all of the information provided. Implausibly low or high energy intakes were defined as less than 1.1 times basal metabolic rate (calculated according to Henry equation [16]) (1.1*BMR)), and greater than 2.5 times basal metabolic rate respectively; the latter being the upper limit of

sustained energy expenditure defined by the Scientific Advisory Committee for Nutrition [17]. These participants were excluded from analyses (n=12,189).

Heel BMD was measured, by trained staff, via ultrasound densitometry (Sahara bone sonometer) using the following formula:

$BMD = 0.002592 \times (BUA + SOS) - 3.687 g/cm^2$

Where BUA is the broadband ultrasound attenuation (dH/MHz) and SOS is the speed of sound (m/s). Single left and right calcaneus measurements were taken and the average used in analysis. The sonometer was turned on for at least one hour prior to any measurements, with a quality control (QC) phantom inserted and the system QC procedures followed at this point. Further details are available on the UK Biobank Website (http://www.ukbiobank.ac.uk).

Height was measured to the nearest centimetre (cm) using a Seca 202 height measure. The duration of light, moderate and vigorous physical activity undertaken over the previous 7 days was self-reported using the International Physical Activity Questionnaire (IPAQ), as described previously [18]. In addition, participants were asked three questions: 'In a typical day, how many hours do you spend watching TV, using a PC, and driving?', and the combined figure was used as a proxy for overall sedentary behaviour [18]. Grip strength was measured as previously described [19] and the mean of the right and left values was expressed in absolute units (kg) for subsequent analysis. Ethnicity was self-reported and categorized into: white, South Asian, black, Chinese, other and mixed. Smoking status was self-reported and classified as: never, former and current. Area-based socioeconomic status was derived from postcode of residence using the Townsend score [20]. Medical history, including menopause status, was collected from the self-completed baseline questionnaire. Further details of these measurements can be found in the UK Biobank online protocol (http://www.ukbiobank.ac.uk).

Statistical analyses

Of the 321,778 people with BMD data we excluded from all analyses individuals who reported any of the following conditions (Chronic Obstructive Pulmonary Disease, chronic asthma, chronic liver diseases, cancer, alcohol problems, substance abuse, eating disorders, sleep apnoea, schizophrenia, cognitive impartment, Parkinsons, dementia, chronic pain syndrome, heart diseases, rheumatoid arthritis, other inflammatory polyarthropathies, osteoporosis, and those who indicated they were unable to walk) and restricted analysis to those who also had data on all co-variates (n=70,215) **(Supplementary figure S1)**.

All analyses were performed stratified by sex. Firstly, to explore a possible linear association between protein intake and BMD, protein intake was first modelled as a continuous variable and changes in BMD were estimated per 1 g/kg/day higher protein intake. Secondly, to explore a potential non-linear dose-response relationship between protein intake and BMD, protein was categorised into <0.8 g/kg/day, 0.8-1.2 g/kg/day, 1.2-1.6 g/kg/day, 1.6-2.0 g/kg/day and >2.0 g/kg/day. Associations of protein intake (continuous or categorical variable) with BMD were investigated using regression analysis. The following statistical adjustments were made: model 0 = unadjusted; model 1 = adjusted for age, ethnicity and Townsend score; model 2 = model 1+ adjusted for smoking, body weight, physical activity, grip strength, sedentary behaviours and dietary intake (total energy, alcohol, fruit and vegetable, calcium and potassium). As a sensitivity analysis, the associations (model 0-2) were performed again but including participants with the aforementioned conditions and adjusted for these as covariates in model 2.

We investigated whether the associations of protein intake with BMD differed by sex by performing a 2-way interaction analysis and fitting a protein*sex term into our model. We then investigated whether the associations of protein intake with BMD differed by sex and other factors by performing a 3-way interaction analysis and fitting a protein*sex*age or physical activity or smoking term into our model. In women only, we investigated whether the associations of protein intake with BMD differed by menopausal status by performing a 2-way interaction analysis by fitting a protein*menopausal status term into our model. We also investigated whether there were interactions between protein intake and calcium intake by performing a 2-way interaction analysis by fitting a protein*calcium intake term into our models.

