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INCREASED CONSUMPTION OF PLANT FOODS IS ASSOCIATED WITH INCREASED BONE MINERAL DENSITY

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Abstract: *Objectives:* To determine the relationship between plant food consumption and bone mineral density (BMD) in a healthy population when age, gender, BMI and physical activity are accounted for. *Design:* Cross-sectional study. *Setting:* Participants were recruited from the Sydney Adventist hospital and the University of New South Wales, Sydney, Australia. *Participants:* 33 males and 40 females (total n=73) participated in this study. The mean age was 56.1 ± 8.5 years. All participants were non-diabetic and in general good health. *Measurements:* A principle component analysis (PCA) was performed on 12 month self-report food intake data, gathered using the Cancer Council Victoria Dietary Questionnaire for Epidemiological Studies Version 2. Dual-energy X-ray absorptiometry was used to measure total BMD. Fasting plasma total protein, calcium and 25-Hydroxy Vitamin D levels were analysed by the Sydney Adventist Hospital pathology laboratory. Anthropometric measures were obtained using a standardized protocol. Self-reported physical activity levels were assessed using the International Physical Activity Questionnaire. *Results:* The PCA revealed three principle components. These were termed 'Meat Based', 'Junk Food' and 'Plant Based.' After controlling for age, gender, physical activity and BMI, the Plant Based component correlated positively with BMD (p=0.054, R2=0.439) and T-score (p=0.053, R2=0.221). Using a similar model no association between the Meat Based component and BMD or T-score was found. However, when the Plant Based component was included the Meat Based component correlated positively with BMD (p=0.046, R2=0.474) and T-score (p=0.046, R2=0.279). There was no significant association between the Junk Food component and BMD or T-score. People in the third Plant (927 ± 339 vs 751 ± 255 g/day, p=0.025) and Meat Based (921 ± 270 vs 676 ± 241 g/day, p=0.002) tertile had higher calcium intakes than those in the first. People in the second Plant Based tertile had higher plasma Vitamin D levels than those in the first (63.5 ± 16.8 vs. 52.3 ± 22.1 nmol/L, p=0.053) while those in the third Junk Food tertile had lower levels than those in the first (52.4 ± 18.5 vs. 65.4 ± 19.8 nmol/L, p=0.027). No association between Plant Based tertiles and protein intake was observed, however those in the third Meat Based (99.7 ± 25.1 vs. 50.9 ± 13.8 g/day, p=0.000) and Junk Food (87.4 ± 30.7 vs. 56.6 ± 22.2 g/day, p=0.000) tertile had higher protein intake compared to those in the first tertile. *Conclusion:* In a healthy middle aged population with normal BMD, an increase in plant food consumption, either alone or in combination with a diet containing meat, is associated with improved bone mineralisation markers. This positive relationship is most likely due to the extensive range of micronutrients and phytochemicals packaged within plants.

Key words: Bone mineral density, osteoporosis, dietary, vegetables, vegetarian.

Introduction

Osteoporosis is estimated to affect 200 million people worldwide (1). Due to its high global prevalence and significant impact on morbidity and mortality rates, this major epidemic is of serious public health concern.

Literally meaning 'porous bone', osteoporosis is a disease characterised by reduced bone formation, excessive bone loss, or a combination of both, leading to bone fragility and an increased risk of fractures (2). Bone mineral density (BMD) is the most robust and consistent predictor of osteoporotic fracture (3, 4). It is a non-invasive marker of bone mass and is considered a surrogate for bone strength, enabling an individual's future fracture risk to be predicted.

Several factors affect BMD including age, gender and physical activity levels (5, 6). A large number of nutritional components can also positively or negatively influence bone mass. These range from inorganic minerals e.g. potassium, calcium, phosphorus and magnesium (7-9), vitamins e.g. vitamins A, C, D, K, and certain B vitamins (10-14) and macronutrients e.g. protein and fatty acids (8, 15, 16) to phytonutrients including carotenoids such as β-carotene and lycopene (17).

The effect of nutritional components on bone health may occur through either direct mechanisms, such as vitamin K2 stimulation of 1,25-dihydroxyvitamin D3 induced osteoblastic mineralization (10), or indirect mechanisms. For example inadequate copper intake has been shown to reduce bone strength through IGF-1 mediated pathways (18, 19). The consequence of these endocrine /paracrine systems or bone metabolism modifications is alteration in bone structure and ultimately strength.

In addition to direct intake concentrations, the relative proportion of nutritional components may also affect bone health and thus osteoporosis risk. For example while sufficient protein ingestion is considered essential for bone formation and

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maintenance (20), if consumed in high disproportion to other nutrients (i.e. complex carbohydrates) the effect on bone health may be adverse (21).

In terms of broader dietary patterns, plant based diets (i.e. vegan/vegetarian) are generally higher in complex carbohydrates and lower in protein as well as calcium, than those that contain meat. On the other hand adherence to a plant based diet is associated with higher intake of other nutrients such as vitamin K2 and isoflavones known to positively influence osteoblastic activity and mineralization (22). The long term effect of plant-based diets on bone health is thus difficult to assess and somewhat contentious with a recent meta-analysis linking vegetarian and vegan diets to lower BMD and higher risk of fracture (23).

