What to eat for healthy bones?

Epidemiological studies on diet, bone health and frailty

Ester Anthonina Leida de Jonge

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

Impaired bone health: a major health concern in the middle-aged and elderly

Impaired bone health, as for example reflected by low bone mineral density (BMD) or high risk of fractures, is a major health concern in the middle-aged and elderly population. Impaired bone health may lead to disability and reduced quality of life (1). Is has been estimated that within the European Union 3.5 million fractures occur per year (2).

Bones must endure voluntary physical activities without breaking or causing pain, and in the meantime they must be strong enough to maintain their load-bearing capacity. Therefore, a "healthy" bone might be best described as bone which is highly adaptive to physiological challenges. Physiological challenges differ across the life course. During childhood, bones must adapt to growth, and during puberty and menopause bones must adapt to hormonal changes (3). Therefore, adaptations to changes are present throughout the lifecourse (Fig. 1). The studies described in the present thesis focus on the middle-aged and elderly. In this age group, loss of BMD is a main measure of impaired bone health that reflects the bone's degree of mineralization. When a participants' femoral neck BMD is 2.5 standard deviations below the peak bone mass of a healthy reference population matched for gender and ethnicity (T-scores), this person fulfils the criteria of having osteoporosis (5). Currently, it has been estimated that 200 million people suffer from osteoporosis worldwide and that approximately 30% of postmenopausal women suffer from osteoporosis in Europe(6). However, it can be considered a "silent disease" which is typically not diagnosed until a fracture occurs.

Novel approaches to measure bone health in the general population

Although BMD is one of the major determinants of bone strength and fracture risk, it has been debated that considerable overlap in BMD values occurs between individuals who develop fractures and those who do not. Based on this observation, there has been a growing interest in studies on other factors that might influence bone strength and fracture risk, including the microarchitecture of trabecular bone (Fig. 2) and the macro geometry of cortical bone (14). These novel measures will be addressed in the present thesis.

Dietary intake as modifiable determinant of bone health

Low BMD can be caused by endocrine and metabolic disorders such as diabetes mellitus and inherited disorders of collagen metabolism. Also, disorders related to nutrition and lifestyle such as malnutrition and vitamin D deficiency might contribute

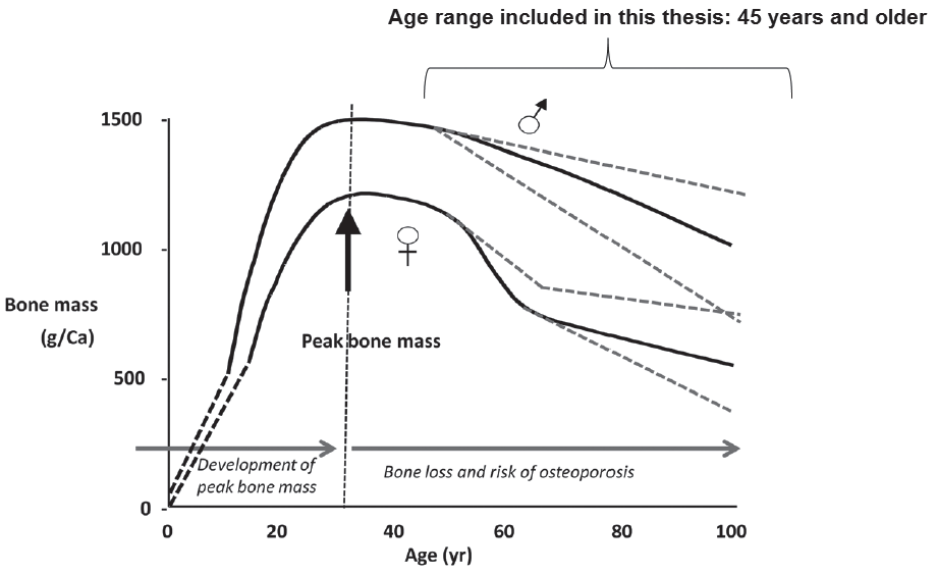

Figure 1. Changes in bone mass across the life course

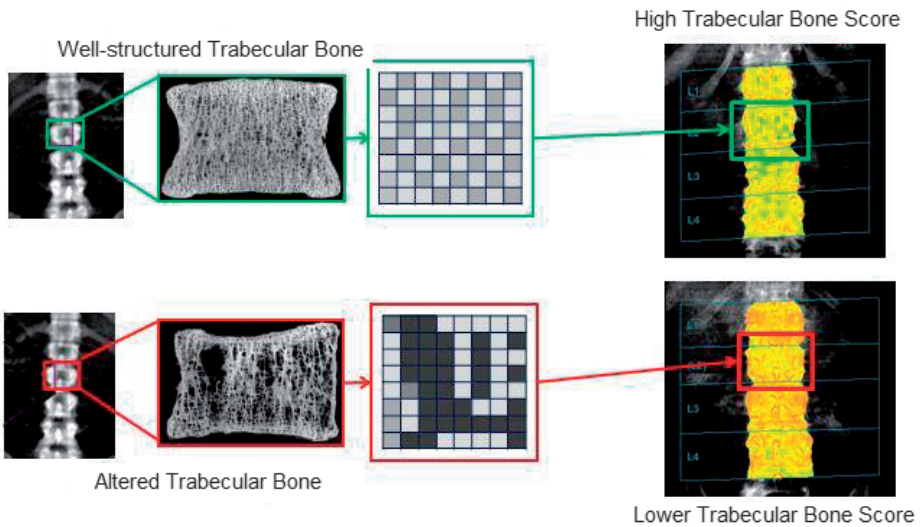

Figure 2. Trabecular Bone Score; a novel measure of trabecular bone integrity.

to low BMD(7). In the general population, age, body weight or changes in body weight, chronic diseases, use of medication, hormonal factors, and lifestyle factors are the main determinants of BMD(8). In the ageing population, weight loss is an important concern(4). Among lifestyle factors and next to physical activity and smoking, dietary intake is an important modifiable determinant of BMD.

Diet might influence bone health by affecting body weight and therefore mechanical loading of the weight bearing bones. Moreover, dietary intake might affect the metabolism of calcium, the main constituent of bone, via two mechanisms. First, dietary components might influence the intestinal absorption of dietary calcium and the uptake of serum calcium into the bone tissue. For example, vitamin D is known to enhance both dietary and serum calcium absorption, whereas dietary fibres are suggested to bind to calcium and therefore inhibit its intestinal intake(9). Second, dietary intake might affect modelling and remodelling(10). That is, dietary components might influence formation and functioning of the bone forming osteoblast and bone resorbing osteoclast (11), which results in altered serum calcium uptake by the bone or calcium release from the bone, independent of mechanical loading. For example, dietary vitamin A has been shown that retinoic acid, the biologically active form of vitamin A, inhibits osteoblast activity and stimulates osteoclast formation in animals(12, 13).

A shift in dietary guidelines and potential consequences for nutritional research

Eventually, epidemiological studies on dietary intake and bone health might facilitate the optimalization of dietary guidelines for the general population. In the Netherlands, the national dietary guidelines were recently (04-11-2015) updated. These guidelines were designed by the Health Council of the Netherlands and based on review of the available evidence with respect to nutrients, food products and dietary patterns in relation to chronic diseases. For this review process, the chronic diseases included were limited to the top 10 diseases based on mortality, life years lost, and burden of disease. This implies that coronary heart diseases, stroke, heart failure, type 2 diabetes, chronic obstructive pulmonary disease (COPD), several types of cancer (breast, intestines and lung), dementia or cognitive decline and depression were included, whereas outcomes of bone health were not. Moreover, one of the most important differences between earlier versions of the guidelines and the new ones was the shift from a nutrient-based approach towards an approach focused on food groups and overall diets(17). In line with this shift in guidelines, various statistical approaches have been developed to study overall dietary patterns next to studying single nutrients. Different approaches will be applied in the present thesis.

Calcium is the main constituent of the human bone. The Dutch population traditionally has intakes of dietary calcium which are relatively high (ranging from

817-1.136 mg/day for adults (15) and with medians of 900 to 995mg/day for older adults(16), making this population particularly interesting for investigating dietary intake that might favourably affect bone health, beyond calcium.

Study design: The Rotterdam Study

The Rotterdam Study is a prospective, population-based cohort study comprising middle-aged and elderly participants from the well-defined neighbourhood Ommoord in Rotterdam, the Netherlands.

The Rotterdam Study started in 1990 and targets cardiovascular, endocrine, hepatic, neurological, ophthalmic, psychiatric, dermatological, oncological, and respiratory diseases. Initially, 7983 participants aged 55 years or older were enrolled in the first study cohort, which was 78% of the 10215 invitees. In 2002, 3011 (out of 4472 invitees) participants who had become 55 years of age or moved into the study district were additionally enrolled in the second cohort of the study. In 2006, a third cohort was initiated, in which 3932 (out of 6057 invitees) younger participants aged 45 years and over were additionally included (18). Dietary intake was assessed at baseline of each of these cohorts, using food frequency questionnaires. Additionally, an additional dietary intake measurement was performed at the fifth visit of the first cohort (between 2009 and 2011). Radiological examination of the bones was performed at every single visit to the study centre using dual X-ray absorptiometry (DXA) scans. Fractures were reported by local general practitioners in the research area (covering 80% of the cohort partcipants) by means of a computerized system. All reported events were verified by two trained research physicians, who independently reviewed and coded the fractures according to the International Classification of Primary Care (ICPC) and International Classification of Diseases, 10th edition (ICD-10).

Overall aim of this thesis

The overall aim of this thesis was to evaluate dietary intake in relation to various aspects of bone health. In **chapter 2**, we will study associations between socio-economic indicators of dietary quality, defined as adherence to national dietary guidelines. In **chapter 3**, we will study associations between dietary intake of single nutrients, nutrient-based indices and metabolic end products of nutrients in relation to measures of bone health. In **chapter 4** we will study overall dietary patterns in relation to bone health. Moreover, since impaired bone health might contribute to overall frailty during ageing, we will study associations with frailty in this chapter as well. Lastly, in **chapter 5**, methodological considerations, practical implications of our finding and suggestions for future research will be addressed in a general discussion.

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SOCIO-ECONOMIC INDICATORS AND DIETARY QUALITY IN AN ELDERLY POPULATION

ABSTRACT

Background

Socio-economic inequalities in dietary quality of the elderly are poorly understood. We aim to examine the strength and independence of associations between three major socio-economic indicators (income, education and occupation) and dietary quality. Furthermore, we aim to evaluate the influence of socio-economic indicators on changes in dietary quality during a 20-year follow-up period.

Methods

Data were collected in the framework of the Rotterdam Study: a prospective population-based cohort comprising subjects aged 55 years or older living in Rotterdam, the Netherlands (n=5434). Socio-economic indicators (education, occupation and household income) were measured at baseline (1989-1993) and each classified into categories. Dietary quality was assessed at baseline (1989-1993) and after 20 years of follow-up (2009-2011) and quantified using the Dutch Healthy Diet Index. This index reflects adherence to the Dutch guidelines for a healthy diet with a theoretical range of 0 (no adherence) to 80 (optimal adherence) points. Linear regression models were adjusted for age, smoking status, BMI, physical activity level, total energy intake and mutually adjusted for the other socio-economic indicators. To study changes in dietary quality, quality at follow-up was used as the outcome in models adjusted for dietary quality at baseline.

Results

A higher level of education was associated with higher dietary quality at baseline. We observed a 2.29 points higher index in the highest educated participants than in the lowest educated participants (95%CI=1.23-3.36). In addition, the highest educated participants were more likely to have higher dietary quality at follow-up (β=3.10, 95%CI=0.71-5.50), after adjustment for baseline dietary quality. In contrast, higher income was associated with lower dietary quality at follow up (β=-1.92, 95%CI = -3.67, -0.17), whereas occupation was not independently associated with dietary quality at baseline nor at follow-up.

Conclusion

In our cohort of Dutch elderly, high education was the most pronounced socio-economic indicator of high dietary quality at baseline and at follow-up. Our results highlight that different socio-economic indicators influence dietary quality in different manners. Future studies are required to explore potential barriers of adhering to the dietary guidelines in the lowest educated elderly.

INTRODUCTION

The prevalence of chronic diseases is associated with socio-economic inequalities [1, 2]. These inequalities might partly be explained by unevenly distributed dietary quality. Several studies reported a poorer quality among those in than in higher socio-economic groups [3-8]. Yet, the strength of the observed associations differs and information in elderly people is scarce.

Income, education, occupation and wealth are the most commonly studied socio-economic indicators. Nevertheless, often only one socio-economic indicator is used, providing little information on why low socio-economic individuals are more likely to engage in more unhealthy dietary patterns. Income, education, occupation and wealth are conceptually different and as such might influence nutrition and lifestyle via different mechanisms [9]. A high education enables people to understand the complexity of a healthy diet, to understand food labels and respond better to nutritional interventions [10-12]. The proposed association between occupation and dietary quality can be explained by socio environment and work cultures [13, 14]. For example, night shift workers have reported to have more eating moments and snack intake than day workers and professionals and intermediate professionals indicated to eat more fish, fruits and vegetables and less fried foods than people with manual or lower occupations [13]. Aldo, sufficient income could be required to afford healthy food products.-

Studies on dietary quality usually take into account the totality of diet, including the food items, food groups, and nutrients consumed, their variety, and the frequency and quantity in which they are consumed [16]. Previous studies showed that in general, lower socio-economic position is associated with lower overall dietary quality [8, 17, 18]. Even so, most studies use only a single socio-economic indicator or combine indicators to one socio-economic status. For example, Martikainen et al. [18], found that a low employment grade was associated with unhealthy diets- defined using population specific dietary patterns. Similarly, Norhtstone et al., observed participants with a high education adhered more often to a health conscious dietary pattern. Despite growing interest in socio-economic inequalities in dietary quality, only a few longitudinal studies exist [17] [19]. Longitudinal studies provide the opportunity to address the effect of socio-economic indicators on changes in dietary quality. This topic is of special interest in the elderly population, because the ability to meet their nutritional needs may be affected by psychological and physiological factors related to ageing as well as economical and societal factors.

We hypothesize that high socio-economic class is associated with better dietary quality. To study this, the first aim of this study is to examine the strength and independence of three major socio-economic indicators (income, education and occupation) and dietary quality among older people in the Netherlands. Secondly,

we aim to evaluate the influence of socio-economic indicators on changes in dietary quality during a 20-year follow-up period.

METHODS

Study population

This study was performed in the framework of the Rotterdam Study, an ongoing population-based prospective cohort study in Ommoord, a district in Rotterdam, the Netherlands. The rationale and design of the Rotterdam Study are described elsewhere [20]. Briefly, all residents aged 55 years and over in the Ommoord district were invited to participate (n=10,215) of whom 7983 (78%) participated in the first cohort. The current study used data from the first examination of the original cohort of the Rotterdam Study (RS-I-1, 1989-1993) and data from the fifth re-examination of the original cohort (RS-I-5), which took place between March 2009 and January 2011. Home interviews were held to collect data on current health status, use of medication, medical history, lifestyle, and risk factors for chronic diseases. Subsequently, participants visited the research center for extensive clinical examination and dietary assessment. Every 2-3 years participants were invited for a follow-up visit.

Ethics, consent and permissions

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Assessment of socio-economic indicators

At baseline education, occupation and household income were measured using questionnaires. Income in Guilder (the Dutch currency at the moment of data collection; 1 Guilder corresponded to approximately €0.45) per annum per household was reported as falling to one of 13 categories, which in the analyses were collapsed into four categories: <28000, 28000-39999, 40000-54999, >54999. Education was asked for in 8 categories, and in the analysis collapsed in four categories: primary education with or without a partially completed higher education; lower vocational or lower secondary education; intermediate vocational education and general secondary; and higher vocational or university education. Current or last occupation was categorized into five groups: routine non-manual employees in administration and commerce lower and higher grade professionals, small proprietors, and manual workers. Lastly, 18% (n= 584) of the women indicated to take care of the household full-time and therefore categorized as “not working”.

Assessment of dietary intake and dietary quality

Dietary intake

At baseline, dietary intake was assessed using a validated food frequency questionnaire (FFQ) in a two-stage approach. In the first stage, participants completed a self-administered checklist of 170 food items in which they were asked which foods they consumed at least twice a month in the preceding year, forming a basis for the second stage in which a trained dietician collected data on the frequencies and amounts of the foods. The 170-item FFQ was validated in a subsample of the Rotterdam Study (n=80) using multiple food records and 24 hour urine urea [21]. The validation demonstrated that although the FFQ overestimated the intake of protein, dietary fiber and micronutrient intake, it was able to adequately rank participants according to their intake (Pearson correlation ranging between 0.44 and 0.85). From the full cohort at baseline (n=7983), 6521 participants visited the research center and were eligible for a dietary interview. From all eligible participants, 1086 had no dietary assessment for several reasons: they participated in the pilot phase (n=271), were suspected of dementia and were not interviewed because of expected recall difficulties (n=122), logistical reasons (n=481), or unreliable dietary reports (n=212, determined by a trained dietician). In total 5435 participants had reliable dietary data.

At follow-up (RS-I-5), an FFQ based on 389 items was used which was previously validated in two other Dutch populations using a 9-day dietary record [22] and a 4 week dietary history [23]. Information on portion size, type of food item, and preparation method were collected. From the full RS-I-5 cohort (n=2140), 1441 completed an FFQ (n=435 did not attend the research center, n=264 did not return the FFQ). We identified those participants whose questionnaires indicated an unfeasible total energy intake per day (<500kcal or >5000kcal; n=79), and excluded these participants from analysis as we could be confident that these data were unreliable and not indicative of the nutritional status of the individuals in question. Nutrient data were calculated from the Dutch Food Composition Table, using 1993's version for RS-I-1 and 2011's update for RS-I-5 to account for the changes in nutritional composition of foods.

Dietary quality: The Dutch Healthy Diet Index

Information from the FFQ was used to estimate dietary quality with The Dutch Healthy Diet Index (DHDI), developed by Van Lee *et al.* [36] reflecting adherence to the Dutch Guidelines for a Healthy Diet (2006). Briefly, the original DHDI includes 10 components, each with a score ranging between zero and ten, where ten indicates that a participant meets the Dutch recommendations or has an optimal intake. A total DHDI is calculated by adding all component scores together, resulting a score

between zero (no adherence to recommendations) and 100 (complete adherence to recommendations). After exclusion of the non-dietary component "physical activity" and exclusion of "acidic drinks and foods" due to missing information, the DHDI for the Rotterdam Study assessed eight components: vegetables, fruit, fiber, fish, saturated fat, trans fat, sodium and alcohol, and a total score between 0 and 80 was calculated for each participant at baseline and follow-up. Details regarding the cut-offs used to compose this index and the food items included per FFQ are shown in Supplemental table 1.

Assessment of covariates

At baseline, weight (kg) and height (cm) were measured at the research center, and BMI (kg/m^2) was calculated and categorized according to the WHO criteria for overweight (25< BMI <30) and obesity (BMI >30). Cigarette smoking status was collected through self-report. Physical activity levels were assessed in RS-I-3 with an adapted version of the Zutphen Physical Activity Questionnaire [24], including questions regarding walking, cycling, sports, gardening, hobbies and housekeeping. Answers were translated into metabolic equivalent of task (MET) values and participants were classified in tertiles: high, middle and low physical activity [25]. Household composition was defined as living with a partner, alone or other than a partner. "Diseased" was defined as being hospitalized in the past year, experienced a heart attack with admission during the past years of being diabetic as study entrance.

Statistical analyses

All analyses were performed in the full population and stratified by gender if significant interaction with gender was observed [5, 6, 17]. Education and income were analyzed using dummy variables with the lowest groups as reference groups. As most participants' current or last occupation was 'routine non manual employees in administration and commerce', this category was used as the reference group and dummy variables for the three other occupation groups were created. The correlation between the DHDI score at baseline and follow-up was calculated with the Pearson correlation coefficient. Overall stability of the diet was estimated by calculating the percentage of participants that remained within the same energy adjusted quartile of the DHDI. Additionally, stability was assessed for each item of the DHDI.

Cross-sectional analysis

First, cross-sectional analyses were performed to assess the associations between socio-economic indicators and dietary quality at baseline (RS-I-1). Three multivariate linear regression models were created based on: crude analysis for each socio-economic indicators as exposure and DHDI-score as outcome adjusted for age and gender (model 1) multivariate analysis for each socio-economic indicator adjusted for

baseline characteristics related to lifestyle (smoking, physical activity, living situation and BMI) and total energy intake (model 2) and multivariate analysis additionally adjusted for all other socio-economic indicators (occupation, income, education, model 3). The second and third models were adjusted for total energy intake because by design of the DHDI, it might be easier to adhere to the guidelines at higher levels of energy intake.

Longitudinal analyses

Moreover, longitudinal analyses were performed. To investigate indicators that influence dietary quality 20 years later, the same three models were created for the longitudinal analysis, using dietary quality at RS-I-5 as the outcome and an additional adjustment for dietary quality at baseline. These analyses was performed in the full population with available dietary data at both RS-I-1 and RS-1-5. Only if significant longitudinal associations between a specific socio-economic indicator with and dietary quality were observed, we further explored which food groups might have contributed to these associations. Therefore, we ran model 3 with fruit, vegetables, whole grains, fish or meat as the outcome measures. Fruit, vegetables and fish are components of the DHDI. The food group whole grains was selected to reflect a main contributor to the DHDI component fibre and meat as contributor to the DHDI component SFA and sodium.

Imputation of covariates and additional analyses

For all independent variables, multicollinearity was checked with the variance inflation factor (VIF). VIF values above 10 indicated multicollinearity, provided that variables were not interaction terms or dummy variables. A multiple imputation procedure to impute missing values of socio-economic indicators and the covariates of linear regression analyses [26]. We used the fully conditional specification (Markov chain Monte Carlo method), with the maximum number of iterations set at 100. Normally distributed continuous variables were predicted using linear regression, non-normally distributed variables using predictive mean matching and binary or categorical variable using logistic regression.

Several additional analyses were performed. First, we adjusted the analyses for the presence of chronic diseases at baseline. Second, baseline analyses were stratified by completeness of dietary intake data. We evaluated if missing items of the FFQ influenced our results by repeating the analyses for participants with no missings on the FFQ. Third, since the measurement of sodium intake using an FFQ is prone to measurement error, we performed a longitudinal sensitivity analyses in which the sodium component was excluded from the DHDI at baseline and at follow-up. IBM's SPSS Statistics (Version 21) software package was used to analyze the data and values of $p < 0.05$ were considered significant.

RESULTS

Description of the study population

Baseline characteristics of female and male participants are provided in Table 1. From the 7983 included participants at baseline, 5434 (68%) had dietary data available of whom 3210 were female (59%). The median baseline age was 66.8 years for the full population (IQR=11.8), 67.3 years (IQR=12.4) for female participants and 66.3 (IQR=10.9) for male participants. The mean dietary quality score was 45.2 (SD=9.95) for all and 42.3 (SD=9.81) for males and 47.25 (SD=9.53) for females.

For 1247 participants, of whom 750 were female, dietary quality was available at baseline and follow-up. Overall, there was an improvement in dietary quality over the assessment period. The mean DHDI-score at baseline was 46.7 (SD=9.89) and 55.4 (SD=11.3) at follow-up with a Pearson's correlation coefficient of 0.20. For 28.9% the dietary quality was considered to be stable (Figure 1a), and stability was slightly higher in males (31.2%) than in females (27.5%) ($p < 0.001$). Stability of dietary quality was similar within the different categories of education and income, whereas routine non manual employees and small proprietors were less likely to have a stable dietary quality. Stability of intake for each component of the DHDI is shown in Figure 1b. For some components, the absolute median sub- scores changed substantially between baseline and follow-up, but stability was relatively high. For example for SFA, the median values is substantially higher at follow-up than at baseline, but stability of this DHDI component was 92%. This implies that overall intake of the population went down over time, but the ranking of participants remained stable.

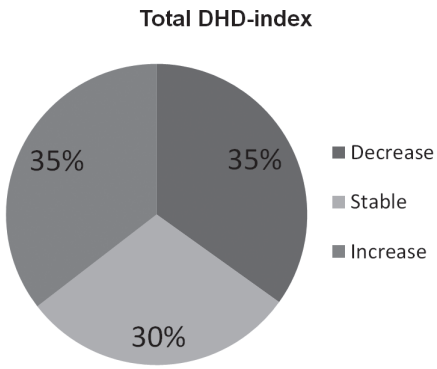

Figure 1a. Distribution of participants with stable, increasing or decreasing overall dietary quality over time. Dietary quality was considered "stable" if a participant was in the same quartile of the energy-adjusted DHD-index at follow-up as at baseline. Baseline DHDI (median) = 45.1, follow-up DHDI (median) = 57.1.

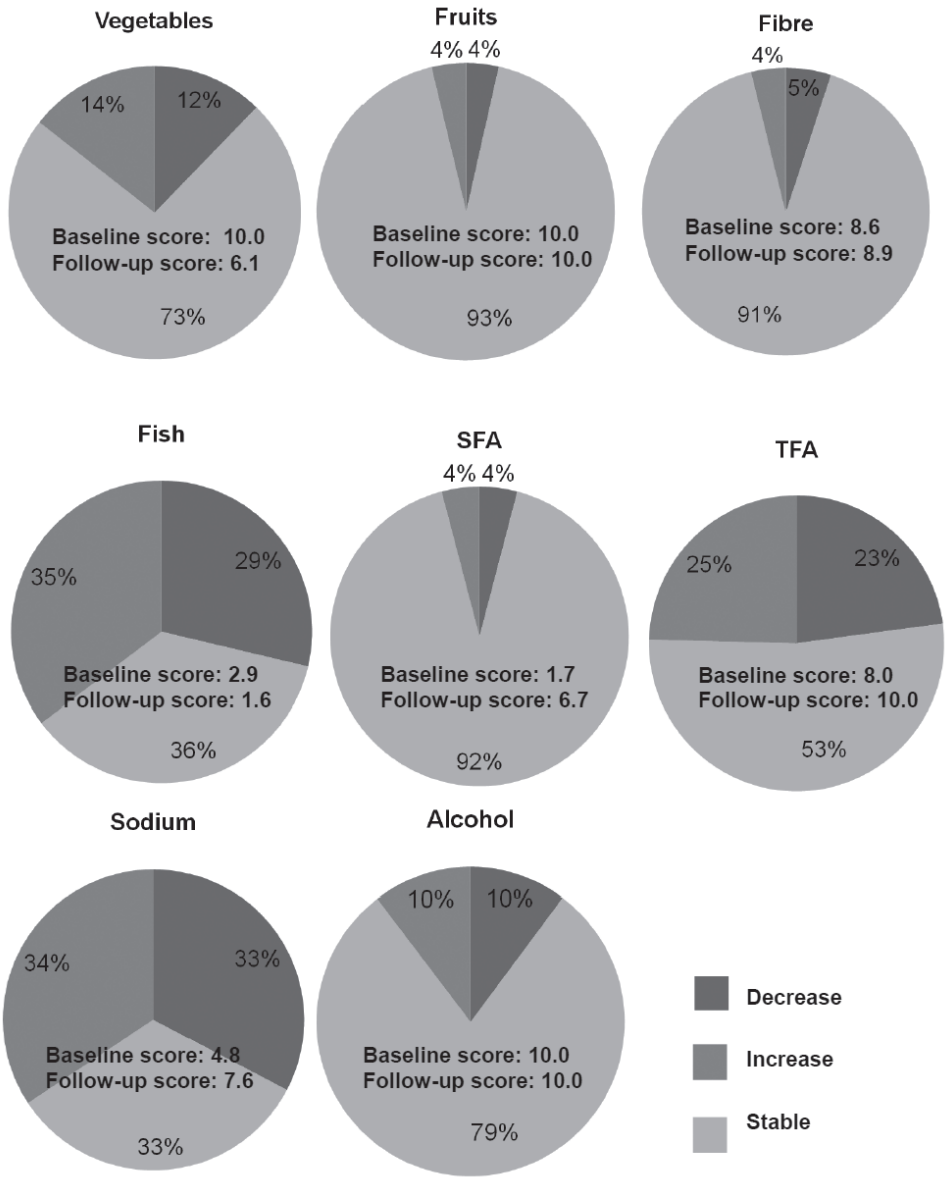

Figure 1b. Distribution of participants with stable, increasing or decreasing intake per component of the DHDI. Dietary quality was considered "stable" if a participant was in the same quartile of the energy-adjusted DHD-index at follow-up as at baseline. Scores reflect the median sub scores per component based on the cut-off values published by van der Lee *et al* [36].

Table 1. Baseline characteristics of the study population stratified by gender

		All		Survivors	
		Males (n=2224)	**Females (n=3210)**	**Males (n=497)**	**Females (n=750)**
		n (%)	**n(%)**	**n(%)**	**n(%)**
Dutch Healthy Diet Index mean (SD)		42.3 (9.8)	47.3 (9.5)	43.5 (9.28)	48.9 (9.7)
Age categories	55-64 years	947 (43%)	1331 (42%)	377 (76%)	591 (79%)
	65-74 years	901 (40%)	1194 (37%)	117 (24%)	155 (21%)
	75-84 years	352 (15%)	612 (19%)	3 (1%)	4 (1%)
	≥85 years	24 (1%)	73 (2%)	0 (0%)	0 (0%)
Smoking	Current smoker	647 (29%)	615 (19%)	122 (25%)	126 (17%)
	Non smoker	1566 (71%)	2576 (81%)	373 (75%)	620 (83%)
BMI	Underweight (<20 kg/m^2)	17 (1%)	26 (1%)	2 (0%)	591 (79%)
	Normal weight (20-25 kg/m$^{2)}$	886 (40%)	1140 (36%)	206 (42%)	155 (21%)
	Overweight (25-30 kg/m^2)	1146 (52%)	1411 (44%)	260 (53%)	4 (1%)
	Obesity (> 30 kg/m^2)	158 (7%)	614 (19%)	27 (5%)	0 (0%)
Physical activity	Low	522 (33%)	771 (33%)	97 (20%)	138 (19%)
	Average	522 (33%)	771 (33%)	178 (37%)	275 (38%)
	High	521 (33%)	770 (33%)	209 (43%)	314 (43%)
Household income (Guilders per year)	< 28000	499 (25%)	1427 (49%)	52 (12%)	203 (31%)
	28000-39999	697 (34%)	742 (26%)	138 (31%)	180 (27%)
	40000-54999	564 (28%)	528 (18%)	163 (37%)	189 (29%)
	> 54999	254 (13%)	201 (7%)	94 (21%)	92 (14%)
Education	Primary education	534 (24%)	1351 (42%)	85 (17%)	209 (28%)
	Lower secondary	533 (24%)	1011 (32%)	109 (22%)	265 (36%)
	Intermediate	819 (37%)	695 (22%)	197 (40%)	218 (29%)
	Higher	326 (15%)	138 (4%)	104 (21%)	54 (7%)
Current/last occupation	Routine non manual employee	583 (26%)	1097 (34%)	120 (24%)	265 (35%)
	Lower and higher professional	79 (36%)	299 (9%)	219 (44%)	78 (10%)
	Small proprietors	93 (4%)	78 (2%)	18 (4%)	14(2%)
	Manual workers	755 (34%)	1155 (36%)	140 (28%)	149 (20%)
	Not working	2 (0%)	582 (18%)	0 (0%)	244 (33%)

BMI=Body Mass Index

Cross-sectional analysis: indicators of dietary quality at baseline

The association between baseline dietary quality scores and socio-economic indicators are presented in Table 2. In the fully adjusted models, only education was positively associated dietary quality (Table 2). Those who attained higher education had on average a 2.29 (95% CI= 1.23, 3.36) points higher dietary quality index than those who attained primary education (Table 2) independent of baseline characteristics, income and occupation. Although we did find differences in dietary quality for different income and occupation groups in the crude models, after adjusting for baseline characteristics these differences became non-significant (Table 2). No interaction between gender and any of the socio-economic indicators was observed in relation to dietary quality at baseline.

Longitudinal analysis: indicators of changes in dietary quality over time

A higher level of education was associated with higher dietary quality at the follow-up visit in both crude and multivariate models (p for trend <0.001, Table 3). As these models were adjusted for baseline dietary quality, this positive association might be interpreted as an increase in dietary quality over time. In contrast, participants with the highest income at baseline tended to have lower dietary quality at follow-up (Table 3). No interaction with gender was observed with any of indicators in relation to dietary quality at follow-up (p for interaction all >0.28). Positive longitudinal associations of education with dietary quality were accompanied by positive associations with vegetable intake, whereas negative associations with income were accompanied by negative associations with fish intake (data not shown).

Additional analyses

The baseline associations between socio-economic indicators and dietary quality among the survivors showed less pronounced results (data not shown). We found no major changes if additionally adjusting for chronic diseases or for participants with incomplete dietary intake data. Lastly, removal of the sodium-component from the DHDI revealed similar results (data not shown).

Table 2. Cross sectional association between education and dietary quality at baseline among older person in Rotterdam, the Netherlands in 1989-1993 in the full population (n = 5434)

Socio-economic indicator		Model 1		Model 2		Model 3	
		B (95%CI)*[1]	P for trend	B (95%CI)	P for trend	B (95%CI)	P for trend
Income (Guilders per month)	< 28000	Reference	**<0.01**	Reference	0.07	Reference	0.31
	28000-39999	0.40 (-0.30, 1.09)		0.22 (-0.45, 0.88)		0.06 (-0.61, 0.73)	
	40000-54999	**1.25 (0.47, 2.03)**		0.71 (-0.07, 1.49)		0.22 (-0.58, 1.02)	
	> 54999	**1.16 (0.24, 2.08)**		0.69 (-0.20, 1.57)		0.08 (-0.86, 1.03)	
Highest education attained	Primary education	Reference	**<0.01**	Reference	**<0.01**	Reference	**<0.01**
	Lower secondary	**1.03 (0.38, 1.68)**		0.70 (-0.08, 1.32)		0.62 (-0.01, 1.25)	
	Intermediate	**1.41 (0.73, 2.08)**		**1.01 (0.37, 1.64)**		**0.81 (0.13, 1.48)**	
	Higher	**3.53 (2.52, 4.54)**		**2.58 (1.62, 3.54)**		**2.29 (1.23, 3.36)**	
Last occupation	Routine non manual	Reference	N.A.*[2]	Reference	N.A.	Reference	N.A.
	Lower and higher	0.63 (-0.11, 1.37)		0.46 (-0.24, 1.17)		0.05 (-0.70, 0.79)	
	Small proprietors	-0.20 (-1.69, 1.30)		0.20 (-1.27, 1.67)		0.36 (-1.12, 1.84)	
	Manual workers	**-0.97 (-1.57, -0.37)**		**-0.66 (-1.23, -0.08)**		-0.45 (-1.04, 0.15)	
	Not working	-0.18 (-1.11, 0.76)		-0.54 (-1.59, 0.49)		-0.48 (-1.36, 0.41)	

Model 1: adjusted for age and sex
Model 2: model 1 + smoking status, BMI, physical activity level and total energy intake
Model 3: model 2+ other socio-economic indicators
*[1]: Regression coefficients represent differences in (absolute) dietary quality indices (range DHDI 0-80)
*[2]: Since occupation is a nominal rather than an ordinal variable, no p for linear trend was computed (N.A= not applicable).

Table 3. Longitudinal association between household income at baseline (1989-1990) and dietary quality at follow-up (2009-2011) among older person in Rotterdam, the Netherlands, full population (n = 1247)

Socio-economic indicator		Model 1		Model 2		Model 3	
		B (95%CI)*[1]	P for trend	B (95%CI)	P for trend	B (95%CI)	P for trend
Income (Guilders per month)	< 28000	Reference	**0.05**	Reference	**0.04**	Reference	**<0.01**
	28000-39999	-0.96 (-2.51, 0.60)		-1.14 (-2.73, 0.45)		-1.38 (-2.95, 0.20)	
	40000-54999	-1.13 (-2.65, 0.40)		-1.27 (-2.82, 0.30)		**-1.92 (-3.54, -0.29)**	
	> 54999	**-1.73 (-3.39, -0.07)**		**-1.92 (-3.67, -0.17)**		**-2.87 (-4.65, -1.09)**	
Highest education attained	Primary education	Reference	**0.03**	Reference	0.06	Reference	0.09
	Lower secondary	**1.56 (1.13, 3.00)**		**1.57 (0.16, 2.97)**		**1.72 (0.26, 3.17)**	
	Intermediate	0.93 (-0.47, 2.34)		0.89 (-0.49, 2.26)		1.09 (-0.39, 2.57)	
	Higher	**2.42 (0.58, 4.27)**		**2.21 (0.43, 3.99)**		**2.12 (0.05, 4.19)**	
Last occupation	Routine non manual	Reference	N.A.*[2]	Reference	N.A.		N.A.
	Lower and higher	**1.53 (0.13, 2.94)**		**1.51 (0.10, 2.91)**		**1.52 (0.04, 3.01)**	
	Small proprietors	-0.21 (-0.23, 2.82)		-0.18 (-3.22, 2.86)		-0.32 (-3.37, 2.74)	
	Manual workers	-0.34 (-1.65, 0.98)		-0.08 (-1.40, 1.24)		-0.08 (-1.42, 1.26)	
	Not working	-0.12 (-1.59, 1.35)		-0.24 (-1.72, 1.25)		-012 (-1.60, 1.37)	

Model 1: adjusted for age and DHDI at baseline.
Model 2: model 1 + smoking status, BMI, physical activity level and total energy intake
Model 3: model 2 + total energy intake and other socio-economic indicators
*[1]: Regression coefficients represent differences in (absolute) dietary quality indices (range DHDI 0-80)
*[2]: Since occupation is a nominal rather than an ordinal variable, no P for linear trend was computed (N.A= not applicable).

DISCUSSION

Main findings

We used a validated index to assess dietary quality and evaluate the association with education, income and occupation. Higher educational levels were associated with a better dietary quality at baseline. Moreover, dietary quality at follow-up was shown to be higher among the most educated elderly and lower among those with the highest income at baseline, after adjustment for baseline dietary quality.