Ethical Approval

The UK Biobank study was approved by the North West Multi-Centre Research Ethics Committee and all participants provided written informed consent to participate in the UK Biobank study. The study protocol is available online (http://www.ukbiobank.ac.uk/). This research has been conducted using the UK Biobank resource under application number 7155.

Role of funding source

The UK Biobank was supported by the Wellcome Trust, Medical Research Council, Department of Health, Scottish Government and the Northwest Regional Development Agency. It also had funding from the Welsh Assembly Government and the British Heart Foundation. The research was designed, conducted, analysed and interpreted entirely by the authors.

Results

The baseline characteristics of the participants are presented in **Tables 1 (by sex in supplementary tables 1 and 2)** by categories of protein intake and sex. Overall those with higher protein intakes were younger, had lower body weight, were more active and less sedentary, have higher energy, calcium and potassium intake, and more likely to be of non-white ethnicity and never to have smoked.

No interaction (p=0.082) between protein intake*sex on BMD was observed but due to clear sex differences in BMD all analysis was carried out stratified by sex. Within women no interaction (p=0.319) between protein intake*menopausal status on BMD was evident. No interactions were observed between protein intake and calcium (males: p=0.939 and females: p=0.440).

When protein intake was treated as a continuous variable in our model 0 (unadjusted) and model 1 (sociodemographic adjustment) protein intake was negatively associated with BMD in both men and women. However, after adjustment for diet and lifestyle factors protein intake (model 2) was positively associated with BMD in both men and women (women β-coefficient 0.010 [95% CI 0.005; 0.015, p<0.0001] and men (β-coefficient 0.008 [95% CI 0.000; 0.015, p=0.044]); per 1.0 g/kg/day increment in protein intake (**Table 2)**. Similar findings were observed when protein intake was expressed as a categorical variable and these findings, fully adjusted, are visualised in **Figure 1**. These associations appear linear with no obvious evidence of a plateau effect or an inverted U shaped in the relationship. When participants with co-morbidities at baseline were included, similar results were observed **(Supplementary table S3)**.

Our three way interaction analysis revealed no significant interactions between protein intake*sex*smoking (p=0.947) or protein intake*sex*physical activity (p=0.407) but a significant protein*sex*age interaction (p=0.001) was found. Associations between protein intake and BMD were then examined in age tertiles (<54 years, 54-61 years and >61 years) **(Supplementary figure S2 and Supplementary table S4)**. This analysis found that in women the positive associations between protein intake and BMD were similar across the age groups (<54 years β-coefficient 0.008 [95% CI 0.000; 0.016, p=0.030]; 54-61 years β-coefficient 0.014 [95% CI 0.005; 0.023, p=0.003]; >61 years β-coefficient 0.012 [95% CI 0.000; 0.024, p=0.044]) However, in men, when stratified by age, no associations between protein intake and BMD were found (<54 years β-coefficient 0.010 [95% CI -0.001; 0.021, p=0.082;_54-61 years β-coefficient 0.004 [95% CI -0.010; 0.017, p=0.563]; >61 years β-coefficient 0.009 [95% CI -0.006; 0.024, p=0.253]).

Discussion

The main finding of the current study is that higher reported protein intakes are associated with a higher BMD with no evidence of a plateau or inverted U relationship. These relationships were observed after adjustment for sociodemographic, health and lifestyle factors. Previous studies have generally found that high protein intakes were associated with higher BMD and/or a better maintenance of BMD over time, [21–24] although some studies have found no such associations [25,26]. These studies have primarily been carried out in women and so there has been little investigation of whether sex differences in the associations between protein intake and BMD exist. Indeed current data in this area is ambiguous. One small study (n=1280 men and n=1639 women; age range 29-86 years) did report that whilst a higher protein intake was associated with a higher BMD in women no associations were seen in men [13]. However, the opposite has also been shown with a positive association between protein intake and BMD seen in men (n=1168) but not women (n=1164), all over 70 years old [27]. Other work (n=1919 men and n=4591 women) has found that in those over 50 years of age protein intake was positively associated with BMD in both sexes [28].