In Western countries a growing proportion (24, 25) of the population choose to follow a predominately plant based diet and increased consumption of fruit and vegetables is advocated by a number of health professionals and associations. However it has yet to be established whether the consumption of more plant foods, in addition to or in replacement of animal products, is beneficial to bone health in mid-life.

Importantly the majority of studies in the current literature focus on singular components without taking into account other factors, such as physical activity or BMI, that are known to effect BMD. Further, few simultaneously quantitate dietary intake or physiological concentrations of the more complex array of relevant nutritional components. In order to address these limitations this cross-sectional study sought to determine whether increased plant consumption, regardless of other dietary influences, was associated with BMD in a healthy population while accounting for age, gender, BMI and physical activity.

Methods

Participants

In this cross-sectional study male (n=48) and female (n=52) participants aged between 40 and 75 years were recruited at the Sydney Adventist hospital and the University of New South Wales, Australia. After principle component analysis only 73 participants remained (male, n=33; female, n=40) (see Statistical Analysis below). Participants were non-diabetics and considered in general good health. Blood collection and dual-energy x-ray absorptiometry (DXA) scanning were performed on the same day in a fasted state (approximately 12 hours). Physical activity and nutritional questionnaires were completed no more than 2 weeks prior.

Ethical approval was obtained from The Adventist HealthCare Limited Ethics Committee, Australia (EC00141). Written informed consent was obtained from all participants.

Total Body Bone Mineral Density & T-score

Dual-energy X-ray absorptiometry (DXA) (Lunar iDXA GE Healthcare, Madison, WI, USA) with automatic total body scan mode and enCORE software (version 16, GE Healthcare, Madison, WI, USA) was used to measure total body bone mineral density (BMD).

Prior to scanning participants were asked to change into a standard cloth gown and remove all metal items. Participants were scanned by trained operators and were correctly centred on the scanning table in a supine position with arms at sides slightly separated from the trunk and the palms facing the thighs. Quality control scans were obtained daily using the manufacturer supplied spine calibration phantom.

Total body cuts were automatically drawn by the software according to anatomical landmarks and then double-checked by a trained operator and adjusted if required.

Ethnically adjusted T-scores were derived using the manufacturer's software by comparison to a healthy young adult reference population on a standard deviation scale. A negative T-Score indicates an individual's BMD is below the Young Adult value. A positive T-Score indicates the patient's BMD is above the Young Adult value. T-scores below -1.0 are indicative of low bone density or osteopenia.

Plasma Protein, Calcium & Vitamin D

Fasting plasma total protein, calcium and 25-Hydroxy Vitamin D levels were analysed by the Sydney Adventist Hospital pathology laboratory. Fasting plasma total calcium and protein levels were determined by a photometric method on a Roche/Hitachi Cobas c system. 25-Hydroxy Vitamin D levels were quantitated on an IDS-iSYS instrument using a chemiluminescence immunoassay.

BMI

Anthropometric measures were obtained using a standardized protocol. Weight (kg) and height (cm) were measured to the nearest 0.5 kg and 0.1 cm, with participants wearing a cloth gown and without shoes. BMI was calculated as weight in kilograms divided by the square of the height in meters.

Physical Activity

Self-reported physical activity levels were assessed using the long version of the International Physical Activity Questionnaire. MET minutes per week (METmin/wk) were calculated accordingly to the developers' instructions and used in statistical analysis (26).

Sun Exposure

Sun exposure was estimated by asking the following question: "About how many hours a day would you usually spend outdoors on a weekday and on the weekend?" with open entry responses for a weekday and weekend day. This question has been previously validated in Australian adults to reflect diary-recorded time outdoors and UVR dosimeter dose (27). Results were used to calculate the average time spent outdoors each week.

Table 1
Food items included in PCA

Food Items Retained	Vegetables	Tomatoes + Capsicum + Lettuce + Cucumber + Celery + Beetroot + Carrots + Cabbage + Cauliflower + Broccoli + Spinach + Peas + Green beans + Pumpkin + Mushrooms + Zucchini
	Beans	Other beans + bean sprouts
	Total Fruit	Oranges + Apples + Pears + Bananas + Melon + Pineapple + Strawberries + Apricots + Peaches + Mango + Tinned fruit
	Pork	Pork
	Chicken	Chicken
	Red Meat	Beef + Veal + Lamb
	Processed Meat	Salami + Sausages + Ham + Bacon
	Total Fish	Fish + Tinned fish
	Total Alcohol	Light beer + Heavy beer + Red wine + White wine + Fortified wine + Spirits
	Total Cheese	Hard cheese + Firm cheese + Soft cheese + Ricotta or cottage cheese + Cream cheese + Low fat cheese
	Soy	Soymilk + Tofu
	Total Nuts	Nuts + Peanut butter
	Cereal	All Bran + Bran flakes + WeetBix + Cornflakes + Porridge + Muesli
	Refined Carbohydrates	White bread + Pasta + Rice
	Fast Food	Meat pies + Pizza + Hamburger
	Sweet Food	Sweet Biscuits + Cakes + Sugar + Chocolate + Ice cream.
	Savoury Snack	Crisps + Crackers + Chips
	Caffeine	Caffeine
Food Items Removed	Dairy	Full cream milk + Butter + Hard cheese + Firm cheese + Soft cheese + Ricotta or cottage cheese + Cream cheese + Low fat cheese + Yoghurt
	Total Margarine	Margarine + Polyunsaturated margarine + Monounsaturated margarine
	Eggs	Eggs
	Bread	White bread + High fibre white bread + Whole meal bread + Ryebread + Multigrain bread.