Comparison with previous findings

In line with previous findings, we observed that socio-economic inequalities explain part of the variation in dietary quality [27-30]. Our results highlight that different socio-economic indicators influence dietary quality in different manners [9]. Our most pronounced associations were between high education and dietary quality in line with previous observations [5, 12, 18, 31-35]. We found that irrespectively of confounders and other socio-economic indicators, participants with a high education scored on average 2.29 points higher on the DHDI than participants with primary education only. In means of daily dietary intake this difference could for example be explained by any of the following: 50g more vegetables, 50g more fruit, one small glass of alcohol less or approximately 10g more fiber. Previously, van Lee et al., reported a significant inverse association between adherence to the DHDI and all-cause mortality. Participants in the highest quartile (average DHDI score [SD]= 74.2 [4.5]) had on average a 23% (HR=0.77 95%CI=0.67-0.85) lower all-cause mortality risk than participants in the lowest quartile (average DHDI score [SD]= 47.5 [5.0]) during a 20-year follow-up [36]. Our results could be explained by a generally higher capacity of educated people to understand dietary guidelines and food labels, and the possession of better cooking skills [10, 12]. In addition, educated people could have higher capacity to understand nutritional interventions and adapt nutritional behavior in response to medical advices and treatments [37].

In general, reported associations between income and dietary quality are attributed to high expenses of healthy foods [11, 12]. Indeed, Dijkstra et al (2014) concluded that the main barrier for not meeting dietary recommendations was the high price of fruit and fish [38]. Nevertheless, we did not find an association between income and dietary quality at baseline. Our lack of results might be explained by the similarities in income in our study population, or because other perceived barriers (e.g. taste preference, difficulties with preparation, poor appetite, habits and traditions) could play a more important than price in our study population. Additionally, income is a socio-economic indicator that is sensitive to chance, especially when retirement is approaching. This might be explained by the socio security system in Netherlands that guarantees elderly people a certain amount of money [39].

Previous studies found that those with manual occupations are more likely to have dietary patterns that are classified as less healthy [13, 40, 41]. Additionally, in Australian adults, an improvement of adherence to dietary guidelines over time was observed for people with higher occupational level [17]. It may be speculated that due workplace cultures, people develop dietary habits that can persist after retirement. Indeed, it has been previously shown that men do not markedly change their dietary habits after retirement [42].

The different associations of income and education with dietary quality may be explained by the differences between cultural and economic capital. Cultural capital includes dispositions and competencies, possessions of books and instruments, and educational qualifications. Whereas income is referred to as economic capital. In a recent review regarding cultural capital and the relation to food choices, Kamphuis et al., concluded that higher educated people had more cultural capital which was related to healthier food choices [15]. As we only included middle-aged and elderly people (aged 50 years and over), the distinction between cultural and economic capital might have been more distinct than observed in younger cohorts, because when our participants were young education was reserved for higher social classes [43].

Strengths and limitations

This study has several strengths. First, it reports results of a large cohort of middle aged and elderly participants who were all from the same district of one city and of whom the vast majority was Caucasian, for whom the FFQ was designed and validated. Because the whole cohort was from the same district, factors such as food availability, accessibility, environment, and culture are less likely to have influenced our results. Second, the study had a longitudinal component, which is in contrast to many studies into dietary quality. Last, nutrient data were calculated from the Dutch Food Composition Table, using 1993's version for RS-I-1 and 2011's update for RS-I-5; this enabled the study to take into account the changing nutritional composition of foods.

However, we do also recognize some limitations. Our most important limitation is the use of two different FFQs. Although the FFQ is a method which is suitable for ranking participants dietary intake, the use of different FFQs and different food composition tables at RS-I-1 and RS-I-5 meant that the absolute difference in two dietary index scores at different time points was not possible to measure. Even so, because new foods become available, and food composition and dietary patterns may change over time, it has been suggested to use an up-to-date FFQ to assess dietary intake in cohort [44]. The FFQ applied at baseline consisted of 170 items, whereas the FFQ used at follow-up was based on 389 items. Previous studies have shown that, comparing a 44-item FFQ with a 273-item FFQ, the additional gain in information using a more extensive FFQ was limited [44]. However, it cannot be excluded that

an increase in the number of items in an FFQ leads to higher reported intakes. With respect to fibre, fish, SFA, TFA and sodium intake this could have led to an overall higher reported intake at follow-up. In contrast, in the first FFQ, more items on fruit, alcohol and vegetables were included (supplement 1). Furthermore, although we used validated FFQ's, results may be influenced by report bias, whereas people with a higher level of education may give more socially desirable answers and exaggerate the consumption of healthy foods [5, 45] thereby increasing our estimate of the effect.

Stability of dietary quality over time?

Overall, the stability of dietary quality was poor, whereas only 30% of the participants was ranked in the same quartile at baseline as at follow-up. However, most individual DHDI items showed a rather high stability over time. The low stability was mainly caused by three individual DHDI items: fish, TFA and sodium. Overall, these items were consumed in very low (fish) or high (salt, TFA) quantities at baseline and highly increased (fish) or decreased (salt, TFA) at follow-up. The largest beneficial differences were observed for participants who had a higher educational background. Dietary guidelines on TFA, salt and fish intake were adapted between baseline and follow-up. These results therefore indicate that mainly participants with a high education are able to adapt their dietary behavior according to new dietary guidelines. Overall, changes in dietary quality can be caused by within-subject measurement error of the instrument and the true variation that occurred over time. The large observed variations found for fish, trans fat and salt intake, could be explained by true variation as dietary guidelines have been changed during the follow-up period. Additionally, for these and other food groups true variation could have occurred by changes induced by among others diseases, losing a spouse, cognitive impairment or physical limitations. Nevertheless, within-subject measurement error could be larger due to the use of two different FFQs, than when the same FFQ would have been applied repeatedly.

Suggestions for future research

In the future, the analysis could have been improved by additional dietary measurements during the follow-up period and information of participants' wealth because wealth is considered an important aspect of socio-economic status. For example, it has been shown that home ownership and having a pension were the strongest predictors for dietary quality [31]. We did not have any data about dietary behavior of the participants. For example, we lacked information on meal preparation (e.g. cooking vs. meals on wheels). The influence of socio-economic indicators on dietary quality could be diminished if participants use a meal service. Last, we have no information about changes in socio-economic indicators over time. It could for example be that income has declined after retirement.

CONCLUSION

We observed that education is the socio-economic indicator most strongly associated with both current dietary quality and changes in dietary quality over time. We therefore suggest that dietary interventions for older adults and elderly people take into account educational differences and pay more attention to those who could have difficulties with understanding nutritional guidelines and recommendations. Future studies are required to explore potential barriers of adhering to the dietary guidelines in the lowest educated elderly to further improve preventative strategies for malnutrition.

Supplemental Table 1. Cut-offs used per component of the Dutch Healthy Diet Index, to compose a score reflecting dietary quality

Component		Reflected recommendation	0 points	10 points
1	Vegetables (day)	Eat 150 to 200 grams of vegetables	0	≥ 200
2	Fruit + fruit juices (day)	Eat 200 grams of fruit a day	0	≥ 200
3	Fibre (day)	Eat 30 to 40 grams a day of dietary fiber, especially from sources such as fruit, vegetables and whole-grain cereal products	0 gram/ 4.2 MJ	≥ 14 gram/ 4.2 MJ*
4	Fish (day)	Eat two portions of fish a week, at least one of which should be oily fish	0 mg EPA + DHA	≥ 450 mg EPA + DHA
5	SFA	Limit saturated fatty acid consumption to less than 10 percent of energy intake.	≥ 16.6 en%	< 10 en%
6	TFA	Limit mono trans-fatty acid consumption to less than 1 percent of energy intake	≥ 1.6 en%	< 1 en%
7	Sodium	Limit consumption of table salt to 6 grams a day	≥ 2.45 mg	< 1.68 mg
8	Alcohol	If alcohol is consumed at all, male intake should be limited to two Dutch units (20 gram ethanol) a day and female intake to one	Male: ≥60 grams Female: ≥40 grams	Male: ≤20 grams Female: ≤10 grams

*: 4.2 MJ corresponds to 1000 kcal.

FFQ baseline (170 items)	FFQ follow-up (389 items)
onions, tomatoes, bell pepper, mushrooms, cabbage, spinach broad beans, string beans, carrots, endive, cauliflower, green beans, beetroot, leek, legume, savoy, white cabbage, green cabbage, red cabbage, sprouts, cooked chicory, kale, sauerkraut, lettuce, carrot salad, chicory salad, endive Salad	raw vegetables, cauliflower, broccoli and other cabbage, spinach, onion or leek, beans (green beans, string beans, peas, broad beans), carrots, lettuce, tomato, raw cabbage
tangerines, oranges, grapefruits, lemons, bananas, apples, pears, strawberries, grapes, canned fruit, orange juice, other fruit juices	apple, banana, pear, orange, strawberries, grapes, berries, cherries, melons, other fruit, orange juice, other juice
Calculated on all items	Calculated on all items
Free space to fill in any type of fish	Ready to use fish (fried fish, fried plaice, calamari, fried clams), clams, lean fish (flounder, brill, cod, coley, haddock, tilapia, sole, tuna, fish fingers, whiting), semi fat fish (trout, mullet, plaice, rainbow trout, catfish, swordfish, shrimp, crab, lobster), herring, fat fish (salmon, mackerel, eel, sardines, herring, halibut, butterfish)
Calculated on all items	Calculated on all items
Calculated on all items	Calculated on all items
Calculated on all items	Calculated on all items
Beer, red wine, white wine, sherry, vermouth, port, compare, Dutch gin, liquer, eggnog, cognac, beerenburg, whiskey, vieux	Beer, red wine, white wine, eggnog, strong liquorish (whiskey, rum, gin, cognac)

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3

NUTRIENTS IN RELATION TO BONE OUTCOMES

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DIETARY VITAMIN A INTAKE AND BONE HEALTH

Supplementary Information accompanies this paper on European Journal of Clinical Nutrition website (http://www.nature.com/ejcn)

ABSTRACT

Background

High vitamin A intake might be associated with a decreased bone mineral density (BMD) and increased risk of fractures.

Objective

To study whether dietary intake of vitamin A (total, retinol or beta-carotene) is associated with BMD and fracture risk and if associations are modified by body mass index (BMI) and vitamin D.

Subjects/ Methods

Participants were subjects aged 55 years and older (n= 5288) from the Rotterdam Study, a population-based prospective cohort. Baseline vitamin A and D intake was measured by a food frequency questionnaire. BMD was measured by dual-energy X-ray absorptiometry at 4 visits between baseline (1989-1993) and 2004. Serum vitamin D was assessed in a subgroup (n =3161). Fracture incidence data were derived from medical records with a mean follow up time of 13.9 years.

Results

Median intake of vitamin A ranged from 684 retinol equivalents (RE)/d (quintile 1) to 2000 RE/d (quintile 5). After adjustment for confounders related to lifestyle and socio-economic status, BMD was significantly higher in subjects in the highest quintile of total vitamin A (mean difference in BMD (95%CI) = 11.53 (0.37- 22.7) mg/cm^2) and retinol intake (mean difference in BMD (95%CI) = 12.57 (1.10- 24.05) mg/cm^2) than in the middle quintile. Additional adjustment for BMI diluted these associations. Fracture risk was reduced in these subjects. Significant interaction was present between intake of retinol and overweight (BMI>25) in relation to fractures (P for interaction =0.05), but not BMD. Stratified analysis showed that these favourable associations with fracture risk were only present in overweight subjects (BMI >25). No effect modification by vitamin D intake or serum levels was observed.

Conclusion

Our results suggest a plausible favourable relation between high vitamin A intake from the diet and fracture risk in overweight subjects whereas the association between vitamin A and BMD is mainly explained by BMI.

INTRODUCTION

Adequate nutrition is an important modifiable factor for maintaining bone mineral density (BMD). Although the emphasis in previous studies has been on the intake of calcium and vitamin D, other micronutrients such as vitamin A have also been suggested to play a role in bone remodeling (1). Through diet, vitamin A can be consumed as pre-formed retinol or as one of the pro-vitamins A; alpha- carotene, beta- carotene or beta-cryptoxanthin (2). Preformed retinol is mainly present in foods from animal origin, such as liver, dairy products and eggs, whereas the pro-vitamins are abundant in foods from plant origin such as fruits and vegetables (3).

Results from observational studies have raised the concern that high dietary intake of vitamin A, above 1500 RE per day, might be associated with 10% lower BMD and up to 2 times higher fracture risk compared to intakes of 800-1000 RE per day (4-6). In addition, data from the Rancho Bernardo Study showed a U-shaped relationship between retinol intake and BMD loss in the elderly (7). It has been discussed that potential harmful effects of vitamin A may be driven by the preformed retinol and not by beta-carotene, potentially because the human body converts beta-carotene into retinol only if there is a higher demand, e.g. if the dietary intake of retinol is very low (8, 9). Also, they might be driven by supplemental rather than dietary intake (4).

Animal studies indicate that retinoic acid, the biologically active form of vitamin A, inhibits osteoblast activity and mineralisation and stimulates osteoclast formation (10, 11). In addition, vitamin A diminishes the ability of vitamin D to increase calcium absorption (12). Therefore, negative associations of high vitamin A intake with bone- related outcomes might vary across vitamin D intake levels or plasma concentrations. Data from the Women's Health Initiative Study supported this hypothesis by showing an increased fracture risk in women with a high intake of retinol (2500 µg/day) in combination with low vitamin D intake (<11 µg/day) only(13). Also, it was shown that risk of osteoporosis was highest in Spanish postmenopausal women with high retinol plasma concentrations combined with vitamin D deficiency (14). Body Mass Index (BMI) is another important determinant of BMD (15). Several studies reported overweight to be positively associated with higher BMD, through increased mechanical loading (16, 17). On the other hand, it could be speculated that the effects of vitamin A intake on BMD are different in overweight than in normal weight subjects, as plasma concentrations of fat soluble vitamins were shown to be significantly reduced in overweight subjects (with BMI>25 kg/m^2)(18).

Most studies on vitamin A and bone health have been carried out in (postmenopausal) women but studies in men are scarce. The primary aim of our study was to assess whether dietary total vitamin A, retinol or beta- carotene is associated with BMD and fracture risk in Dutch elderly males and females. The secondary aim was to assess whether there is any interaction between dietary vitamin A intake and vitamin D intake or status or BMI in these associations.

METHODS

The Rotterdam Study

This study was embedded in the Rotterdam Study, a prospective, population based cohort study among Dutch subjects aged 55 years and older living in the Ommoord district in Rotterdam, The Netherlands. Both the objectives and the study design have been described in detail previously (19). Subjects were invited to participate from January 1990 onwards (response rate 78%). Between 1990 and 1993 a baseline home interview on medical history, risk factors for chronic diseases and medication use and information on age at menopause was taken by trained interviewers. The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports (19).

Assessment of dietary intake

All participants were interviewed at baseline for food intake assessment using an extensive semi- quantitative food frequency questionnaire (FFQ) at the study center by a trained dietician. The questionnaire was validated and adapted for use in the elderly (20-22). It consists of 170 food items in 13 food groups and questions about dietary habits. The ability of the FFQ to rank subjects adequately according to their dietary intakes was demonstrated by a validation study comparing the FFQ to 15 day- food records collected over a year to cover all seasons. Pearson's correlation coefficients of this comparison ranged from 0.4 to 0.8 after adjustment for sex, age, total energy intake, and within-person variability in daily intakes (22).

The dietary intake of nutrients was calculated using the Dutch Food Composition Database (NEVO) from 1993, 1998 and 2006. Beta- carotene and consequently total vitamin A, data were updated in 2004 by the Dutch Institute for Public Health and the Environment (23), by re-estimating retinol equivalents for all foods in the Dutch Food Composition Database (NEVO). The effects of retinol and beta carotene are studied both separately and combined in the variable "total vitamin A" as retinol equivalents (RE). Intake of RE was calculated using the following equation: RE = μg retinol + (μg β-carotene/ 6) + (μg α-carotene/12) + (μg β-cryptoxanthin/ 12). We used RE for our main analyses, to be able to compare our results with previous work. In addition, we calculated total vitamin A in retinol activity equivalents (RAE), using the following equation: RAE = μg retinol + (μg β-carotene/ 12) + (μg α-carotene/24) + (μg β-cryptoxanthin/ 24). This RAE value takes into account more recent insights regarding bio efficacy of the pro- vitamin A carotenoids (23-26). Data on dietary supplement use were collected separately from the FFQ during the home interview. Dietary supplement use was not included in the calculation of dietary intake but used in sensitivity analysis (see "statistical analysis").

"Energy- adjusted" nutrient intakes were computed as the unstandardized residuals from a linear regression model in which total caloric intake served as the independent variable and the nutrient intake as the dependent variable(27). Energy adjusted intakes were divided into quintiles for all nutrients (total vitamin A, retinol and beta- carotene).

Assessment of BMD and fractures

BMD of the femoral neck was measured by dual energy X-ray absorptiometry (DXA) using a Lunar DPX- densitometer (Lunar Radiation Corp., Wadison, WI) and analyzed with DPX-IQ software at four visits between 1989 and 2004. BMD values are expressed in g/cm^2.

Fractures were reported by general practitioners in the research area (covering 80% of the cohort) by means of a computerized system. All reported events were verified by two trained research physicians, who independently reviewed and coded the information according to the International Classification of Primary Care (ICPC) and International Classification of Diseases, 10th edition (ICD-10). Fractures included those of radius/-ulna (ICPC: L72, ICD-10: S52.0 to 52.9), tibia/-fibula (ICPC: L73, ICD-10: S82.0 to 82.9), hand/-foot (ICPC: L74, ICD-10: S62.0 to 52.9 and S92.0 to 92.0), femur (ICPC: L75, ICD-10: S72.0 to 72.9) and other fractures (ICPC: L76, ICD-10: S02.0 to 02.9, S12.0 to 12.9, S22.0 to 22.9, S32.0 to 32.8, S42.0 to 42.9, T08, T10, T12, T14.2 and M84.3 to M84.9). Subsequently, inconsistently coded events were reviewed by a medical expert for final classification. Subjects were followed from their baseline visit until January 2007 or until a first fracture occurred, resulting in a mean fracture follow up of 13.9 (± 0.69) years.

Assessment of covariates

Weight and height were measured at the baseline visit (1989-1993) at the research center. BMI was calculated as weight in kilograms divided by height in meters squared. Smoking habits were coded as "current or "past or never". Dietary intake of calcium, vitamin D and alcohol were obtained similarly by the FFQ as described previously. Socio-economic status (SES) was estimated using education level and net household income. Highest education level was classified as "lower" (primary, primary plus higher not completed, lower vocational, lower secondary) or "higher" (intermediate vocational, general secondary, higher vocational or academic). Net income was classified as "low" (< 2400) or "high" (> 2400) Dutch Florins (≈ 1600 euro's) per month. Disability index was coded 0 to 3 and is a combined variable reflecting subject's ability to perform dressing, rising, eating, walking, hygiene, reach and grip activities, based on the Stanford Health Assessment Questionnaire (28). Age at menopause was defined as the age at which the menstrual cycle was absent for 12 months and use of female hormones (HRT) was coded as "ever "or "never" and collected at the 2nd visit (1993- 1995). Physical activity was

defined in total minutes of activity per week, including household activities and collected at the 3rd visit (1997- 1999) using the Zutphen Questionnaire and LASA Questionnaire (29-31).

Assessment of vitamin D status

At the 3rd visit (1997- 1999), radioimmunoassays (IDS Ltd, Boldon, UK, available at www.idsltd.com) were used to measure serum vitamin D concentrations. Serum 25-hydroxyvitamin D (25(OH)D) were measured in a subgroup of participants (n= 3171). The sensitivity of the test was 3nmol/L which ranged from 4 to 400nmol/L. Intra-assay accuracy was <8% and the inter-assay accuracy was <12%. Subjects were classified to be vitamin D deficient (<50 nmol 25-OH-D3/ l), insufficient (≥ 50 and < 75 nmol/l) or sufficient (≥ 75 nmol/l) (32).

Statistical analysis

The associations between energy adjusted dietary intake of total vitamin A, retinol, beta- carotene and BMD were determined by generalized estimating equation (GEE) modelling using BMD of the femoral neck at four visits between 1993 and 2004. Associations with fracture risk were estimated by Cox proportional hazard modelling. All exposure variables (total vitamin A, retinol and beta- carotene) were assessed in separate models, continuously and in quintiles, and adjusted for potential confounding by age and sex (model 1). We have performed analysis in quintiles to correct for the measurement error of the FFQ (22) and to facilitate comparisons of the effects of the extreme intakes (5th and 1st quintile) to the average intake (3rd quintile) of vitamin A in our population. Potential confounders were selected according to previous literature and univariate analyses. Subsequently, additional confounding by smoking, dietary calcium intake, alcohol intake, education, net income, disability index, and physical activity was tested (model 2). Since BMI can be a confounder as well as an effect modifier, the potential role of BMI in the associations between vitamin A intake and BMD or fracture risk were explored using two approaches. First, we have added BMI as an additional covariate to our multivariate models (model 3). Second, we tested for effect modification by adding interaction terms of the exposures (i.e. vitamin A) with BMI (as continuous variable) or overweight status ('overweight' defined as BMI> 25 kg/m^2 based on cut-offs of the WHO (33, 34)) together with BMI or overweight status as independent variables to a model adjusted for age and sex. Effect modification by vitamin D intake or status was tested similarly, using the interaction terms of the exposure with vitamin D intake or status as continuous variables. Stratified analyses were performed if the P-value for interaction was < 0.10. Strata were computed based on the WHO cut-off for BMI (below and equal to 25 kg/m^2 or above 25 kg/m^2) and based on the median for vitamin D. For the analysis on incident fractures, baseline BMD was added to

the model to assess whether the associations were explained by BMD (model 4). Analyses regarding vitamin D status were additionally adjusted for centre visit, to correct for seasonal influences. Missing data on covariates were multiple imputed (N=5 imputations; Supplemental table 1 and 2). Since results did not differ before and after the imputation procedure, all analyses are reported after the multiple imputation procedure.

Although no detailed data on dietary supplement use was available, several sensitivity analyses were performed to assess whether supplement use could influence our results. First, we have added the use of any dietary supplement over the past year as an additional covariate to our main analysis (using model 2). Second, we have excluded all subjects that reported to use any vitamin A containing supplement (of unknown dosage of either retinol or carotenoids and for unknown duration, n = 59).

Statistical analyses were performed by IBM SPSS statistics version 20. For all analyses, P- values of < 0.05 were considered statistically significant.

RESULTS

Study population

The study population consisted of 2172 male and 3116 female elderly subjects. A flowchart showing sample sizes for our main analysis is shown in Fig. 1. Baseline characteristics including anthropometrics, dietary intake and covariates are presented in Table 1 per quintile of total vitamin A intake. Median (interquartile range (IQR)) dietary intake of vitamin A ranged from 684 (568- 793) RE in the lowest quintile to 2000 (1712- 2485) RE in the highest quintile. Correlation between dietary intake of retinol and beta-carotene was 0.026. The contribution of preformed retinol, beta- carotene and other carotenoids to total vitamin A intake were 41%, 50% and 9% respectively. Dietary intake of all vitamin A variables were significantly correlated with total energy intake (Pearson's r = 0.24 for total vitamin A, 0.23 for retinol and 0.10 for beta- carotene. Median (± SD) of femoral neck BMD in males and females per follow up visit are shown in Supplemental Fig. 1. Between baseline (1989- 1993) and last follow up visit, mean BMD decreased in females (-2.5%), but less in males (-0.2%).

Average follow up was 13.9 (± 0.69) years. Overall, fracture incidence was approximately three- fold higher in females than in males. The majority of all fractures (89% in males, 92% in females) was of osteoporotic origin (defined as any type of fracture excluding high trauma and those at skeletal sites like skull, fingers, toes, and ribs likely caused by trauma) and 22% of all fractures occurred at the hip. Fifteen percent of the participants (n=802) had a history of fractures 5 years prior to study entrance.

Table 1. Characteristics of participants of the Rotterdam Study (n =5288) per quintile of total dietary vitamin A intake

	Quintiles of total vitamin A intake (in RES)	
	Q1	**Q2**
Sex (% males)	45	44
Age (y)[1,2]	67 (61- 73)	67 (61- 73)
Height (cm)[1,2]	168 (161- 174)	167 (161- 175)
Weight (kg)[1,2]	73 (65- 80)	73 (65- 82)
BMI (kg/m^2) [1,2]	25.6 (23.7- 27.8)	25.9 (23.8- 28.1)
% Overweight/ obesity [2,3]	46/ 11	48/ 13
Dietary intake[1,2]		
Total vitamin A (RE/d)[4]	684 (568- 793)	945 (867- 1052)
Total vitamin A (RAE/d)[5]	404 (333- 491)	600 (483 -655)
Retinol (µg/d)	194 (135- 289)	264 (173- 384)
Beta-carotene (µg/d)	2373 (1845- 2909)	3490 (2942- 3964)
Fruits and vegetables (g/d)	356 (268- 467)	417 (328- 522)
Milk and milk products (g/d)	253 (160 -390)	263 (165- 406)
Meat and meat products (g/d)	86 (60- 112)	89 (65- 118)
Fats and oils (g/d)	27 (14- 42)	32 (18-46)
Vitamin D (µg/d)	3.62 (2.68-4.61)	3.29 (2.36- 4.45)
Calcium (mg/d)	1009 (804- 1279)	1046 (857- 1282)
Total energy (kcal/d)	1893 (1600- 2262)	1948 (1644- 2289)
Alcohol (g/d)	3.5 (0.1- 16.9)	3.4 (0.2- 14.4)
Serum 25(OH)D (nmol/l) [1,6]	45 (29-64)	45 (29-64)
Disability (index) [1,2]	0.13 (0.00 -0.38)	0.13 (0.00 -0.38)
Physical activity (hours/day) [1,6]	5.8 (4.2- 7.5)	5.8 (4.1- 7.8)
Of which vigorous (h/day)	0.4 (0.1- 0.9)	0.5 (0.1- 1.0)
Age at menopause (y) [1,3]	50 (47-52)	50 (47-52)
Prevalent osteoporosis (%) [2]	11	10
High education (%) [7]	36	39
High income (% > 1600 euro/mo) [2]	53	53
Current smokers (%) [2]	25	23
History of any fractures[2] (%)	16	14
Current or past HRT use (%)[8]	13	13

[1]Median (interquartile range) [2]Assessed at baseline; [3]'Overweight' was defined as BMI >25 and ≤ 30 kg/m^2, 'Obesity' was defined as BMI>30 kg/m^2; [4] RE = µg retinol + (µg β-carotene/ 6) + (µg α-carotene/12) + (µg β-cryptoxanthin/ 12); [5] RAE = µg retinol + (µg β-carotene/ 12) + (µg α-carotene/24) + (µg β-cryptoxanthin/ 24) [6]Assessed at 3rd visit (1997- 1999)[36]; [6] Including housekeeping,

Quintiles of total vitamin A intake (in RES)		
Q3	**Q4**	**Q5**
41	38	37
67 (61- 73)	67 (61- 73)	67 (61- 73)
167 (161- 174)	166 (161- 173)	166 (160- 174)
73 (66- 81)	73 (66- 81)	74 (67- 82)
25.9 (23.8- 28.4)	26.0 (24.2- 28.5)	26.4 (24.2- 29.1)
49/ 14	46/ 16	47/ 18
1141 (1050- 1257)	1389 (1263- 1551)	2000 (1712- 2485)
689 (583- 819)	868 (716- 1066)	1417 (1096- 1916)
356 (212- 523)	487 (272- 734)	1021 (594- 1518)
3992 (3336- 4575)	4601 (3586- 5461)	5042 (3771- 6586)
451 (351- 567)	478 (371- 587)	512 (398- 638)
277 (165- 439)	281 (165- 450)	260 (156- 257)
89 (64- 120)	91 (68- 124)	102 (75- 134)
33 (18-46)	32 (20- 46)	33 (19- 46)
3.01 (2.18- 4.12)	3.02 (2.20- 4.15)	3.16 (2.25- 4.32)
1097 (878- 1341)	1097 (894- 1340)	1130 (872- 1374)
1929 (1625 -2260)	1918 (1610- 2263)	1923 (1605- 2242)
3.1 (0.2 (14.4)	3.5 (0.2 (14.8)	3.9 (0.2 (15.0)
44 (30-65)	45 (30-62)	45 (30- 67)
0.13 (0.00 -0.38)	0.13 (0.00 -0.38)	0.13 (0.00 -0.50)
5.9 (4.2- 7.8)	5.8 (4.3- 7.9)	5.9 (4.2- 7.9)
0.4 (0.2- 1.0)	0.5 (0.2- 1.0)	0.4 (0.1- 1.0)
50 (45-52)	50 (46-52)	50 (45-52)
10	10	11
37	36	35
53	50	46
22	20	25
15	14	17
13	17	12

[7]Includes intermediate vocational, general secondary, higher vocational or academic education; [8] Assessed at 2nd visit (1993-1995); [9]Quintiles are based on energy adjusted intake values of REs, using the residual method[22]. Abbreviations: RE= retinol equivalents, RAE= retinol activity equivalents BMI= body mass index; HRT= hormone replacement therapy.

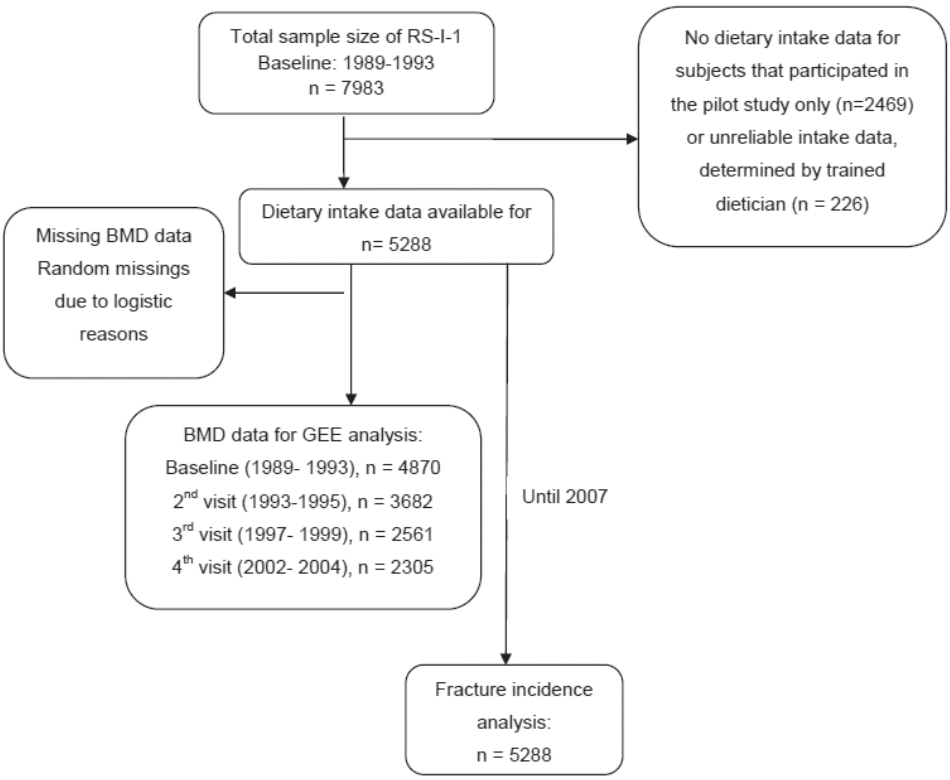

Figure 1. Data availability for main analyses

Table 2. Associations of dietary intake total vitamin A, retinol and beta- carotene with BMD, obtained using GEE analysis.

	Model 1[1]	Model 2[1]	Model 3[1]
Total vit. A[2]	**0.53 (0.06- 0.99)**	**0.46 (0.00- 0.91)**	0.14 (-0.28- 0.56)
Retinol[2]	0.31 (-0.23-0.87)	0.45 (-0.09- 1.01)	0.13 (-0.40- 0.75)
Beta-carotene[2]	**0.21 (0.02-0.40)**	0.10 (-0.07- 0.28)	0.03 (-0.11- 0.18)

[1] Regression coefficients (95% confidence intervals). Regression coefficients represent the change in BMD (mg/cm^2) per unit increase of 100 RE total vitamin A/ day, 100 µg retinol/ day and 100 µg beta- carotene/ day. Considering a population mean BMD of 0.90 g/cm^2, a regression coefficient of 0.52 indicates a 0.06% higher BMD in subjects with 100 RE higher intake of total vitamin A.
[2]: All vitamin A variables are adjusted for total energy intake, using the residual method
Model 1: Adjusted for age and sex
Model 2: Model 1 + calcium intake, smoking, disability index, net income, highest education level, physical activity, alcohol intake and for women only HRT use, age at menopause
P-values < 0.05 in **bold**.
Model 3: Model 2 + BMI
Abbreviations: HRT: hormone replacement therapy; BMD: bone mineral density; GEE: generalized estimating equation

Associations between dietary intake of vitamin A, retinol, beta- carotene and BMD

Regression coefficients and 95% confidence intervals of the association between dietary intake of total vitamin A, retinol, beta-carotene and BMD are shown in table 2. Dietary intake of total vitamin A was associated with a higher BMD, which remained significant after adjustment for age, gender, calcium intake, smoking, disability index, smoking, income and education, physical activity and use of HRT and age at menopause (β (95% CI) = 0.46 (0.00- 0.91) mg/cm^2 BMD per 100 RE) for total vitamin A. For beta-carotene, positive associations with BMD disappeared after adjustment for confounders. For preformed retinol, no significant associations were found in continuous analyses. However, categorical analysis showed that BMD of subjects with intakes in highest quintile of retinol (Q5) was significantly higher than BMD of subjects with intakes in the middle quintile (Q3, reference, β (95%CI) = 12.57 (1.10- 24.05). Median dietary intakes of all vitamin A variables per quintile are shown in Supplemental table 3. Results were diluted after additional adjustment for BMI in continuous (table 2, model 3) and categorical analyses (Fig. 2a).

Associations between dietary intake of total vitamin A, retinol, beta- carotene and fracture risk

Cox proportional hazard ratios and 95% confidence intervals of the association between dietary intake of vitamin A, retinol and beta- carotene and risk of all fractures using model 3 are shown in Fig. 2, lower part. Significantly lower fracture risks were observed in subjects with intakes in the highest quintile (Q5) compared the middle quintile (reference, Q3) of total vitamin A (HR (95% CI) = 0.82 (0.69- 0.97)) and retinol (HR (95% CI) = 0.81 (0.68- 0.96)). These effects remained significant after additional adjustment for BMI (Fig. 2b). However, effects were diluted and lost significance after additional adjustment for baseline BMD.

Interaction with overweight status

At baseline, 61% of the participants (n= 3244) had a BMI of > 25 kg/m^2. No significant interaction was found between total vitamin A, retinol or beta- carotene and overweight status (P for all interactions > 0.32) or BMI as continuous variable (P for all interactions > 0.18) in relation to BMD.

In relation to fracture risk, interaction was observed between intake of total vitamin A and retinol with overweight status as well as with BMI as continuous variable (P for all interactions < 0.06). These interactions with BMI were not present for beta-carotene. After adjustment for confounders and BMI (model 3), stratified analysis showed a significant lower fracture risk (Fig. 3a) in subjects in the highest quintile of retinol intake only in those with a BMI > 25 (HR (95%CI) = 0.78 (0.68 – 0.89) versus 1.04 (0.87- 1.24) with BMI ≤ 25). These results were diluted but remained significant

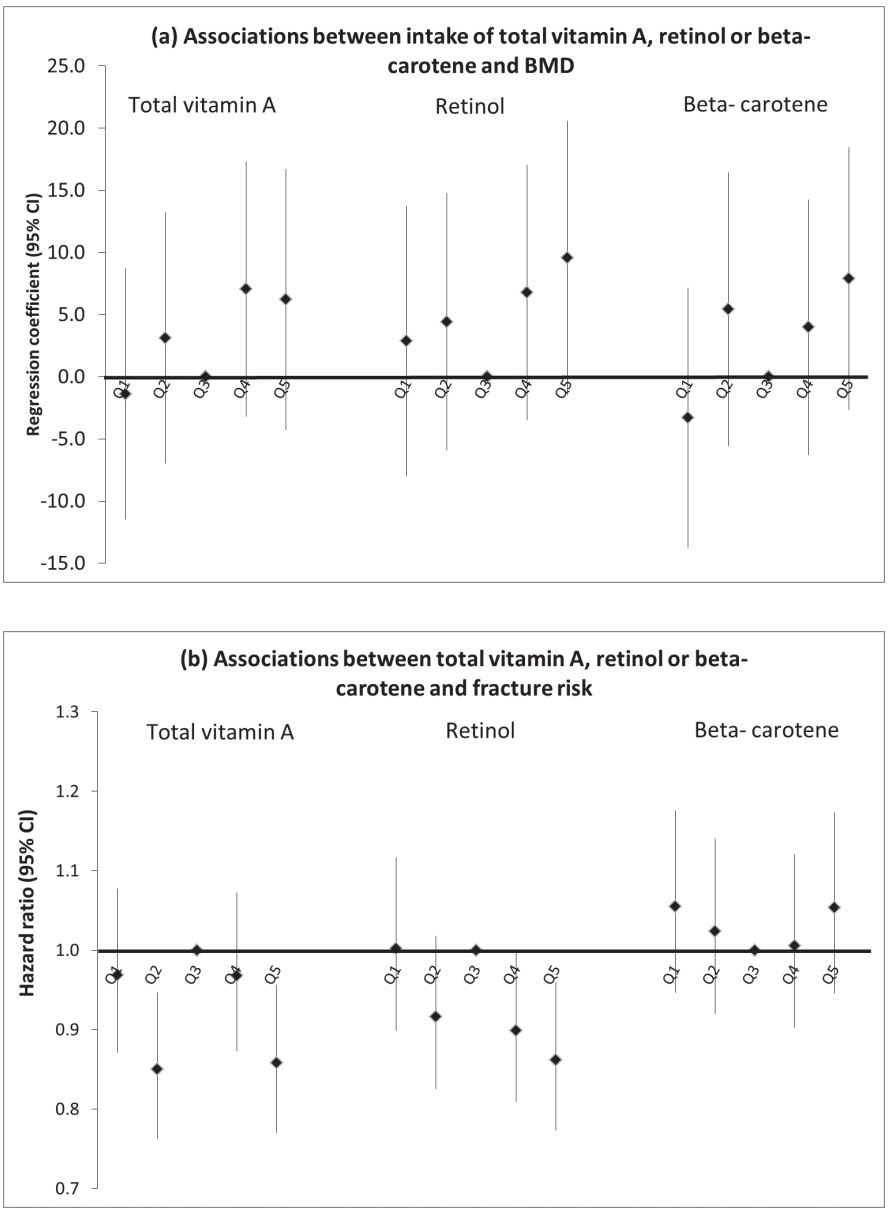

Figure 2. Associations with BMD (a) and fracture risk (b) comparing quintiles of dietary intakes of energy adjusted total vitamin A, retinol and beta- carotene with quintile 3 as reference.
Regression coefficients represent the difference in BMD (mg/cm^2) of each quintile to the reference. Considering a median population BMD of 0.90 g/cm^2, a regression coefficient of 10 represent a 1% higher BMD. See S-table 3 for median intakes per quintile
Model 3: adjustment for age, sex, calcium intake, smoking, disability index, net income, highest education level, physical activity, alcohol intake, BMI and for women only HRT use, age at menopause.
Abbreviation: BMD: bone mineral density, HRT: hormone replacement therapy.