The current study has been able to investigate differences between sexes in the largest study thus far, and we have found that higher reported protein intakes are positively associated with BMD in both men and women, although the strength of the association is weaker in men. This positive association between dietary protein intake and BMD was not affected by menopausal status (women), smoking status, physical activity levels and age group. As a protein*sex*age interaction was observed analysis was carried out stratified by age categories. In women the positive associations between protein intake and BMD were similar across age categories. However, in men whilst a positive association was seen <54 years of age no associations were

evident >54 years of age. Further work is needed to confirm this finding and to investigate the mechanisms underlying it, which the current study is not able to do.

The current findings, along with several aforementioned studies, would strongly refute the acid-ash hypothesis, which posits that high protein diets result in demineralisation of the skeleton and urinary calcium excretion [9]. Similarly, a recent meta-analysis of randomized control trials and prospective cohort studies in this area has suggested that protein intakes above the current recommendations (0.8g/kg/day) may be beneficial for BMD, although the authors acknowledge the lack of large, long-term and well controlled trials in this area [12].

As high protein intakes are frequently recommended for the maintenance of muscle mass and function [e.g] [29] and weight loss [e.g.] [6] then this would suggest that such practices may not have any negative effects on BMD. That is not to say that caution need not be taken when making recommendations to increase protein intake as there are data to indicate that higher protein intakes can be deleterious for metabolic health. For example it has been shown that high protein intakes (1.2 g/kg/day) eliminate the beneficial effects of weight loss on insulin action seen in those with lower protein intakes (0.8g/kg/day) [e.g.] [30]. The associations we have observed between protein intake and BMD, may be altered by the protein source; this was something we were unable to investigate in the current study. In recent studies, in women, it has been found that protein from vegetable, but not animal, sources was associated with a higher BMD [31–33]. However, these findings are not unequivocal as one study has found the opposite with animal protein intake positively and vegetable protein intake negatively associated with BMD [34]. The reason for these difference remains to be established. As well as the protein sources, specific amino acids have also been found, to have differential associations with BMD, in a twin study of women. This study found that whilst total protein intake had a protective effect

on BMD higher intakes of alanine, arginine, glutamic acid, leucine, lysine and proline were specifically associated with higher BMD [35].

The mechanisms which underlie the positive association between protein intake and BMD have yet to elucidated but may relate to the increase in circulating insulin-like growth factor 1 (IGF-1) associated with increases in protein intake [36]. IGF-1 is known to induce osteoblast proliferation, differentiation and mineralisation *in vitro* [37] and is also necessary for normal bone growth in animal models [38]. There is also data indicating that high protein diets increase intestinal absorption of calcium [10] and this may explain the positive association seen between protein intake and BMD in the current study. Further work is needed to confirm the mechanisms underlying the observations of the current study and the previous literature in this area.

Strengths and limitations

UK Biobank aimed to be representative of the general population in terms of age, sex, ethnicity and socioeconomic status but is unrepresentative in terms of lifestyle, with participants less likely to be obese and have lower disease frequency – indicative of a "healthy volunteer" selection bias [39]. Therefore, caution should be heeded in generalizing summary statistics to the general population. This does not detract from the ability to generalize estimates of the magnitude of associations. Our study benefited from a very large number of participants, recruited from the general population, across the whole of the UK. Because of the cross-sectional nature of this study we were unable to determine the temporal relationships between diet and BMD and, as with any observational study, association may not necessarily infer causation.

The greatest sources of uncertainty for all nutritional epidemiology, including the present analyses, lie in the estimation of long-term exposure to food and drinks intakes, and then from the application of standard food composition tables to quantify protein consumption [40]. All methods of dietary assessment can incur extensive errors and biases, which are diminished, but not eliminated, by studying large numbers [40,41]. Dietary intake was self-reported outside the clinic, which may encourage more truthful reporting, and was collected using a 24 h recall questionnaire which has been shown to produce more accurate results than a food frequency questionnaire (the usual approach adopted in large-scale studies) [42]. Accuracy was further improved by administering the questionnaire on four occasions over the course of a year and deriving mean values. In addition, online administration of the questionnaires is expected to minimize any reporting bias due to social desirability. Although not the gold standard method for measuring BMD, heel ultrasound has been shown to correlate strongly with DXA (dual energy x-ray absorptiometry) and is a valid technique in epidemiological studies [43].