Nutrition

Self-report food intake was assessed using the Cancer Council Victoria Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2). This questionnaire asks participants to report their usual consumption of 74 foods and six alcoholic beverages over the preceding 12 months using a 10-point frequency scale. Additional questions are asked about the number and size of serves or type of fruit, vegetables, bread, dairy products, eggs, fat spreads and sugar. Nutrient intakes were computed from NUTTAB 2010 and AUSNUT 2007, national government food composition databases, using software developed by the Cancer Council of Victoria. Both the development of the DQES and its validation in Australian adults have been previously reported (28).

Statistical Analysis

A principal components analysis (PCA), using SPSS version 24.0 for Windows, was performed on standardized data gathered from 100 participants using the Cancer Council Victoria Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2). In addition to self-reported caffeine consumption (mg/day), the intake (g/day) of 80 foods collapsed into 21 individual composite food items were initially assessed (Table 1).

After removal of variables (bread and dairy including total margarine) that did not adequately correlate ($r<0.3$) with at least one other variable a total of 18 variables composed of 78 individual food items were included in the analysis (Table 1).

The overall KMO measure for this analysis was 0.72 ranked as ˜middling˝ according to Kaiser's (1974) classification of measure values. Individual KMO measures were all greater than 0.5. Bartlett's test of sphericity was statistically significant ($p=0.000$), indicating that the data was likely factorizable.

PCA revealed six components that had eigen values greater than one and which explained 27.2%, 14.2%, 9.3%, 7.7%, 6.2% and 5.7% of the total variance, respectively. The screen plot displayed an inflection at both the third and fifth component (Cattell, 1966) however only the three-component solution met the interpretability criterion. As such, three components were

retained.

The three-component solution explained 51.0% of the total variance. A Varimax orthogonal rotation was employed to aid interpretability. The rotated solution generally exhibited 'simple structure' (29). The interpretation of the data was consistent with expected dietary patterns.

The three principle components were named 'Meat Based', 'Junk Food' and 'Plant Based' that, after rotation, accounted for 27.2%, 14.2% and 9.3% of the total variance respectively. Each principle component score is indicative of the quantity of its composite components consumed. For example a high Plant Based score indicates the consumption of comparatively greater quantities of fruit, vegetables, nuts etc. in relation to other participants, while a high Meat Based score indicates the ingestion of proportionately large quantities of meat. Importantly a participant can have a high score on each of the three principle components, a low score on each component or a combination of high and low scores. A high score on each of the three principle components suggests the comparatively large consumption of all food types included in the PCA. On the other hand a low score on each of the principle components suggests a low intake of all food types in the PCA. Component loadings and communalities of the rotated solution are presented in Table 2.

Table 2
Rotated Component Matrix

	Meat Based	Junk Food	Plant Based
Red Meat	.805	.229	-.175
Chicken	.779	.247	-.081
Processed Meat	.752	.254	-.072
Total Fish	.733	.005	.137
Total Alcohol	.681	.217	.092
Pork	.614	.000	-.049
Caffeine	.560	-.042	-.088
Total Cheese	.470	.179	-.017
Soy	-.454	.118	.259
Fast Food	.075	.778	.032
Ref Carb	.006	.776	.029
Sweet Food	.207	.746	.008
Savoury Snack	.426	.562	.111
Total Nuts	-.021	.156	.773
Cereal	.039	.200	.683
Beans	-.227	.085	.677
Vegetables	.131	-.127	.651
Total Fruit	-.196	-.191	.518

Extraction Method: Principal Component Analysis; Rotation Method: Varimax with Kaiser Normalization; a. Rotation converged in 5 iterations.

After PCA only 73 participants remained due to incomplete responses on the Cancer Council Victoria Dietary Questionnaire. For some participants data required for the calculation of physical activity levels as MET minutes per week (METmin/wk) was missing. Outliers, classified as 3 ± standard deviations from the mean, were also removed. This explains the discrepancy in participant numbers for subsequent analyses.