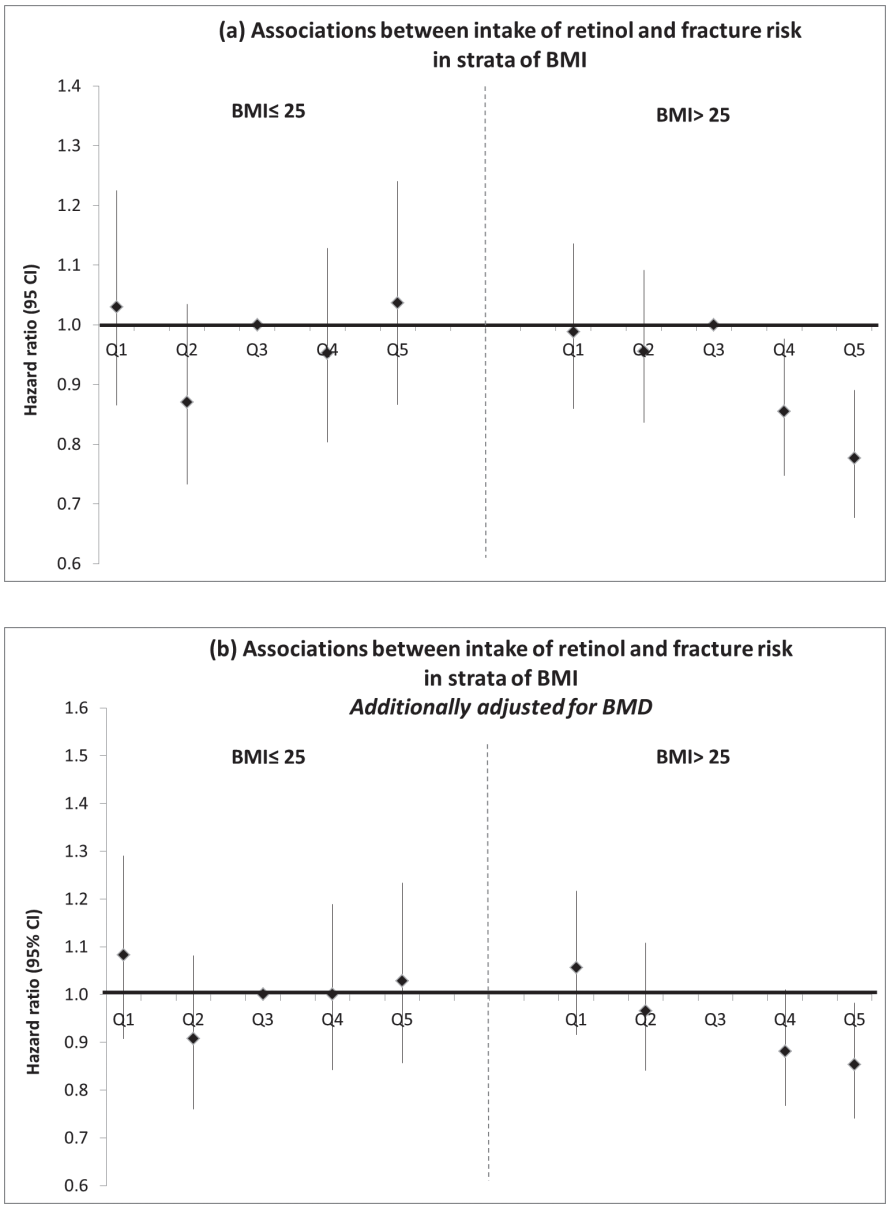

Figure 3. Associations between energy adjusted dietary intake of retinol and fracture risk in strata of BMI, using quintile 3 as reference in model 3 (a) and model 4 (b).
Model 3: Adjusted for age, sex, calcium intake, smoking, disability index, net income, highest education level, physical activity, alcohol intake and for women only HRT use, age at menopause and BMI.
Model 4: Model 3, additionally adjusted for BMD
See S-table 3 for median intakes per quintile.
Abbreviations: HRT= hormone replacement therapy, BMI=body mass index, BMD= bone mineral density.

after additional adjustment for baseline BMD (Fig. 3b). The lowered risk of fractures in subjects with high BMI was also observed in those with high intake of total vitamin A, but not beta- carotene (Supplemental Fig. 2a and 2b).

Interaction with vitamin D intake

Significant interaction was observed between dietary intake of vitamin D and total vitamin A (P for interaction= 0.016) as well as beta- carotene (P for interaction =0.001) in relation to BMD. However, stratified analysis for dietary vitamin D intake above or below the median of 3.2 microgram per day, using model 3, did not show significant associations between total vitamin A or beta- carotene intake and BMD in any of the strata (Fig. 4 and b). In relation to fractures, no significant interaction was found between total vitamin A, retinol or beta- carotene and vitamin D intake (P for all interactions > 0.45)

Interaction with vitamin D plasma concentrations

Vitamin D concentrations in serum were available for 1294 males and 1867 females. Twenty-five percent of males and 39% of females were vitamin D deficient (<50 nmol 25-OH-D3/ l) and only 42% of males and 24% of females had sufficient (≥ 75 nmol/l) vitamin D plasma concentrations. No significant interactions between dietary intake of vitamin A and vitamin D plasma concentrations were present in relation to BMD or fracture risk (P all interactions > 0.31).

Sensitivity analyses

Sensitivity analyses showed that additional adjustment for use of any dietary supplements as well as exclusion of any vitamin A-containing supplement use during the past year did not change the effect estimated of our main analyses (data not shown).

DISCUSSION

In this prospective study among elderly males and females, we showed that high dietary intake of vitamin A was associated with a higher BMD and lower fracture risk. However, favourable associations with BMD disappeared after adjustment for BMI. The relation with fracture risk was only present in overweight subjects. Also, our results suggest that the association between vitamin A intake and fracture risk is explained by differences in BMD. No effect modification by either vitamin D intake or status was observed.

Our observed association between high vitamin A intake and low fracture risk was mainly explained by high intake of retinol and not beta-carotene. We speculate that vitamin A status of our population was sufficient and therefore the conversion of beta-carotene into retinoic acid may have been too limited to exert effects on bone. Furthermore, variation in bioavailability of beta-carotene due to differences in food matrices might have affected our results (8, 9).

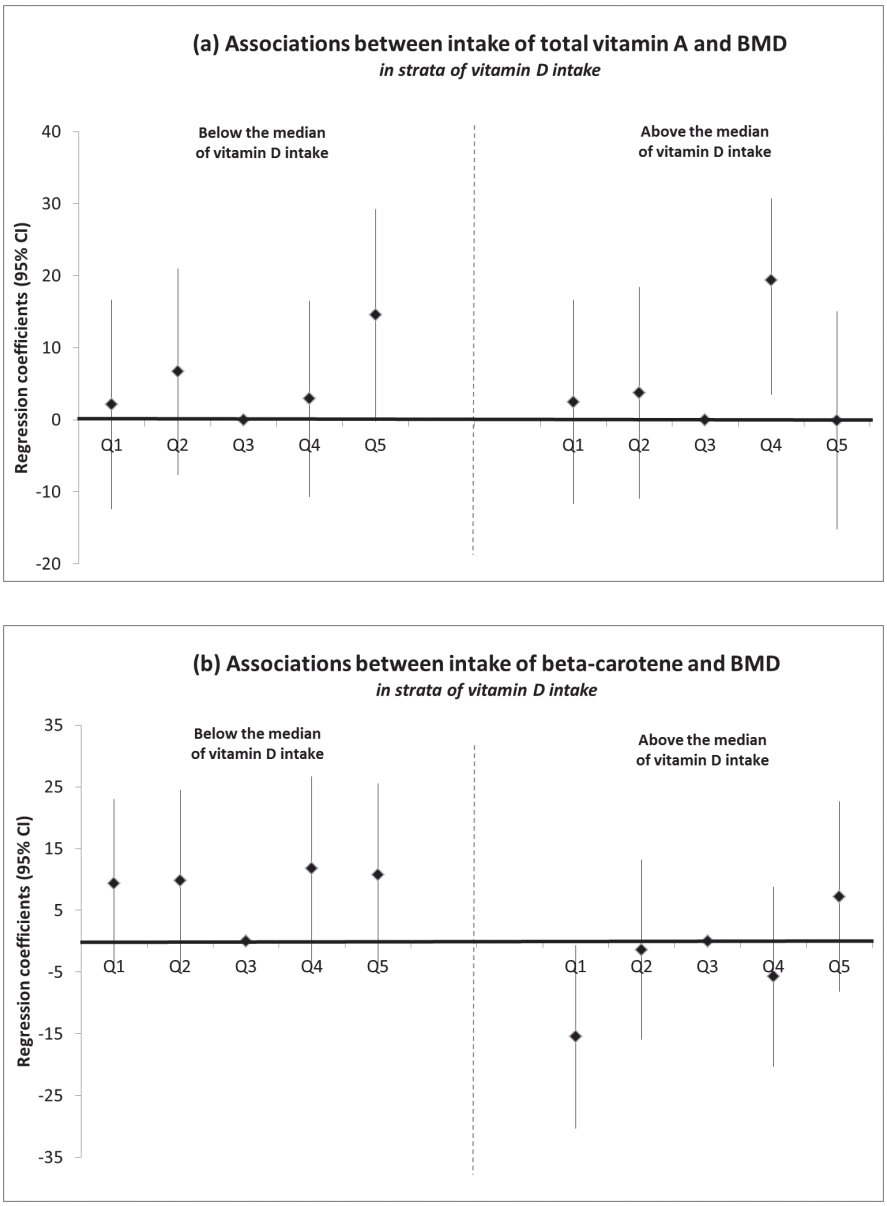

Figure 4. Associations between energy adjusted dietary intake of total vitamin A (a) or beta-carotene (b) and BMD in strata of dietary vitamin D intake (above or below the median of 3.2 microgram/day, using model 3. Model 3: Adjusted for age, sex, calcium intake, smoking, disability index, net income, highest education level, physical activity, alcohol intake, BMI and for women only HRT use, age at menopause. Abbreviations: HRT: hormone replacement therapy, BMI=body mass index, BMD= bone mineral density.

Comparisons with other studies

Our observed association between dietary intake of retinol and high BMD are not in line with earlier findings by Promislow *et al* (7) who found a similar association in Californian women that derived their vitamin A intake from food products only but independent of BMI, whereas the favourable associations with BMD in our study diluted after adjustment for BMI. With respect to fracture risk, we have not been able to confirm earlier findings by Feskanich *et al* (4), who showed an increased risk for hip fracture in the highest quintile of retinol intake from food among postmenopausal women from the Nurse's Health Study (NHS). Possibly, absolute intake of retinol in the NHS is higher than in the Rotterdam Study, or retinol intake might be derived from different food products in the United States than in the Netherlands, due to country-specific food fortification or dietary habits. For example, in the NHS milk is reported as reasonable source of vitamin A intake, whereas in our population, vegetable oils and meat are more important sources. Also the lower calcium intake in NHS that may result in lower BMD may explain differences in fracture risk observed in NHS versus RS.

It can be argued that BMI is a confounder as well as an effect modifier in our analyses. Additional adjustment for BMI diluted any association of vitamin A with BMD, but not with fracture risk. Further analyses stratified for BMI categories showed that the positive association between vitamin A intake and fracture risk was only present in overweight and not in normal weight subjects. Two potential causes could explain our observed effect modification by overweight status. First, more vitamin A can be stored in fat tissue of subjects with a high BMI and therefore less retinoic acid is available for osteoblast inhibition or osteoclast formation. In other words, high BMI might protect against the unfavorable effects of excess vitamin A intake on bone. As intake levels of vitamin A in our study are not extremely high, one could even hypothesize that high BMI creates a very mild vitamin A deficiency, which could explain the positive relation of high intake with BMD in this subgroup. Second, subjects with higher BMI have higher mechanical loading, thereby increasing their BMD. Hence, further studies should clarify the role of BMI in the association between vitamin A and BMD.

Our stratified analysis by vitamin D status did not confirm earlier results from the Women's Health Initiative showing only a modest increase in total fracture risk with high vitamin A and retinol intakes in the low vitamin D-intake group (13). However, variation in vitamin D intake in our population might have been too small to detect an effect in the strata. It has been shown in rats that vitamin A antagonizes the ability of vitamin D to enhance calcium uptake (35). The importance of low vitamin A intake for beneficial effects of vitamin D and calcium has also been described in relation to other health outcomes (36). Replication in larger vitamin D status samples would be necessary to further explore this interaction and its potential interplay with dietary

calcium intake or source. Also complementary interactions, synergistic or antagonistic, could play a role in optimizing BMD and reducing fracture risk. Full dietary pattern analysis in this traditional Dutch population might provide further insights. In addition to earlier studies comparing dietary to supplemental vitamin A, a comparison of different vitamin A fortified food products, e.g. milk versus margarine, would also provide extra insights in these potential nutrient interactions, in line with previous recommendations by Rejnmark *et al*, 2003 (37).

Implications

Currently, a debate is ongoing on the potential safe upper levels of vitamin A. The current level is at 3000 RE/ day (38), but it has been suggested that adverse effects with respect to bone already occur at lower intake levels of 1500 RE/ day. Our findings do no support this hypothesis. However, we recognize that our analyses are limited to average dietary intake levels, with a median intake of approximately 2200 RE in the highest quartile, and that the (excessive) use of dietary supplements which can provide up to 5700 RE per day might provide harmful effects with respect to BMD and fracture risk as shown in earlier studies (39). Hence, studies on extremely high intake of vitamin A deserve further attention.

Strengths & limitations of the study

Our analysis has several strengths. First, we have a large sample size of community-dwelling subjects that increases the external validity of our results. Second, we were able to test for potential confounding effects of an extensive list of covariates. Third, in addition to dietary intake data we had data on vitamin D plasma concentrations which is a better reflection of vitamin D status than assessed from diet. To appreciate our findings, some limitations need to be taken into account. Unfortunately, we did not have extensive information on physical exercise at baseline, which is a suggested determinant of BMD in the elderly (40). However, by adjusting for disability index and physical activity at the 3rd visit (1997- 1999) we attempted to diminish confounding by physical activity but residual confounding still might be present. Although we calculated dietary intake levels of vitamins by using a validated FFQ and we used most recent food composition tables, measurement error may still be present. Although we adjusted the results for total energy intake to account for systematic measurement error, random error still may be present that might have diluted our results (41). We did not have data on plasma concentrations of vitamin A which could have provided additional insight on vitamin A metabolism and interaction with vitamin D. We did not record specific supplement use, but other studies showed that the percentage of Dutch elderly using vitamin A supplements in the early 1990's was very low (<1%) (42).We therefore do not expect our results to be largely biased by supplement use. Lastly, we only had BMD data of the femoral neck and not of other sites of the body.

CONCLUSIONS

Our results do not support earlier findings that high vitamin A intake unfavourably affects BMD and fracture risk, but in contrast suggest that a favourable relation between high vitamin A intake and fracture risk may exist in overweight subjects whereas the association between vitamin A and BMD is mainly explained by BMI. No effect modification by vitamin D intake or plasma concentrations was observed. Further studies are needed to understand the interaction between vitamin A and vitamin D as well as the interplay with BMI in relation to BMD and fracture risk.

Supplementary information is available at the European Journal of Clinical Nutrition's website.

The supplementary materials contain information related to: (1) details of multiple imputation modelling, (2) fracture cases and median intake of total vitamin A, retinol and beta- carotene per quintile of energy adjusted intake, (3) Mean (± SD) bone mineral density over the follow up time in males and females and (4) additional stratified analysis for BMI. This information is provided in 3 tables and 3 supplementary figures.

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3.2

DIETARY ACID LOAD, TRABECULAR BONE INTEGRITY AND MINERAL DENSITY

ABSTRACT

Background

Studies on dietary acid load (DAL) and fractures have shown inconsistent results. Associations between DAL, bone mineral density (BMD) and trabecular bone integrity might play a role in these inconsistencies and might be influenced by renal function and dietary fibre intake.

Purpose

To study: (1) associations between DAL with BMD and the Trabecular Bone Score (TBS) and (2) the potential influence of renal function and dietary fibre in these associations.

Methods

Dutch individuals aged 45 years and over (n= 4672) participating in the prospective cohort of the Rotterdam Study were included. Based on food frequency questionnaires, three indices of DAL were calculated: the net endogenous acid production (NEAP) and the ratios of vegetable or animal protein and potassium (VegPro/K and AnPro/K). Data on lumbar spinal TBS and BMD were derived from dual energy X-ray absorptiometry measurements.

Results

Independent of confounders, NEAP and AnPro/K, but not VegPro/K were associated with low TBS (standardized β (95%)= -0.04 (-0.07, -0.01) and -0.08 (-0.11, -0.04)) but not with BMD. Associations of AnPro/K and VegPro/K with TBS were non-linear and differently shaped. Unfavourable associations between NEAP, BMD and TBS were mainly present in subgroups with high fibre intake.

Conclusions

High NEAP was associated with low TBS. Associations of AnPro/K and VegPro/K and TBS were non-linear and differently shaped. No significant associations of DAL with BMD were observed, nor was any significant interaction between DAL and renal function. Mainly in participants with high intake of dietary fibre, DAL might be detrimental to bone.

INTRODUCTION

Fractures are a major health concern in the ageing population and can result in disability and reduced quality of life [1]. Whereas bone mineral density is the most extensively studied determinant of fracture risk, other factors including micro damage, mineralization, bone turnover, macro geometry of the cortical bone and microarchitecture of the trabecular bone are important determinants as well [2]. A novel measure to assess microarchitecture is trabecular bone score (TBS). In brief, this measure combines information on connectivity density, trabecular separation and trabecular number in a single score [2]. In Canadian postmenopausal women, spinal TBS was shown to predict osteoporotic fractures as did hip BMD, but their use in combination incrementally improved prediction [3]. Moreover, spinal TBS was shown to be associated with prevalent and incident vertebral fractures independently of BMD in Dutch participants of the Rotterdam Study[4]. Therefore, TBS might be a relevant measure of trabecular bone integrity to study in relation to modifiable life style factors, such as dietary intake.

High dietary acid load (DAL) reflects a diet which is rich in nutrients that are metabolized to non-carbonic acids (e.g. sulphuric acid from the metabolism of protein) in amounts that exceed the quantities of alkali bicarbonate produced from combustion of organic salts (such as potassium chloride in vegetables[5]). Therefore, long-term consumption of such a diet might disturb the balance between CO_2 and HCO_3^- in blood and cause mild but chronic systemic acidosis[6]. DAL has been suggested to affect bone because bone might serve as the primary buffering system for alkali components such as calcium and potassium in case of systemic acidosis[7]. Studies on the relation between DAL and vertebral fractures have shown inconsistent results and potential effects are suggested to be mediated by differences in BMD[8,9]. However, the role of TBS in this association is unclear.

On the one hand, by increasing DAL, dietary protein might have catabolic effects on bone. On the other hand, since the amino acids are important substrates for building bone matrix [10], dietary protein has anabolic effects. It could therefore be hypothesized that associations between DAL and bone outcomes are non-linear.

Whereas the lungs are the primary organs used to neutralize acute metabolic acidosis, chronic disturbances of the acid-base balance are mainly regulated by the kidneys[11]. Renal function is an essential determinant of the regulation of acid-base balance via bicarbonate resorption and acid secretion. Impaired renal function is associated with disturbances in mineral and bone metabolism [12] and fracture risk [7,13]. For that reason we hypothesize that participants with altered renal function are less able to maintain a proper acid-base balance when consuming a diet with high acid load and are therefore more likely to develop low BMD and TBS.

Different food groups are known contributors to DAL. Protein sources such as meat, dairy and grain products might contribute to a high DAL, whereas sources of potassium such as vegetables might contribute to a low DAL. It has been suggested that contrasting associations between DAL and bone outcomes might have been influence by dietary fibre intake [14].More specifically, high intake of grains might contribute to high DAL and high fibre intake, whereas high intake of vegetables might contribute to low DAL and high fibre intake. As dietary fibre might reduce intestinal calcium absorption [14], it could be argued that associations between DAL and bone outcomes might be more detrimental to bone in subject with high intake of dietary fibre.

Therefore, our main aim was to study the associations of dietary acid load (DAL) with bone mineral density (BMD) and trabecular bone integrity (reflected by TBS) in middle-aged and elderly subjects of the Rotterdam Study. Moreover, we explored potential non-linear associations. A secondary aim was to assess whether the magnitude of the associations differ according to renal function and intake of dietary fibres.

METHODS

Study design

This cross-sectional analysis was embedded in the Rotterdam Study (RS), a prospective, population-based cohort study. Subjects were middle-aged and elderly people (n =4672, S-Figure 1) from three RS-cohorts. The design and objectives of this study have been described extensively elsewhere[15]. In brief, participating males and females were 45 years or older at the start of the study (1989-1993 for the first cohort (RS-I), 2000-2001 for the second cohort (RS-II) and 2006-2008 for the third cohort (RS-III)). The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and for the study to obtain information from their treating physicians.

Assessment of dietary intake and DAL

Dietary intake was assessed using a validated, semi-quantitative food frequency questionnaire (FFQ) on 389 food items [16,17] at the fifth visit of RS-I (2009-2011), the third visit of RS-II (2011-2012) and the second visit of RS-III (2012-2014). Next, the intake of macro-and micronutrients was calculated using the Dutch Food Composition Database (NEVO) from 2006 [18].The net rate of endogenous non-carbonic acid production (NEAP) is a common measure of DAL which is based on the ratio of dietary protein to potassium. This ratio was shown to explain 71% of the variation in steady state rate of renal net acid excretion, measured as the sum of the excretion rates of titratable acids and ammonium minus that of bicarbonate previously [5]. In our study,

NEAP was calculated based on the following equation NEAP (mEq/day)= 54.4×protein intake (g/day)/potassium (mEq/day)-10.2 [5]. Subsequently, NEAP were adjusted for total energy intake using the residual method [19]. To study whether acid load due to high vegetable or animal protein intake is differently associated with bone outcomes, protein potassium ratios were calculated using energy-adjusted vegetable protein (for VegPro/K) and animal protein (for AnPro/K) in mg/d as the numerator and energy-adjusted potassium (K) intake in mEq/d as the denominator. Two Dutch adult study populations [16,17] have shown that the FFQ was validated to properly rank subjects with respect to high or low intake of nutrients. Pearson's correlation coefficients were 0.62 for calcium, 0.71 for potassium and ranged from 0.59 to 0.68 for total, vegetable, and animal protein after adjustment for total energy intake and sex [16].

The dietary potential renal acid load (dPRAL) is a measure of DAL that can be calculated using the following equation: PRAL (mEq/d) = 0.49 * protein (g/d) + 0.037 * phosphorus (mg/d) - 0.021 * potassium (mg/d) - 0.026 * magnesium (mg/d) - 0.013 * calcium (mg/d) [20]. Although this is theoretically a more precise estimate of DAL, we did not include this measure in our main analyses since we did not have data on phosphate from food additives available. Instead, we used dPRAL as a reflection of DAL in a sensitivity analysis.

Assessment of spinal TBS and BMD

Lumbar Spine (L1-L4) BMD was measured using dual energy X-ray absorptiometry (DXA) (Prodigy, GE Lunar Corp, Madison, WI, USA). TBS was analyzed using TBS iNsight software (Med-Imaps, Geneva, Switzerland) and BMD using GE Lunar software. In brief, TBS is a novel gray-level texture measurement, extracted from DXA images, that correlates with 3D parameters of bone microarchitecture, connectivity density, trabecular separation and trabecular number. For each region of measurement, TBS was evaluated based on gray-level analysis of the DXA images as the slope at the origin of the log-log representation of the experimental variogram. The method of TBS assessment has been described in detail elsewhere[3].

Assessment of covariates

Body weight (kg) and height (cm) were measured at the research center as the subjects wore light clothing and no shoes. Smoking was coded as "current," "past," or "never". Physical activity was estimated using the total number of sports and practicing any sport at a professional level based on the validated LASA-questionnaire[21]. Plasma vitamin D (25-Hydroxyvitamin D in nmol/l) was assessed using electrochemiluminescence immunoassay (COBAS, Roche Diagnostics GmbH, Germany). Estimated glomerular filtration rate (eGFR in ml/min), reflecting renal function, was based on both creatinine and cystatin C. Net household income and highest education attained were categorized into "low," "medium," and "high" as a proxy for socio-economic status.

Dummy variables using the "medium" category as the reference were used for further analyses. Prevalence of type 2 diabetes was based on fasting glucose (>11 mmol/l) or use of antidiabetic mediation. Use of lipid lowering or antihypertensive drugs was collected during the home interview. Intake of alcohol, dietary calcium, and fibre (in grams per day), and information on dietary supplement use were assessed using the FFQ. "Supplement use" was defined as taking calcium, vitamin D, or multivitamin supplements at least once per week, as these nutrients are components of the DAL and/or important for bone health. Menopausal status was assessed using STRAW-criteria [22]. Use of female hormones was collected using questionnaires and coded as "ever" or "never". All covariates were assessed at the baseline visit of our study, similar to the TBS and BMD data.

Statistical analysis

To determine which food groups were the main determinants of each of the DAL measures, stepwise backwards regression was used (P for exclusion > 0.01). Associations of DAL with BMD, TBS were explored using linear regression modelling with NEAP, VegPro/K or AnPro/K as the exposure and TBS, BMD and presence or absence of vertebral fracture as the outcome (all in study-population specific Z-scores). Subjects with extreme total energy intake (<500 kcal per day or more than 5000 kcal per day) were removed from the analyses. All analyses were adjusted for age, sex, total energy intake, body weight and height in a basic model (model 1). Based on literature[9] and previous analyses on diet and bone in the Rotterdam Study[23,8], a second model was developed which was further adjusted for smoking, physical activity, socio-economic status, use of lipid lowering drugs, use of dietary supplements and intake of alcohol and calcium (model 2). Natural cubic splines were computed to explore potential non-linear associations in our most adjusted models (model 2), of which the degrees of freedom were determined based on the lowest Akaike's 'An Information Criterion (AIC) value. Likelihood ratio tests (LRT) were performed to determine whether a non-linear model fitted the data significantly better than a lineal model. If the results of the LRT was significant, effect estimates were calculated for separate intervals of DAL, using the knots defined in the cubic splines as cut-offs.

To assess the influence of sex, kidney function and dietary fibres on the relation between DAL, TBS and BMD, we tested for interaction by adding the product term of each DAL variable with sex, eGFR or dietary fibre plus eGFR or dietary fibre as independent variables to our model 2. Only if the P for interaction was <0.10, results were stratified according to the population median of eGFR or dietary fibre intake.

We performed several sensitivity analyses. First, despite missing data on phosphate from food additives, we used dPRAL as a reflection of DAL in model 2. Also, we reran our main analyses in model 2 after exclusion of participants with incomplete dietary intake data. Moreover, we excluded participants with type 2 diabetes mellitus

because incidence has been shown to be higher in people with high DAL[24] and diabetes patients tend to have lower TBS [25,26]. We used the multiple imputation procedure to deal with missing covariates (details in S-table 1 and 2). All analyses were performed using SPSS (version 22, IBM Corp, New York, United States of America) and R (Version 0.99.484 – 2009-2015 RStudio Inc., Vienna, Austria) statistical software. A p-value of 0.05 was considered to be significant.

RESULTS

Study population

Overall, characteristics of our study population did not markedly differ between the three cohorts of the Rotterdam Study (table 1). However by study design, participants in the third cohort were younger (median (IQR = 57 (52, 60) versus 71 (69,75) in the second and 78 (75, 82) years in the first cohort). Moreover, these younger participants were more likely to have higher total energy intakes, be less physically active and to have better renal function (table 1). Whereas the majority of females in the first and second cohort (>90%) were postmenopausal, this was only 77% in the third cohort. The median spinal TBS was consistent across cohorts, but younger participants were more likely to have higher BMD (table 1). NEAP was significantly correlated with AnPro/K (Pearson's r = 0.40) and to VegPro/K (Pearson's r= 0.23). However, the two different ratios of protein to potassium were not significantly correlated to each other. Moreover, NEAP and the AnPro/K were negatively correlated with dietary fibre intake (Pearson's r = -0.47 and -0.30), whereas fibre was weakly positively correlated to the VegPro/K (Pearson's r=0.13, P for all significant correlations <0.001). An overview of food groups explaining most of the variance in NEAP, VegPro/K and AnPro/K is shown in S-table 3.

Associations between DAL, TBS and BMD

High NEAP was significantly associated with low TBS in our basic models (β = -0.04, 95% CI = -0.08, -0.01) table 2, model 1). Also the ratios of vegetable protein and animal protein to potassium were significantly associated with TBS, but in opposite directions. Whereas high VegPro/K was associated with high TBS (β = 0.06 (95% CI = 0.04, 0.07), high AnPro/K was associated with low TBS (β = -0.07 (95% CI = -0.10, -0.04). These associations were independent of confounders (table 2, model 2). For both protein to potassium ratios, LRT indicated the presence of a non- linear relationship, of which are visualised in Fig. 1. Positive associations between VegPro/K and TBS reached a plateau at the population mean VegPro/K (≈ 0.25 g/mEq). In contrast, negative associations between AnPro/K and TBS become prominent at the population mean (≈ 0.34 g/ mEq) only. No significant associations between any of our DAL measures and BMD were observed in any of our models (table 2), nor were there any indications for non-linearity of these associations.

Table 1. Characteristics in 3 cohorts of the Rotterdam Study (RS), total n = 4672

	1st cohort of the RS N = 1229		2nd cohort of the RS N = 1440		3rd cohort of the RS N = 2003	
Age (y)[1]	78	(75, 82)	71	(69, 73)	57	(52, 60)
Height (cm) [1]	165	(159,173)	167	(161, 175)	170	(164, 178)
Weight (kg) [1]	74	(66, 83)	77	(68, 86)	78	(69, 87)
Spinal TBS	1.29	(1.22, 1.36)	1.30	(1.23, 1.36)	1.30	(1.21, 1.37)
Spinal BMD (g/cm^2)	1.11	(0.97, 1.27)	1.12	(0.98, 1.28)	1.15	(1.02, 1.30)
Total energy intake (kcal/d)[1]	1934	(1549, 2387)	1955	(1559, 2369)	2233	(1852, 2720)
Plasma vitamin D (nmol/l) [1]	54	(37, 73)	62	(43, 84)	61	(43, 82)
NEAP (g/mEq)	35	(28, 42)	35	(29, 42)	36	(30, 43)
VegPro/K (g/ mEq	0.23	(0.19, 0.28)	0.24	(0.19, 0.28)	0.26	(0.21, 0.31)
AnPro/K (g/ mEq)	0.33	(0.25, 0.43)	0.33	(0.25, 0.43)	0.33	(0.25, 0.43)
Physical activity (METh/week)	90	(64, 123)	81	(55, 108)	43	(18, 81)
eGFRcrcys (ml/min)	65	(56, 76)	74	(64, 83)	87	(78, 95)
Sex (% males)	42		44		42	
Education (%)						
Low	9		28		25	
Middle	12		20		20	
Middle- high	16		25		21	
High	63		24		35	
Income (%)						
Low	64		38		23	
Middle	28		63		24	
Middle- high	3		12		13	
High	5		15		40	
Current smokers (%)	7		10		13	
Type 2 diabetes (%)	12		12		6	
Menopausal status (% postmenopausal)[2]	97		92		77	
Use of any dietary supplement (%)[3]	60		59		60	
Use of lipid lowering drugs (%)	34		32		27	
Use of antihypertensives (%) [4]	23		17		6	

[1] Median (interquartile range); [2]Applicable to females only; [3]: Use of any dietary supplement ≥1 time/ month; [4]: including diuretics. Abbreviations: BMD= Bone mineral density; BMI: Body Mass Index; HRT: Hormone replacement therapy; METh= metabolic equivalent of tasks in hours; TBS: Trabecular Bone Score, dPRAL = dietary potential renal acid load, TPro/K = total protein/ potassium ratio, VegPro/K = vegetable protein/ potassium ratio, AnPro/K = animal protein/ potassium ratio.

Table 2. Linear associations between DAL, TBS and BMD

	Model 1 Basic		Model 2 Confounders	
	β	95% CI	β	95% CI
		Trabecular Bone Score		
NEAP	**-0.04**	**(-0.08, -0.01)**	**-0.04**	**(-0.07, -0.01)**
VegPro/K	**0.06**	**(0.04, 0.07)****	**0.05**	**(0.01, 0.08)**
AnPro/K	**-0.07**	**(-0.10,-0.04)****	**-0.08**	**(-0.11, -0.04)**
		Bone mineral density		
NEAP	-0.02	(-0.05, 0.01)	-0.02	(-0.05, 0.01)
VegPro/K	-0.01	(-0.04, 0.01)	-0.00	(-0.04, 0.03)
AnPro/K	-0.02	(-0.03, 0.04)	-0.02	(-0.05, 0.02)

Regression coefficients represent changes in Z-score of BMD or TBS for each Z-score increase in DAL.
Model 1: Adjusted for age, sex, total energy intake, body weight and height, and Rotterdam Study cohort
Model 2: Model 1 + education and smoking, dietary calcium intake and alcohol consumption
Significant associations (P-value < 0.05) **in bold**.
**: Presence of a non-linear relationship, based on a likelihood ratio test comparing the linear model to a non-linear model.

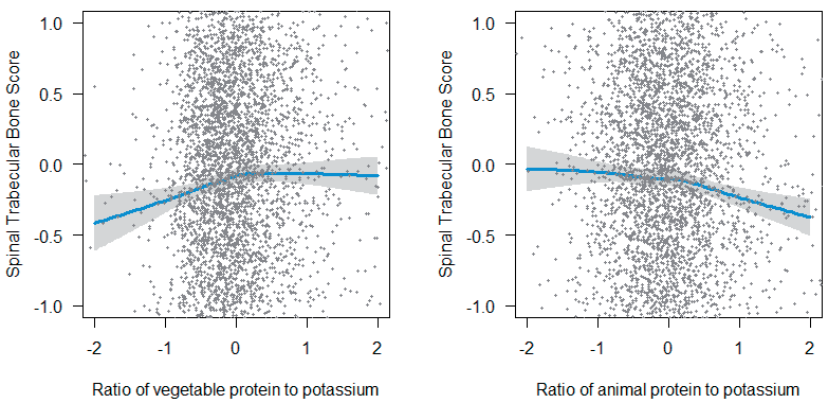


Figure 1. Non- linear associations between ratios of vegetable or animal protein to potassium and spinal trabecular bone score, reflecting trabcular bone integrity, using basic models adjusted for age, sex, body weight and height and total energy intake.

Additional analyses

Associations of dPRAL with TBS and BMD were similar to those of NEAP (β = -0.04, 95% CI = -0.07, -0.01 for TBS and β = -0.02, 95% CI = -0.05, 0.00 for BMD in model 2). Also, we observed a potential interaction between renal function and VegPro/K in relation BMD (P for interaction= 0.06). Stratified analyses suggested that VegPro/K might be associated with low BMD in subjects with renal function below the median (β = -0.05, 95% CI = -0.15, 0.05) but not in subjects with renal function ≥ the median (β = 0.01, 95% CI = -0.03, 0.04). No evidence for other interactions between DAL and renal function in relation to TBS or BMD were observed (P for interaction all> 0.16).

Moreover, we observed evidence for a potential interaction between NEAP and dietary fibre intake in relation to TBS (P for interaction = 0.06) and BMD (P for interaction < 0.01). Stratified analyses were suggestive for a stronger association between NEAP and low TBS in participants with high fibre intake ((β: -0.03; 95% CI: -0.08, 0.02) than in those with low fibre intake ((β: -0.01; 95% CI: -0.05, 0.03, S-table 4). Also, data suggested that NEAP was associated with low BMD in subjects with high fibre intake only (β (95% CI) =-0.03 (-0.08, -0.02)). No other interactions between DAL and dietary fibre in relation to TBS or BMD were observed (S-table 4b). Analyses in subgroups with complete FFQ data (n= 3170) or without type 2 diabetes (n= 4696) showed similar results as our main analyses (S-table 5).

DISCUSSION

Summary of our main findings and comparisons to other studies

In our population of middle-aged and elderly participants, we observed a negative association between NEAP and trabecular bone integrity reflected by the novel Trabecular Bone Score. This negative association was also observed for a high AnPro/K but not for a VegPro/K.

In contrast, we observed no significant association between DAL and BMD. Although both TBS and BMD are derived from the same DXA images, they do reflect different characteristics of the bone. With BMD, the degree of mineralization is quantified, whereas TBS rather combines information on connectivity density, trabecular separation and trabecular number [2]. The results of our main analyses indicate that DAL (NEAP and AnPro/K) might be detrimental to bone health by influencing the trabecular integrity, without necessarily altering BMD.

To our knowledge, we are the first to study associations between DAL and TBS. However, treatment with K-citrate with the aim to neutralize dietary acid load for 24 months was shown to improve bone microarchitecture, reflected by trabecular thickness and number in 201 elderly [28]. Associations between DAL and BMD have been studied extensively with conflicting results[29,8,30,31]. Protein intake is an

important contributor to DAL. A recent meta-analyses on 12 prospective cohort studies showed that total dietary protein consumption may decrease the risk of hip fracture, but concluded that evidence was insufficient to draw the same conclusion for animal or vegetable protein. No significant overall associations were observed of total, animal or vegetable protein in relation to all fractures and limb fractures in this study and the lack of studies on vertebral fractures was emphasized by the authors[32].