In conclusion, the current study has shown that higher dietary protein intake is associated with higher BMD in both men and women. These data are supported by the findings of a recent meta-analysis [12] although the lack of long term trials were highlighted. Together this data indicates the need for large scale randomised controlled trials in this area.

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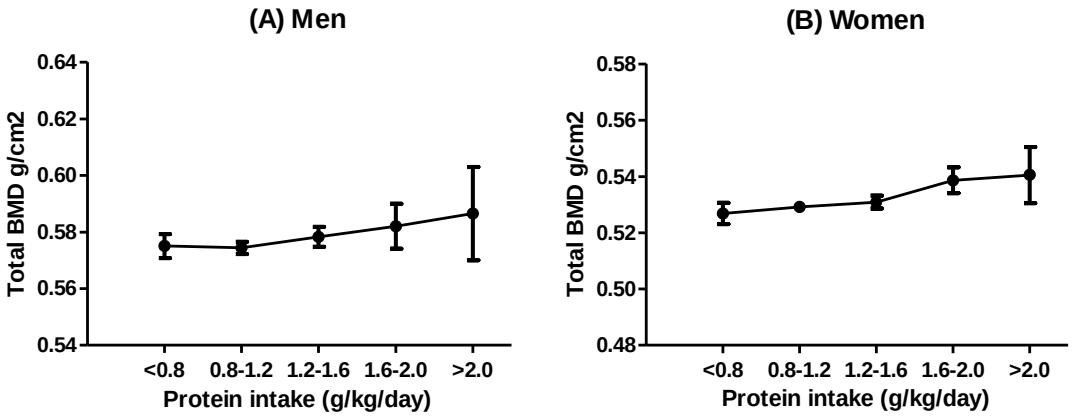

Figure 1 Heel BMD by protein intake in men (A) and women (B)

Data presented as adjusted mean and their 95%CI. Analyses are model 2, so adjusted for age, ethnicity, Townsend score, smoking, body weight, physical activity, sedentary behaviours, total energy intake, alcohol intake, fruit and veg intake, calcium intake and potassium intake.

Table 1 Cohort descriptive characteristics by reported protein intake categories in all participants