Successive analysis included multiple linear regression to assess the influence of nutrition (quantified by the three components generated using PCA), physical activity, age and gender on BMD and T-scores. Unless otherwise stated our 'typical regression model' included age, gender, physical activity, BMI and the diet component(s) of interest. So that the influence of physical activity, measured in MET minutes per week (METmin/wk), and diet composition could be compared, the standardized residuals were analyzed. Normality of the studentized residuals of each regression model was assessed using both Kolmogorov-Smirnov and Shapiro-Wilk tests in addition to histogram displays. When required the Levene's Test of Equality was used to check homogeneity of variances between groups. The adjusted R-squared and Bonferroni adjusted significance values are provided throughout with test significance set at P-value ≤0.05.

In order to compare mean levels of nutritional parameters and time spent outdoors between principle component tertiles,' analysis of variance (ANOVA) with Fisher's Least Significant Difference (LSD) post hoc test was performed. The independent T test was used to assess the difference in BMD and T-score between genders. Normality of each variable was assessed as previously described with test significance set at P-value ≤0.05.

Results

Gender

No statistically significant difference in T-score was observed between males (1.1 ± 1.0 units, n=32) and females (0.9 ± 1.1 units, n=40, p>0.05). All T-scores were classified as normal being -1.0 or higher.

The BMD of females (1.18 ± 0.11 g/cm2, n=40) was found to be significantly lower compared to males (1.31 ± 0.10 g/cm2; n=32, p=0.000) (Table 3).

Age

The mean age for this cohort was 56.1 ± 8.5 years (n=73) (Table 3).

As expected we found that increased age was significantly associated with decreased BMD and T-scores. Using the typical regression model (see statistical methods above) when the Meat Based, Junk Food, or Plant Based components were separately controlled for; a one year increase in age resulted in a 0.005 (R2=0.429, n=66, p=0.003), 0.005 (R2=0.411, n=66, p=0.002) and 0.006 (R2=0.429, n=66, p=0.003) g/cm2 decrease in BMD respectively. Similarly a one year increase in age corresponded with a 0.054 (R2=0.183, n=66, p=0.002), 0.049 (R2=0.206,

n=66, p=0.003) and 0.057 (R2=0.221, n=66, p=0.001) unit decrease in T-score respectively.

Table 3
Physiological measures

	Gender	n	Mean	SD
Age (years)	Male	33	56.2	9.18
	Female	40	55.9	8.09
	Total	73	56.1	8.5
BMI (kg/m^2)	Male	33	25.7	3.3
	Female	40	26.4	5.3
	Total	73	26.1	4.5
BMD (g/cm^2)	Male	32	1.31	0.10
	Female	40	1.18	0.11
	Total	72	1.23	0.12
T-score (units)	Male	32	1.1	1.0
	Female	40	0.9	1.1
	Total	72	1.0	1.0
Physical Activity (METmin/wk)	Male	30	4890	3622
	Female	36	3770	3171
	Total	66	4279	3404

BMI

The mean BMI for this cohort was 26.1 ± 4.5 kg/m2 (n=73).

We observed a positive association between BMI and both BMD and T-score. Using the typical regression model when the Meat Based, Junk Food, or Plant Based components were separately controlled for; a one unit increase in BMI resulted in a 0.006 (p=0.043, R2= 0.204, n=66), 0.007 (p=0.020, R2= 0.408, n=66) and 0.007 (p=0.017, R2= 0.436, n=66) g/cm2 increase in BMD respectively. Similarly when the Meat Based, Junk Food or Plant Based components were accounted for a one unit increase in BMI resulted in a 0.056 (p=0.039, R2= 0.427, n=66), 0.066 (p=0.021, R2= 0.179, n=66) and 0.065 (p=0.018, R2= 0.217, n=66) unit increase in T-score respectively.

Physical Activity

On average participants performed 4279 ± 3404 MET minutes of physical activity each week (n = 66) (Table 3).

Using the typical regression model no association between physical activity levels and BMD or T-scores was observed. Further no association between physical activity levels and BMD or T-scores was found after each principle component was separately included into the typical regression model.

Diet

Interestingly, as presented in Figure 1, we found a statistically significant association between the Plant Based component and both BMD and T-scores. Using the typical regression model a one unit increase in the Plant Based component resulted in a 0.024 g/cm2 unit increase in BMD (p=0.054, R2=0.439, n=66) and a 0.243 increase in T-score (p=0.053, R2=0.221, n=66).

Figure 1
A one unit increase in adherence to a Plant Based diet resulted in a) a 0.024 g/cm2 increase in BMD (p=0.054, R2=0.439, n=66) and b) a 0.243 unit increase in T-score (p=0.053, R2=0.221, n=66). Analysed using multiple linear regression controlling for age, gender, physical activity (METmin/wk) and BMI. The adjusted R-squared and Bonferroni adjusted significance values are provided