Potential non-linearity of the associations

Moreover, our results suggest that the shape of associations of the VegPro/K and AnPro/K with TBS are non-linear. Whereas the negative associations are only observed at the highest AnPro/K the positive associations were only observed at the lowest VegPro/K only. This difference might be due to the difference in sulphur containing amino acids [27], since animal proteins may contain more sulphur (e.g. methionine and cysteine) therefore produce more acid than do vegetable proteins. The non-linear shape of the association between VegPro/K and TBS in our population might reflect the importance of the anabolic effects of protein at acid loads below the mean, since amino acids are important substrates for building bone matrix[10]. Moreover, DAL has been suggested to affect bone outcomes via increased calcium excretion by the kidneys since bone minerals (mainly calcium) can be used as a base to neutralize low blood pH[34]. However, Cao and Nielsen recently concluded that, although a diet with a high acid load due to high intakes of meat and protein might increase renal acid load and urinary calcium excretion, demineralized bone was not necessarily the source of this extra calcium lost in urine. In contrast, the authors suggest that in case of diet-induced renal calcium loss, the body will compensate by promoting intestinal calcium uptake as primary mechanism to neutralize low blood pH [34].

DAL; only adversely associated with bone outcomes in subgroups?

Since the kidneys are the primary organs to regulate chronic systemic acidosis, it could be argued that high DAL might be detrimental only in specific subgroups, such as those with low renal function.

We observed no significant interaction between DAL and renal function in relation to bone outcomes in our cohort of Dutch elderly, despite a trend towards more Also in a cohort of Swedish elderly, stratified analyses based on renal function using a more stringent cut-off of 60 ml/min showed no associations between DAL and risk of fractures (from the neck down) in both strata [8]. Some studies have suggested that the potential adverse effects of DAL on bone outcomes might be present only in subjects with the lowest intake of alkali forming nutrients. For example, an inverse association between dietary potential renal acid load (dPRAL) and proximal femur

BMD was detected among men with low dietary calcium intake (<800 mg/d) only [29]. Traditionally, the Dutch diet is rich in dairy products and calcium[33], which is also reflected by the mean of 1010 mg of daily calcium intake in our population. Therefore, we were unable to study the association between DAL and bone outcomes in participants with low calcium intake in our population. In addition to interaction between DAL and acid forming or alkali-forming nutrients, we hypothesised that interaction between DAL and other nutrients in the overall diet might occur. More specifically and as suggested by Cao *et al.* previously [14], dietary fibre might inhibit intestinal absorption of dietary calcium and dietary acid load might reduce uptake of calcium from the bloodstream by the bones. DAL might therefore be more strongly associated with unfavourable bone outcomes in subgroups with high fibre intake. Indeed, our data were suggestive for an interaction between NEAP and dietary fibre in relation to BMD and TBS. Dietary fibre derived from grains might be presents in high DAL diets and derived from fruits and vegetables in low DAL diets. Altogether, these findings might imply that DAL might be adversely associated with bone outcomes in subgroups only, and that the food groups that determine DAL in a specific population (e.g. grains, fruits and vegetables) matter.

Strengths and limitations

Our study has several strengths. To our knowledge, we were the first to investigate DAL in relation to trabecular integrity of the bone in a large population based study. Moreover, we have used different measures of DAL, allowing us to separately investigate which components of the DAL might be important for bone. However, we also recognize some limitations. The use of an FFQ to assess dietary intake is prone to measurement error. To account for systematic measurement error, we adjusted our analyses for total energy intake using the residual method [19]. Also, we performed sensitivity analyses to account for incomplete dietary intake data. Results from our sensitivity analysis showed similar effect sizes before and after exclusion of participants with incomplete data. Unfortunately, we did not have biomarkers of acid load, such as urinary pH or serum bicarbonate levels to validate our findings. Random error might have still been present and diluted our associations. Since we did not have data on phosphate from food additives available, we were unable to calculate dPRAL, another common measure of DAL in a reliable matter. TBS data were only available in a subsample of the Rotterdam Study. However, characteristics of the participants included in our study did not markedly differ from those included in the full third cohort of the Rotterdam Study. Hence, we believe that our results are valid for our full cohort. Lastly, we did not have sufficient participants with an eGFR < 60ml/min to study the influence of impaired versus normal renal function, limiting any conclusions on specific associations in those with impaired renal function or renal failure.

Practical implications and recommendations for future research

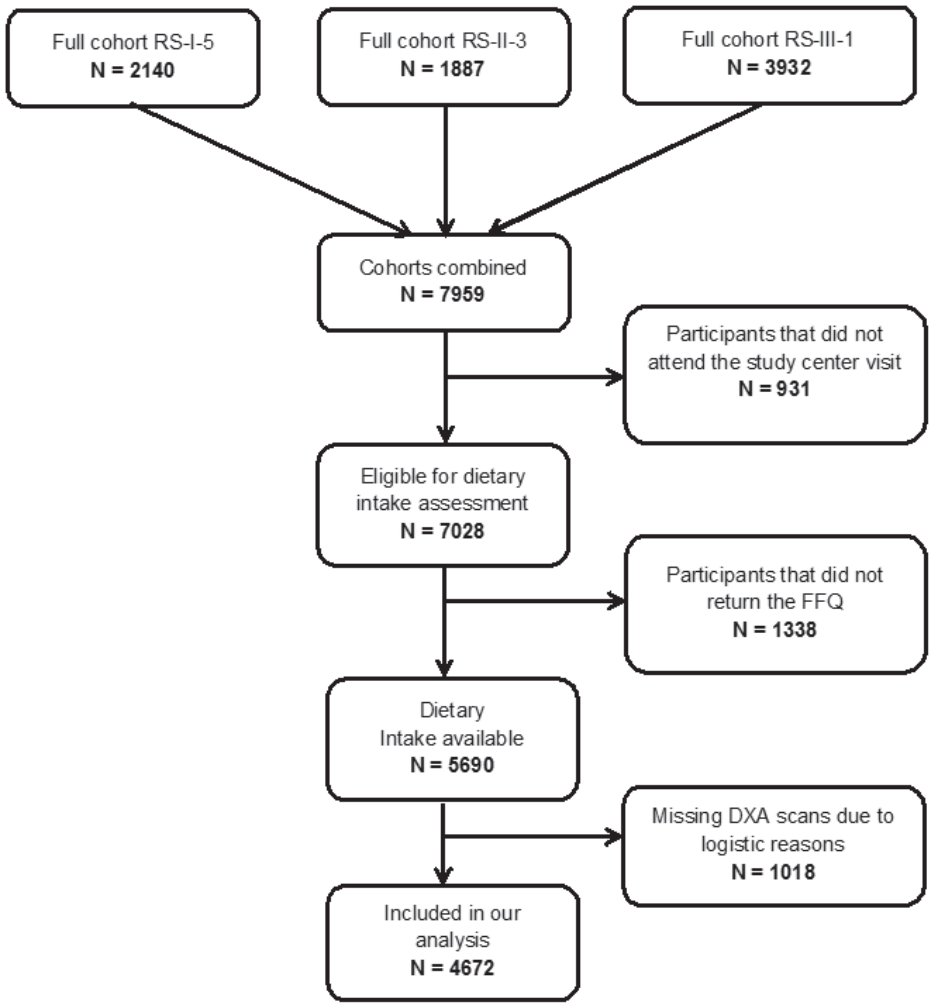
Our results do not support the hypothesis that high DAL is associated with low BMD. In contrast, we observed significant associations with low TBS. Moreover, they are suggestive for differently shaped, non-linear associations between AnPro/K and VegPro/K with TBS. Future studies are needed to confirm our results in other populations. Moreover, our population had low median DAL and small variance compared to other studies[27,35]. Future studies in populations with more extreme ranges of DAL might provide additional insights. Lastly, we hypothesized that associations with TBS might add to the explanation of conflicting results on DAL and fracture risk. Long term studies will be needed to confirm this hypothesis. Due to its contribution to high DAL, protein has been suggested to adversely affect skeletal health[7,34]. However, when combined with exercise, protein intake, an important component of the acid load variables in our study, also has positive musculoskeletal effects, for example for the prevention of sarcopenia (loss of muscle mass) [36,37]. Therefore, one can argue that recommendations to reduce animal protein intake to facilitate a reduction in DAL in the ageing population might be undesired. In contrast, a proper balance between protein from animal sources by protein from vegetable sources might be beneficial for overall musculoskeletal health. Future research would be needed to further clarify the role of protein sources in skeletal health.

CONCLUSIONS

In our population of middle-aged and elderly, high NEAP was associated with low trabecular bone integrity. Associations of AnPro/K and VegPro/K and TBS were non-linear and differently shaped.

No significant associations with BMD were observed, nor was any interaction between DAL and renal function in relation to TBS or BMD. Only in participants with high intake of dietary fibre, NEAP might be detrimental to bone outcomes. These findings imply that nutrients that characterize a high DAL diet but are not incorporated in the DAL equation might influence associations of DAL with TBS and BMD.

SUPPLEMENTAL MATERIALS



Supplementary Figure 1. Data availability and sample sizes included in our analyses

Supplemental table 1. Details on the multiple imputation procedure

Multiple imputation procedure		
Software used		SPSS 21 for windows.
Imputation method and key settings		Fully conditional specification (Markov chain Monte Carlo method); Maximum iterations: 5000 (MAXMODELPARAM)
Number of imputed data sets created		10
Variable included in the imputation procedure and used in main analyses	Imputed and used as predictor	Smoking behavior Vitamin D status Household income, highest level of education attained and household size Renal function Physical activity Menopausal status Prevalent type 2 diabetes Medication use: diuretics, calcium blockers, lipid lowering agents, drugs for treatment of bone disease and other drugs for the musculoskeletal system, antihypertensive drugs, hormone replacement therapy (females only)
	Used as predictor only (no missings or outcome variables)	Dietary acid load (NEAP and protein/ potassium ratio), total energy intake, age, Body height and weight Use of dietary supplements Means of spinal TBS or BMD Rotterdam Study cohort Age, sex
Variables not used in main analyses but used as predictors of missing data to increase plausibility of missing at random assumption		Ethnicity of all grandparents Pack years of smoking
Treatment of non-normally distributed variables		Predictive mean matching
Treatment of binary/categorical variables		Logistic regression

Supplemental Table 2. Values of imputed covariates before and after the multiple imputation process

		Original data		After multiple imputation N= 4672	
Continuous variables					
Plasma vitamin D (nmol/l)[1]		59	(41, 81)	58	(40, 79)
	Missing (%)	7%		-	
Glomerular Filtration Rate (ml/min)[1]		77	(65, 88)	78	(66, 88)
	Missing (%)	*4%*		-	
Physical activity (METh total)[1]		71	(39, 105)	68	(36, 100)
	Missing (%)	*7%*		-	
Categorical variables					
Income (% low/ middle/ middle- high/ high)		38/ 29/ 10/ 23		38/ 28/ 10/ 23	
	Missing (n)	*10%*		-	
Education (%low/ middle/ Middle-high/high)		21/18 / 21/ 39		23/ 18/ 21/ 37	
	Missing (n)	*1%*		-	
Smoking (% current)		11		13	
	Missing (n)	*5%*		-	
Prevalent diabetes (%)		10		14	
	Missing (n)	*8%*		-	

[1]: Median (interquartile range) [2]: applicable to females only
Main exposures (DAL) and outcomes (TBS and BMD) were not imputed
Abbreviations: TBS = trabecular bone score; BMD = bone mineral density
METh= metabolic equivalent of tasks in hours per week

Supplemental Table 3. Food groups contributing to high or low dietary acid low, expressed as NEAP or protein potassium ratios

Food group	Low NEAP	High NEAP	Low VegPro/K	High VegPro/K	Low AnPro/K	High AnPro/K
Fruit	-0.53	-	-0.15	-	-0.34	-
Potatoes	-0.23	-	-	-	-0.12	-
Milk and milk products	-0.05	-	-0.12	-	-	0.14
Yoghurts	-	-	-0.20	-	-	0.14
Soy products	-	-		0.07	-	-
Cheese	-	0.30	-	-	-	0.30
Vegetables	-0.25	-	-	-	-0.17	-
Eggs	-	0.07	-	-	-	0.07
Fish	-	0.16	-	-	-	0.26
Grains	-	0.17		0.31	-	-
Meat	-	0.24	-0.10	-	-	0.40
Poultry	-	0.12	-	-	-	0.18
Nuts and seeds	-	-		0.10	-0.10	-
Vegetable oils	-	-	-0.26	-	-0.21	-
Animal fats	-	-	-0.11	-	-	0.32
Explained variance (adjusted R^2)	**0.67**		**0.51**		**0.72**	

Food groups that are significantly contributing to high DAL (corresponding to a positive standardized regression coefficient for high and a negative one for low contribution)

Supplemental Table 4. Linear associations between DAL, TBS and BMD in strata of dietary fibre intake

	Trabecular Bone Score					Bone Mineral Density				
	fibre < the median		fibre ≥ the median		P Int	fibre < the median		fibre ≥ the median		P Int
	β	95% CI	β	95% CI		β	95% CI	β	95% CI	
NEAP	**-0.01**	**(-0.05, 0.03)**	**-0.03**	**(-0.08, 0.02)**	**0.06**	**-0.00**	**(-0.04, 0.04)**	**-0.03**	**(-0.08, -0.02)**	**<0.01**
VegPro/K	0.02	(-0.03, 0.06)	0.02	(-0.02, 0.05)	0.49	-0.02	(-0.06, 0.02)	-0.01	(-0.05, 0.03)	0.86
AnPro/K	-0.06	(-0.12, 0.01)	-0.03	(-0.06, 0.00)	0.81	-0.02	(-0.07, 0.05)	0.00	(-0.03, 0.03)	0.53

Regression coefficients represent changes in Z-score of BMD or TBS for each Z-score increase in DAL.
Model 3: Adjusted for age, sex, total energy intake, body weight and height, education, smoking, dietary fibre, calcium and alcohol
Associations with P for interaction <0.10 **in bold**.
Abbreviations: BMD= Bone Mineral Density; DAL= dietary acid load; eGFR= estimated Glomerular Filtration Rate; TBS= Trabecular Bone Score
*1: P for interaction = 0.001

Supplemental Table 5. Sensitivity analyses in subgroups with complete FFQ data and without type 2 diabetes

	Full population		Subjects with complete FFQ data[2]		Subjects without type 2 diabetes	
	N= 4672		N= 3170		N= 4696	
	Trabecular Bone Score (TBS)					
	β	95% CI	β	95% CI	β	95% CI
NEAP	**-0.04**	**(-0.07, -0.01)**	**-0.06**	**(-0.10, -0.02)**	**-0.03**	**(-0.06, -0.00)**
VegPro/K	**0.05**	**(0.01, 0.08)**	0.05	(-0.00, 0.09)	0.03	(-0.01, 0.06)
AnPro/K	**-0.08**	**(-0.11, -0.04)**	**-0.10**	**(-0.15, -0.04)**	**-0.03**	**(-0.06, -0.00)**
	Bone Mineral Density (BMD)					
NEAP	-0.02	(-0.05, 0.01)	-0.02	(-0.06, 0.02)	0.01	(-0.03, 0.03)
VegPro/K	-0.00	(-0.04, 0.03)	-0.03	(-0.07, 0.02)	-0.00	(-0.04, 0.03)
AnPro/K	-0.02	(-0.05, 0.02)	-0.02	(-0.07, 0.03)	0.00	(-0.03, 0.03)

1: Regression coefficients represent changes in Z-score of TBS for each Z-score increase in DAL.
[2:] Participants with <1% missing data in the FFQ
Models were adjusted for age, sex, body weight and height, total energy intake, education, smoking, calcium and alcohol (model 2).

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SERUM URIC ACID IN RELATION TO BONE MINERAL DENSITY, HIP GEOMETRY AND FRACTURE RISK

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ABSTRACT

Context

Uric acid (UA) is a metabolic end product of purine breakdown which in access might cause gouty arthritis. The role of uric acid in skeletal metabolism remains to be unraveled.

Objective

First, we prospectively investigated the association between serum UA, bone mineral density at the femoral neck (FN-BMD), hip bone geometry parameters, and fracture risk. Second, we examined whether the associations were modified by age and vitamin C intake.

Participants and Setting

We included 5074 elderly participants of The Rotterdam Study, a prospective population-based cohort.

Exposure

Serum UA was assessed at baseline.

Main Outcomes and Measures

FN-BMD was measured at baseline (1989-1993), and at second (1993-1995), third (1997-1999), and fourth visits (2002-2004) of the Rotterdam Study. Hip bone geometry parameters were measured at baseline and at the second and third visits. Bone geometry measures included cortical thickness, bone width, section modulus (reflecting bending strength) and buckling ratio (reflecting bone instability). BMD and geometry measures were derived from dual energy x-ray absorptiometry (DXA) images. Fracture data were reported by general practitioners.

Results

After adjustment for confounders, serum UA levels (per SD increase) were associated with higher FN-BMD ($\beta = 0.007$ g/cm^2; 95% CI = 0.004–0.013), thicker cortices ($\beta = 0.002$ cm; 95% CI = 0.000–0.003), lower bone width ($\beta = -0.013$ cm; 95% CI = –0.023 to –0.003), and lower cortical buckling ratio ($\beta = -0.192$; 95% CI = –0.327 to –0.058). The associations of UA with FN-BMD and cortical buckling ratio tended to become stronger over time. Also, high levels of serum UA were associated with lower risk of any fractures and non-vertebral fractures (HR=0.93, 95% CI= 086, 1.00 for both) and of osteoporotic fractures (HR= 0.91, 95% CI= 0.58, 0.98). Associations with fractures were more prominent in older individuals (age >65 y) and in participants with high intakes of vitamin C (> median).

Conclusions

Higher levels of serum UA were associated with higher BMD (at the expense of thicker cortices and narrower bone diameters) and may be a protective factor in bone metabolism. However, interactions with age and vitamin C may be present.

INTRODUCTION

Uric acid (UA) is the final breakdown product of purine metabolism. RNA and DNA are the main endogenous sources of purines, whereas diet is the main exogenous source of purines. UA has been traditionally viewed as a metabolic by-product, which in excess may cause gouty arthritis and renal stones (1). Furthermore, UA is recently regarded as a risk factor for cardiovascular diseases (2). However, high UA levels were also suggested to have beneficial effects. UA accounts for approximately half of the antioxidant properties of human plasma (3) and high serum levels of UA may play physiologically beneficial roles because of their antioxidant properties (4). They were e.g. shown to be associated with slower progression of Parkinson's disease, Huntington's disease, and mild cognitive impairment (5, 6).

The effect of UA in skeletal metabolism remains to be unraveled. On the one hand, experimental and clinical studies have shown that low circulating levels of antioxidants have detrimental effect on bone metabolism (7). On the other hand, high UA levels have been associated with metabolic syndrome (8), diabetes (9), and obesity (10), conditions that have been shown to exert both beneficial and detrimental influences on bone outcomes (11). Recent literature suggests that UA may actually be beneficial for bone metabolism. In a cross-sectional cohort study in 1705 older men, Nabipour et al (12) showed that higher serum UA levels were associated with higher bone mineral density (BMD) and lower prevalence of vertebral and non-vertebral fractures. Also, two recent studies showed that UA is a protective factor against incident osteoporotic and non-vertebral fractures in cohorts consisting of men only (13, 14). However, evidence remains unclear on whether this relationship is also present in women. Also, the longitudinal associations between serum UA and bone outcomes are unknown.

In the present study, we therefore investigated the association between UA, BMD at the femoral neck (FN-BMD), hip bone geometry parameters (HBGPs), and fracture risk in both men and women using a longitudinal design. UA increases with advancing age (15). Vitamin C intake increases UA excretion, and therefore lowers the plasma levels of UA (8, 15). We therefore evaluated whether associations between UA and bone outcomes were modified by age and vitamin C intake.

SUBJECTS AND METHODS

The Rotterdam Study is a population-based cohort study, including 7983 participants age 55 years and older living in Ommoord, a district of Rotterdam. The rationale and design of the Rotterdam Study was described elsewhere (16). The Rotterdam Study started in the early 1990s, and periodical examinations were performed every 3 to 5 years. In addition, participants were continuously followed for vital status and medical outcomes, obtaining information regularly from the municipal health authorities in

the Rotterdam area. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, and written informed consent was obtained from all participants.

MEASSUREMENTS

Uric Acid

Values of serum UA were obtained from baseline (1989–1993) nonfasting blood samples that were centrifuged for 10 minutes at 3000 rotations per minute. Subsequently, the serum was stored at –20°C for 1 week, until UA activity was determined with a Kone Diagnostica reagent kit and a Kone autoanalyzer. To check the calibration, three control samples were included for every 10 samples. If the average values of the control samples of each run (100 samples) were not within 2.5% of the true value, the run was repeated.

Skeletal assessments

FN-BMD (g/cm^2) at baseline (1989–1993) and at the second (1993–1995) and third visits (1997–1999) was measured by dual-energy x-ray absorptiometry (DXA) using a Lunar DPX-L densitometer (Lunar Radiation Corp) (18) and analyzed with DPX-IQ version 4.7d software, whereas at the fourth visit (2002–2004), FN-BMD was measured by using a GE Lunar Prodigy bone densitometer (General Electric). Hip structural analysis (19) was used to measure HBGP from the DXA scans of the femur narrow neck region in the three visits of the Rotterdam Study as described previously (20). HBGP included cortical thickness (cm), bone width, section modulus and buckling ratio. Mean cortical thickness was calculated as the difference between subperiosteal and endocortical radii, which were obtained by modeling the narrow neck region as a circular annulus, under the assumption that the proportion of cortical versus trabecular bone was 60:40. Section modulus was calculated as the ratio of the cross sectional moment of inertia and the maximum distance from the center of mass to the medial or lateral surface (dmax) and is a reflection of bending strength (24) standardized to size. Buckling ratio was estimated as dmax divided by the mean cortical thickness estimate. A high buckling ratio indicates cortical bone instability.

All events, including incident fractures and death, were reported by general practitioners in the research area (covering 80% of the cohort) by means of a computerized system. Research physicians regularly followed participant information in the general practitioners' records outside the research area and made an independent review and encoding of all reported events. Subsequently, a medical expert reviewed all coded events for the final classification using the guidelines for International Classification of Diseases (ICD)-10. Additional information on hip fractures was gathered through the Dutch National Hospital Registration System. An osteoporosis

expert reviewed all coded events for final classification. Subjects were followed from their baseline visit until January 1, 2007, or until a first fracture or death occurred or until they were lost to follow-up.

Assessment of covariates

The information on current health status, medical history, medication use and smoking were assessed at baseline by means of a home interview at baseline. Participants were asked whether they were current smokers of cigarettes, cigars, or pipe. Cardiovascular disease was defined as a history of myocardial infarction, coronary artery bypass, or percutaneous transluminal coronary angioplasty. Type 2 diabetes mellitus was diagnosed if a non-fasting serum glucose level was ≥11 mmol/L or if a person used glucose lowering drugs. Information on medication use included the use of diuretics, hormonal replacement therapy, systemic corticosteroids, drugs for bone diseases, drugs for other musculo-skeletal diseases, thyroid therapy and anti-gout drugs use. Serum measures were all determined in non-fasting blood samples. Estimated glomerular filtration rate (eGFR) was calculated using the simplified Modification of Diet in Renal Disease (MDRD) equation(22). At baseline, a computerized validated 170-item semi-quantitative food frequency questionnaire (FFQ) was used to asses dietary intake(23). The Dutch Healthy Diet (DHD)-index was used to take into account overall dietary quality(24). The following food components were included within the DHD-index in this study: intake of vegetable, fruit, dietary fiber, fish, trans fat, polyunsaturated fat, saturated fat, alcohol and sodium (intake of acidic drinks and foods were not available in current study). At the third visit to research center, the total weekly duration of physical activity (including housekeeping activities) was assessed by an adapted version of the Zutphen Physical Activity Questionnaire and the LASA Physical Activity Questionnaire(25, 26)

POPULATION FOR ANALYSIS

Serum UA and FN-BMD

Of 5150 individuals with available information on serum UA, 1077 participants were excluded because FN-BMD was not measured at baseline (1990–1993), leaving 4073 participants for the cross-sectional analysis on serum UA and FN-BMD. Among them, 781 participants did not have any follow-up measurement and were therefore excluded from the longitudinal analysis, leaving 3292 participants for inclusion. (Supplemental Figure S1).

Serum UA and hip bone geometry

There were 1828 participants who did not have measures of HBGP at baseline. Hence, 3322 participants were included in the cross-sectional analysis on serum UA and hip bone geometry. Among them, 604 participants did not have HBGP measured at both

the second and third visits, therefore leaving 2718 participants for the longitudinal analysis (Supplemental Figure S1).

Serum UA and fracture risk

Data on fracture follow-up were not available for 76 participants. Therefore, 5074 men and women were enrolled in the final analysis and were observed for occurrence of incident fractures comprising a follow-up of 10.9 years (Supplemental Figure S1).

Statistical Analysis

Cross-sectional associations

Intraclass correlation coefficient was used to assess the within-subject correlations of the repeated measures of FN-BMD and HBGP in the same individual. To examine the cross-sectional association between serum UA (per SD increase) and FN-BMD and HBGP, linear regression models were fitted in generalized estimated equations (GEE). We used exchangeable correlation structure to adjust for the within-subject correlations due to the repeated measurements of FN-BMD and HBGP in the same individual. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated from the Cox proportional hazard regression models to test the association of UA (per SD or per quintile) with risk of fracture. Associations were first examined in our basic model (model 1), which included age, gender, height, weight, and estimated glomerular filtration rate (eGFR). The multivariable model adjustment (model 2) included the factors from the base adjustment model plus smoking status, Dutch Health Diet Index, physical activity, prevalent diabetes mellitus, prevalent cardiovascular disease, history of hip or knee surgery, diuretic drug use, hormone replacement therapy, corticosteroid drug use, drugs for other musculoskeletal diseases, thyroid therapy, anti-gout drug use, serum phosphate, serum total calcium, and energy-adjusted dietary intake of vitamin C. Models for FN-BMD and HBGP were additionally adjusted for the type of DXA scans.

Longitudinal associations; interaction with time

To examine the longitudinal effect of UA on FN-BMD and HBGP, the cross-product (interaction) between UA and a time variable t (t = 1, 4, and 8 for HBGP analysis; and 1, 4, 8, and 13 for FN-BMD analysis) was tested in the multivariable model. Significant interaction indicates that a potential association became stronger or less strong over time.

Additional analyses

We tested for possible nonlinear effects by adding a quadratic term of serum UA in the multivariable model. To test for effect modification, product terms of serum UA with age or dietary intake of vitamin C were added as independent variables to the multivariable models. Analysis stratified by age (≤65 or >65 y), or by vitamin C intake (≤ or > the median) was performed in case of significant effect modification. Because

FN-BMD was not cross-calibrated between the first three measures and the fourth, we repeated all analyses excluding the fourth measure. Furthermore, diabetes mellitus has been shown to affect UA levels and BMD might be altered in diabetes patients. Therefore, a sensitivity analysis was performed excluding subjects with type 2 diabetes mellitus. Also, we measured serum UA levels after 8 years in a subgroup of study participants and observed high correlations with baseline UA measure (partial Pearson correlation = 0.70; intraclass correlation = 0.82), supporting internal consistency and validity. To adjust for potential bias associated with missing data, we used a multiple imputation procedure (n = 5 imputations). Rubin's method was used for the pooled regression coefficients (β) and 95% CIs. A P value < .05 was considered as statistically significant, but to account for multiple testing, we adjusted the P value from .05 to .005 by applying the Bonferroni correction for the number of outcomes studied (n = 10). All analyses were done using SPSS statistical software, version 20.0 (SPSS Inc).

RESULTS

Table 1 shows the selected characteristics of study participants according to the outcome of interest. There was no significant difference between study groups with regard to serum UA levels or fracture incidence. Table 2 shows the FN-BMD and HBGP characteristic and the within-subject correlations between measures. A within-subject correlation coefficient of 0.91 was observed between the first and second measurements of FN-BMD and of 0.87 between the first and third measurements of FN-BMD. Anthropometrics, lifestyle factors, and other characteristics of the excluded participants did not differ substantially from the participants included in the study (data not shown).

Association of UA with FN-BMD and hip bone geometry

After adjustment for potential confounders, in the cross-sectional analysis, each SD increase in serum UA levels was associated with higher FN-BMD (β = 0.007 g/cm^2; 95% CI = 0.004, 0.013), thicker cortices (β = 0.002 cm; 95% CI = 0.000, 0.003), lower bone width (β = −0.013 cm; 95% CI = −0.023, −0.003), and lower cortical buckling ratio (β = −0.192; 95% CI = −0.327, −0.058, Table 3). No associations with section modulus were observed. The longitudinal analysis revealed that the effect of serum UA levels on FN-BMD tended to become stronger over time (Table 3). In other words, serum UA was associated with trajectories of FN-BMD. More specifically, per SD increase in UA, there was an annual increase of 0.0003 (95% CI = 0.000, 0.001) g/cm^2 in FN-BMD). In cortical buckling ratio, an annual decrease of 0.02 (95% CI = −0.03, −0.01) was observed. In contrast, no change on the effect of UA on cortical thickness and bone width over time was observed (P-interaction UA with the time variable > 0.05; data not shown). No significant quadratic term was detected for any of the associations (data not shown), suggesting no evidence for non-linear associations.

Table 1. Baseline characteristics of subjects in each of the three study populations

	Fracture (n=5,074)	**FN- BMD (n=4,073)**	**Hip Geometry (n=3,322)**
Serum UA (µmol/l)	324.0 ± 82.3	321.0 ± 78.9	319.5 ± 77.5
Age (years)	70.3 ± 9.1	68.6 ± 7.8	68.2 ± 7.7
Women (%)	61.5	59.7	59.3
Height (cm)	166.1 ± 9.2	166.7 ± 9.1	167.0 ± 9.1
Weight (kg)	72.7 ± 11.9	73.3 ± 11.6	73.4 ± 11.4
BMI (kg/m^2)	26.3 ± 3.8	26.4 ± 3.7	26.3 ± 3.1
Physical activity (min/week)	2543 ± 1176	2550± 1180	2623 ± 1164
Dutch Healthy Diet-index	48.0 ± 10.1	47.9 ± 10.1	47.9 ± 10.1
Vitamin C intake (mg/day)	112 ± 53.5	119 ± 52.1	116 ± 50.1
Serum Calcium (mmol/l)	2.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.2
Serum phosphorous (mmol/l)	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
Glomerular Filtration Rate (mL/ min/1.73m^2)	76.9 ± 17.4	77.9 ± 16.7	77.8 ± 16.2
Smoking Status (%)			
Current	22.9	24.6	24.7
Never or Former	77.1	75.4	75.4
Diabetes Mellitus (%)	11.1	10.2	10.2
Cardiovascular disease (%)	32.7	29.1	27.9
Hip and Knee operations (%)	9.7	9.1	8.9
Diuretic use (%)	10.3	14.4	13.1
Hormone replacement therapy (%)	1.3	1.4	1.4
Corticosteroids (%)	2.0	2.0	1.9
Thyroid drug use (%)	2.4	2.1	1.9
Antigout preparation (%)	0.6	0.6	0.6
Other drugs for disorders of the musculo-skeletal system	0.2	0.2	0.2
All fractures (%)	25.6	25.5	25.5
Vertebral Fractures (%)	5.0	5.6	5.5
Non-vertebral fractures (%)	22.8	22.3	22.3
Osteoporotic Fractures (%)	23.4	23.1	22.8
Hip fractures (%)	6.9	6.2	5.9

BMD, Femoral neck bone mineral density

Table 2. Bone mineral density at femoral neck and hip bone geometry characteristics and the within subject correlations between follow-up measurement visits

FN-BMD (g/cm²)	Mean ± SD	2nd visit	3rd visit	4th visit
1st visit (N=4,073)	0.86 ± 0.14	*r*=0.95	*r*=0.94	*r*=0.92
2nd visit (N=2,916)	0.86 ± 0.14		*r*=0.95	*r*=0.93
3rd visit (N=2,052)	0.86 ± 0.15			*r*=0.78
4th visit (N=1,664)	0.85 ± 0.14			
Cortical thickness (cm)				
1st visit (N=3,322)	0.13 ± 0.03	*r*=0.84	*r*=0.77	
2nd visit (N=2,387)	0.14 ± 0.03		*r*=0.74	
3rd visit (N=1,827)	0.14 ± 0.04			
Bone width (cm)				
1st visit (N=3,322)	3.19 ± 0.32	*r*=0.84	*r*=0.83	
2nd visit (N=2,387)	3.09 ± 0.36		*r*=0.84	
3rd visit (N=1,827)	3.11 ± 0.38			
Section Modulus (cm³)				
1st visit (N=3,322)	1.12 ± 0.34	*r*=0.92	*r*=0.89	
2nd visit (N=2,387)	1.16 ± 0.36		*r*=0.87	
3rd visit (N=1,827)	1.15 ± 0.39			
Cortical Buckling Ratio				
1st visit (N=3,322)	13.95 ± 3.50	*r*=0.77	*r*=0.72	
2nd visit (N=2,387)	12.84 ± 4.22		*r*=0.77	
3rd visit (N=1,827)	13.33 ± 4.64			

r, Interclass correlation coefficient; FN-BMD, Femoral neck bone mineral density

Fracture free survival analysis

During the follow-up, 1297 subjects developed any type of fracture, 1156 developed non-vertebral fractures, and 254 developed clinical vertebral fractures, whereas 1185 and 348 individuals developed osteoporotic and hip fractures, respectively. After adjustment for potential confounders, each SD of serum UA was significantly associated with lower risk of any type of fractures (HR= 0.925, 95% CI = 0.86,0.995), non-vertebral fractures (HR =0.924, 95% CI = 0.856,0.998), and osteoporotic fractures (HR=0.905, 95% CI = 0.838,0.977) (Table 4). No association was found between serum UA and risk of vertebral fractures or hip fractures (Table 4). No significant quadratic relationship between serum UA and the risk for any type of fractures or fracture subtypes was found (data not shown).

Influence of age and dietary intake of vitamin C

Significant interaction with age was observed for the association between UA and cortical buckling ratio (P-interaction < 0.001). After stratification by age, an inverse association was observed between UA and cortical buckling ratio only among subjects > 65 years old (per SD increase, β = −0.23 versus -0.15 for subjects ≤ 65 years) No effect modification by age was found for BMD and the other HBGPs (Table 3).

Also, effect modification by age was observed for the association between serum UA and any type of fractures (P-interaction = 0.01) and vertebral fracture (P-interaction = 0.01) (Table 4). After stratification by age, there was an inverse association between serum UA and any fracture risk among subjects > 65 years old (HR = 0.91; 95% CI = 0.84,0.99; P = 0.03), whereas no significant association was found in participants ≤ 65 years old (HR = 0.96; 95% CI = 0.83,1.11; P = 0.61) (Figure 1A). No association

Table 3. The association of serum uric acid (μmol/l) with femoral neck bone mineral density

FN-BMD (g/cm²)[a]	*Continuous*	*P-value*	*P for int. with age*	*P for int. with vitamin C intake*
Model 1: β, 95% CI	**0.007 (0.002; 0.011)**	**0.002**		
Model 2: β, 95% CI	**0.007 (0.004; 0.013)**	**0.001**	0.09	0.96
Cortical thickness (cm)	*Continuous*	*P-value*		
Model 1: β, 95% CI	**0.001 (0.0003; 0.002)**	**0.016**		
Model 2: β, 95% CI	**0.002 (0.0003; 0.002)**	**0.014**	0.17	0.22
Bone width (cm)	*Continuous*	*P-value*		
Model 1: β, 95% CI	**-0.014 (-0.024; -0.005)**	**0.003**		
Model 2: β, 95% CI	**-0.013 (-0.023; -0.003)**	**0.008**	0.84	0.21
Section Modulus (cm^3)	*Continuous*	*P-value*		
Model 1: β, 95% CI	0.002 (-0.007; 0.012)	0.63		
Model 2: β, 95% CI	0.004 (-0.006; 0.013)	0.48	0.21	0.45
Cortical Buckling Ratio[b]	*Continuous*	*P-value*		
Model 1: β, 95% CI	**-0.184 (-0.313; -0.055)**	**0.005**		
Model 2: β, 95% CI	**-0.192 (-0.327; -0.058)**	**0.005**	**<0.001**	0.51

FN-BMD: femoral neck bone mineral density
Model 1: age, gender, height, weight, eGFR, time when the measurements were performed
Model 2: Model 1 + smoking status, Dutch Healthy Diet Index, physical activity, prevalent diabetes mellitus, prevalent cardiovascular disease, history of hip or knee surgery, diuretic drug use, hormone replacement therapy, corticosteroid drug use, thyroid therapy, antigout drugs, serum phosphate, serum total calcium and dietary intake of vitamin C.
[a] inclusion of the interaction between UA and the time variable in the multivariable model revealed that: interaction UA x time variable: β= 0.0003, (95%CI: 0.000; 0.001), *P*=0.03
[b] inclusion of the interaction between UA and the time variable in the multivariable model revealed that: interaction UA x time variable: β= -0.02 (95%CI: -0.03; -0.01), *P*=0.048. Significant associations (P<0.05) are displayed in bold.

was observed between serum UA and the risk for vertebral fractures in either age group after stratification by age (Figure 1B).

No effect modification by dietary intake of vitamin C in relation to BMD and bone geometry was observed (Table 3). Effect modifications by dietary intake of vitamin C were observed only for the association of serum UA with the risk of any type of fractures (P for interaction = 0.01), non-vertebral fractures (P-interaction = 0.02), or osteoporotic fractures (P-interaction = 0.01) (Table 4). After stratification by median vitamin C intake, serum UA was associated with a lower risk of developing any type of fracture (HR = 0.865; 95% CI = 0.778–0.962; P = .008), non-vertebral fractures (HR = 0.873; 95% CI = 0.78–0.997; P = .018), or osteoporotic fractures (HR = 0.849; 95% CI = 0.761–0.949; P = .004) among participants with higher intakes of vitamin C, whereas no associations were observed among subjects with low intake of vitamin C (Figure 1, C–E).