	Category of Daily Protein Intake (g.kg^{-1}.day^{-1})					
	Overall	**<0.8**	**0.8 – 1.2**	**1.2 – 1.6**	**1.6 – 2.0**	**>2.0**
Socio-demographics						
Total, n	70215	11121	34024	19724	4481	865
Women, n (%)	39066 (55.6)	5382 (48.4)	17727 (52.1)	12315 (62.4)	3069 (68.5)	573 (66.2)
Age (years)	55.2 (7.8)	55.0 (7.7)	55.5 (7.7)	55.2 (7.8)	54.5 (8.0)	53.0 (8.0)
Ethnicity, n (%)						
White	68637 (97.8)	10838 (97.5)	33409 (98.2)	19273 (97.7)	4331 (96.7)	786 (90.9)
Black	364 (0.5)	77 (0.7)	147 (0.4)	91 (0.5)	26 (0.6)	23 (2.7)
South Asian	399 (0.6)	79 (0.7)	171 (0.5)	104 (0.5)	32 (0.7)	13 (1.5)
Chinese	159 (0.2)	10 (0.1)	41 (0.1)	56 (0.3)	32 (0.7)	20 (2.3)
Mixed background	308 (0.4)	59 (0.5)	124 (0.4)	92 (0.5)	24 (0.5)	9 (1.0)
Other	348 (0.5)	58 (0.5)	132 (0.4)	108 (0.5)	36 (0.8)	14 (1.6)
Deprivation Index	-1.93 (2.85)	-1.64 (2.99)	-1.96 (2.84)	-2.06 (2.77)	-1.90 (2.90)	-1.66 (3.08)
Deprivation, n (%)						
1 (Least Deprived)	22459 (32.0)	3191 (28.7)	10985 (32.3)	6572 (33.3)	1448 (32.3)	263 (30.4)
2	19054 (27.1)	2954 (26.6)	9214 (27.1)	5469 (27.7)	1199 (26.8)	218 (25.2)
3	16020 (22.8)	2594 (23.3)	7808 (23.0)	4419 (22.4)	993 (22.2)	206 (23.8)
4 (Most Deprived)	12682 (18.1)	2382 (21.4)	6017 (17.7)	3264 (16.6)	841 (18.8)	178 (20.6)
Smoking Status, n (%)						
Never	41491 (59.1)	6075 (54.6)	19666 (57.8)	12354 (63.4)	2840 (63.4)	556 (64.3)
Former	23905 (34.0)	4117 (37.0)	12058 (35.4)	6128 (31.1)	1356 (30.3)	246 (28.4)
Current	4819 (6.9)	929 (8.4)	2300 (6.8)	1242 (6.3)	285 (6.4)	63 (7.3)
Body Composition & physical activity						
Body weight (kg)	76.1 (14.8)	85.8 (16.5)	78.0 (13.5)	70.2 (11.9)	65.8 (11.9)	64.1 (13.0)
Heel BMD (g.cm^{-2})	0.551 (0.130)	0.600 (0.137)	0.552 (0.129)	0.544 (0.128)	0.545 (0.131)	0.551 (0.142)
Total Sedentary Time (hours.day^{-1})	4.9 (2.1)	5.2 (2.3)	4.9 (2.1)	4.6 (2.0)	4.5 (2.0)	4.5 (2.1)
Total PA (MET-min.wk^{-1})	2345.1 (2971.1)	2197.2 (3114.5)	2278.7 (2890.1)	2459.8 (2958.4)	2591.3 (2994.0)	2970.2 (3985.3)
Handgrip strength (kg)	32.2 (10.7)	33.7 (10.9)	32.9 (10.8)	30.8 (10.2)	29.7 (9.9)	29.7 (10.6)
Dietary Intake						
Total energy Intake (kcal.day^{-1})	2169.6 (547.1)	1797.9 (424.5)	2093.5 (470.6)	2373.6 (533.9)	2639.4 (591.9)	2855.0 (622.5)
Fruit & vegetable Intake (portions.day^{-1})	4.2 (2.3)	4.1 (2.4)	4.2 (2.2)	4.3 (2.2)	4.4 (2.4)	4.6 (2.7)
Alcohol Intake (g.day^{-1})	2.7 (1.4)	2.8 (1.5)	2.6 (1.4)	2.6 (1.4)	2.8 (1.4)	2.9 (1.5)
Protein Intake (% of TE)	15.5 (3.3)	13.1 (2.9)	15.3 (3.0)	16.5 (3.1)	17.8 (3.5)	20.4 (4.5)
Fat Intake (% of TE)	32.3 (6.4)	31.3 (7.1)	32.1 (6.3)	32.8 (6.1)	33.3 (6.4)	33.5 (6.6)

Carbohydrate Intake (% of TE)	47.0 (7.8)	49.2 (8.5)	47.1 (7.5)	46.1 (7.4)	45.0 (7.8)	42.2 (9.0)
Calcium Intake ($mg.day^{-1}$)	978.1 (328.3)	778.5 (242.2)	936.4 (278.1)	1088.0 (355.6)	1243.8 (408.4)	1298.4 (444.2)
Potassium Intake ($mg.day^{-1}$)	3772.8 (1050.8)	3025.0 (825.4)	3624.0 (867.6)	4172.7 (1013.9)	4726.8 (1219.6)	5180.2 (1311.5)

Data presented as mean (SD) or % (n) for continuous and categorical variables as appropriate. TE: total energy intake; SD: standard deviation; MET: metabolic equivalent; BMD: bone mineral density.

Table 2 Association between protein intake and BMD

Men	**Total N**	**β-coefficient (95%CI)**	**p-value**
Model 0	31,149	-0.009 (-0.137; -0.004)	0.001
Model 1	31,149	-0.010 (-0.015; -0.005)	<0.0001
Model 2	31,149	0.008 (0.000; 0.015)	0.044
Women			
Model 0	39,066	-0.004 (-0.008; -0.001)	0.017
Model 1	39,066	-0.005 (-0.008; -0.001)	0.005
Model 2	39,066	0.010 (0.005; 0.015)	<0.0001

Model 0 = unadjusted

Model 1 = adjusted for age, sex, ethnicity and Townsend score

Model 2 = model 1 + adjusted for smoking, body weight, physical activity, grip strength, sedentary behaviours, total energy intake, alcohol intake and fruit and veg intake.