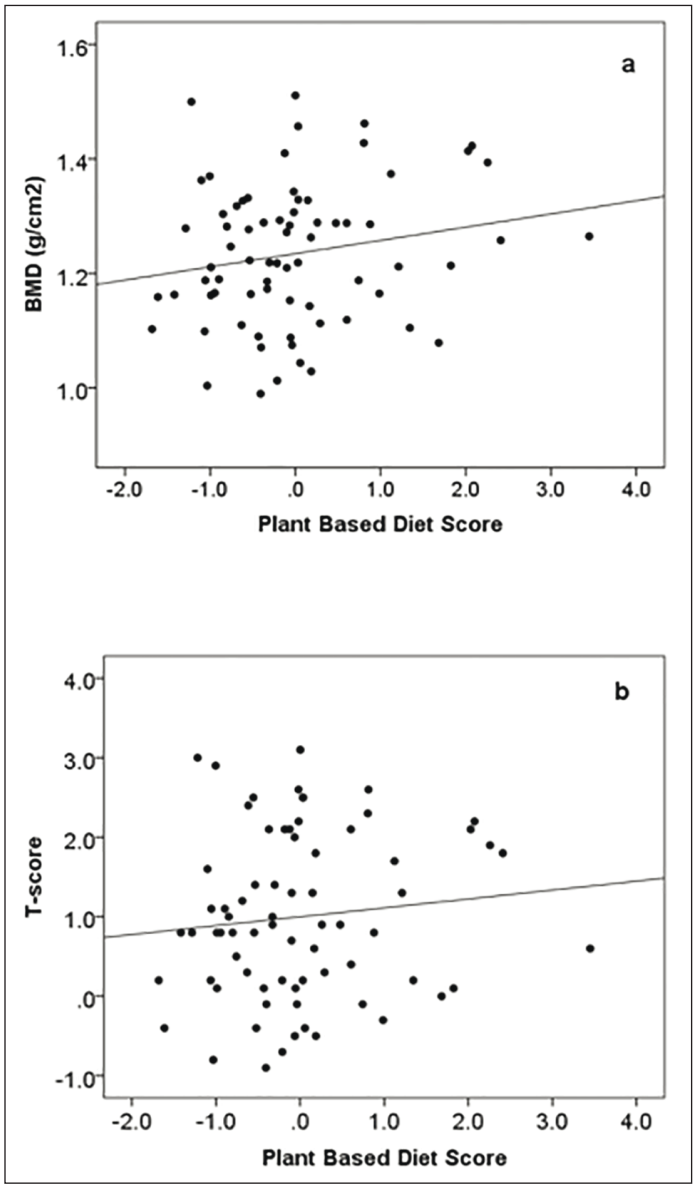

Table 4
Mean levels of nutritional parameters between principle component tertiles

			Plant Based			Meat Based			Junk Food		
	Tertile	**n**	**Mean (SD)**	**P vs. T2**	**P vs. T3**	**Mean (SD)**	**P vs. T2**	**P vs. T3**	**Mean (SD)**	**P vs. T2**	**P vs. T3**
Calcium (g/day)	T1	24	751 (255)	0.819	0.025*	676 (241)	0.079	0.002*	772 (311)	0.597	0.090
	T2	25	733 (185)	-	0.013*	810 (273)	-	0.146	731 (213)	-	0.026*
	T3	24	927 (339)	0.013*	-	921 (270)	0.146	-	907 (279)	0.026*	-
Vitamin D (nmol/L)	T1	24	52.3 (22.1)	0.053	0.066	58.7 (18.5)	0.922	0.572	65.4 (19.8)	0.432	0.027*
	T2	25	63.5 (16.8)	-	0.918	58.1 (24.1)	-	0.511	70.0 (19.9)	-	0.133
	T3	24	62.9 (19.2)	0.918	-	62.0 (17.1)	0.511	-	52.4 (18.5)	0.133	-
Protein (g/day)	T1	24	66.2 (19.5)	0.653	0.279	50.9 (13.8)	0.001*	0.000*	56.6 (22.2)	0.009*	0.000*
	T2	25	72.0 (29.3)	-	0.517	65.2 (15.2)	-	0.000*	71.5 (20.6)	-	0.059
	T3	24	77.3 (32.3)	0.517	-	99.7 (25.1)	0.000*	-	87.4 (30.7)	0.059	-

Analysis of Variance (ANOVA) with Fisher's Least Significant Difference post hoc test was used to assess differences in mean levels of nutritional parameters between tertiles. * $p \leq 0.05$.

No significant association between the Meat Based component and either BMD or T-score was observed when using the typical regression model. Importantly however, when the Plant Based component was included, a one unit increase in the Meat Based component resulted in a 0.024 g/cm2 unit increase in BMD (p=0.046, R2=0.474, n=66) and a 0.238 increase in T-score (p=0.046, R2=0.279, n=66). In this same model a one unit increase in the Plant Based component resulted in a 0.025 g/cm2 unit increase in BMD (p=0.043, R2=0.474, n=66) and a 0.248 increase in T-score (p=0.043, R2=0.279, n=66).

No significant association between the Junk Food component and either BMD or T-score was observed; either individually or when included together with the Plant Based component in the typical regression model.

>> Vitamin D

We subsequently analyzed the data to determine if there was a relationship between the consumption of plant foods and plasma levels of vitamin D. While a statistically significant association was not found using multiple linear regression, when the Plant Based component was divided into tertiles, those who had higher scores (i.e. ate comparatively greater quantities of plant foods) were observed to have higher levels of plasma Vitamin D (Table 4).