Table 4. The association of serum uric acid (µmol/l) with fracture risk

All fracture	**RR, 95% CI**	**P for int. with age**	**P for int. with vitamin C intake**
Cases/No. at risk	1297/5074		
Model 1	**0.932 (0.870-0.998)**		
Model 2	**0.925 (0.860-0.995)**	**0.01**	**0.01**
Non-vertebral fractures	**RR, 95% CI**		
Cases/No. at risk	1156/5074		
Model 1	0.933 (0.868-1.003)		
Model 2	**0.924 (0.856-0.998)**	0.46	**0.02**
Vertebral fractures	**RR, 95% CI**		
Cases/No. at risk	254/5074		
Model 1	0.911 (0.777-1.069)		
Model 2	0.932 (0.786-1.105)	**0.01**	**0.02**
Osteoporotic Fractures	**RR, 95% CI**		
Cases/No. at risk	1185/5074		
Model 1	**0.913 (0.849-0.982)**		
Model 2	**0.905 (0.838-0.977)**	0.08	**0.01**
Hip Fractures	**RR, 95% CI**		
Cases/No. at risk	348/5074		
Model 1	0.897 (0.797-1.022)		
Model 2	0.896 (0.78-1.029)	0.12	0.80

Model 1: age, gender, height, weight, eGFR, index time
Model 2: Model 1 + smoking status, Dutch Healthy Diet-index, physical activity, prevalent diabetes mellitus, prevalent cardiovascular disease, history of hip or knee surgery, diuretic drug use, hormone replacement therapy, corticosteroid drug use, thyroid therapy, antigout drugs, serum phosphate, serum total calcium and dietary intake of vitamin C. Significant associations (P<0.05) are displayed in bold.

Sensitivity analysis

Exclusion of the fourth measurement of FN-BMD or of the subjects with prevalent diabetes mellitus from our analysis did not affect our results (data not shown). Moreover, after we applied the Bonferroni correction, the association of serum UA with FN-BMD and HBGP remained significant in all study participants as well as the association of serum UA with risk of developing any type of fracture and osteoporotic fractures in participants with higher intakes of vitamin C.

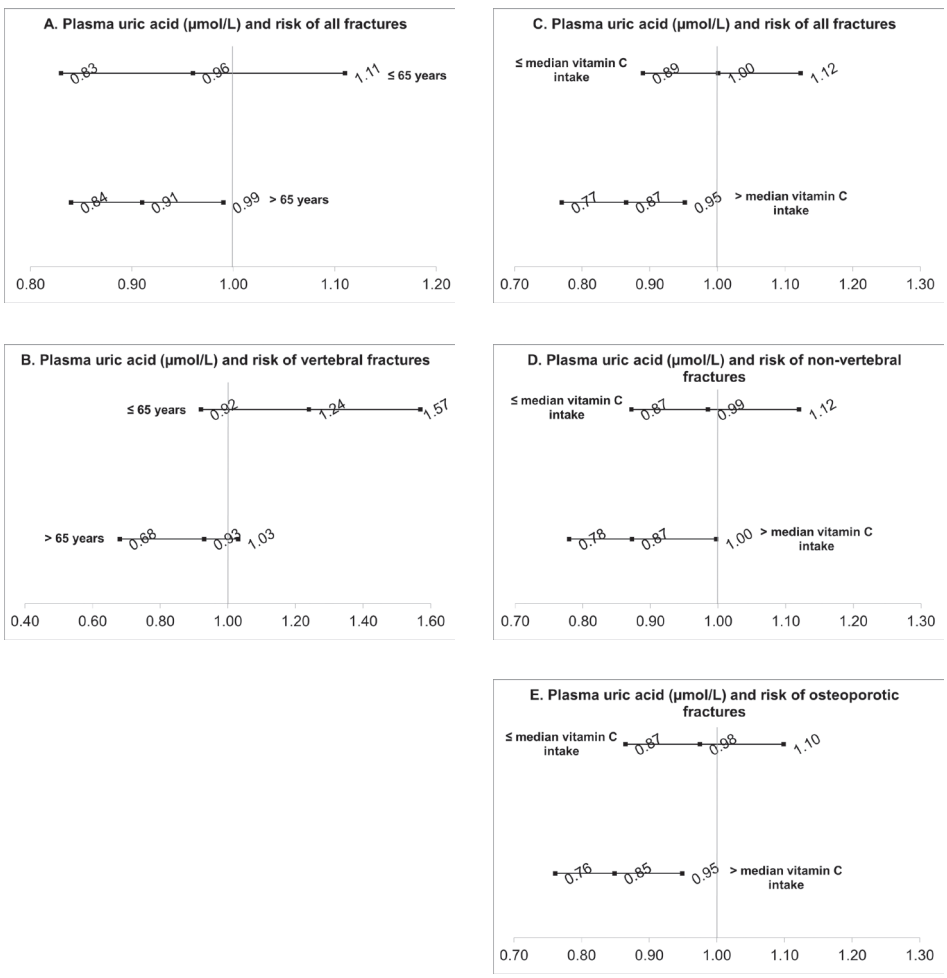

Figure 1. The association between serum uric acid (µmol/l) and fracture risk in strata of age (A and B) and intakes of vitamin C(C,D and E).
Numbers respresent hazard ratios and 95% confidence intervals.

DISCUSSION

Main findings

In this large prospective study, higher serum UA concentrations were associated with higher BMD at the femoral neck, thicker cortices, lower bone width and lower cortical buckling ratio (reflecting more bone stability). In addition, we noted that high serum UA was associated with a lower risk of osteoporotic fractures.

Comparison to other studies

Our results on serum UA and BMD are similar to those reported by Nabipour et al (12) and Sritara et al (21). They found that higher serum UA levels in men were associated with higher BMD. Also, the positive association between UA and BMD was observed in women by Makovey and colleagues(22). They showed that higher serum UA was associated with less annual loss of BMD at the lumbar spine, forearm and total body but not at the hip(22). Moreover, Ahn et al (23) observed that in 7,502 healthy postmenopausal women, higher serum UA levels were also associated with higher bone mass, lower bone turnover and lower prevalence of vertebral fractures. Although the evidence shows an association between UA and bone health, a recent Mendelian Randomization study reported no causal effect of UA on BMD (24). However, several issues may have comprised their approach in assessing causality (eg, use of a weak instrument, pleiotropic effect of the genetic variants, and lack of a sufficiently powered setting). Furthermore, Zhang *et al*. (2015) reported no association between UA and FN-BMD in the general population, which can be due to differences in the study design (cross-sectional versus our longitudinal design), the relatively young population and inclusion of different ethnic groups in the study population (25). As shown in our study, UA may have a protective effect mainly in older individuals who are at higher risk for bone loss. Also, the levels of UA and its effect on health may vary across different ethnicity groups (26).

To our knowledge, the present study is the first to show that serum UA levels are associated with favorable hip bone geometry and also with a reduction in incident fracture risk of non-vertebral and osteoporotic-fractures. We did not find an association between serum UA and hip or vertebral fractures, which may be due to the low number of cases. So far, most of the studies on the topic are cross-sectional and used as primary endpoint BMD or prevalent fracture without evaluating the association with hip bone geometry or incident fractures. Very recently, two longitudinal studies showed that in men, UA is associated with a reduction in incident osteoporotic and non-spine fractures, but not with incident hip fractures (13,14). These results are consistent with ours and further support the hypothesis that UA may act as a protective factor against metabolic bone diseases not only in men but also in women.

Potential mechanisms

UA is a biomarker commonly measured to diagnose gout. Also, UA is regarded as a risk factor for cardiometabolic diseases due to stimulation of smooth muscle cell proliferation, increased inflammation, and increased endothelial dysfunction (8, 27). However, there is growing evidence indicating that higher serum levels of UA may have beneficial effects because of its role as an antioxidant and cytoprotectant. UA accounts for a substantial part of the anti-oxidative capacity of the plasma (28) and is capable of scavenging intracellular free radicals during metabolic stress such as nitric oxide, peroxyl radicals, and hydroxyl radicals (29). Given this, it is also plausible that high UA levels may exert a protective effect in bone metabolism. Oxidative stress seems to attenuate osteoblastogenesis and bone formation (30), and it has been associated with bone mass (31). Moreover, an *in vitro* study demonstrated that UA treatment decreased osteoclastogenesis and reduced the production of reactive oxygen radicals in osteoclast precursors (32).

Potential influence of age and vitamin C

Also, a novel finding of this study is the role that age and vitamin C may play in the effect of UA on musculoskeletal outcomes. Although this is the first study to note the interaction between age and UA on bone, a similar interplay of age and UA has been reported before for other health outcomes, e.g. blood pressure (15, 33). Supplemental vitamin C intake has been reported to have a uricosuric effect by increasing renal fractional clearance of UA, inhibiting UA synthesis, and thus lowering the plasmatic levels of UA (34). Under this contention, vitamin C would tend to lower the beneficial effect of UA on bone. In contrast, in the current investigation, we observed a synergistic effect of vitamin C and UA. However, recent evidence shows that vitamin C intake from diet, in contrast to vitamin C supplementation, is not associated with lower serum UA levels, but to the contrary, it can be positively associated with UA levels (35). High vitamin C intake is associated with lower bone loss and may have a protective role for bone health due to its antioxidant properties (36). Therefore, vitamin C from diet may strengthen the effect of UA on bone. Another explanation for the apparent paradox may also be the switch from antioxidant to pro-oxidant properties of UA, particularly when it is present in blood at supernormal levels (8).We postulate that vitamin C intake may help to regulate the role of UA as anti- or pro-oxidant. However, in the current investigation, the interplay between UA and vitamin C was observed only for the risk of fractures and not for BMD or hip bone geometry. Therefore, other mechanisms may be involved. Further studies are thus needed to replicate our findings and to shed more light on the interplay between age, vitamin C, and UA in relation to bone health.

Strengths and limitations

This study has several strengths. This is a large, prospective, population-based study of 5074 individuals with a comprehensive follow-up of 10 years on average. In addition, in this setting we had the possibility to adjust for a broad spectrum of anthropometric, dietary, clinical, biochemical, and biophysical bone-related confounders. Also, it is the first prospective study to use BMD and hip bone geometry measures in multiple time points. Additionally, to our knowledge, this is the first study on the topic to enroll both men and women. Moreover, our cohort was recruited from community and not clinical practices such that the sample was not selected for comorbid diseases that could influence serum UA levels or the relation with bone parameters. However, there are also shortcomings. We only report results on older individuals and those of Dutch-Northern European background, which is the reason these results are not generalizable to younger individuals or individuals of very distinct ethnical background. Furthermore, blood levels of major endogenous components, exogenous antioxidants (e.g. vitamins C and E), and antioxidant enzymes were not examined, which can differ from the dietary intake of these nutrients. We did not have PTH or NTX N-terminal telopeptide of type 1 collagen (a sensitive marker of overall bone resorption) measures in our study, which has been reported to correlate with UA, and therefore, we could not determine these associations to be worthy of further investigation. Moreover, we did not have measures of BMD at the total hip or lumbar spine. Lastly, selection bias may be present due to missing data on bone measurements. However, using a selected source population for a cohort usually leads to bias toward the null rather than a false-positive association (37).

CONCLUSION

In conclusion, in this large, prospective, population based cohort of elderly men and women, serum UA levels were shown to have a protective effect on BMD, favorable configuration of hip bone geometry, and lower fracture risk. Additional studies are warranted to establish causality and the precise mechanisms of action and to give more insight into the interplay of UA with age and intake of vitamin C as determinants of bone health.

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4

OVERALL DIETARY PATTERNS IN RELATION TO BONE HEALTH AND FRAILTY

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4.1

DIETARY PATTERNS IN AN ELDERLY POPULATION AND THEIR RELATION WITH BONE MINERAL DENSITY

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ABSTRACT

Purpose

Our aim was to identify dietary patterns that are associated with bone mineral density (BMD) against a background of relatively high dairy intake in elderly Dutch subjects.

Methods

Participants were 55 years of age and older (n= 5144) who were enrolled in The Rotterdam Study, a population-based prospective cohort study. Baseline intake of 28 pre-defined food groups was determined by a validated food frequency questionnaire. Dietary patterns were identified using principal component analysis. BMD was measured by dual energy X-ray absorption at baseline and at three subsequent visits (between 1993 and 2004). Linear mixed modelling was used to longitudinally analyse adherence to each pattern with repeatedly measured BMD (both in Z-scores).

Results

After adjustment for confounders, two dietary patterns were associated with high BMD: a "Traditional" pattern, characterized by high intake of potatoes, meat and fat (β= 0.08; 95% CI: 0.05, 0.11) and a "Health conscious" pattern, characterized by high intake of fruits, vegetables, poultry and fish (β= 0.06; 95% CI: 0.04, 0.09). The "Processed" pattern, characterized by high intake of processed meat and alcohol was associated with low BMD (β= -0.03; 95% CI: -0.06, -0.01). Associations of adherence to the "Health conscious" and "Processed" pattern with BMD were independent of body weight and height, whereas the association between adherence to the "Traditional" pattern with BMD was not.

Conclusions

Against a background of high dairy intake and independent of anthropometrics, a "Health conscious" dietary pattern may have benefits for BMD, whereas a "Processed" dietary pattern may pose a risk for low BMD.

INTRODUCTION

In the recent decades, the role of individual nutrients such as calcium and vitamin D in healthy bone remodelling in elderly has been studied extensively [1]. However, people do not eat isolated nutrients but, rather, a combination of different foods. Hence, nutritional research is shifting from a traditional approach of investigating the effects of single nutrients (e.g. calcium or vitamin D) and foods (e.g. dairy products) to a more holistic approach investigating overall dietary patterns. Studying dietary patterns might help us to identify potential additive, synergistic, or antagonistic effects between components of the full diet that may affect bone mineral density (BMD) [2]. Also, cumulative effects of a combination of nutrients on BMD might be easier to identify than the effect of a single nutrient, which might be too small to detect [3].

Dietary patterns differ between populations and depend on cultural habits and food availability. Identifying the dietary patterns associated with high or low BMD in different populations might help to identify common combinations of food groups or food products that are important for bone health. The current food-based dietary guidelines for maintaining BMD in populations where low BMD is prevalent recommend sufficient intake of calcium and vitamin D [4], mainly by dairy consumption. However, evidence on the effect of dietary patterns on BMD in populations with high dairy intake is scarce. Since average dairy consumption in the Netherlands is relatively high (ca. 350 g dairy/day [5] including milk, yoghurt and cheese), studying the full dietary patterns of the Dutch elderly can provide insights into the relationship between overall diet composition and BMD against a background of high dairy intake.

Mechanical loading of the weight-bearing bones is an important determinant of BMD [6]. Weight loss might decrease mechanical loading, whereas weight gain might increase mechanical loading [7,8]. In response to a decrease or increase in mechanical loading, altered remodeling will result in a lower or higher BMD. Diet might influence BMD by affecting body weight and thus mechanical loading.

In addition, diet has the potential to modify the bone's response to mechanical loading [9,10], by either favourably or unfavourably affecting bone remodelling directly. It could be speculated that when mechanical loading is compromised due to weight loss, a diet-induced stimulation of remodelling will be more important to maintain a high BMD than when loading remains stable. Summarized, we hypothesize that body weight-induced changes in mechanical loading and diet-induced modifications in response to mechanical loading might interact in relation to BMD.

Hence, our primary aim was to identify dietary patterns that are associated with BMD in middle-aged and elderly subjects against a background of high dairy intake. Moreover, we explored whether the effect of dietary patterns on BMD might be influenced by body weight or changes in body weight over time.

METHODS

Design

This study was embedded in the Rotterdam Study. Details on the objectives and design have been described previously [11]. In brief, Dutch subjects of 55 years and older living in the Ommoord district of Rotterdam, the Netherlands, were included in this prospective population based cohort study. The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Centre and by the review board of The Netherlands Ministry of Health, Welfare and Sports.

Baseline assessment of dietary intake

Baseline dietary intake of 170 food items was assessed by a trained dietician using a validated, semi-quantitative food frequency questionnaire (FFQ). The reliability of dietary intake was determined during this assessment by the dietician. For example dietary data was considered as unreliable when patients had difficulties with recall or when they did not cooperate during the interview. The questionnaire was validated and adapted for use in the elderly [12,13].The ability of the FFQ to rank subjects adequately according to their dietary intakes was demonstrated by a validation study (n =80) comparing the FFQ to 15 day-food records collected over a year to cover all seasons [14]. Pearson's correlation coefficients of this comparison ranged from 0.4 to 0.8 for macro-and micronutrients after adjustment for sex, age, total energy intake, and within-person variability in daily intakes.

Identification of dietary patterns and assignment of pattern-adherence scores

All food items were categorized into 28 pre-defined food groups to reduce the complexity of dietary data. An overview of these food groups, which were based on similarities in product composition or culinary use, is shown in Supplemental table1. Next, dietary patterns were derived by Principal Component Analysis (PCA) on intake of these food groups in grams per day, unadjusted for total energy intake. We used Varimax rotation and Kaiser Normalization to obtain patterns with simpler structure [15] and optimal interpretability. Factor loadings, which reflect the correlation between a food group and a dietary pattern, were used to characterize a pattern using a cut-off of 0.2, similar to comparable studies [16,17]. Food groups with a factor loading > 0.2 indicate a positive contribution and < -0.2 a negative contribution to a specific pattern. Adherence to patterns with an Eigenvalue (a measure of explained variance) of > 1.5 only was studied in relation to BMD. For each participant, pattern adherence scores were constructed by summing up observed intakes of the pattern's food groups weighted by the corresponding factor loading for each of the three dietary patterns separately.

Longitudinal assessment of BMD

BMD of the femoral neck was measured by dual energy X-ray absorptiometry (DXA) using a Lunar DPX-densitometer (Lunar Radiation Corp., Wadison, WI) at baseline and at 3 subsequent visits (1993-1995, 1997-1999 and 2002–2004). DXA scans were analysed with DPX-IQ (visit 1 to 3) and PRODIGY (visit 4) software and BMD values are expressed in g/cm^2.

Longitudinal assessment of anthropometrics

Body weight (kg) and height (cm) were assessed at the research centre repeatedly, during the same visits as at which the BMD measurements were assessed. Body weight was measured using a digital scale and body height was measured using a stadiometer, while subjects wore light clothing and no shoes.

Assessment of covariates

The selection of covariates was based on previous studies investigating the associations between dietary pattern-adherence and BMD [18-20]. A schematic overview of the data collection relevant to this study is shown in Supplemental Fig.1.

Covariates assessed at baseline

Smoking was identified as "current" or "past" or "never". Highest education and net household income were used as proxy for socio-economic status (SES). Education was coded as "low" (primary education, primary + higher not completed, lower vocational and lower secondary education) or "high" (intermediate vocational, general secondary, higher vocational education & university). Household income was coded "above" or "below" the average of 2400 net Dutch Guilders (≈ 1600 euro) per month. Lower limb disability index, a combined index reflecting a subject's ability to stand up, walk, climb and bend, was based on the Stanford Health Assessment Questionnaire [20]. Prevalent Type 2 diabetes Mellitus was determined as baseline serum glucose concentrations >11 mmol / l or use of glucose lowering drugs. Prevalent CVD included prevalent coronary heart disease, heart failure, stroke and arterial fibrillation. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study have been described in detail elsewhere [21]. The use of serum lipid reducing agents and antihypertensive drugs was registered during the home interview by trained research assistants [22].

Covariates assessed at other visits

Use of hormone replacement therapy (HRT) in females was assessed at the 2nd visit and coded as "never" or "ever." Physical activity was assessed on the 3rd visit, using the Zutphen Study Physical Activity Questionnaire including questions on walking, cycling, gardening, diverse sports, hobbies, and housekeeping. Total time spent on physical activity was calculated by summing minutes per week for each type of activity [23-25]. Serum 25-hydroxyvitamin D (25(OH)D) was measured in a subgroup of participants (n= 3171)

during the 3rd visit to the research centre using radioimmunoassay's (IDS Ltd, Boldon, UK, available at www.idsltd.com). The sensitivity of the test was 3nmol/L which ranged from 4 to 400nmol/L. Intra-assay accuracy was <8% and the inter-assay accuracy was <12%.

Status of body weight change; definitions of weight gain and weight loss

Weight loss and weight gain were defined as > 5% decrease or increase in baseline body weight during the full follow up period (1989 to 2004). All other values were considered to indicate a stable body weight.

Population of analysis

Of the full cohort of the Rotterdam (n=7983), 1462 subjects did not attend the study centre and 271 were not offered an FFQ since they participated in the pilot phase of the Rotterdam Study only. Moreover, 122 participants were excluded due to suspected dementia, 2012 due to unreliable dietary intake data defined by the dietician and 481 were excluded for logistic reasons, leaving 5435 subjects with reliable intake data. Subjects were included for analysis when both reliable dietary intake data and at least one BMD measurement was available (n =5144). Of these subjects, 4870 had measurements of BMD at baseline, 3682 at the second visit, 2561 at the third visit and 2305 at the fourth visit.

Statistical analysis

Characteristics of the study population

Differences in characteristics between the tertiles of adherence to each dietary pattern were assessed using one way Kruskal-Wallis tests for (non-normally distributed) continuous variables and Chi-square tests for categorical variables. These values are presented as median and interquartile range (IQR) for continuous variables and as percentages for categorical variables.

We used the multiple imputation procedure for missing covariates using the Markov chain Monte Carlo method. Normally and non-normally distributed variables were predicted using predictive mean matching and binary or categorical variables using logistic regression.

Longitudinal associations between dietary pattern adherence and BMD

The association between adherence to the dietary patterns and BMD trajectories was studied using linear mixed modelling (LMM), a technique that takes the correlation between the repeated BMD measurements within each subject into account by including random effects in the model [26]. Specifically, we used a random intercept and slope (for time) model and assumed independent error terms. We used Z-scores of adherence to each dietary pattern as exposure variables and sex-specific Z-scores of BMD as the outcome. Despite using different

densitometers in time we have shown in previous work no cross-calibration is required [27]. The centre visit (1, 2, 3 or 4) was used as time variable and recoded as 0, 2, 6 and 10 years to adjust for differences in mean time interval between visits. Covariates were added to the model step wisely as independent variables to test for potential confounding and were kept in the multivariable model when they changed the regression coefficient of the associations between the dietary pattern adherence and BMD by >10% [28].

Accordingly, three models were developed. The first was a basic model adjusted for age, sex and total energy intake and adherence to the other PCA derived patterns (model 1). The second model was further adjusted for confounders and additionally included smoking, net household income, education, prevalent diabetes, physical activity and use of HRT (model 2). Since anthropometrics could be both confounders and intermediates in our analyses, we developed a third model that was further adjusted for body weight and height, which were measured repeatedly (model 3). Also, we have studied longitudinal associations between dietary pattern adherences and body weight using model 2 with body weight (in kg) instead of BMD as the outcome, which was additionally adjusted for height. To assess whether adherences to dietary patterns were associated with trajectories of BMD, we tested the interaction with time by adding the product term of time x adherence score to the dietary pattern to model 3.

Influence of sex and changes in body weight

Effect modification by sex was tested by adding sex and the product term of sex x adherence score to the dietary pattern as independent variables to model 1.

We assumed that participants that experience weight loss have more reduction of BMD and those that experience weight have less reduction of BMD over the follow-up period than those with stable weight. To test this assumption, we performed linear mixed models with BMD as the outcome and interaction between weight loss or weight gain and time in models with body weight change (> 5% loss, stable (reference) or >5% gain), age and sex. Only when our assumption was statistically confirmed, effect modification by body weight change was further evaluated.

Stratified analyses were only performed if the P for interaction was <0.10, using model 1. Stratified analyses for body weight were additionally adjusted for baseline body weight height.

Sensitivity analyses

We performed two sensitivity analyses to compare the results of (1) our main analyses with and without using imputed covariates and (2) our stratified analyses using a more stringent cut-off to define weight gain or loss (+/-10% instead of +/-5% change in body weight). LMM was performed using R statistical software version 3.2.1. (The R Foundation for Statistical Computing, Vienna, Austria). All other analyses were performed using SPSS software version 22 (IBM, Chicago, IL, USA).

RESULTS

Dietary pattern identification

Three dietary patterns with an Eigenvalue of > 1.5 were identified (scree plot in Supplemental Fig. 2), with a cumulative explained variance of 19%, namely: (1) a "Traditional" dietary pattern characterized by high intake of potatoes, meat and fat and low intake of soy products; (2) a "Processed" dietary pattern characterized by high intake of processed meat, alcohol, mixed dishes like pizza, and low intake of fruit and yoghurt; and (3) a "Health conscious" dietary pattern characterized by high intake of fruits and vegetables, poultry, fish, and alcohol, and low intake of sweets. A description and label of each pattern and the corresponding factor loadings per food group are shown in table 1. None of the patterns has a factor loading for milk and milk products or cheese > 0.2 or < -0.2. However, the factor loading for milk-and milk products was close to this cut-off (-0.19) for the "Processed" pattern, which was low in yoghurt, another source of dairy products. However, despite a negative factor loading for yoghurt, also participants in the highest tertile of adherence to the "Processed" dietary pattern had relatively high intakes of total dairy products (2.3 serving per day versus a median intake of 2.7 servings in the full study population), including milk, milk products and cheese as well as yoghurt.

Study population for investigating associations between pattern adherence and BMD

The median total dairy intake of our study population was 2.7 servings per day, and was mainly determined by daily consumptions of milk and milk products (1.4 servings) and cheese products (0.9 servings, Supplemental table 2).

Characteristics of subjects in each tertile of adherence to the three dietary patterns are shown in table 2. Briefly, subjects with high adherence to the "Traditional" and "Processed" patterns were more often males (59% vs. 26% and 62 vs. 24% for the highest versus the lowest tertile respectively (P for difference <0.001)). Smoking was more prevalent in subjects with high adherence to the "Processed" pattern. Females with high adherence to the "Health conscious" pattern were more likely to have used HRT. No clear differences in age, physical activity or indicators of SES were observed. Calcium intake was constant over the tertiles of adherence to the "Traditional" pattern (P for difference= 0.59) and time spent on vigorous physical activity was constant over the tertiles of the "Processed" pattern (P for difference = 0.15). Between baseline and the 4th visit, mean BMD slightly decreased in females (1.2%), but not in males. At the same time, mean body weight increased in both males (+3.4 %) and females (+ 2.3%). Median intake of food groups in the lowest an highest tertile are shown in Supplemental table 2.

Table 1. Factor loadings-matrix and labels for the three dietary patterns that explained most of the variance in food group intake

Pattern	**1**	**2**	**3**
High factor loadings for:	Meat, fat, potatoes, eggs	Processed meat, alcohol, mixed meals, eggs	Fruit, vegetables, poultry, fish, alcohol, eggs
Low factor loadings for:	Soy products, mixed meals	Fruit, yoghurt	Sweets
Label	*"Traditional"*	*"Processed"*	*"Health conscious"*
Fruit and fruit products	-.036	**-.548**	**.219**
Vegetables & vegetable products	.182	-.187	**.240**
Pulses & legumes	-.046	-.010	-.110
Milk-and milk products	.014	-.192	.054
Yoghurt	-.038	**-.506**	.114
Cheese products	-.037	.086	-.001
Soy products	**-.498**	.159	-.031
Refined grain products	.005	.170	.082
Whole grain products	.063	.011	-.052
Soft drinks & lemonades	.097	-.082	.149
Eggs	**.280**	**.258**	**.257**
Unprocessed meat	**.641**	.086	-.076
Processed meat	**.520**	**.451**	.054
Poultry	-.023	-.022	**.494**
Fatty fish	-.071	.137	**.524**
Lean and battered fish	-.029	-.150	**.629**
Shell fish	.032	-.024	**.326**
Savory snacks	.015	-.073	-.006
Sweets	.131	-.177	**-.211**
Nuts and seeds	-.007	.089	.051
Vegetable oils and fats	**.296**	.072	.006
Animal fats	**.243**	.092	-.090
Coffee tea and water	.027	-.135	.081
Alcoholic drinks	.154	**.558**	**.202**
Mixed meals[1]	**-.208**	**.372**	.117
Soups and sauces	.089	.077	.192
Potatoes	**.582**	.095	-.185
Porridges	-.064	-.008	.007

Extraction Method: Principal Component Analysis, Rotation Method: Varimax with Kaiser Normalization, a. Rotation converged in 17 iterations.
Factor loadings represent the correlations between the food groups and the dietary patterns.
Factor loadings > 0.2 or < -0.2 are **in bold** and were used to label the dietary patterns.
[1]: Mixed meals included Pizza, Nasi and Bami Goreng.
Bami and Nasi are traditional Indonesian dishes with meat, vegetables and rice (Nasi) or pasta (Bami) and could reflect either home-made or take-away food)

Table 2. Characteristics of participants of the Rotterdam Study (N=5435) per tertile of adherence to the "Traditional", "Processed" or "Health conscious" dietary pattern

	"Traditional" pattern		
	1st tertile	**2nd tertile**	**3rd tertile**
Age (y)[1]	67 (61-74)	67 (62-74)	66 (61-72)
Total energy intake (kcal/d)[1]	1684 (1453-1987)	1877 (1616-2161)	2210 (1922-2538)
Physical activity (h/day) [1]	5.9 (4.3-7.9)	5.6 (4.0-7.4)	5.9 (4.4-8.0)
Of which vigorous (h/week)[1]	3.3 (1.3-6.5)	3.1 (1.0-6.7)	3.5 (1.0-7.0)
Dutch Healthy Diet Index [1,2]	52 (45-59)	49 (43, 56)	44 (38-51)
Calcium intake (mg/day) [1]	1090 (869, 1331)	1075 (863, 1304)	1066 (857, 1331)
25 (OH) D3 (nmol/l) [1,3]	41 (27-57)	46 (30-65)	47 (31-69)
BMD at 1st visit (mg/cm^2) [1,6]	0.84 (0.75, 0.94)	0.85 (0.77, 0.94)	0.89 (0.79, 0.99)
BMD at 2nd visit (mg/cm^2) [1,6]	0.84 (0.74, 0.94)	0.85 (0.76, 0.95)	0.89 (0.79, 0.98)
BMD at 3rd visit (mg/cm^2) [1,6]	0.84 (0.74, 0.93)	0.86 (0.76, 0.96)	0.89 (0.79, 0.99)
BMD at 4th visit (mg/cm^2) [1,6]	0.84 (0.74, 0.92)	0.85 (0.76, 0.94)	0.88 (0.78, 0.98)
Body weight at 1st visit (kg)[1]	70 (63, 78)	73 (66, 80)	76 (69, 84)
Body weight at 2nd visit (kg)[1]	70 (63, 78)	74 (66, 81)	77 (69, 85)
Body weight at 3rd visit (kg)[1]	71 (62, 79)	74 (66, 81)	77 (69, 85)
Body weight at 4th visit (kg)[1]	72 (63, 81)	75 (67, 83)	78 (70, 87)
Baseline body height (cm)[1]	164 (159, 171)	166 (160, 173)	171 (164, 177)
Sex (% males)	26	38	59
Body weight change (% loss/ gain) [4]	15/ 36	17/ 34	18/29
Prevalent osteoporosis (%)	14	12	8
Prevalent type 2 diabetes (%)	9	9	11
Prevalent CVD (%)	12	13	13
High education (%)	36	36	38
High income (%>1600 euro/ mo)	47	51	54
Current smokers (%)	19	20	28
Current or past HRT use (%)[5]	9	10	8
Lipid lowering drug use (%)	3	2	2
Antihypertensive drug use (%)	12	13	13
Lower limb disabled (%)	20	18	15

[1]Median (interquartile range), [2] The Dutch Healthy Diet Index reflects adherence to the Dutch guidelines for a healthy diet and included information on intake of vegetables, fruit, fiber, fish, saturated fatty acids, trans fatty acids, acidic drinks and foods, sodium and alcohol (van der Lee, 2012). [3] Measured at the 3rd visit,

"Processed" pattern			"Health conscious" pattern		
1st tertile	**2nd tertile**	**3rd tertile**	**1st tertile**	**2nd tertile**	**3rd tertile**
68 (62-74)	68 (62-74)	66 (61-72)	68 (63-75)	66 (61-73)	67 (61-72)
1886 (1602-2233)	1861 (1581, 2191)	2027 (1687, 2351)	1929 (1616-2262)	1887 (1599-2208)	1955 (1634-2304)
6.0 (4.3, 8.0)	5.7 (4.0, 7.4)	5.7 (4.2, 7.8)	5.6 (3.9-7.6)	6.0 (4.3-7.8)	5.9 (4.4-7.9)
3.2 (1.5-7.3)	3.3 (1.0, 6.5)	3.5 (1.0-7.0)	3.0 (1.0-6.3)	3.5 (1.3-7.0)	3.6 (1.5-7.2)
53(46-59)	49 (42-55)	44 (37, 50)	44 (37-51)	49(42-56)	52 (45-58)
1208 (997, 1475)	1053 (858, 1286)	968 (758, 1203)	1017 (814-1260)	1077 (870-1319)	1141 (899-1390)
43 (28-62)	43 (29, 62)	48 (32, 69)	41(26-60)	46(30-64)	47 (32-68)
0.85 (0.76-0.94)	0.86 (0.77-0.95)	0.88 (0.78-0.97)	0.85 (0.76-0.94)	0.86 (0.77-0.96)	0.88 (0.78-0.97)
0.85 (0.75-0.95)	0.85 (0.76-0.95)	0.88 (0.78-0.98)	0.85 (0.74-0.94)	0.86 (0.76-0.96)	0.88 (0.78-0.97)
0.85 (0.75-0.95)	0.85 (0.76-0.95)	0.88 (0.78-0.98)	0.84 (0.74-0.95)	0.86 (0.77-0.96)	0.88 (0.79-0.97)
0.84 (0.75-0.94)	0.85 (0.76-0.94)	0.86 (0.76-0.93)	0.83 (0.74-0.92)	0.85 (0.76-0.95)	0.87 (0.77-0.96)
72 (65-81)	73 (65-81)	75 (67-83)	72 (65-80)	72 (65-80)	74 (67-83)
72 (65-81)	73 (64-80)	76 (68-83)	72 (65-80)	73 (65-81)	75 (68-83)
73 (65-83)	73 (65-81)	77 (68-84)	73 (64-80)	73 (65-81)	75 (68-83)
73 (65-82)	4 (66-83)	77 (68-86)	74 (65-82)	74 (66-83)	76 (69-85)
164 (160-171)	166 (160-173)	171 (164-177)	167 (160-174)	166 (161-173)	167 (161-174)
24	36	62	58	61	58
17/35	17/32	16/31	19/29	16/33	15/35
11	13	10	14	11	9
9	9	11	10	9	10
11	14	12	13	12	12
32	35	44	33	37	40
48	48	56	46	51	55
14	22	34	24	22	23
9	9	8	8	8	11
3	3	2	2	3	3
12	14	12	13	13	13
20	19	15	20	16	17

[4]: Weight loss or gain is defined as >5 % reduction or increase in body weight, [5] Females only, [6]: Sample sizes of BMD were N = 4870 for visit 1, n= 3682 for visit 2, n= 2561 for visit 3 and n= 2305 for visit 4. Abbreviations: CVD = cardiovascular disease, HRT = Hormone replacement therapy.

Associations between adherence to identified dietary patterns and BMD

Regression coefficients and 95% CI of the associations between adherence to all dietary patterns and repeatedly measured BMD (all in Z-scores) are shown in table 3. After adjustment for potential confounders (model 2), the "Traditional" pattern and the "Health conscious" pattern were significantly associated with higher BMD (β: 0.06; 95% CI: 0.03, 0.09 for "Traditional" and β: 0.06; 95% CI: 0.03, 0.08 for "Health conscious" dietary pattern). In contrast, the "Processed" pattern was significantly associated with lower BMD (β: -0.03; 95% CI: -0.06, -0.01). The P-value for interaction with time was only significant for the "Health conscious" pattern (P=0.01), which reflects that high adherence to this dietary pattern is associated with less decline of BMD over time.

Influence of body weight and height or changes in body weight status

The "Traditional" and "Health conscious" pattern were associated with high body weight ((β: 1.79; 95% CI: 1.50, 2.09) and (β: 0.84; 95% CI: 0.60, 1.11) kg per Z-score of pattern adherence). In contrast, the "Processed" pattern was not significantly associated with body weight. After additional adjustment for body weight and height in the analyses of dietary patterns and BMD (table 3, model 3), a significant association between adherence to the "Health conscious" pattern and high BMD remained. However, the magnitude of the effect was diluted (β: 0.04; 95% CI: 0.02, 0.07 in model 3 versus β: 0.06; 95% CI: 0.03, 0.08 in model 2). In contrast, the significant association between adherence to the "Traditional" pattern and BMD was lost after adjustment for body weight and height, whereas the association between adherence to the "Processed" pattern and low BMD was not affected by additional adjustment for body weight and height.

We observed significant interaction between weight loss and weight gain with time in relation to BMD. This substantiates our assumption that participants that lost weight experienced more reduction of BMD and participants that gained weight experienced less reduction of BMD over the follow-up period that participants with stable body weight. Interaction with body weight change was only observed for adherence to the "Processed" pattern (P for interaction = 0.06), but not for both other patterns (P for interactions > 0.55). Data may suggest a stronger association between adherence to the "Processed" pattern and low BMD in subjects that experienced ≥5% weight gain (β; -0.07, 95% CI: -0.17, -0.02) than in those with ≥5% weight loss (β; -0.03, 95% CI: -0.12, 0.06, Fig. 1). No interaction between adherence to any dietary pattern with sex in relation to BMD was observed (P all interactions > 0.60).

Table 3. Dietary pattern adherence and BMD of the femoral neck, obtained using linear mixed modelling with random intercept and slope.

Adherence to the:	Model 1 [1]		Model 2[1]		Model 3[1]		P for interaction with time[2]
"Traditional" pattern	**0.05**	**(0.02, 0.08)**	**0.06**	**(0.03, 0.09)**	0.01	(-0.01, 0.04)	0.48
"Processed" pattern	**-0.05**	**(-0.08, -0.02)**	**-0.03**	**(-0.06, -0.01)**	**-0.03**	**(-0.06,-0.00)**	0.99
"Health conscious" pattern	**0.06**	**(0.04, 0.09)**	**0.06**	**(0.03, 0.08)**	**0.04**	**(0.02, 0.07)**	**0.01**

[1]: Regression coefficients (95% confidence intervals) of the fixed effects. Regression coefficients represent differences in BMD (in sex-specific Z-scores) for each SD of increase in dietary pattern adherence. [2]: The P-value for interaction with time was tested using model 1, to study the association between dietary pattern adherence and BMD trajectories. A significant P for interaction reflects that high adherence to a specific dietary pattern is associated with less decline of BMD over time.
Model 1: Adjusted for age, sex, total energy intake and adherence to other dietary patterns (basic model)
Model 2: Model 1 + additional adjustment for SES, smoking, prevalent T2DM at baseline, total physical activity and use of lipid lowering drugs
Addition of lower limb disability, prevalent CVD at baseline, use of HRT or antihypertensive drugs and plasma vitamin D did not change the effect estimate by ≥ 10%.
Model 3: Model 2 + additional adjustment for body weight and height
Abbreviations: BMD = bone mineral density, CVD = Cardiovascular disease, HRT= Hormone replacement therapy, SD= standard deviation
In bold: P-value < 0.05

Sensitivity analyses

Multiple imputation of missing covariates did not markedly affect the effect estimates of adherence to all dietary patterns in relation to BMD (data not shown). Also, the use of a more stringent cut-off to define weight gain or loss (+/-10% instead of +/-5% change in body weight) did not change the results of our stratified analysis (Fig.1).