Specifically the mean plasma vitamin D level of participants in the first tertile was 52.3 ± 22.1 nmol/L (n=23). This was lower than the mean vitamin D levels of those in the second (63.5 ± 16.8 nmol/L, p=0.053, n=24) as well as the third tertile (62.9 ± 19.3 nmol/L, p=0.066, n=24), which contained participants who consumed more plant foods.

No difference in plasma vitamin D levels was observed between tertiles in the Meat Based component. However participants in the first Junk Food tertile (i.e. ate comparatively less quantities of 'Junk' food) were found to have significantly higher plasma levels of vitamin D compared to those in the third tertile (65.4 ± 19.8 vs 52.4 ± 18.5 nmol/L, p=0.027, n=23).

The majority of participants (67%) were found to have sufficient plasma vitamin D levels (≥ 50 nmol/L). Twenty eight percent had insufficient levels (30 – 50 nmol/L) and 5% were classified as deficient (< 30 nmol/L).

Participants classified as deficient were observed to spend significantly less time outdoors compared to those who had sufficient vitamin D levels (9 ± 7 hours , n = 5; vs. 16 ± 8 hours, n = 64, p = 0.046). No significant association between time outdoors and tertiles within the Plant Based or Junk Food dietary components was observed. However those in the third Meat Based component (i.e. highest meat consumption) reported spending significantly longer time outdoors compared to those in the first tertile (13 ± 7 hours vs. 17 ± 7 hours, n = 23, p = 0.034).

>> Calcium

We also sought to determine if mean plasma calcium levels differed between the Plant Based component tertiles. While no difference in mean plasma calcium levels was found we did observe that those in the third Plant Based tertile (927 ± 339 g/day, n=24) had a significantly higher dietary calcium intake compared to those in the second (733 ± 185 g/day, p=0.013, n=25) and first tertile (751 ± 255 g/day, p=0.025, n=24) (Table 4).

Likewise individuals in the third Meat Based component tertile (921 ± 270, n=24) had significantly higher levels of dietary calcium intake compared to those in the first tertile (676 ± 241 g/day, p = 0.002, n = 24); and individuals in the third Junk Food tertile (907 ± 279 g/day, n=24) had significantly higher levels of dietary calcium intake compared to those in the second tertile (731 ± 213 g/day, p = 0.03, n = 25). Dietary calcium intake was not found to be significantly different between other tertiles within the Plant Based, Meat Based or Junk Food components.

Importantly dairy intake was not found to be significantly

different between tertiles within the Plant Based, Meat Based or Junk Food components.

>> Protein

We performed further statistical analysis to determine if a relationship was present between each principle component and both plasma total protein and dietary protein intake. Interestingly no association between plasma total protein and either the Plant Based, Meat Based or Junk Food components were found.

Regarding dietary protein 82% of participants were found to meet the US recommended dietary allowance of 0.75 grams per kilogram of body weight per day. Dietary protein intake was found to be significantly different between those in the first (50.9 ± 13.8 g/day, n=24) and second (65.2 ± 15.2 g/day, p = 0.001, n=25), as well as second and third (99.7 ± 25.1 g/day, p = 0.000, n=24) Meat Based component tertiles. Similarly those in the second (71.5 ± 20.6 g/day, p = 0.009, n = 25) and third (87.4 ± 30.7, p = 0.009, n = 24) Junk Food tertile were observed to have a significantly higher dietary protein intake compared to those in the first Junk Food tertile (56.6 ± 22.2 g/day, n = 24). While protein intake increased between the first (66.2 ± 19.5 g/day, n = 24), second (72.0 ± 29.3 g/day, n = 25) and third tertiles (77.3 ± 32.3 g/day, n = 24) of the Plant Based component this was not statistically significant (Table 4).

Discussion

This cross-sectional study sought to clarify the relationship between plant food intake on BMD and T-scores, surrogate measures of bone strength, in a healthy population while accounting for other known influences.

As previously observed by others, younger age (5), higher BMI (30) and a male gender (31) were generally associated with increased BMD and T-score. While physical activity is also often reported to accompany increased BMD (29) we did not observe any influence of physical activity on either BMD or T-scores. This may be because the modest number of participants prevented a more detailed statistical analysis of the influence of exercise type on bone mineralisation measures. Thus the known positive effects of weight bearing and resistance exercise on BMD (33, 34) may have been diluted by the inclusion of low impact activities in our measure of physical activity.

Despite this lack of association, physical activity as well as BMI, gender and age where controlled for when investigating the possible relationship between diet and bone health. Using this approach we observed a positive association between increased consumption of plant foods and both BMD and T-score. Importantly while both the Meat Based and Junk Food components alone were not associated with either BMD or T-score, a positive association between both BMD and T-score and increased consumption of foods in the Meat Based component was found when the Plant Based component was included in the model. This suggests that in a healthy population with normal BMD, an increase in consumption of plant based foods, either alone or as part of a diet containing meat, leads to increases in bone mineralisation. However the lack of association between BMD or T-scores and the Junk Food component, alone or in combination with the Plant Based component, suggests that the bone health promoting benefits associated with increased plant food consumption cannot negate the detrimental effects of consuming energy dense nutrient poor discretional food items.