DISCUSSION

Summary of main findings

In this Dutch population of middle-aged and elderly subjects, we identified two dietary patterns that were associated with higher BMD; a "Traditional" pattern (high in potatoes, meat and fat), and a "Health conscious" dietary pattern (high in fruits, vegetables, poultry, fish and alcohol). In contrast, adherence to a "Processed" pattern (high in processed meat, mixed meals and alcohol) was associated with low BMD. The associations between adherence to the "Traditional" pattern and BMD were explained, at least partly, by differences in body weight and height.

Comparison with published dietary pattern analyses

The observed associations are to some extent similar with those reported in previous studies. Data from the Canadian Multicentre Osteoporosis Study (CAMOS) suggest that

a nutrient-dense diet high in fruit, vegetables, whole grains and fish was associated with high BMD (β: 0.01 (95% CI: 0.00, 0.02) in g/cm^2 per Z-score of pattern adherence) after adjustment for BMI[17]. This dietary pattern was similar to the "health conscious" pattern that we identified in our study population. The combined intake of fruits, vegetables, and fish was also shown to be associated with high BMD in Japanese farmwomen, when consumed in a pattern with soy products [29]. The existing Mediterranean Diet Score (MDS), developed by Trichopoulou et al [30], was shown to be associated with high BMD [31]. Studies on the MDS and fracture risk showed both unfavorable[2] and favorable [32] results. The MDS reflects high intake of cereals, legumes, fruits & nuts, vegetables, oils and fish and low intake of dairy and meat products. Although none of the dietary patterns that were defined in our population exactly reflects the Mediterranean diet, it could be argued that it has similarities to our "Health conscious" pattern, due to its high factor loadings for fruits, vegetables and fish.

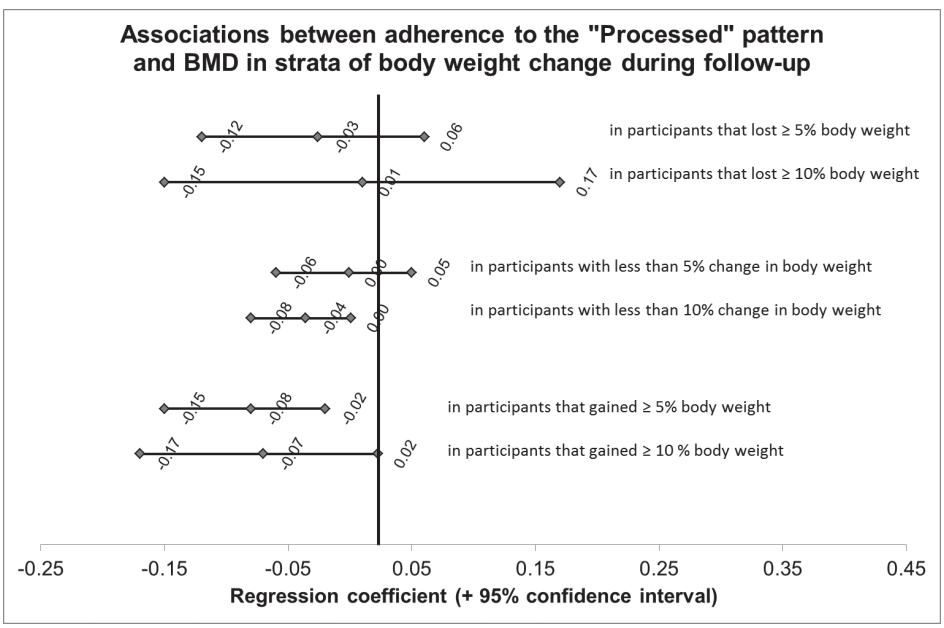

Figure 1. Associations between adherence to the "Processed" dietary pattern and BMD of the femoral neck, in strata of body weight change between baseline and visit 4 (n = 2532).
[1]: Regression coefficients and 95% confidence intervals of the fixed effects.
Regression coefficients represent differences in BMD (in sex-specific Z-scores) for each SD of increase in adherence to the "Processed" dietary pattern using a cut-off of 5% (solid line) or 10% (dashed line) change in body weight to define weight loss or weight gain.
Models are adjusted for age, sex, initial body weight and height, total energy intake and adherence to the other two dietary patterns
Abbreviations: BMD = bone mineral density, SD: standard deviation.

The "Processed" pattern which was high in processed meat, alcohol, and mixed meals, and low in yoghurt was associated with low BMD. The association of patterns high in meat and unhealthful, energy-dense food products with low BMD was also observed in several other populations, including Iranian women [33] and Canadian men (0.009 g/cm^2 decrease per Z-score of pattern adherence) and women (0.004 g/cm^2 decrease per Z-score of pattern adherence) [17]

Explanation of our results

To identify important dietary components underlying the observed associations between the "Processed" and "Health conscious" pattern and BMD, it is not only relevant to study the factor loadings of our food groups to these patterns, but also the absolute intakes of these food groups. For example, the factor loading of mixed meals for the "Processed" pattern (0.37) indicates a strong correlation, but the intake of mixed meals in the highest tertile of adherence to the "Processed" pattern is < 1 serving per month (Supplemental table 2). It is therefore unlikely that these individual food groups explain our results. More plausibly, the intake of fruits, vegetables, or fish could have contributed in either an additive or synergistic manner to the observed relation with high BMD. Vitamin D intake from sources such as fatty fish could explain the relation with high BMD, as it is well established that vitamin D is needed for calcium uptake by the intestine and important for bone health [34]. Fruits and vegetables contain a variety of nutrients that might explain positive associations of a diet with a high factor loading for these food groups, such as magnesium, vitamin C, carotenoids and potassium [35]. Magnesium might contribute to healthy bone remodelling [36] via its favourable impact on osteoblastic and osteoclastic activity and vitamin C and carotenoids might explain the association via anti-oxidant related mechanisms [1]). Moreover, poultry and fish, rather than red meat, might be sources of protein that are beneficial for bone remodelling. Negative associations of red and organ meat, but not poultry, with bone outcomes was also shown in Chinese elderly[37], a finding which may be explained by differences in fat or amino acid content or quality. Also, a potential interplay between calcium, sodium, magnesium, and phosphorus could play a role. For example, an excess intake of phosphorus, especially from processed food products as found in the "Processed' pattern has been suggested previously to disrupt hormonal regulation of calcium and vitamin D, thereby leading to low BMD [38].

There is general consensus that body weight is a main determinant of BMD [39], as it influences mechanical loading of the weight bearing bones. In our analysis, we took two approaches to investigate the influence of body weight on the associations between dietary pattern adherence and BMD. First, we built an additional model adjusted for repeatedly measured body weight and height and second, we tested for interaction between dietary pattern adherence and status of body weight change in

relation to BMD. The "Traditional" and the "Health Conscious" dietary pattern both showed positive associations with BMD, despite their highly different food group composition (potatoes, meat and fat versus fruits, vegetables, poultry and fish). Since the association between the "Traditional" pattern and BMD was mainly explained by differences in body weight and height, we can hypothesize that adherence to the "Traditional" dietary pattern influence BMD by increasing body weight and consequently mechanical loading. In contrast, the association between adherence to the "Health Conscious" dietary pattern and BMD was independent of body weight and height. We therefore hypothesize that adherence to this dietary patterns might have influenced BMD by influencing the bone's response to mechanical loading, rather than loading itself (in line with the Mechanostat Theory proposed by H. Frost [9]). We found no evidence that associations between adherence to any of the dietary patterns and BMD was different for people that lost or gained body weight than for those with stable body weight.

Strengths and limitations

Our study has several strengths. First, we had repeated measurements of BMD and anthropometrics, allowing longitudinal analyses on dietary patterns and BMD with precise adjustment for body weight and height. Second, we had a large sample which included both males and females. Third, to our knowledge, we are the first to investigate the relationship between dietary patterns and BMD against a background of high dairy intake (median intakes 19 servings per week). We also recognize some limitations. Dietary intake, assessed using FFQ, was self-administered and therefore susceptible to measurement error. However, the ability to properly rank subjects into categories of low to high intake was established in a validation study that compared the FFQ to a 24-h recall in a random sample of The Rotterdam Study[14]. Also, dietary intake was assessed at baseline only. Changes in dietary behaviour over time might have affected the results. However, it has been shown in a comparable cohort that ranking of individuals is fairly similar when using a single FFQ measurement than when using repeated measurements [40]. Participants with dietary intake data were slightly younger, more often non-smokers, less likely to have prevalent type 2 diabetes and more likely to use hormone replacement therapy than participants of the full cohort (n = 7983). It could therefore be stated that our study population was slightly healthier than our full cohort, and was therefore more likely to adhere to a healthy diet and to have high BMD. This does not necessarily imply that our association under study cannot be translated to the full cohort and general population due to selective participation. The latter assumption was supported by recent findings of Winding *et al.* 2014 in a Danish youth cohort [41]. Hence, we believe that our results are still valid. Despite our effort to adjust for a number of

confounders, residual confounding related to an overall healthy lifestyle might still be present. Also the single measurement of physical activity and plasma vitamin D only at the third visit may have led to residual confounding by physical activity and vitamin D levels.

The use of a PCA to determine dietary patterns has some methodological limitations. Although the 'a posteriori' nature of the patterns identified provides a realistic reflection of dietary patterns in our study population, it does not necessarily provide the most optimal dietary pattern [3] in relation to BMD and may affect the external validity of the results. In addition, several decisions such as the clustering of food items into groups and extraction of the patterns from the PCA are to some extent subjective to the investigator and may affect the final dietary patterns that are analysed. The three patterns identified in this study explain 20% of the overall variance, which is similar to some [18] but lower than other studies [33,42] investigating dietary patterns in relation to bone, This shows the complexity of efficiently using dietary intake data and may affect the external validity of our results. Lastly, data was only available on BMD of the femoral neck, and not of the spine. Some studies have shown that dietary patterns were associated with BMD of the lumbar spine, but not of the femoral neck [33,42], so we might have not been able to detect additional associations between our dietary patterns and spinal BMD.

Implications, recommendations and future perspectives

Contributing to the development of food-based dietary guidelines, a systematic review on the relationship between dietary patterns and health outcomes has been published by the United States Department of Agriculture. These food-based dietary guidelines were based mainly on studies on overweight and underweight, cardiovascular disease and type 2 diabetes. However, some studies on osteoporosis have been included [43]. With that in mind, we believe that our study could contribute to further improvement of food-based dietary guidelines in relation to bone health. Our results indicate that, against a background of high dairy intake, different dietary patterns may have an influence on BMD. Although food groups such as fruits and vegetables are included in many dietary guidelines[44], specific advice on high consumption of poultry, eggs, and limited consumption of processed meat are not always included.

In addition, it would be worthwhile to investigate further the effects of different food groups beyond calcium-rich foods, such as dairy, on bone mineralization. It would be beneficial to investigate the effects of the different food groups both at the population level and the mechanistic level. Another field of research could focus on the role of fat quality and potential differences in effects between diets rich in meat versus poultry and fish.

CONCLUSION

Against a background of high dairy intake in this population, a "Health conscious" dietary pattern, characterized by high intake of fruit, vegetables, fish and poultry may have additional benefits for BMD independent of anthropometrics. In contrast, adherence to a "Processed" dietary pattern characterized by high intake of processed meat, mixed meals and alcohol may pose a risk for low BMD.

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4.2

DEVELOPMENT OF A FOOD GROUP-BASED DIET SCORE AND ITS ASSOCIATION WITH BONE MINERAL DENSITY

Supplemental materials accompany this paper on http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4555156/

ABSTRACT

Background

No diet score exists that summarizes the features of a diet that is optimal for bone mineral density (BMD) in the elderly.

Aim

Our aims were (a) to develop a BMD-Diet Score reflecting a diet that may be beneficial for BMD based on the existing literature, and (b) to examine the association of the BMD-Diet Score and the Healthy Diet Indicator, a score based on guidelines of the World Health Organization, with BMD in Dutch elderly participating in a prospective cohort study, the Rotterdam Study (n = 5144).

Methods

Baseline dietary intake, assessed using a food frequency questionnaire, was categorized into food groups. Food groups that were consistently associated with BMD in the literature were included in the BMD-Diet Score. BMD was measured repeatedly and was assessed using dual energy X-ray absorptiometry. The BMD-Diet Score considered intake of vegetables, fruits, fish, whole grains, legumes/beans and dairy products as "high-BMD" components and meat and confectionary as "low-BMD" components.

Results

After adjustment, the BMD-Diet Score was positively associated with BMD (β (95% confidence interval) = 0.009 (0.005, 0.012) g/cm^2 per standard deviation). This effect size was approximately three times as large as has been observed for the Healthy Diet Indicator.

Conclusions

The food groups included in our BMD-Diet Score could be considered in the development of future dietary guidelines for healthy ageing.

INTRODUCTION

Osteoporosis, characterized by low bone mineral density (BMD), is a major determinant of fracture risk and can lead to a decreased quality of life and loss of independency in the elderly [1]. An important and modifiable risk factor for osteoporosis is an inadequate diet [2]. Although studies on single nutrients, such as calcium and Vitamin D, have provided important insights on the relationship between diet and bone health [3], investigating full dietary patterns has additional benefits because additive or antagonistic nutrient-interactions might occur [4]. Two main approaches of dietary pattern identification can be distinguished. The first is an *a posteriori* approach, in which statistical data reduction techniques, such as factor or cluster analysis, are used to identify dietary patterns in a specific population [4]. This approach can be particularly useful to identify the local and existing dietary patterns as they are shaped by a variety of lifestyle factors, including individual preferences and beliefs, cultural traditions, and food availability and affordability [5]. Second, an *a priori* approach can be used, in which diet scores or diet indices are developed based on current knowledge from literature and guidelines.

Examples of diet scores are the Alternate Healthy Eating Index (AHEI) and the Recommended Food Score (RFS), which reflect diet quality based on the Dietary Guidelines for Americans and the food guide pyramid developed by researchers at the US Department of Agriculture. However, these scores were recently shown not to be associated with BMD in pre-menopausal women [6]. Accordingly, it may be argued that existing dietary scores based on existing dietary recommendations may not fully capture or consider foods that influence bone health.

Adherence to the dietary guidelines of the World Health Organization (WHO) [1] has been translated into the Healthy Diet Indicator (HDI) by Jankovic *et al.*, (2014) [7]. This score reflects the overall quality of a subject's diet based on single nutrients (e.g., sodium) and some food groups (e.g., fruits and vegetables). The guidelines, and therefore the score, were developed based on existing evidence on dietary intake and chronic diseases, which included limited data from osteoporosis-related studies [1]. Moreover, as dietary guidelines are in transition to become food-group-based rather than nutrient-based, it would be valuable to develop a BMD-Diet Score based on the intake of food groups. By deriving these food groups from full dietary pattern analyses, this BMD-Diet Score might account for potential nutrient interactions. Eventually, it might serve the development of future food-group-based guidelines that sufficiently account for bone health.

In the present study, the first aim was to develop a BMD-Diet Score reflecting an overall diet that may be beneficial for BMD based on a narrative review of previously published *a priori* and *a posteriori* dietary pattern analyses on BMD. A second aim was to examine the association of the BMD-Diet Score and the Healthy Diet Indicator, a

diet score based on current dietary guidelines of the WHO, with measured BMD and to compare these associations.

Experimental Section

Study Population

This study was embedded in the Rotterdam Study I (RS-I-1), a prospective cohort study among subject from the Ommoord district in Rotterdam, the Netherlands. Participants were elderly males and females of 55 years and older at baseline (1989–1993). Details on the design and main objectives of the Rotterdam Study have been published elsewhere [8]. The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee, MEC 89.230) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports [8]. Written informed consent was obtained from all subjects.

Dietary Assessment

All participants were interviewed at baseline for food intake assessment using an extensive semi-quantitative food frequency questionnaire (FFQ) at the study center, administered by a trained dietician. The questionnaire was validated and adapted for use in the elderly [9,10]. It consists of 170 food items and questions about dietary habits. The ability of the FFQ to rank subjects adequately according to their dietary intakes was demonstrated by results from a validation study comparing the FFQ to 15-day food records collected over a year to cover all seasons. Pearson's correlation coefficients of this comparison ranged from 0.4 to 0.8 after adjustment for sex, age, total energy intake, and within-person variability in daily intakes [9]. The dietary intake of nutrients was calculated using the Dutch Food Composition Database (NEVO) from 1993 and 2006.

Development of the BMD-Diet Score

We searched PubMed for publications (through March 2015) on studies examining the relationship between dietary patterns and BMD using the following search terms: "Dietary patterns" OR "diet score" AND "bone" OR "BMD" OR "osteoporosis". Studies included dietary patterns, derived by either cluster or factor analysis, or dietary indices as exposure and bone mineral density or loss thereof, or osteoporosis as outcome in adult populations. Selected studies on single food groups, single dietary nutrients or nutrient biomarkers as exposure and outcomes other than bone mineral density or osteoporosis were excluded. Furthermore, we excluded specific diseased populations, such as celiac disease patients and studies in children (because their dietary patterns may differ of those from adults and they are still undergoing bone accrual). We only considered original research (observational and experimental) and no case reports.

We extracted food groups from dietary patterns and labelled them as "high-BMD" or "low-BMD" food groups if significant associations ($p < 0.05$) were reported with high or low BMD, respectively. Characterization as well as labelling of dietary patterns derived by principal component analysis was based on their factor loadings, which represent the correlation between the food groups and the dietary patterns. However, different studies might use different factor loading- thresholds. Because not all studies reported smaller factor loadings, we only included food groups with a factor loading of >0.3 for positively correlated food groups and <–0.3 for negatively correlated food groups. Next, we created bar charts presenting the count of dietary patterns in which any of these food groups occurred. Only those with the highest frequency of occurrence (>25th percentile of cumulative count) were included for the BMD-Diet Score. The direction of the association (favorable or unfavorable) was considered consistent when more than two thirds (67%) of the studies showed an effect in the same direction. Only food groups with consistent associations with BMD were included in the BMD-Diet Score.

For each participant, the newly developed BMD-Diet Score was calculated as follows: first, dietary intake of all relevant food groups was categorized into quartiles. Next, each subject was assigned ascending values (1,2,3,4) for food groups that are assumed to increase BMD and descending values (4,3,2,1) for those assumed to decrease BMD, based on their quartiles of intakes. Only if the distribution of intake of a food group did not allow computation of quartiles (e.g., for groups with a high number of non-consumers such as legumes and beans), values were dichotomized. Intake of alcoholic beverages was not included in the BMD-Diet Score but considered a potential confounder in our analysis, because the relationship with BMD might be non-linear [11,12].

Computation of HDI, Based on Dietary Guidelines of the WHO

The computation of the HDI for each participant was based on WHO dietary guidelines of 2003. Briefly, the HDI consists of 12 dietary components, of which 5 are recommended to be consumed in moderation: saturated fatty acids (SFA), mono-and disaccharides, cholesterol, trans fat and sodium, three components which are recommened to consume within a specific range: polyunsaturated fatty acids (PUFAs), protein, total fat, *n*-6 PUFAs and *n*-3 PUFAs, and two components for which an adequate intake is recommended: dietary fiber and fruits and vegetables. Cut-offs and more detailed information regarding the scoring system are presented in Table 1. The HDI is coded as a continuous variable, proportionally ranging from 10 to 0 between the optimal intake and the lower or upper limit respectively per component. Therefore, the theoretical range of HDI is 0 to 120.

Table 1. Cut-offs used for computation of the Healthy Diet Indicator (HDI) (Jankovic, 2014 [7], adapted).

	Lower Limit	Optimal Intake *	Upper Limit **
Components of the Healthy Diet Indicator	**0 Points**	**10 Points**	**0 Points**
Moderation (unfavorable) components			
Saturated fatty acids	N.A.	<10	>15
Monosaccharides and disaccharides	N.A.	<10	>30
Cholesterol	N.A.	<300	>400
Trans fatty acids	N.A.	<1	>1.5
Sodium (grams, not sodium chloride)	N.A.	<2	>3.0
Moderation range components			
Polyunsaturated fatty acids (PUFAs)	0	6 to 10	>10
Protein	0	10 to 15	>20
Total fat	0	15 to 30	> 43
n-6 PUFA	0	5 to 8	>8.5
n-3 PUFA	0	1 to 2	N.A. **
Adequacy (favorable) components			
Dietary fiber (g)	0	>25	N.A.
Fruits and vegetables (g)	0	>400	N.A.

*: Representing the World Health Orgnization (WHO) recommendation; ** For *n*-3 PUFA's no upper level could be calculated as the 85th percentile of intake falls within the range of optimal intake in our population; Abbreviations: N.A. = not applicable, PUFA = polyunsaturated fatty acid; The Healthy Diet Indicator (HDI) is coded as a continuous variable, proportionally ranging from 10 to 0 between the optimal intake and the lower or upper limit respectively.

Assessment of BMD

BMD of the femoral neck was measured by dual energy X-ray absorptiometry (DXA) using a Lunar DPX- densitometer (Lunar Radiation Corp., Madison, WI, USA) at baseline (1989–1993) and at 3 subsequent visits (1993–1995, 1997–1999 and 2002–2004). DXA scans were analyzed with DPX-IQ software (v.4.7d) and BMD values are expressed in g/cm^2. A flowchart showing the numbers of subjects with available BMD data for each visit is shown in Figure S1.

Assessment of Covariates

We included covariates related to body composition, lifestyle, socio-economic status (SES), prevalent metabolic diseases, use of medication and other indicators of overall health, of which the majority was assessed at baseline (1989–1993). Body height and weight were measured at the research center at baseline and three follow up visits (1993–1995, 1997–1999 and 2002–2004). Regarding lifestyle factors, smoking at baseline was calculated as "current" or "past or never". Physical activity was assessed

at the 3rd visit (1997–1999), using the Zutphen Study Physical Activity Questionnaire including questions on walking, cycling, gardening, diverse sports, hobbies, and housekeeping [13–15]. Total time spend on physical activity was calculated by the sum of minutes per week for each type of activity. Dietary intake of alcoholic beverages and calcium were derived from the FFQ. Baseline use of any dietary supplement was assessed during the home interview, without specific questions on dose or duration and coded as "never" or "ever". Highest education and net household income were used as proxy for SES. Education was coded as "low" (primary education, primary + higher not completed, lower vocational and lower secondary education) or "high" (intermediate vocational, general secondary, higher vocational education and university). Household income was coded "above" or "below" the average of 2400 net Dutch Guilders (≈1600 Euro) per month. Regarding prevalent diseases at baseline, type 2 diabetes mellitus was determined as baseline serum glucose concentrations >11 mmol/L or use of glucose lowering drugs and cardiovascular disease included prevalent coronary heart disease, heart failure, stroke and arterial fibrillation at baseline. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study have been described in detail elsewhere [16]. Regarding medication, the use of serum lipid reducing agents, antihypertensive drugs, or drugs taken for calcium homeostasis and disorders of the musculoskeletal system was registered during the home interview by trained research assistants [17]. Use of hormone replacement therapy (HRT) in females was coded as "never" or "ever". Lower limb disability and Vitamin D status were included as remaining measures of overall health. Lower limb disability index, a combined index reflecting a subject's ability to stand up, walk, climb and bend [18] was based on the Stanford Health Assessment Questionnaire. Serum 25-hydroxyvitamin D (25(OH)D) was measured in a subgroup of participants (n = 3171) at the 3rd visit of the cohort to the visiting center using radioimmunoassays (IDS Ltd, Boldon, UK). The sensitivity of the test was 3 nmol/L which ranged from 4 to 400 nmol/L. Intra-assay accuracy was <8% and the inter-assay accuracy was <12%.

Statistical Analysis

Characteristics of the study population were provided for subjects with a BMD Diet-Score below or above the median separately. Median values (+ interquartile ranges) for continuous variables and percentages of the total population for categorical variables were provided. The association between the BMD-Diet Score and HDI with BMD was studied using linear mixed modelling with the diet scores, expressed in standard deviations (SDs) or in quartiles, as exposure and longitudinal measurements of BMD (expressed in g/cm^2 and sex-specific z-scores) as the outcome. Analyses in quartiles, using the lowest quartile as the reference category, were performed to explore potential non- linear relationships. We coded the time- variable in the mixed model 0, 2, 6.5 and 11, to correct for differences in the length of time-

intervals between subjects. Basic models (model 1) were adjusted for age and sex only. Potential confounding was tested by adding covariates to the models separately. Only covariates that changed the effect estimates by >10% were kept in the final adjusted models [19]. Based on this criterion, analyses were adjusted for age, sex and total kilocalorie intake, plus body weight and height (model 2), education, household income, current smoking behavior, physical activity, prevalent type 2 diabetes at baseline, and use of lipid lowering drugs, alcohol consumption and dietary supplement use (model 3). To assess whether BMD Diet-score had additional value upon the HDI, we further adjusted the final model for the HDI diet score (model 4). The aim of this paper is to study associations between diet scores reflecting full dietary patterns, not single nutrients, in relation to BMD. However, as the nutrient calcium is one of the most important constituents of the bone, we investigated the effects of additional adjustment for calcium intake in a separate model (model 5). To be able to study whether the trajectories of BMD were different in subjects with low or high diet scores, we tested for interaction with time by adding the product term of BMD-Diet Score or HDI with time to model 3.

We used a multiple imputation procedure to estimate missing values for covariates (details in Table S1 and S2). To facilitate proper comparison of the effect estimates of associations between the BMD-Diet Score (ranging from 0 to 30) with BMD with that of the HDI (ranging from 0 to 120) with BMD, the regression coefficients were shown per SD increase for both diet scores.

As the majority of studies that served as a basis for our BMD-Diet Score were performed in women only, we tested for interaction with sex, by adding the product term of the our main exposures (the two diet scores) and sex to our basic models. Additionally, we performed a sensitivity analysis excluding participants with type 2 diabetes at baseline. All analyses were performed using SPSS 22 (IBM, Chicago, IL, USA) and R 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria) statistical software.

RESULTS

Food Groups Included in Our BMD-Diet Score

In summary, we identified 15 papers to be used for the development of our BMD-Diet Score. The majority of these studies investigated *a posteriori* defined dietary patterns using principal component analysis [20–31] or cluster analysis [32]. Details on these studies regarding their design, sample size and food group extracted are shown in Table S3 and S4. Studies on *a priori* defined diet scores and BMD showed positive effects for the Mediterranean Diet Score [33], the Dietary Diversity Score [23,34] and the Diet and Lifestyle Score, based on guidelines of the American Heart Association [35] (Table S5).

After careful evaluation of the available evidence, eight food groups were included in the BMD Diet-score: vegetables, fruits, dairy products, whole grain products, fish and legumes & beans as "High-BMD" components and meat (including red, processed and organ meat) and confectionary (including candies, cakes and cookies) as "Low-BMD" components (Figure 1). An overview of food items included in each food group is shown in Table S6.

Characteristic of the Study Population

Characteristics of the study population are shown in Table 2. Subjects with a BMD-Diet Score above the median were more likely to be female (62% *vs*. 56%) and to have a higher income (54% *vs*. 49%) than those with a BMD-Diet Score below the median. Furthermore, they were less likely to be smokers (27% *vs*. 19%) and had higher calcium intakes (median of 1248 mg/day *vs*. 960 mg/day).

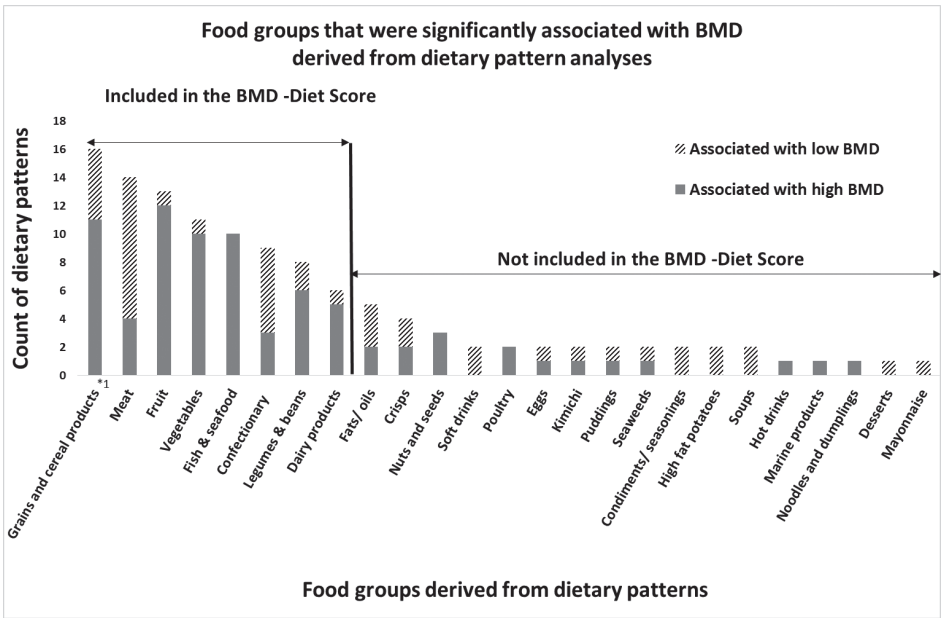

Figure 1. Results of the narrative review: Food groups that were associated with high or low bone mineral density (BMD) in dietary pattern analyses; The X-axis displays the food groups, derived from dietary patterns that were significantly associated with high or low BMD in the reviewed literature. The Y-axis displays the number of dietary patterns in which corresponding food group occurred (count of dietary patterns). As some studies report more than one dietary pattern to be associated with BMD, the number of patterns that was counted is slightly different from the number of studies that was counted. *1: Although not all studies distinguished between refined and whole grains, those that did found particularly beneficial associations with bone for whole grains only.

Table 2. Characteristics of the study population in participants with a BMD-Diet Score below or above the median.

	BMD-Diet Score below or Equal to the Median [2]	BMD-Diet Score above the Median	Total
n	**2903**	**2241**	**5144**
Age (year) [1]	68 (61, 73)	65 (60, 71)	67 (61, 73)
Total energy intake (kcal/day) [1]	1926 (1613, 2265)	1921 (1617, 2254)	1923 (1615, 2261)
Dietary calcium intake (mg/day) [1]	960 (769, 1170)	1248 (1032, 1497)	1079 (863, 1324)
Physical activity (h/day)	5.6 (4.0, 7.5)	6.0 (4.4, 8.0)	5.8 (4.2, 7.7)
Of which vigorous (h/day) [1]	0.4 (0.1, 0.9)	0.6 (0.2, 1.1)	0.5 (0.2, 1.0)
Height (cm) [1]	167 (161, 174)	167 (160, 174)	162(157,166)
Weight (kg) [1]	73 (65, 80)	74 (66, 81)	73 (66, 81)
Healthy Diet Indicator [1]	74 (66, 82)	79 (70, 86)	76 (68, 84)
Plasma Vitamin D (nmol/L) [1,3]	44 (29, 64)	45 (31, 65)	45 (30, 64)
Sex (% females)	56	62	57
Prevalent osteoporosis (%)	12	10	11
Prevalent type 2 diabetes (%)	9	10	10
Prevalent cardiovascular disease (%)	13	12	13
High education (%)	35	39	37
Monthly income > 1600 Euro (%)	49	54	51
Current smokers (%)	27	19	23
Current or past HRT use (%) [4]	8	11	9
Lipid lowering drug use (%)	2	3	3
Antihypertensive drug use (%)	13	13	13
Lower limb disabled (%)	19	16	17

[1]: median (interquartile range), [2] the median of the BMD-Diet Score in our population is 19; [3]: assessed at the 3rd visit; [4]: expressed as percentages of the female population; Abbreviations: BMD = Bone mineral density; HRT = hormone replacement therapy.

The BMD-Diet Score and the HDI were weakly but significantly correlated (Pearson's ρ = 0.18). The HDI, but not the BMD-Diet Score, was significantly correlated with lower total energy intake (Pearson's ρ = −0.23). Median intake of each food group included in the BMD-Diet Score is shown in Table S7.

Longitudinal Associations between BMD-Diet Score, HDI and BMD

Associations between the BMD-Diet Score or the HDI and BMD, are shown in Table 3. Adjusted for age, sex and total energy intake (model 1), a high BMD-Diet Score was significantly associated with higher BMD (β (95% confidence interval (CI)) = 0.012 (0.008, 0.015) g/cm^2 per SD increase in the diet score). This association was slightly attenuated (β (95% CI) = 0.010 (0.007, 0.013) after adjustment for body height and weight (model 2) and after including additional confounders (β (95% CI) = 0.009 (0.005, 0.012), model 3). Additional adjustment for adherence to the HDI did not change the results (model 4). After further adjustment for dietary calcium intake effect sizes were diluted, but remained significant (β (95% CI) = 0.004 (0.001, 0.009) g/cm^2 per SD increase in the diet score). No significant interaction with time was observed (*p* for interaction = 0.25), indicating that the trajectories of BMD were comparable between subjects with high or low BMD-Diet Scores. The HDI was significantly associated with higher BMD in the basic model. However, after adjustment for age, sex, height and weight (model 2) the standardized effect size decreased (β (95% CI) = 0.005 (0.002, 0.008) and was of a lesser magnitude than that of the BMD-Diet Score. After adjustment for confounders the association was diluted and became non-significant (β (95% CI) = 0.003 (−0.000, 0.007) in model 3). Further adjustment for adherence to the BMD-Diet Score did not change this effect (model 4), while a positive association was observed after additional adjustment for calcium intake (model 5). No significant interaction with time was observed (*p* for interaction = 0.18). Categorical analyses, using the lowest quartile as the reference group, did not indicate the presence of a non- linear relationship between the BMD- Diet Score or the HDI with BMD (Table 3).

Additional Analysis

No interaction between the BMD-Diet Score or the HDI with sex was observed in relation to BMD (*p* all interactions > 0.12). Additionally, stratification by gender did not show different associations for males and females. Additional analyses with BMD in sex- specific *z*-scores as the outcome did not change the results. In addition, sensitivity analyses in which participants were excluded if they had type 2 diabetes at baseline did not change the results.

Table 3. Associations between the BMD-Diet Score or Healthy Diet Indicator and femoral neck BMD, using linear mixed modelling.

		Model 1		Model 2	
		Basic		*Model 1+*	
				Height and Weight	
		β [1]	95% CI	β	95% CI
Food group-based BMD-Diet Score	**Per SD**	**0.012**	**(0.008, 0.015)**	**0.010**	**(0.007, 0.013)**
	Q2 *vs.* Q1	0.007	(–0.004, 0.018)	0.007	(0.003,0.016)
	Q3 *vs.* Q1	**0.024**	**(0.014, 0.034)**	**0.020**	**(0.011, 0.030)**
	Q4 *vs.* Q1	**0.029**	**(0.020, 0.040)**	**0.024**	**(0.016, 0.033)**
	***P* for trend**	**<0.001**		**<0.001**	
WHO guidelines-based HDI	**Per SD**	**0.004**	**(0.001, 0.008)**	**0.005**	**(0.002, 0.009)**
	Q2 *vs.* Q1	–0.006	(–0.017, 0.005)	–0.006	(0.016,0.005)
	Q3 *vs.* Q1	**0.006**	(–0.026, 0.016)	**0.007**	(0.012,0.016)
	Q4 *vs.* Q1	**0.011**	(–0.000, 0.021)	**0.012**	**(0.002, 0.022)**
	***P* for trend**	**0.014**		**0.003**	

[1] Regression coefficients (+95% confidence intervals) are shown for the fixed effects of the linear mixed model per SD increase or per quartile, using the first quartile as the reference, in the corresponding diet score. As the median BMD in this population is 0.86 g/cm^2, a regression coefficient of 0.012 g/cm^2 approximates a 1.4% higher BMD; Model 1: Adjusted for age, sex and total energy intake; Model 2: Model 1, additionally adjusted for body weight and height; Model 3: Model 2, additionally adjusted for education, household income, smoking behavior, physical activity, use of lipid lowering drugs + use of any dietary supplement + alcohol intake.

DISCUSSION

Summary of Main Findings

This is the first study in which a food group-based BMD-Diet Score based on existing evidence from previous studies on full dietary patterns and BMD in several populations has been developed. We found that this newly developed BMD-Diet Score was significantly associated with high BMD, independent of adherence to the dietary recommendations of the WHO as assessed by the HDI. Our findings suggest that there is room for improvement of current dietary guidelines seeking optimal bone health.

Comparison to Existing Scores That were Shown to Favorably Affect Markers of Bone Turnover

Our BMD-Diet Score was developed based on studies investigating the effects of dietary patterns on BMD. However, the associations between existing diet scores have also been studied in relation to other bone- related outcomes, such as markers of bone turnover. For example, the "Dietary Approaches to Stop Hypertension" (DASH)-Diet score was shown to favorably affect osteocalcin, a serum marker of bone formation, which, if sustained, may improve bone mineral status [36] and reduce bone loss.

Model 3		Model 4		Model 5	
Model 2+		*Model 3+*		*Model 3+*	
Confounders		*Other Score*		*Calcium Intake*	
β	95% CI	β	95% CI	β	95% CI
0.009	**(0.005, 0.012)**	**0.008**	**(0.004, 0.011)**	**0.004**	**(0.001, 0.008)**
0.005	(–0.004, 0.015)	0.005	(–0.004, 0.015)	0.001	(–0.009,0.012)
0.019	**(0.009, 0.028)**	**0.018**	**(0.008, 0.028)**	**0.019**	**(0.002, 0.022)**
0.022	**(0.013, 0.031)**	**0.021**	**(0.012, 0.030)**	**0.010**	**(0.000, 0.020)**
	<0.001		**<0.001**		**0.016**
0.003	(–0.000, 0.007)	0.003	(–0.000, 0.007)	**0.005**	**(0.003, 0.010)**
–0.008	(–0.018, 0.003)	–0.009	(–0.020, 0.002)	–0.007	(–0.018, 0.003)
0.004	(–0.004, 0.013)	0.002	(–0.007, 0.010)	0.005	(–0.004, 0.013)
0.007	(–0.004, 0.017)	0.002	(–0.009, 0.012)	0.008	(–0.002, 0.018)
	0.067		0.377		**0.038**

Additional adjustment for plasma vitamin D, *use of antihypertensive drugs, drugs for calcium homeostasis or for disorders of the musculo-skeletal system*, *HRT*, *lower limb disability or CVD prevalence did not change these results*; Model 4: Model 3, additionally adjusted for the other diet Score (HDI for the BMD-Diet Score analysis and vice versa); Model 5: Model 3, additionally adjusted for calcium intake; Significant findings ($p < 0.05$) in **bold**, Abbreviations: BMD = bone mineral density, HDI = Healthy Diet Indicator, HRT = hormone replacement therapy, CVD = cardiovascular disease, SD = standard deviation; CI = Confidence interval; Q = quartile.

The DASH-Diet score and our BMD-Diet Score share common components, namely fruits, vegetables, fish, and whole grains as favorable (high-BMD) food groups and (red) meat as unfavorable (low-BMD) food groups. However, the DASH-Diet score does include dairy products as favorable components, similar to our BMD-Diet Score, but uses a more specific definition by including only low fat dairy products [36]. Additionally, the study by Karamati *et al.*, (2012) [28] showed a dietary pattern including low fat dairy to be associated with high BMD, and a pattern including high fat dairy to be associated with low BMD. Based on these findings, it could be argued that the BMD-Diet Score might be refined further by using low fat instead of all dairy products as a favorable component. The DASH-Diet score includes total fat as unfavorable nutrient-component (Table S8). Our BMD-Diet Score was based solely on food groups and therefore has no specific fatty acid-component. However, it includes foods as pork, cake, and chocolate bars, products high in saturated fatty acids, in the unfavorable "low BMD" components (Table S6), and fish, rich in polyunsaturated fatty acids, as favorable component. Therefore, our BMD-Diet Score could be considered a score in which the existing DASH-diet score was covered, but was fully translated into food groups.

Potential Nutrients Involved

The aim of this paper is to study the associations between complete dietary patterns, reflected by different diet scores in relation to BMD. However, calcium is a vital element of bone and a well-established dietary factor that influences BMD [3,37,38]. Our analysis showed the associations between the BMD-Diet Score and BMD were diluted, but remained significant after additional adjustment for dietary calcium. This indicates that calcium intake is important, but does not fully explain the favorable association between the BMD-Diet Score and BMD. This finding is in line with an earlier review by Ahmadieh *et al.*, (2001) who highlighted the positive contributions of a variety of nutrients to BMD, such as Vitamin B2, B6, Vitamin C and Vitamin K, in addition to calcium [2]. These nutrients can underlie our associations, since vitamin B2 and B6 might be reflected by the whole grain component of our BMD-diet Score and Vitamin C and K1 by the fruits and vegetable components.

Strengths and Limitations

Our study has several strengths. Firstly, the development of our BMD-Diet Score was based on a variety of study populations, including both Caucasian and Asian subjects. Despite the differences in dietary habits between these populations, we were able to identify common food groups that were consumed and were shown to be associated with BMD across populations. Secondly, by using full dietary pattern analyses as a basis for the BMD-Diet Score, we were able to take into account strong correlations and potential interactions between foods and nutrients. Thirdly, we had the opportunity to include repeated measurements of BMD, body weight, and height. Repeated BMD measurements provided more insights into long-term associations between dietary intake and BMD and the opportunity to study associations with BMD trajectories. Repeated measurements of body weight and height enabled a precise adjustment for changes in anthropometric measures, which are known to be important determinants of BMD. Lastly, our sample included both males and females, increasing the external validity of our results since most studies on dietary patterns and BMD focused on women only. We do, however, also recognize some limitations. Our study population consisted of Dutch participants from one specific neighborhood, in which the vast majority of inhabitants were of Caucasian background, an aspect that is important to consider when extrapolating our findings to other populations. The absolute intakes of some components of the BMD-Diet Score (such as fish and legumes) were very low in our population, which might have affected the strength of our associations. However, for the main food groups, including fruits, vegetables, fish and whole grain products, we believe this concern is limited since items in these food groups are widely consumed in our population. It could be argued that using results from Rotterdam Study for the development of the BMD-Diet Score while

subsequently testing the association between this score with BMD in the same cohort might have led to bias. However, the composition of the BMD-Diet Score would be similar with or without inclusion of our own previous results [24] (Table S3 and S4) in its development. Therefore, we believe that inclusion of our previous results did not lead to bias in this study.

Future Steps and Implications

This is the first study that developed a BMD Diet Score that has been associated with BMD in a Dutch population of elderly subjects. Although the score is based on data from different populations, it is essential to study its performance in other populations, including Asian and other non-Caucasian populations. For example populations with (a) low dairy intake or (b) higher levels of Vitamin D or (c) high intake of foods that were hardly consumed in our population such as fish or legumes would be particularly interesting for replication. If future studies replicate positive associations with BMD, this BMD-Diet Score could help to shape food group-based dietary guidelines aiming to contribute to healthy ageing while considering a healthy BMD as important aspect of ageing. However, since dietary guidelines aim to promote overall healthy ageing by preventing all chronic diseases such as cardiometabolic diseases and cancer, our BMD-Diet Score should be studied in relation to these health outcomes as well. Calcium might favor BMD while adversely affecting cardiovascular disease risk [39], whereas an approach which evaluates the full diet, such as the BMD-Diet Score, might indicate benefit for various aspects of healthy ageing simultaneously. For the development of our BMD-Diet Score we only used studies with BMD, and not fracture risk, as the primary outcome. However, as adherence to the Mediterranean Diet Score, for example, has been shown to be favorably associated with fracture risk in a cohort of adults from eight European countries [40], consumption of the food groups in our proposed BMD-Diet Score might favorably affect fracture risk as well.

CONCLUSIONS

We developed a new BMD-Diet Score composed of components representing high intake of vegetables, fruits, fish, whole grains, legumes and beans, and dairy products, and low intake of and meat and confectionary. This BMD-Diet Score is positively associated with BMD in our cohort of middle-aged and elderly subjects independent of adherence to the HDI based on dietary guidelines from the WHO. The food groups included in our BMD-Diet Score could be considered in the development of future dietary guidelines for healthy ageing.

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4.3

DIETARY PATTERNS EXPLAINING DIFFERENCES IN BONE MINERAL DENSITY AND HIP STRUCTURE

Supplemental materials accompany this paper on http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4555156/

ABSTRACT

Background

Evidence on the association between dietary patterns, measures of hipbone geometry, and subsequent fracture risk are scarce.

Objective

To evaluate whether dietary patterns that explain most variation in bone mineral density (BMD) and hipbone geometry are associated with fracture risk.

Design

Participants were 4028 subjects aged ≥ 55 years included in the Rotterdam study. Intake of 28 food groups was assessed using food frequency questionnaires. BMD, bone width, section modulus (SM, reflecting bending strength) and cortical Buckling Ratio (BR, reflecting bone instability) were measured using dual energy x-ray absorptiometry. BMD and geometry-specific dietary patterns were identified using reduced rank regression. Fracture data were reported by general practitioners (median follow-up 14.8 years).

Results

We identified four dietary patterns. Of the four, we named two patterns "Fruit, Vegetables and Dairy" and "Sweets, animal fat and low meat," respectively. These two patterns were used for further analysis. Independent of confounders, adherence to the "Fruit, Vegetables and Dairy" pattern was associated with high BMD, high SM, low BR and lower risk of fractures (HR (95% CI) for osteoporotic fractures = 0.90 (0.83, 0.96) and for hip fractures= 0.85 (0.81, 0.89) per Z-score of dietary pattern adherence). Adherence to the "Sweets, animal fat and low meat" pattern was associated with high Bone Width, high SM, high BR, and higher risk of fractures (HR (95% CI) for osteoporotic fractures = 1.08 (1.00, 1.06) and for hip fractures =1.06 (1.02, 1.12) per Z-score).

Conclusion

A "Fruit, Vegetables and Dairy" pattern might be associated with low fracture risk due to high BMD, high bending strength and more stable bones. A "Sweets, animal fat and low meat" pattern might be associated with high fracture risk due to widened, unstable bones, independent of BMD. Dietary recommendations associated with optimal bone geometry in addition to BMD might influence long term risk of fractures.

INTRODUCTION

Compromised bone health might lead to disability and reduced quality of life in the ageing population(1). Whereas bone mineral density (BMD) is the most commonly studied determinant of bone health, other parameters including bone micro damage, mineralization, bone turnover, microarchitecture of the trabecular bone, and macro geometry of the cortical bone are important determinants as well (2). In a previous study, Rotterdam Study participants who developed hip fractures during follow-up had lower BMD, thinner cortices, greater bone width, lower strength, and higher instability at baseline than those who did not. This study highlighted that the buckling ratio portrays the critical balance between cortical thickness and bone width. It suggests that extreme thinning of the cortices in expanded bone might play a key role in susceptibility to fractures (3). Among several modifiable determinants of BMD bone geometry in the ageing population, diet plays a key role (4).

Different approaches are available to identify dietary factors relevant to bone health. Besides evaluating the role of single nutrients (e.g. calcium and vitamin D), the number of studies investigating the associations between adherence to overall dietary patterns in relation to bone outcomes such as BMD and fracture risk is growing (5-16). An advantage of studying dietary patterns is that it accounts for potential nutrient interactions within the dietary pattern. Thereby the effect of dietary patterns might be larger than the summed effects exerted by individual nutrients(17). Also, results of food group-based pattern analyses can be more directly translated into dietary guidelines for the general public (18). Dietary patterns affecting bone health have been explored mainly using principal component analyses (PCA).

The PCA method thereby aims to identify dietary patterns that specifically affect parameters of bone health. Although several previous studies examined PCA-derived dietary patterns in relation to bone health, not many studies have attempted to identify dietary patterns specifically affecting bone outcomes. Furthermore, several studies have examined adherence to dietary patterns in relation to BMD (5-15) and fracture risk(13, 19, 20). Summarized, these studies showed that dietary patterns high in fruits, vegetables, fish, whole grains, legumes, and dairy products, and low in meat and confectionary might favour BMD(21). However, less evidence is available regarding their associations with macro geometry of the bone. Identifying associations between dietary patterns and the macro geometry of the bone might result in definitions of new dietary patterns that specifically affect parameters of bone health and which cannot be explained by BMD alone. To the best of our knowledge, dietary patterns have not been investigated in relation to measures of bone geometry in population-based studies yet.

Therefore, the aim of our study was to identify dietary patterns that explain maximal variance in BMD and maximal variance in geometry measures of the hip, using

Reduced Rank Regression. We studied associations of adherence to these patterns with BMD and bone geometry independent of confounders and with subsequent risk of osteoporotic fractures.

Subjects

Participants were men and women from the first cohort of the Rotterdam Study. Details regarding the design of the Rotterdam Study are described elsewhere(22). In brief, 4028 **(Supplemental Figure 1**) subjects who were 55 years and older at the onset of the study (1989- 1993) and who were living in the Ommoord district of Rotterdam, The Netherlands, were included in this prospective population based study. They were examined during follow-up visits every 3 to 4 years. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All participants provided written informed consent to participate in the study and to obtain information from their treating physicians(22).

METHODS

Assessment of dietary intake

A trained dietitian assessed baseline dietary intake using a validated, semi- quantitative food frequency questionnaire (FFQ) composed of 170 food items. The questionnaire was validated and adapted for use in the elderly(23). Nutrient intakes estimated with the FFQ were compared to nutrient intakes obtained with 15 day-food records collected over a year to cover all seasons in a validation study (n=80)(23). Pearson's correlation coefficients of this comparison ranged from 0.4 to 0.8 for macronutrients and micronutrients after adjustment for sex, age, total energy intake, and within-person variability in daily intakes. The 170 food items were categorized into 28 pre-defined food groups, based on similarities in nutrient composition (e.g. apples and pears) or culinary use (e.g. mixed meals). An overview of these pre-defined food groups and the corresponding food items derived from our FFQ are shown in Supplemental Table 1.

Baseline measurements of BMD and bone geometry

Baseline BMD measurements of the right proximal femur were performed using Dual-Energy X-ray Absorptiometry (DXA) using a Lunar DPX-L densitometer (Lunar Radiation Corp., Madison, WI, USA) and analyzed with DPX-IQ v.4.7d software. We used the Hip Structural Analysis (HAS) software developed by Beck *et al*.(24) to measure hip bone geometry from the DXA scans(3). BMD and Bone Width were measured directly from mineral mass distributions, using algorithms and precision properties

described previously(25, 26). Mean cortical thickness was calculated as the between subperiosteal and endocortical radii, which were obtained by modeling the narrow neck region as a circular annulus, under the assumption that the proportion of cortical versus trabecular bone was 60:40. Section Modulus (SM) was calculated as the ratio of the cross sectional moment of inertia and the maximum distance from the center of mass to the medial or lateral surface (dmax) and is a reflection of bending strength (24) standardized to size. Buckling Ratio (BR) was estimated as dmax divided by the mean cortical thickness estimate. A high BR indicates cortical bone instability. Details on the precision of different hip structural measures compared to the traditionally applied BMD measurement are described elsewhere(25).

Assessment of fractures

General practitioners reported fractures with a computerized system, covering 80% of the cohort. Two trained research physicians verified all reported events and independently reviewed and coded the information according to the International Classification of Primary Care (ICPC) and International Classification of Diseases, 10th edition (ICD-10). Osteoporotic fractures were defined as those fractures which were unlikely to be caused by trauma and included the following ICD-10 codes: S22.1 to S22.4 (rib fractures), S32.0 to S32.5 and S32.8 (single fractures of the lumbar spine and pelvis) S42.0 to S42.4 and S42.7 to S42.9 (upper arm fractures), S52.0 to S52.9 (fractures of the forearm), S72.0 to S72.4 and S72.7 to S72.9 (fractures of the femur) S82.0 to S82.9 (fractures of the lower leg) and S92.3 (fractures of metatarsal bone). Hip fractures included ICD10 codes S72.0, S72.1 and S72.2. A medical expert reviewed events that were inconsistently coded for final classification. Subjects were followed from their baseline visit until December 2012, until they were lost to follow-up, or until a first fracture occurred.

Covariate assessment

Covariates assessed at baseline

Basic information was assessed at baseline, including sex and age at study entry. Body weight (kg) and height (cm) were assessed at the research centre. Body weight was measured using a digital scale and body height was measured using a stadiometer, while subjects wore light clothing and no shoes.

Self-reported smoking status was assessed during the home interview and categorized as “current” or “past” or “never”. Highest education and net household income were used as proxy for socio-economic status (SES). Education was coded as “low” (primary education, primary + higher not completed, lower vocational and lower secondary education) or “high” (intermediate vocational, general secondary, higher vocational education or university). Household income was coded “above”

or "below" the average at that time of 2400 net Dutch Guilders (approximately 1600 Euros) per month. Lower limb disability index, a combined index reflecting a person's ability to stand up, walk, climb and bend, was based on the Stanford Health Assessment Questionnaire (11). Prevalent Type 2 Diabetes Mellitus was determined as non-fasting serum glucose concentrations >11 mmol / l or use of glucose lowering drugs. Information on prevalent CVD, including prevalent coronary heart disease, heart failure, stroke or atrial fibrillation was collected as described in detail elsewhere (27). The use of serum lipid reducing agents and antihypertensive drugs was registered during the home interview by trained research assistants (28).

Covariates assessed at other visits

Use of hormone replacement therapy (HRT) in women was assessed at the 2nd visit and coded as "never" or "ever." Physical activity was assessed on the 3rd visit, using the Zutphen Study Physical Activity Questionnaire including questions on walking, cycling, gardening, diverse sports, hobbies, and housekeeping. Total time spent on physical activity was calculated by summing of minutes per week for each type of activity (29-31). Serum 25- hydroxyvitamin D (25(OH)D) was measured in a subgroup of participants (n= 3171) during the cohort's 3rd visit to the visiting centre using radioimmunoassay's (IDS Ltd, Boldon, UK, available at www.idsltd.com). The sensitivity of the test was 3nmol/L which ranged from 4 to 400nmol/L. Intra-assay accuracy was <8% and the inter-assay accuracy was <12%. Missing values of covariates were predicted using the multiple imputation (MI) procedure. We used the Markov Chain Monte Carlo method. Non-normally distributed continuous variables were predicted using predictive mean matching and categorical variables using logistic regression.

Statistical analyses

Identification of dietary patterns

Dietary patterns were identified using Reduced Rank Regression (RRR). The use of this data reduction technique for identification of dietary patterns has been described elsewhere (32). In brief, the RRR identifies linear functions of predictors explaining most of the variation in the response variable. The response variables are selected by the researcher and typically include nutrients, biomarkers of dietary intake or determinants of a particular health outcome. Energy adjusted intakes of 28 pre-defined food groups (33) were computed as the unstandardized residuals of linear regression models with total energy intake (in kcal/day) as the exposure and intake of the food group (in grams per day) as the outcome (residual method(34)). Next, we used these energy adjusted food group intakes as predictors and the following variables as response variables for our RRR analysis: femoral neck BMD, Bone Width, SM and cortical BR. We performed two separate analyses: one using femoral neck BMD only and one using femoral neck BMD plus all measures of bone geometry described above

as response variables. This approach was chosen to determine if the use of bone geometry variables would reveal different dietary patterns than the use of BMD only. We aimed to identify patterns that explain variation in bone measures independent of their most important determinants, so we standardized all our response variables for age, sex, body weight and height. The output variables of the RRR procedure (e.g. the factors) will be further referred to as "dietary patterns". For each participant and for each dietary pattern, a pattern adherence score was calculated by summing up their standardized intake per food group multiplied by the factor loading of the corresponding food group. Factor loadings represent the standardized correlations between the food groups and the dietary patterns. The number of dietary patterns derived from RRR is equal to the number of response variables, thus our two RRR analyses resulted in one dietary pattern predicting BMD and in four dietary patterns predicting BMD and the four geometry outcomes. Therefore, all participants received four different pattern adherence scores (Z-scores). The number of patterns that was used for further analyses was based on their explained variance of total dietary intake and of BMD and bone geometry.

Associations between dietary pattern adherence and bone outcomes

Cross-sectional associations of dietary pattern adherence with BMD and bone geometry were assessed using linear regression modelling. We used Z-scores of pattern adherence as the exposure and Z-scores of BMD, Bone Width, SM and BR standardized for age, sex, body weight and height (the response variables of our RRR analyses) as the outcome.

We studied potential confounding by step-wisely adding covariates to our basic model (model 1), which was adjusted for age, sex, body weight and height. Only covariates that changed the regression coefficient of the main effect (i.e. the association between dietary pattern adherence and BMD or bone geometry outcomes) by >10% were kept in the final model (35). Following this approach, model 2 was adjusted for vitamin D plasma concentrations, the month of its measurement, and for the use of lipid lowering drugs. Lastly, since calcium in the main constituent of bone, we further adjusted for dietary calcium intake to study whether observed associations between dietary patterns, BMD, and bone geometry were independent of calcium intake (model 2a).

Associations between dietary pattern adherences and hip fracture risk were studied using Cox Proportional Hazard Regression modeling. The proportional hazard assumption of the Cox model was checked by performing a test for heterogeneity of the exposure over time. Similar to our linear regression models, Cox models were adjusted for age, sex, body weight and height (model 1) plus vitamin D plasma concentrations, the month of its measurement, and for the use of lipid lowering drugs (model 2).We built an additional model that was further adjusted for baseline

Bone Width, SM, and cortical BR to explore whether dietary pattern adherence and fracture risk were explained by differences in bone geometry (model 3).

We performed several additional analyses. First, we tested the interaction between dietary pattern adherence and sex in our basic models to assess whether the associations were different by sex. We also tested for interaction between dietary pattern adherence and prevalent type 2 diabetes since specific skeletal characteristics, such as bending strength, may be more important predictors of 12 to 14 year-fracture risk in subjects with diabetes than in healthy subjects (36, 37). Lastly, we excluded participants who had used any kind of dietary supplements to (n=1436 (36%)), because the effect of dietary patterns may be influenced by supplement use. Analyses were performed using SPSS (version 21, IBM Corp, New York, United States of America), SAS (version 9.3, Huizen, The Netherlands) and R (Version 0.99.484 – 2009-2015 RStudio, Inc, Vienna, Austria) statistical software. A P-value of less than 0.05 was considered to be statistically significant.

RESULTS

Study population

Characteristics of men and women participating in this study are shown in **table 1**. Median age was 66 years. During follow-up, 1155 (29%) participants experienced an osteoporotic fracture and 317 (8%) a hip fracture. The mean (± SD) follow-up time was 13.3 (± 6.5) years for osteoporotic and 15.1 (± 6.2) years for hip fractures. Participants with incident fracture were less frequently men than women (26% versus 50%, P<0.001). Baseline BMD was significantly and positively correlated with SM (Pearson's r= 0.76) and negatively correlated with BR (Pearson's r= -0.89) and Width (Pearson's r= -0.15).

Dietary patterns identified

Table 2 displays three of the dietary patterns that were identified. One pattern was identified using only BMD as response variable and two patterns were identified using BMD and parameters of macro geometry (width, SM and BR) as the response variables. All response variables were standardized for age, sex, body weight and height. The pattern explaining most variance in BMD alone was almost identical to the first pattern explaining the most variance in BMD plus geometry (table 2). Of the four patterns explaining most of the variance in BMD and geometry, the first pattern explained 1.5%, the second an additional 0.4%, and the remaining three patterns explained less than 0.02% (data not shown).

Therefore, only the first two patterns explaining variance in both BMD and geometry were used for further analyses. The first pattern was referred to as a "Fruit, Vegetables and Dairy" pattern because it had high factor loadings for intake of fruits,

vegetables, milk and yoghurt, and low factor loadings for sweets and animal fats. The other pattern was named a "Sweets, animal fat and low meat" pattern and had high factor loadings for refined grains, sweets, animal fats and porridge, and low factor loadings for soy, meat and poultry intake. These two patterns explained 7.7% and 9.6% of the total variance in BMD or any of the geometry parameters (table 2).

Table 1. Characteristics of the study population (n 4028)

Characteristic	Men N=1705	Women N=2323
Age (y)[1]	66 (61-72)	66 (61-73)
Total energy intake (kcal/d)[1]	2202 (1916-2526)	1745 (1516-2030)
Adherence to the "Fruit, vegetable and dairy" pattern (Z-score)	0.15 (-0.75-0.55)	-0.02 (-0.59-0.60)
Adherence to the "Sweets, animal fat and low meat" pattern (Z-score)	0.08 (-0.69-0.50)	0.16 (-0.36-0.64)
Physical activity (h/day) [1]	5.7 (4.1-7.6)	6.2 (4.6-8.0)
Of which vigorous (h/week)[1]	4.0 (1.0-8.0)	3.3 (1.5- 6.3)
25(OH)D3 (nmol/l) [1,2]	54 (37, 73)	40 (26-58)
Calcium intake (mg/day)	1100 (871-1364)	1075 (868-1308)
Baseline body weight (kg)	79 (63-86)	71 (73- 79)
Baseline body height (cm)	175 (171-180)	165 (160-172)
Baseline BMD (g/cm^2)	0.92 (0.84-1.01)	0.83 (0.75-0.92)
Baseline Bone Width	3.45 (3.28-3.63)	3.01 (2.87-3.15)
Baseline Section Modulus	1.37 (1.17-1.58)	0.93 (0.80-1.10)
Baseline Buckling Ratio	13 (12-16)	13 (11-15)
Prevalent osteoporosis (%)[3]	7	12
Prevalent type 2 diabetes (%)	10	10
Prevalent CVD (%)	20	7
High education (%)	53	28
High income (%>1600 euro/ mo)	66	45
Current smokers (%)	28	20
Lipid lowering drug use (%)	3	3
Antihypertensive drug use (%)	14	11
Lower limb disabled (%)[4]	10	20
Current or past HRT use (%)[5]	Not applicable	8

1: Median (interquartile range), 2: Measured at the third visit (1997-1999), 3: Defined as sex-specific T-scores <-2.5, using NHANES "non-Hispanic whites" as the reference category, 4: Lower limb disability index, a combined index reflecting a person's person's ability to stand up, walk, climb and bend, was based on the Stanford Health Assessment Questionnaire (11) higher than 3; 5: Applicable to women only, abbreviations: 25(OH)D3=plasma vitamin D, BMD=bone mineral density, CVD=cardiovascular disease, HRT=hormone replacement therapy.

Table 2. Dietary patterns identified using RRR and factor loadings per food group

Response variable used in RRR:	BMD	BMD and hip geometry	
Dietary pattern (label):	**"Fruits, Vegetables and Dairy"**	**"Fruits, Vegetables and Dairy"**	**"Sweets, animal fat and low meat"**
Food groups	Factor loading	Factor loading	Factor loading
Fruit	**0.26**	**0.26**	0.14
Vegetables	**0.23**	**0.25**	-0.07
Pulses	0.07	0.09	-0.14
Milk	**0.59**	**0.56**	-0.07
Yoghurt	**0.37**	**0.38**	0.11
Cheese	-0.03	-0.07	-0.14
Soy	-0.11	-0.09	**-0.35**
Refined Grains	-0.18	-0.20	**-0.42**
Whole Grains	0.15	0.15	**0.21**
Soft drinks/ lemonades	0.11	0.09	0.03
Eggs	0.04	0.04	0.04
Unprocessed Meat	-0.05	-0.05	**-0.21**
Processed Meat	-0.18	-0.19	**-0.22**
Poultry	0.14	0.17	**-0.37**
Fatty Fish	0.12	0.12	-0.14
Lean & battered Fish	0.13	0.15	-0.03
Shellfish	-0.09	-0.08	-0.17
Savory snacks	-0.06	-0.06	-0.07
Sweets	**-0.28**	**-0.24**	**0.30**
Nuts	0.06	-0.03	0.14
Vegetable oils	-0.01	0.01	-0.06
Animal fats	**-0.24**	**-0.25**	**0.26**
Coffee & Tea	0.18	0.15	0.03
Alcohol	0.17	0.20	-0.01
Mixed meals	-0.01	0.02	-0.15
Soups	0.08	0.09	-0.04
Potatoes	-0.01	-0.02	-0.19
Porridges	-0.04	-0.05	**0.23**
Explained variance (all in %)			
in dietary intake	4.4	4.4	4.1
in BMD*1	2.3	2.3	2.3
in Bone Width	-	0.0	1.3
In Section Modulus	-	1.4	1.8
In Buckling Ratio	-	1.7	1.9
BMD plus geometry (total)	*Not applicable*	**7.7**	**9.6**

Factor loadings represent the correlation between a dietary pattern and a food group. Factor loadings <-0.20 and >0.20 are indicated **in bold**; BMD: bone mineral density; RRR: Reduced rank regression. *1: The variance explained by the patterns represent the squared values of the correlation between the dietary pattern and the bone outcomes (R^2). The reduced rank regression identified dietary patterns based on the maximum explained variance of our response variables; BMD, bone width, section modulus and buckling ratio.

Cross-sectional associations between dietary pattern adherence, BMD and bone geometry

Regression coefficients (β) and their 95% confidence intervals (CI) of associations between dietary pattern adherence and BMD, Bone Width, SM and BR are shown in **table 3**. As expected, in our basic models (model 1), the identified dietary patterns were associated with BMD and the bone geometry parameters. Each Z score of higher adherence to the "Fruit, Vegetables and Dairy" pattern was associated with higher BMD (β(95% CI) = 0.16 (0.12, 0.19)), higher SM (β(95% CI) = 0.12 (0.09, 0.15) and lower BR(β(95% CI) = -0.13 (-0.16, -0.10) (all in Z-scores standardized for age, sex, body weight and height). Adjustment for confounders or dietary calcium did not change these effect estimates (table 3, model 2 and 2a). Considering these results and the information provided in Supplemental table 2, our results indicate that a daily intake of 37 grams more of fruits, 24 grams more of vegetables, 135 grams more milk, 29 grams more yoghurt, 13 grams less sweets, and 4 grams less animal fats was associated with a 0.02 mg/cm^2 higher BMD, 0.05 cm^3 larger SM and a 0.50 smaller BR.

Table 3. Cross-sectional associations between dietary pattern adherence, BMD and one geometry

Dietary pattern	Outcome	Model 1 *Basic*		Model 2 *Confounders*		Model 2a *Dietary calcium*	
		β	95% CI	β	95% CI	β	95% CI
"Fruit, Vegetables and Dairy"	BMD	**0.16**	**(0.12, 0.19)**	**0.15**	**(0.13, 0.17)**	**0.14**	**(0.12, 0.17)**
	Width	-0.01	(-0.85, 0.40)	0.00	(-0.02, 0.02)	0.02	(-0.01, 0.04)
	SM (Bending strength)	**0.12**	**(0.09, 0.15)**	**0.12**	**(0.10, 0.14)**	**0.13**	**(0.11, 0.16)**
	BR (Instability)	**-0.13**	**(-0.16, -0.10)**	**-0.12**	**(-0.14, -0.10)**	**-0.12**	**(-0.14, -0.09)**
"Sweets, animal fat and low meat"	BMD	0.00	(-0.03, 0.03)	0.01	(-0.01, 0.03)	0.02	(-0.00, 0.04)
	Width	**0.12**	**(0.08, 0.15)**	**0.11**	**(0.09, 0.13)**	**0.11**	**(0.09, 0.13)**
	SM (Bending strength)	**0.06**	**(0.03, 0.10)**	**0.07**	**(0.05, 0.09)**	**0.08**	**(0.06, 0.10)**
	BR (Instability)	**0.04**	**(0.01, 0.10)**	**0.02**	**(0.01, 0.04)**	0.02	(-0.00, 0.04)

Model 1: Basic model, adjusted for age, sex, total energy intake, body weight and height
Model 2: Model 1, additionally adjusted for vitamin D status plus the month in which it was measured and use of lipid lowering drugs
Additional adjustment for smoking, education, income, prevalent type 2 diabetes, disability, physical activity, age at menopause, female hormone use of use of antihypertensive drugs did not change the effect estimate by >10%.
Model 2a: Model 2, additionally adjusted for dietary calcium
Regression coefficients represent the difference in Z-scores of BMD, width, SM or BR per Z-score increase of dietary pattern adherence. The Z-score of dietary pattern adherence was calculated by summing up a participant's standardized intake per food group multiplied by the factor loading of the corresponding food group. One Z-scores of each bone geometry outcome correspond with:0.14 mg/cm^2 BMD, 0.32 cm width, 0.35 cm^3 of SM, and a BR of 3.3. Abbreviations: BMD: bone mineral density; BR: Buckling ratio (higher values reflecting instability); SM: Section Modulus (higher values reflection more bending strength)
Significant associations (P< 0.05) in **bold**.

Each Z-score of adherence to the "Sweets, animal fat and low meat" pattern was associated with higher Bone Width (β(95% CI) = 0.12 (0.08, 0.15), SM (β(95% CI) = 0.06 (0.03, 0.10) and BR(β(95% CI) = 0.04 (0.01, 0.10) (all in Z-scores standardized for age, sex, body weight and height) but not with BMD in our basic models. The association with BR was mainly explained by confounders and was no longer significant after adjustment for dietary calcium (model 2 and 2a), whereas associations with Bone Width and SM did not change after adjustment.

Considering these results and the information displayed in Supplemental Table 4, our results indicate that a daily intake of 18 grams less refined grains, 5-7 grams less meat and poultry, 4 grams more animal fat and 15 grams more sweets and 11 grams more porridge per day was associated with 0.04 cm wider bones and a 0.03 cm^3 larger SM.

Associations between dietary pattern adherence and fracture risk

After adjustment for confounders adherence to the "Fruit, vegetable and dairy" pattern was associated with lower risk of osteoporotic (HR (95% CI) = 0.92 (0.89, 0.96) and hip fractures (HR (95% CI) 0.81 (0.70, 0.93), **table 4**, model 2). These favorable associations were explained by differences in baseline measures of bone geometry (HR (95% CI)= 0.97 (0.93, 1.00) for osteoporotic and 0.87 (0.74, 1.03) for hip fractures, table 4, model 3). BMD was the most important measure explaining these favorable associations; the addition of this variable to our models caused the largest reduction in our effect estimate (change in HR from 0.92 to 0.97 osteoporotic fractures and from 0.81 to 0.89 for hip fractures).

Table 4. Associations between dietary pattern adherence and risk of osteoporotic fractures and hip fractures

		Model 1		**Model 2**		**Model 3**	
		Basic		***Confounders***		***Bone geometry***	
Dietary pattern	**Outcome**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
"Fruit, vegetable and dairy"	Osteoporotic fractures	**0.90**	**(0.85, 0.96)**	**0.92**	**(0.89, 0.96)**	0.97	(0.93, 1.00)
	Hip fractures	**0.83**	**(0.73, 0.93)**	**0.81**	**(0.70, 0.93)**	0.87	(0.74, 1.03)
"Sweets, animal fat and low meat"	Osteoporotic fractures	**1.10**	**(1.03, 1.17)**	**1.12**	**(1.07, 1.16)**	**1.10**	**(1.06, 1.15)**
	Hip fractures	1.12	(0.99, 1.26)	**1.14**	**(1.05, 1.23)**	**1.10**	**(1.01, 1.19)**

Model 1: adjusted for age, sex, total energy intake, body weight and height
Model 2: Model 1, additionally adjusted for vitamin D status and season of vitamin D measurement
Additional adjustment for smoking, education, income, prevalent type 2 diabetes, disability, physical activity, age at menopause, female hormone use of use of antihypertensive drugs did not change the effect estimate by >10%.
Model 3: Model 2, additionally adjusted for BMD, Width, SM and BR
Significant associations (P< 0.05) in **bold**.
Abbreviations: BMD: bone mineral density; BR: Buckling ratio (higher values reflecting instability); SM: Section Modulus (higher values reflection more bending strength)
Hazard ratios represent the difference in instantaneous risk of fracture per one Z-score difference in dietary pattern adherence. The Z-score of dietary pattern adherence was calculated by summing up a participant's standardized intake per food group multiplied by the factor loading of the corresponding food group.

In contrast, adherence to the "Sweets, animal fat and low meat" pattern was associated with higher hazards of osteoporotic (HR =1.12, 95% CI = 1.07, 1.16) and hip fractures (HR (95% CI) =1.14 (1.05, 1.23), table 4, model 2). These unfavorable associations were only explained in part by differences in baseline measures of bone geometry (HR (95%)= 1.10 (1.06, 1.15) for osteoporotic and 1.10 (1.01, 1.19) for hip fractures, table 4, model 3). Differences in Bone Width (change in HR from1.12 to 1.04 for osteoporotic fractures and from 1.14 to 1.04 for hip fractures) and in BR (change in HR from 1.12 to 1.01 for osteoporotic fractures and from 1.14 to 1.06 for hip fractures) were the most important measures of geometry explaining this association with a higher fracture risk.

Additional analyses

We observed significant interaction between adherence to the "Fruit, vegetable and dairy" pattern and sex in relation to osteoporotic fractures (P= 0.02) and a trend towards interaction with sex in relation to hip fractures (P= 0.06). Stratified analyses (model 2) showed slightly more favorable associations for men than for women with osteoporotic fractures (HR (95% CI)= 0.86 (0.76, 0.98) for men versus 0.92 (0.88, 0.96) for women) and with hip fractures (HR (95% CI) = 0.73 (0.54, 0.98) for men versus 0.84 (0.70, 0.99) for women). No interaction between sex and adherence to the "Sweets, animal fat and low meat pattern" was observed. No interaction with prevalent type 2 diabetes was observed, nor were associations different when we restricted our analyses to only non-users of dietary supplements (n= 2592 (64%) *data not shown*).

DISCUSSION

Summary of main findings

We identified two dietary patterns that explained variation in BMD and bone geometry independently of age, sex, total energy intake, body weight and height in a Dutch elderly population. A "Fruit, Vegetables and Dairy" pattern high in fruits, vegetables, milk and yoghurt, and low in sweets and animal fats was associated with higher BMD, higher bending strength (measured as SM), less bone stability (measured as BR) and lower risk of fractures. In addition, a "Sweets, animal fat and low meat" pattern low in meat, poultry and soy products and high in sweets, animal fats and porridge was associated with wider bones, higher bending strength and higher risk of fractures.

Explanations of our results

To the best of our knowledge, this is the first study that identified dietary patterns that explain variation in novel measures of bone macro geometry and BMD using a RRR approach in a population-based study. Therefore, we cannot directly compare our results to existing evidence. Especially, the relation of dietary patterns with measures of

bone geometry is unknown. However, associations between dietary patterns, BMD and fracture risk were studied in other populations. In these studies, dietary patterns were identified using other data reduction techniques, of which the PCA was most often applied. Favourable associations of the "Fruit, Vegetable and Dairy" pattern with BMD and a lower fracture risk are aligned with results from previous studies that identified similar dietary patterns to be beneficial for bone health in other populations (6, 7). A "Dairy and Fruit" pattern with high factor loadings for intake of fruits, milk and dairy food, flour and bread was shown to be associated with lower risk of osteoporosis in Korean postmenopausal women(6). Similarly, a "Calcium Food" pattern high in dairy products, eggs, beans, nuts, marine products and eggs was shown to be associated with lower prevalence of osteoporosis in Chinese male students(7). Moreover, dietary patterns rich in cheese, milk, and charcuteries were related to a lower risk of wrist and hip fractures in a three-city cohort of elderly adults in France(38). In addition to a positive association with BMD, we also studied novel parameters of bone health and we observed a favourable association with bending strength and bone stability (reflected a low BR). This finding suggests that dietary patterns characterized by high dairy, fruit and vegetable intake may not only be beneficial for density of bones, but also for the hip bone's macro geometry.