When considering nutritional influencers of bone mineralisation calcium, essential for building and maintaining bone, is often the first to come to mind. As expected, we observed that blood calcium concentrations were maintained across all tertiles in each dietary component. Owing to its vital role as a second messenger ionised calcium in extracellular fluids is tightly regulated through the action of vitamin D, parathyroid hormone as well as calcitonin and calcitriol hormones which explains this finding.

We subsequently sought to determine if calcium ingestion changed across tertiles. It was observed that calcium intake increased as total food consumption increased in each dietary component. Surprisingly this included the Plant Based component despite no apparent parallel rise in dairy consumption from one tertile to the next. Although unforeseen, as vegetarian and vegan diets often contain comparatively lower levels of calcium (35, 36), a number of plant foods such as legumes, seeds and nuts, green leafy vegetables as well as fortified food stuffs, are rich sources of calcium; the increased ingestion of which would contribute to the calculated calcium intake in the Plant Based component tertiles. In addition it should also be noted that the Plant Based component in this study delineates an increase in plant food ingestion but not necessarily a reduction in the ingestion of other food groups; with participants able to fall in the third tertile of each of the dietary components (see Statistical Analysis above). Combined these reasons likely explain the incremental rise in calcium ingestion we found across tertiles in the Plant Based component. However as calcium intake was generally found to increase across tertiles in each of the dietary components it is unlikely that variations in calcium account for the positive association we observed between plant food consumption and markers of bone mineralisation.

As mentioned above adequate levels of active vitamin D (1,25-dihydroxycholecalciferol) are required to maintain extracellular fluid calcium concentrations. This is accomplished by increasing calcium absorption from the intestine. When levels of vitamin D are sufficient approximately 30% of dietary calcium is typically absorbed. When vitamin D levels are insufficient this figure may reduce by as much as half (37, 38). Remarkably we observed that people in the third tertile of the Plant Based component (i.e. consumed comparatively high quantities of plant foods) had significantly greater concentrations of plasma vitamin D than those in the second

or first. On the other hand, people in the third Junk Food component tertile (i.e. consumed comparatively high quantities of Junk Food), had significantly lower levels of plasma vitamin D compared to those in the second or third tertile. No difference was found across the Meat Based component tertiles.

This is an interesting finding; considering that up to 90% of available vitamin D is derived from the sun (39) and that, while participants who reported spending more time outdoors did have higher plasma vitamin D levels, outdoor time did not vary across Plant Based or Junk Food component tertiles. Thus while sunlight exposure clearly influences vitamin D stores, our results suggest that the higher vitamin D plasma concentrations observed in the third Plant Based and first Junk Food component tertiles are likely due to diet. But food sources containing vitamin D are scarce. Apart from fortified foods animal products are traditionally considered the main source for the more 'bio-effective' cholecalciferol (vitamin D-3). However, vitamin D-3 and its metabolites are also formed in certain plant foods. Specifically the Solanaceae, or night shade, family of plants (including tomatoes, potatoes, eggplant, bell and chili peppers) have been found to contain relatively high quantities of Vitamin D3 and its provitamin 7-dehydrocholesterol (40, 41, 42, 43, 44). While the leaves of these plants have only been studied to date and the availability of vitamin D3 metabolites in plants remain unknown, this does suggest that plant foods may be a more important source of vitamin D3 than previously thought. This may at least partially explain why increased plant food consumption was linked to greater plasma vitamin D concentrations. In contrast the scarcity of vitamin D in discretionary (i.e. junk) food items is the most plausible reason for the reduced plasma vitamin D concentrations observed in ardent Junk Food eaters. Importantly however as the majority of participants were found to have sufficient plasma vitamin D levels it is unlikely that vitamin D status would be a significant influencer of BMD in this cohort.

In addition to calcium and an accompanying sufficient intake of vitamin D, dietary proteins are also required to provide the essential amino acids necessary to maintain bone health. Protein effects bone maintenance through several mechanisms. Accounting for roughly 50% of bone volume, protein forms part of the bone structural matrix. Protein also influences IGF-1 concentrations as well as calcium absorption and excretion. While there are numerous reports of a positive association between protein ingestion and BMD, (45, 46, 47) the optimal quantity of protein required to support bone health has yet to be confirmed and is highly contentious (48, 49, 50, 51, 52, 53, 54, 55). Overall however it is largely agreed that at least adequate protein consumption is required for normal calcium and bone metabolism. As the majority of participants were found to easily meet the US recommended daily allowance for protein it is therefore unlikely that the quantity of protein consumed each day explains our observations.

In addition to debate regarding the optimal quantity of protein required to support bone health a number of attempts to demonstrate the superiority or inferiority of vegetable versus animal protein have been made. While a detailed review of the evidence and purported mechanisms is beyond the scope of this article (for reviews see 56, 57, 58) it is generally felt that any advantage of one protein source over another, particularity in epidemiological studies, may be due to other nutritional components that accompany the whole food source rather than the characteristics of the protein per se. In agreement we found no association between the Meat Based or Junk Food components and BMD or T-scores, this was despite protein ingestion increasing across tertiles. Conversely protein ingestion was not found to differ across tertiles in the Plant Based component.

Despite the apparent lack of increased protein consumption across Plant Based tertiles we observed that when the Plant Based component was modelled together with the Meat Based component, the lack of association between meat ingestion and BMD converted into a significant positive association. As alluded to above, and when considering the data as a whole, this strongly indicates that other nutritional components, besides calcium, vitamin D and protein, within plant foods are powerful protectors and promoters of BMD.

Plant foods contain a complex abundance of nutritional components that have been shown to positively influence bone health. Notably vitamin K has been shown to play a pivotal role in maintaining adequate bone mineralisation. Multifunctional in its action, vitamin K promotes bone construction by stimulating osteoblastogenesis (59). It also is an essential cofactor for the γ-glutamyl carboxylation of osteocalcin, enabling the enzyme to bind calcium ions for use in extracellular matrix mineralization (60, 61). What's more, vitamin K has been shown to prevent bone resorption by inhibiting osteoblast apoptosis as well as osteoclastogenesis (59, 62).

Vitamin K exists in two forms. Phylloquinone (also called vitamin K1), is present in all photosynthesising plants (63) and is particularly concentrated in green leafy vegetables. The population intake of phylloquinone has been found to range from about 60-375 μg/day (64, 65). The daily intake of menquinones (also called vitamin K2) on the other hand, found in dairy products and fermented soybeans, is only about 10-45 μg/day (64). While phylloquinone accounts for the majority of our dietary Vitamin K intake, reports indicate that menquinones are 10 times more bioavailable (66) and unlike phylloquinone, are extensively distributed throughout the body following absorbtion. However in 2008 Okano and colleagues demonstrated that orally ingested phylloquinone is converted to menquinone-4 in a dose dependant manner (67). Okano concluded that the accumulation of menquinone analogues in tissue is, at least in part, a metabolic product of ingested phylloquinone (65). In a separate study Thijessen et al (68), sought to determine the effect of maternal phylloquinone supplementation on phylloquinone and menquinones-4 levels in breast milk. Compared to controls, 16 days of 2 or 4 mg/day phylloquinone supplementation resulted in a 2.5 and 7

fold increase in breast milk menquinones-4 levels (68). Thus evidence suggests that dietary phylloquinone consumption significantly impacts menquinone stores. Therefore it can be speculated that at least adequate intake of phylloquinone significantly impacts, and may be essential for, bone health.

In addition to vitamin K, plant foods contain a large range of other nutritional components and properties shown to improve bone health. For example the estrogenic activity of legume isoflavones has been found to stimulate osteoblast differentiation and mineralization (22). Similarly the carotenoid lycopene, responsible for the red colour of many fruits and vegetables, has been shown to stimulate osteoblast proliferation (69), inhibit basal as well as parathyroid hormone-stimulated osteoclast formation (70), and prevent mineral resorption mediated by reactive oxygen species (70). Further, as reviewed by Schwalfenberg (71) and more recently demonstrated by Gunn (72), increased consumption of plant based foods increases renal pH (i.e. reduces renal acid load), facilitating significant reductions in urinary calcium loss. These positive effects, as well as the bone health promoting benefits of vitamin K, likely explain why the increased consumption of plant foods was found to be significantly associated with increased BMD and T-scores.

This study has a number of limitations. As mentioned above the relatively low number of participants prevented a more comprehensive statistical evaluation in which the influence of smaller details, such as exercise type, could be investigated. Additionally total body BMD and not the BMD of specific regions such as the femoral neck or lumbar spine was quantified in this study using DXA. When Graat-Verboom and colleagues compared total body versus local DXA-scan, a higher prevalence of osteoporosis was found using local DXA (73). However despite potentially reduced sensitivity due to quantitation of total body BMD we still observed a positive association between increased consumption of plant foods and both BMD and T-score. This association would likely be more robust if local DXA was utilised. Further, as this was a cross-sectional study, only associations can be inferred from the data, the actual effect of food consumption on BMD cannot be established.

In conclusion data from this study suggests that in a healthy middle aged population with BMD within the normal range, an increase in plant food consumption, either alone or in combination with a diet containing meat, is associated with improved bone mineralisation markers. This relationship is most likely due to the extensive range of micronutrients and phytochemicals packaged within plants that have previously been shown to be powerful promoters of bone health. Subsequently healthcare professionals should be encouraged to advise the increased consumption of plant based foods, particularly in mid-life, irrespective of the clients' underlying dietary pattern, to prevent the development of osteoporosis and help curb the growing epidemic of bone disease.

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