The "Sweets, animal fat and low meat" pattern is characterized by high factor loadings for sweets and porridge and low factor loading for meat, poultry and soy products (meat substitutes). Nutrients accompanying high adherence to this pattern may affect Bone Width, bending strength, instability and fracture risk. For example, meat and poultry are important sources of protein. On one hand, low protein diets could adversely affect bone homeostasis by reducing calcium absorption, bone turnover, and the reduction of production of insulin-like growth factor 1(39). On the other hand, it could be argued that adherence to this pattern is associated with poor bone health *per se*, but is, rather a reflection of another chronic condition such as difficulties with chewing or swallowing meat and poultry.

Strengths and limitations

One of our study's strengths is the use of Reduced Rank Regression (RRR), a dimension-reduction method. Most studies on dietary patterns and bone outcomes have used PCA, a method that selects factors that explain as much variation in food intake as possible. In contrast, RRR extracts factors that explain the most variation in a particular response variable (32). As a result, RRR-derived dietary patterns are more strongly associated with the response variables, BMD and measures of geometry, than are PCA-derived patterns.

In addition to the use of RRR, our study has several other strengths. Specifically, we standardized the geometry variables for their main determinants (age, sex and anthropometrics(40)) before adding them as response variable to our RRR analyses.

This approach allowed us to identify dietary patterns that influence bone outcomes via pathways independent of body weight and height. We had detailed information on fracture types and dates that had been prospectively collected. The information allowed us to investigate associations between dietary pattern adherence and fracture incidence rather than prevalence to minimalize the risk of recall bias or reversed causality. However, analyses of *a posteriori*- defined dietary patterns are also accompanied by some limitations. Since the dietary patterns we identified are population-specific and the maximum explained variances in BMD and measures of geometry were relatively low, the generalizability of our findings may have been reduced (17, 32). We had a single measurement of dietary intake, so we were not able to investigate the stability of dietary pattern adherence over time. Also, details on dietary supplement use, such as dose and duration were not collected. Physical activity and plasma vitamin D were collected at the third visit only. Our data suggest that associations between adherence to the "Fruit, Vegetable and Dairy" pattern and fractures are slightly more favorable for men than for women, despite a similar distribution of adherence to this pattern. Although we adjusted for several covariates related to lifestyle and health, gender-specific residual confounding might still be present. Lastly, the ability to properly rank participants according to their dietary intake was validated in a study comparing the FFQ to food records. However, the FFQ was not validated against biomarkers of biomarkers of specific food groups or nutrients, such as urinary urea as estimate of protein intake, therefore data on the exact dietary intake using this FFQ should be interpreted with caution.

Suggestions for future research

This study provides evidence that dietary patterns might affect fracture risk not only via BMD, but also via measures of bone geometry. Future research is needed to explore potential mechanisms underlying our observed associations between the sum of foods and nutrients represented in our "Fruit, Vegetables and Dairy" pattern with bending strength or instability and represented in our "Sweets, animal fat and low meat" patterns with Bone Width.

To conclude, two dietary patterns were identified that explained most of the variance in BMD plus measures of bone geometry, independent of age, sex, body weight and height, in a population of Dutch middle-aged and elderly individuals. A "Fruit, Vegetables and Dairy" pattern was associated with higher BMD, higher bending strength, more bone stability and lower fracture risk. In addition, adherence to a "Sweets, animal fat and low meat" pattern was associated with higher fracture risk and wider, more unstable bones, independent of BMD. This study provides evidence that dietary patterns might affect fracture risk not only via influencing BMD, but also via measures on bone geometry. Therefore, dietary recommendations associated with optimal bone geometry in addition to BMD might influence long term risk of fractures.

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SUPPLEMENTAL MATERIALS

Supplemental Table 1. Pre-defined food groups for Principal Component Analysis

Food group	Food items included
Fruit (products)	Fresh fruits, dried fruits, fruit cocktail in syrup, fruit juices (unsweetened apple juice, grapefruit juice and orange juice)
Vegetable (products)	Vegetables raw or boiled, gherkins, vegetable juices, mushrooms
Pulses & legumes	Beans broad, Beans white or brown, Pea marrow fat legumes
Milk (products)	Buttermilk, (semi) skimmed and whole milk chocolate milk, coffee creamer, custards and puddings, dairy based ice cream and whipping cream
Yoghurts	Low fat, half fat and full fat yoghurt, plain and with fruits, fromage fraise
Cheese (products)	Cheese and cheese spreads varying in fat content (>48, >40 and >20% fat) and sodium content
Soy products	Soya chunks, Tahoe soya curd
Refined grains	Bread white, Currant bread, Macaroni cooked, Rice white boiled, Rusk Dutch
Whole grains	Bread brown wheat/ whole meal, crisp bread, Muesli, Rice brown Rye bread, Wheat bran & germ
Potatoes	Potatoes, boiled
Soft drinks & lemonades	Cola soft drink with/ without caffeine, fruit drink several flavors, carbonated mineral water
Eggs	Eggs, boiled
Unprocessed meat	Beef raw, Hamburger, Horsemeat, Lamb, Liver chicken/ ox/ pork, Mutton, Pork, Veal
Processed meat	Bacon, Beef salted and smoke dried, Corned beef, Croquette meat ragout deep fat fried, ham, sausages and salami
Poultry	Chicken fillet with and without skin
Fatty fish	Eel, Fish, 2-10 g fat and > 10 g fat raw, Herring, Mackerel, Plaice Salmon Sardines/pilchards (fresh and canned)
Lean and battered fish	Cod, Fish fingers, Fish lean 0-2 g fat raw, Haddock fillet in batter fried
Shell fish	Mussels boiled, Shrimps, peeled, boiled
Savory snacks	Biscuit salted average, Crisps, Liquorice Dutch type salted
Nuts and seeds	Nuts mixed unsalted, Peanut butter, Peanuts coated, Peanuts salted, Peanuts unsalted, Linseed
Mixed meals	Bami Goreng, Nasi, Pizza *(Bami and Nasi are traditional Indonesian dishes with meat, vegetables and rice (Nasi) or pasta (Bami) and could reflect either home-made or take-away food)*
Soups and sauces	Salad dressings, salad creams
Sweets	Sweet bread toppings, pie, biscuits, cake, chocolate bars, spiced honey cake, gateau, honey, popsicle ice cream, candy, pancakes, praline and sugar
Coffee tea and water	Coffee or tea prepared, water

Supplemental Table 1. Pre-defined food groups for Principal Component Analysis (Continued)

Food group	Food items included
Vegetable oils and fats	Cooking or frying fat 0-50 mg cholesterol, Margarine, Oils (corn germ, olive, peanut, safflower, soy and sunflower)
Animal fats	Butter unsalted, Frying fat > 50 mg cholesterol
Alcoholic drinks	Beer pilsner, Gin young Dutch, Sherry, Wine
Porridges	Porridge buttermilk with wheat flour paste, Porridge oatmeal, Porridge rice pasteurized

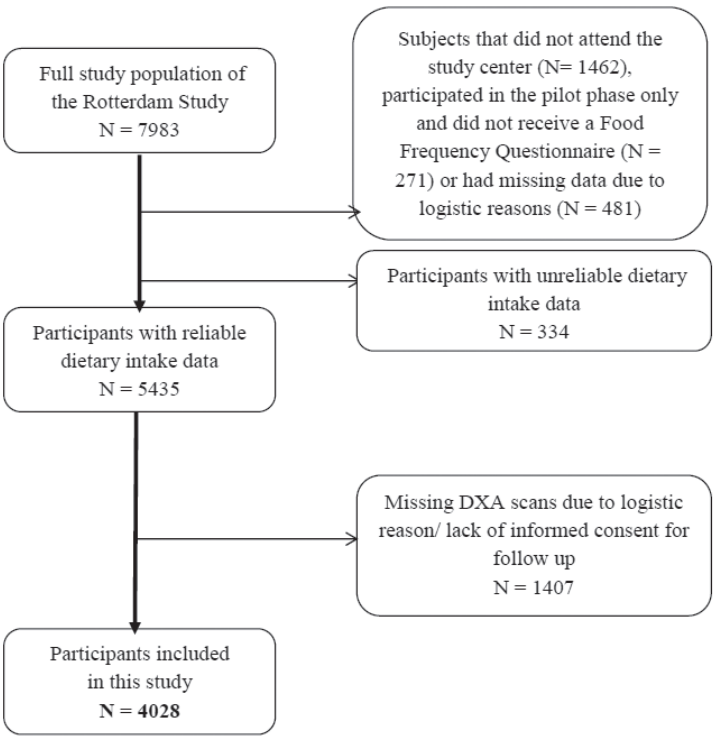
Supplemental Table 2. Mean values and standard deviations of food group intakes and bone outcomes and their relation to 1SD difference in dietary pattern adherence

			Fruit, Vegetable and dairy pattern		Sweets, animal fat and low meat pattern	
Food groups	**Mean (g/day)**	**SD (g/day)**	**FL**	**Difference per SD (g/day)*1**	**FL**	**Difference per SD (g/day)*1**
Fruit	265	149	**0.26**	**37**	0.14	21
Vegetables	194	97	**0.25**	**24**	-0.07	-6
Pulses	8	13	0.09	1	-0.14	-2
Milk	313	236	**0.56**	**135**	-0.07	-14
Yoghurt	70	77	**0.38**	**29**	0.11	8
Cheese	37	23	-0.07	-1	-0.14	-3
Soy	0	3	-0.09	0	**-0.35**	**-1**
Refined Grains	35	44	-0.20	-9	**-0.42**	**-18**
Whole Grains	119	62	0.15	9	**0.21**	13
Soft drinks/ lemonades	136	236	0.09	24	0.03	7
Eggs	13	8	0.04	0	0.04	0
Unprocessed Meat	66	34	-0.05	-2	**-0.21**	**-7**
Processed Meat	31	23	-0.19	-4	**-0.22**	**-5**
Poultry	13	14	0.17	2	**-0.37**	**-5**
Fatty Fish	5	10	0.12	1	-0.14	-1
Lean & battered Fish	11	14	0.15	2	-0.03	0
Shellfish	0	1	-0.08	0	-0.17	0
Savory snacks	2	6	-0.06	0	-0.07	0
Sweets	80	51	**-0.24**	**-13**	**0.30**	**15**
Nuts	7	13	-0.03	-1	0.14	2
Vegetable oils	26	19	0.01	0	-0.06	-1
Animal fats	8	16	**-0.25**	**-4**	**0.26**	**4**
Coffee & Tea	1108	457	0.15	73	0.03	14
Alcohol	77	162	0.20	31	-0.01	0
Mixed meals	7	13	0.02	0	-0.15	-2
Soups	65	69	0.09	6	-0.04	-3
Potatoes	129	70	-0.02	-1	-0.19	-13
Porridges	12	46	-0.05	-2	**0.23**	**11**

Supplemental Table 2. Mean values and standard deviations of food group intakes and bone outcomes and their relation to 1SD difference in dietary pattern adherence (Continued)

			Fruit, Vegetable and dairy pattern		Sweets, animal fat and low meat pattern	
Food groups	**Mean (g/day)**	**SD (g/day)**	**FL**	**Difference per SD (g/day)*1**	**FL**	**Difference per SD (g/day)*1**
Bone outcomes (sex-specific m/w)			β*2	**Difference per SD**	β*2	**Difference per SD**
BMD (g/cm^2)	0.70	0.14	**0.14**	**0.02**	N.S.	**-**
Width (cm^2)	3.20	0.32	N.S.	-	**0.11**	**0.04**
Section Modulus (cm^3)	1.15	0.35	**0.13**	**0.05**	**0.08**	**0.03**
Buckling Ratio	13.7	3.4	**-0.12**	**-0.50**	N.S.	**-**

Abbreviations: FL= factor loading; the standardized correlation coefficient between the dietary pattern and the food group. N.S: Non-significant associations between a dietary patter and bone outcome.
*1: The difference in intake per food group are calculated by multiplying the corresponding factor loading by the value of one SD of that food group in grams per day. Food groups with factor loadings for a specific dietary patterns of <-0.20 or >0.20 are displayed in **in bold**. *2: Differences in bone outcomes are estimated by multiplying the regression coefficients (table 2, model 2a) by the SD of that bone outcome.



Supplemental Figure 1. Sample sizes of participants included in each analysis
Abbreviations: DXA: dual x-ray absorptiometry
The reliability of dietary intake was determined during this assessment by the dietician. For example dietary data was considered as unreliable when patients had difficulties with recall or when they did not cooperate during the interview

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4.4

DIETARY PATTERNS AND THEIR ASSOCIATIONS WITH FRAILTY

ABSTRACT

Background

Frailty has been defined as a state of increased vulnerability to adverse health outcomes at old age. Diet and lifestyle could play a key role in preventing frailty, but little is known about the association between adherence to overall dietary patterns and frailty.

Research aim

To determine the associations between *a priori* and *a posteriori* derived dietary patterns and a general state of health, measured as the accumulation of deficits in a frailty index.

Design

Cross-sectional analysis embedded in the Rotterdam Study cohort (n=2563 males and females aged 45 years and over). Dietary patterns were defined *a priori* using an existing index reflecting adherence to national dietary guidelines and *a posteriori* using principal component analysis (PCA). A frailty index was composed of 45 health deficits. Linear regression analyses were performed using adherence to each of the dietary pattern as exposure and the frailty index as outcome (all in Z-scores). Models were adjusted for age, sex, smoking, level of education, income, physical activity, supplement use, body mass index and total energy intake.

Results

The PCA revealed three dietary patterns that we named a "Traditional" pattern, high in legumes, eggs and savoury snacks; a "Carnivore" pattern, high in meat and poultry; and a "Health Conscious" pattern, high in whole grain products, vegetables and fruit. In crude models, higher adherence to the national dietary guidelines and to the "Traditional" pattern were associated with less frailty (β (95%)= -0.06 (-0.11, -0.01); -0.05 (-0.09, -0.01), respectively). However, these associations were mainly explained by confounders (socio-economic indicators, physical activity). No other significant associations were observed.

Conclusion

In this population of middle-aged and elderly participants, we observed no consistent cross-sectional associations between dietary pattern adherence and frailty. Future studies with a longitudinal design are needed to study associations with changes in frailty during follow-up.

INTRODUCTION

Studies taking into account holistic approaches of health in the elderly are essential to better understand the ageing process and identify strategies to maintain health. One of the methods to estimate the overall health status of an individual, is the frailty index [3].

Although there is no complete consensus on the conceptualization of frailty, experts agree that frailty is a state of increased vulnerability to adverse health outcomes [4]. The frailty index, developed by Mitnitski and Rockwood, appraises frailty as the accumulation of health-related and age-related deficits [3]. The included deficits cover a broad range of health aspects including cognition, disabilities, laboratory abnormalities, and diseases [5]. Several studies, among different age-categories and populations, show that a high frailty index is associated with an increased risk for disability, falls, hospitalization, comorbidity and mortality [6-9]. Prevention of frailty is important because it is difficult to recover from a frail state to a non-frail state [10]. One important modifiable factor that might either positively or negatively influence frailty is diet [11].

Most research on nutrition and frailty or overall health focussed on single nutritional components [11], such as macronutrients and micronutrients. Although these studies have provided valuable knowledge towards possible nutritional strategies to prevent frailty (e.g. high protein intake), people do not eat single nutritional components but meals, combined into patterns. Dietary pattern approaches take into account the totality of the diet and allow for possible interactions and synergetic effects of nutrition [12]. One way to define a person's dietary pattern is via an *a priori* approach, studying adherence to existing dietary guidelines or recommendations in relation to health outcomes. Alternatively, an *a posteriori* approach allows the identification of naturally occurring dietary patterns of populations [13].

Although a few previous studies evaluated dietary patterns and frailty, the majority of studies on frailty and nutrition use the frailty phenotype as an outcome [11]. The frailty phenotype defines frailty as the presence of three out of five physical frailty symptoms (weight loss, weakness, exhaustion, slowness and low activity) [6]. Although this method has great advantages for clinical practice, due to its physical orientation, it is less useful as a measure of overall health and in general less strongly associated with adverse health outcomes (e.g. mortality and hospitalization) [14-19]. Information on how dietary patterns are associated with the frailty index is scarce. To our knowledge, only one previous study, by Woo et al., examined dietary patterns and the frailty index and found that better dietary quality was associated with a lower frailty index [20].

We therefore aimed to examine the association between adherence to national dietary guidelines (*a priori* defined dietary pattern) and population-specific (*a posteriori* defined) dietary patterns and the frailty index in middle-aged and elderly populations.

METHODS

Study population and design

This cross-sectional analysis was embedded in the Rotterdam Study (RS), an ongoing prospective cohort study in the Netherlands [21]. The Rotterdam Study started in 1990 in response to ongoing demographic changes resulting in an increased percentage of elderly people. Men and women aged 45 years and older, living in the Ommoord district in Rotterdam, were invited to participate. During an extensive home interview, trained research assistants collected data on a broad range of health variables including, activities of daily living, current health status, use of medication, depression and lifestyle. Subsequently, participants visited the study center for detailed examinations with an emphasis on imaging, collection of body fluids, and physical functioning. The Rotterdam Study was approved by the Medical Ethics Committee of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports. All participants signed an informed consent. This study adherence to the Declaration of Helsinki for research involving human subjects.

For the current study, we included the first visit of the third cohort of the Rotterdam Study (RS-III-1) comprising of 3,932 participants. Data of the RS-III-1 cohort were collected from February 2006 till December 2008 [21]. For 2570 participants, valid dietary intake and a frailty index were available (Figure 1).

Dietary assessment

Dietary intake was measured with a self-administrated semi-quantitative food frequency questionnaire (FFQ) developed by Wageningen University & Research centre, adapted for the Rotterdam Study. The ability of the FFQ to rank people according to their intake was previously shown in two validation studies [22-24]. The FFQ includes 389 items about the frequency and amount of consumed food items in days, weeks and months according to the previous year and was filled in at home [22]. For the estimation of the portion sizes in grams standardized household measures were applied [25]. For calculation of nutrient and total energy intakes the Dutch Food Composition Table (NEVO) of 2006 was used [26]. Participants with extremely high (>5000 kilocalories) or low (<500 kilocalories) daily energy intake were excluded as it was expected that their questionnaire was unreliable (Figure 1).

Dietary patterns

Two different approaches to determine dietary patterns were applied: 1) an *a priori* defined index for dietary quality and 2) *a posteriori* defined dietary patterns using principal component analysis (PCA).

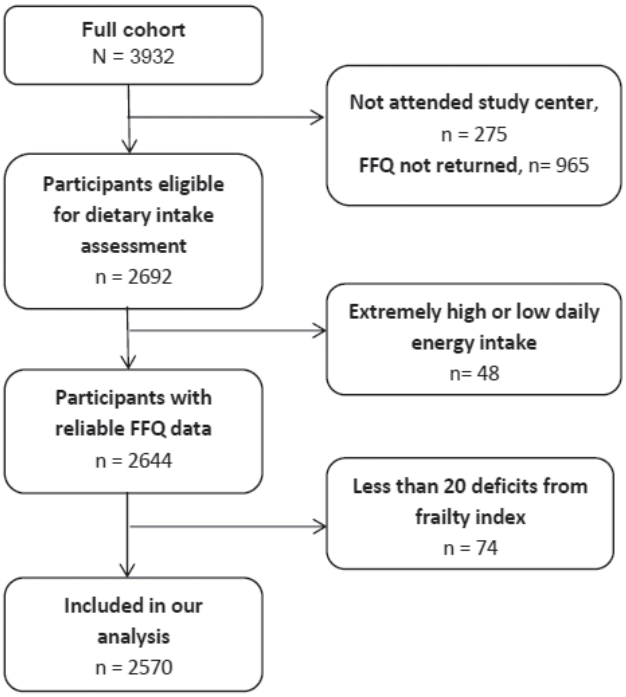

Figure 1. Flowchart of the study population

A priori defined patterns and assignment of pattern-adherence scores

We applied The Dutch Healthy Diet index (DHD-index), developed by van Lee et al. [27]. The DHD-index is a validated index, examining adherence to the Dutch Guidelines for a Healthy Diet of 2006 by the Dutch Health Council [28, 29]. The original DHD-index included 10 guidelines based on the recommendations of the Dutch Health Council (supplementary table 1). Participants received a sub-score, using a 10 point scale that reflected their adherence to each of these 10 guidelines. These sub-scores were then summed to obtain a single index for each participant. Due to limited information on physical activity and acidic drinks and foods in our cohort, we created an adapted version of this original index, with a theoretical range of 0 till 80 points. A higher score represented higher adherence to the national guidelines.

A posteriori defined patterns and assignment of pattern-adherence scores

All food items were categorized into 28 pre-defined food groups to reduce the complexity of dietary data. An overview of these food groups, which were based on similarities in product composition (for example lean versus fat dairy products)

or culinary use (for example readymade meals), is shown in Supplementary table 2. Next, dietary patterns were derived by Principal Component Analysis (PCA) on intake of these food groups in grams per day, unadjusted for total energy intake. We used Varimax rotation and Kaiser Normalization to obtain patterns with simpler structure [30] and optimal interpretability. Factor loadings, which reflect the correlation between a food group and a dietary pattern, were used to characterize and label a pattern using a cut-off of 0.2. Food groups with a factor loading > 0.2 indicate a positive contribution and < -0.2 a negative contribution to a specific pattern. Adherence to patterns with an Eigenvalue (a measure of explained variance) of > 1.5 only was studied in relation to the frailty index. For each participant, pattern adherence scores (Z-scores) were constructed by summing up observed intakes of the pattern's food groups weighted by the corresponding factor loading for each of the three dietary patterns separately.

Frailty index

Frailty was measured using a frailty index, an instrument based on the accumulation of health deficits [31]. In general, deficits can be symptoms, signs, diseases, disabilities and laboratory measurements as long as they are age-related and health-related and are not too exceptional or too common [5]. Previously, a frailty index was designed and validated for the Rotterdam Study consisting of 45 items covering several health domains: functional status (n=13: dressing & grooming, arising, eating, waking, hygiene, reach, grip, riding a bike, telephone, meal preparation, gardening, doing laundry, doing finances), health conditions (n=12: liver enzymes, homocysteine, sex hormone-binding globulin, CRP, creatinine, uric acid, proBNP, systolic blood pressure, hospitalization, falling, mobility, joint complaints), cognition (n=6: forgetfulness, aphasia, mini-mental state examination, letter digit substitution test, word fluency test, the Stroop color and word test), diseases (n=6: cancer, lung conditions, CVD, stroke, diabetes, vision), nutritional status (n=4: vitamin D, hyperlipidemia, HDL, BMI) and mood (n=4: depressed affect, positive affect, somatic and retarded activity, interpersonal) [32]. Deficits were all determined during baseline measurements of the Rotterdam Study and fulfilled all predefined criteria. Deficits were dichotomized or categorized into a score ranging from 0 (not present) till 1 (present). Per person, the number of present deficits was divided by the total number of deficits (45), providing a continuous score ranging from 0 (no deficits present, least frail) till 1 (all deficits present, extremely frail). Previously it was shown that multiple imputation increased the precision of the results. Details about the construction of the frailty index and the imputation are provided elsewhere, in brief, missing values on the deficits were imputed using multiple imputation by chained equations [32]. Individuals with less than 20 observed items were determined to have insufficient information to considerably contribute to a valid frailty index and were excluded from the analyses (Figure 1).

Covariates

Height (cm) and body weight (kg) were measured at the research center using a stadiometer wearing light clothing. BMI was calculated by weight (kg)/height $(m)^2$. Age was calculated as the amount of years calculated from the day of birth till the visit to the Rotterdam Study research center.

Smoking status was classified as never, former or current smoker. Level of education was determined by the highest attained education and classified as low (primary education and lower vocational education), middle (secondary general education and secondary vocational education), middle-high (higher general education) or high (higher vocational education or university education). Monthly household income was classified as low (<€1.500), middle (€1.500-2.900) or high (>€2.900). Physical activity was assessed with the LASA physical activity Questionnaire (LAPAQ) and MET scores were calculated as the sum of hours a week spent in light, moderate or vigorous activity (walking, cycling, gardening, sports, and hobbies), expressed in metabolic equivalent of task (MET) score. MET scores represent the energy that is required for an activity divided by the energy necessary at rest [33]. Total energy intake in kilocalories per day and use of dietary supplements (yes/no) were retrieved from the FFQ.

Statistical analysis

Characteristics of the study population were shown in strata of frailty ≥ or < the median. Linear regression analysis were performed to examine the associations between adherence to each dietary pattern and the frailty index (all in Z-scores). Analyses were performed as a basic model, adjusted for age and sex (model 1), followed by a model that was additionally adjusted for smoking, level of education and income, physical activity, BMI, and supplement use (model 2). Confounders were added to the models based on previous studies or a substantial change in effect estimate (>10%).

Some components of the *a priori* defined DHDI are standardized for total energy intake, whereas others are not. For example, daily dietary fibre intake is expressed per 1000 kcal, whereas daily vegetable intake is expressed in grams. Therefore, a subject's total energy intake influences its index.

Moreover, as we used food groups which were unadjusted for total energy intake as input for our PCA, *a posteriori*- defined dietary patterns might be driven by differences in total energy intake, if patterns have high factor loadings for energy-dense foods. To disentangle potential influence of total energy intake on our associations between dietary patterns and frailty we used two approaches. First, we added total energy as additional covariate to our adjusted models (in model 3). Second, we tested for potential interaction by adding the product term of adherence to each of our dietary patterns with total energy intake to model 3. A similar approach was used to study interaction with sex and age. Stratified analyses were only performed if the P for interaction was < 0.10.

Additionally, we performed several sensitivity analyses. First, we studied whether excluding nutritional associated deficits (BMI, vitamin D, HDL, cholesterol) from the frailty index affected the observed associations. Second, we performed our main analyses in subgroups after exclusion of (1) participants with incomplete dietary intake data (>1% missing items in the FFQ), (2) participants who deceased within 3 years after baseline and (3) dietary supplement users. Analyses were performed using SPSS statistical software (IBM, version 23). A p-value of 0.05 was considered statistically significant.

RESULTS

Dietary patterns derived by principal Component Analysis

A posteriori, we derived three population-specific dietary patterns that we labeled: 1) A *"Traditional" pattern*, characterized by a high intake of savory snacks, legumes, eggs, fried potatoes, alcohol, processed meat and soup; 2) a *"Carnivore" pattern*, characterized by a high intake of red meat and poultry with a low intake of meat replacements; and 3) a *"Health Conscious"* pattern, characterized by a high intake of whole grains, vegetables, fruit and nuts. The factor loadings of the food groups are presented in table 1. The "Traditional" pattern explained 10.0%, the "Carnivore" pattern 7.7% and the "Health Conscious" pattern 5.4% of the total variance in food group intake (Table 1). The DHDI was positively correlated with the "Traditional" pattern (Pearson's r = 0.39) and with the "Health Conscious" pattern (Pearson's r = 0.13), and negatively associated with the "Carnivore" pattern (Pearson's r = -0.25).

Subject characteristics

The median (interquartile range) frailty index of our population was 0.16 (0.09, 0.14). Characteristics of our study population in strata of the frailty index above and below the median are show in Table 2. Participants with a high frailty index were more likely to have a high BMI (29.0 vs. 26.0) and to be less physically active (53 vs. 63 METh/week). Moreover, they were less likely to have a high household income (30 vs. 49%).

Associations between dietary pattern adherence and the Frailty Index.

After adjustment for age and sex, *a priori* defined higher adherence to the DHDI was associated with a lower frailty index (standardized β (95% CI) = -0.06 (-0.11, -0.01), table 3, model 1). Additional adjustment for confounders and total energy intake diluted these results (standardized β (95% CI) = -0.05 (-0.08,0.00), Table 3, model 2).

After adjustment for age and sex, only the population-specific "Traditional" pattern was significantly associated with a lower frailty index (standardized β (95% CI) = -0.05 (-0.09, -0.01), Table 3, model 1). However, this association was mainly

explained by confounders (model 2). No consistent associations between adherence to the "Carnivore" or the "Health conscious" pattern in relation to the frailty index were observed (Table 3, model 1 to 3).

Table 1. *A posteriori* defined dietary derived from Principal Component Analysis.

Food groups	"Traditional" pattern	"Carnivore" pattern	"Health Conscious" pattern
Whole grain products	*	*	0.76
Refined grain products	0.24	*	-0.44
Lean dairy products	*	*	0.27
Fat dairy products	*	*	*
Fruit	-0.25	*	0.42
Vegetables	*	*	0.50
Legumes	0.51	*	*
Potatoes	0.21	0.25	0.24
Fried potatoes	0.45	*	*
Poultry	*	0.48	*
Unprocessed red meat	*	0.65	*
Processed meat	0.33	0.60	*
Meat alternatives	0.24	-0.63	0.21
Eggs	0.47	*	*
Lean fish	*	*	*
Fatty fish	*	*	*
Readymade meals	*	*	*
Tea	*	*	0.28
Coffee	*	*	*
Water and diet soda	*	*	*
Sugar sweetened beverages	*	*	*
Alcohol	0.41	*	*
Sweet snacks	*	*	*
Savory snacks	0.59	0.23	*
Nuts	0.26	-0.21	0.39
Vegetable oils and spreads	0.20	*	*
Animal fats	*	*	*
Soup, sauce, gravy and dressing	0.32	0.22	*
Eigenvalue	**2.8**	**2.2**	**1.5**
Explained variance (%)	**10.0**	**7.7**	**5.4**

* Food groups with a factor loading between -0.20 and 0.20 were not shown.

Table 2. Characteristics of the study sample

	Low Frailty index (≤ the median*)	High Frailty Index (> the median)
N	1207	1363
Age (years)[1]	55.8 (5.3)	58.3 (7.4)
Dutch Heathy Diet Index (Z-scores)[1]	0.07 (0.95)	-0.07 (1.04)
Adherence to "Traditional" Pattern (Z-scores)[1]	0.11 (1.04)	-0.10 (0.95)
Adherence to "Carnivore" Pattern (Z-scores)[1]	-0.05 (0.94)	0.04 (1.05)
Adherence to "Health Conscious" Pattern (Z-scores)	-0.04 (1.01)	0.04 (0.98)
BMI (kg/m²)	26.0 (3.5)	29.0 (4.9)
Energy intake (kcal)	2371 (821)	2260 (913)
Physical activity: METh/ week	63 (54)	53 (64)
Sex (% men)	43	41
Supplement use (% yes)	48	48
Smoking (%)		
- Never	33	29
- Former	43	46
- Current	24	25
Income (%)		
- Low	11	24
- Middle	41	46
- High	49	30
Level of education (%)		
- Low	19	32
- Middle	43	39
- High	38	29

[1]: Mean + SD
BMI = body mass index. METh = metabolic equivalent of task in hours
*: Our population- specific median is 0.12

Influence of total energy intake and other potential effect modifiers

Total energy intake was significantly correlated with the all dietary patterns (Pearson's r= 0.15 for DHDI, 0.37 for the "Traditional" pattern, 0.33 for "Carnivore" pattern and 0.14 for the "Health conscious" pattern). Additional adjustment for total energy intake did not markedly change our results for any of the dietary patterns under study (table 3, model 3). Total energy intake did not interact with any of the dietary patterns in relation to the frailty index (P for interaction all> 0.11), nor did age.

In contrast, we did observe a trend towards signficant interaction between gender and adherence to the "Carnivore" pattern (P for interaction <0.10). Analyses in strata of sex showed that the association between adherence to this "Carnivore" pattern and

Table 3. Associations between adherence to dietary patterns and the frailty index

	Model 1		Model 2		Model 3	
Dietary pattern	β	**(95% CI)**	β	**(95% CI)**	β	**(95% CI)**
***A priori* defined**	**Reflection of adherence to national dietary guidelines**					
Dutch Healthy Diet Index (DHDI)	**-0.06**	**(-0.11, -0.01)**	-0.05	(-0.08, 0.00)	-0.04	(-0.08, 0.00)
***A posteriori* defined**	**Reflection of population-specific dietary patterns**					
Traditional pattern	**-0.05**	**(-0.09, -0.01)**	0.01	(-0.03, 0.05)	0.03	(-0.01, 0.08)
Carnivore pattern	0.05	(-0.00, 0.10)	0.03	(-0.02, 0.08)	0.05	(-0.00, 0.10)
Health conscious pattern	0.03	(-0.01, 0.08)	0.01	(-0.03, 0.05)	0.02	(-0.02, 0.07)

Model 1: Model adjusted for age and sex
Model 2: Model 1 additionally adjusted for smoking, level of education, income, physical activity, dietary supplement use and body mass index.
Model 3: Model 2 additionally adjusted for total energy intake.
Adherences to *a posteriori* defined patterns were additionally adjusted for each other
Regression coefficients represent the differences in frailty index (in Z-scores) per Z-score increase in dietary pattern adherence.
As the frailty index ranges from 0.00 to 0.65 in our population, one Z-score corresponds with ≈0.10 absolute points on the frailty index.

the frailty index was stronger in males than in females (standardized β = 0.07 versus 0.02). Gender was not interacting with any of the other dietary patterns in relation to frailty (P for interaction all> 0.77).

Sensitivity analysis

Excluding participants that died within 3 years after baseline (n = 38), with incomplete FFQ data (n =867) or dietary supplements users (n=1323) did not change the results of our main analyses, nor did the use of an adapted version of the frailty index (Table 4).

DISCUSSION

Main findings and comparison to other studies

Overall we found no consistent association between dietary patterns and frailty after adjusting for other lifestyle factors and energy intake. Nevertheless, we found indications that an *a posteriori* defined pattern high in meat could be associated with higher a frailty index, and adherence to an *a posteriori* defined traditional pattern and the *a priori* defined DHDI to be associated with lower frailty inices. Nevertheless, our results do not support a convincingly strong association between dietary patterns and frailty.

To the best of our knowledge, we were the first to investigate the association between dietary patterns and a frailty index in a Dutch middle-aged and elderly population. Direct comparison of our results with published data is challenging for

Table 4. Sensitivity analyses

			Excluding:	
	Full population		Died within 3 years	
Dietary pattern	β	**(95 % CI)**	β	**(95 % CI)**
Dutch Healthy Diet Index	-0.04	(-0.08, -0.00)	-0.04	(-0.08, 0.01)
Traditional pattern	0.03	(-0.01, 0.08)	0.03	(-0.01, 0.08)
Carnivore pattern	0.05	(-0.00, 0.10)	0.05	(-0.01, 0.10)
Health conscious pattern	0.02	(-0.02, 0.07)	0.02	(-0.02, 0.06)

Models are adjusted for age, sex, smoking, level of education, income, physical activity, supplement use, body mass index and total energy intake (model 3).
Adherences to *a posteriori* defined patterns were additionally adjusted for each other.
*1: From the original 45-item frailty index BMI, HDL, cholesterol and vitamin D were excluded.

several reasons. First, studies used other outcome measures than the frailty index, such such as physical frailty (e.g. the frailty phenotype), or measures of healthy ageing and vitality. Although these measures show similarities to the frailty index, the also show important differences. For example, social health, self-perceived health and resilience are identified to be important for healthy ageing and are therefore included in several healthy ageing instruments, but are no part of the frailty index [34]. The frailty phenotype has been defined as the presence of three out of five physical frailty symptoms: weight loss, self-reported exhaustion, weakness, slow walking speed, and low physical activity [6]. Thereby, the frailty phenotype is physically orientated and is distinct from disabilities, chronic diseases, cognition and mental health, whereas the frailty index does includes these health domains. Last, other studies used different measures of exposure. National dietary guidelines and population-specific dietary patterns differ per country and per study population, as they are shaped by local or cultural habits and availability of food products [13].

In our study, participants with low adherence to the national dietary guidelines- the *a priori* defined DHDI score- were slightly frailer than participants with higher adherence, but these results were explained by other life style factors and energy intake. Woo et al., (2010) observed that adherence to the Diet Quality Index International (DQI-I), an index based on (1) overall food group variety, (2) adequacy of vegetables, fruit, grains, fiber, protein, iron, calcium and vitamin C, (3) moderation of total fat, saturated fat, cholesterol, sodium and empty calorie foods, and (4) overall balance in macronutrient intake and fatty acid ratio [35] was associated with a lower frailty index. However they did not adjust for other lifestyle factors or energy intake. In general, a protective effect dietary quality and physical frailty have been reported [11], whereas studies on overall health as an outcome reported inconsistent results [39]

Excluding:					
Participants with incomplete FFQ data		Dietary supplement users		Using FI without nutritional deficits*1	
β	(95 % CI)	β	(95 % CI)	β	(95 % CI)
-0.06	**(-0.11, -0.00)**	-0.04	(-0.10, 0.02)	-0.04	(-0.08-0.003)
-0.01	(-0.06, 0.05)	0.03	(-0.03, 0.09)	0.04	(-0.01-0.08)
0.06	(-0.00, 0.12)	**0.06**	**(-0.02, 0.14)**	**0.05**	**(0.00-0.09)**
0.01	(-0.04, 0.06)	0.02	(-0.05, 0.09)	0.02	(-0.02-0.06)

Regression coefficients represent the differences in frailty index (in Z-scores) per Z-score increase in dietary pattern adherence.
As the frailty index ranges from 0.00 to 0.65 in our population, one Z-score corresponds with ≈0.10 absolute points on the frailty index.

[40]. Furthermore, several papers report that adherence to a healthy diet (defined by different dietary guidelines) is generally associated with better cognitive functioning, less depressive symptoms and better physical functioning [11], all components of the frailty index.

Contrary to our expectations, we did not observe an association between the "Health Conscious" pattern and the frailty index. Previously, "Health Conscious" or "Prudent" patterns did show associations with different aspects of healthy ageing including self-perceived health, cognition and depression [11, 41-44]. Hodge *et al.*, concluded that a dietary pattern high in fruit was positively associated with overall health [46], and that a "Meat and fatty foods" pattern showed an inverse association with overall health, defined as maintaining a good mental health with the absence of major chronic diseases and limitations in physical functioning [46]. The cross-sectional nature of our analysis could have led to reversed causation (e.g. people in poor health tend to adapt their life style in order to improve their health status). Probably, our population might have been too young and not frail enough to detect a potential association yet.

Potential influence of total energy intake and BMI

In a recent study, Assmann *et al.* observed that a healthy dietary pattern (characterized by high intake of micronutrients, fibres and antioxidants) was associated with better health, but only among French elderly with low energy intake [45]. In our analyses, no significant interaction between adherence to any of our population-specific patterns and total energy intake was observed. Moroever, our results were suggestive for an association between adherence to the "Carnivore" pattern and more frailty in subjects with high BMI only.

Strengths and limitations

Our study has several strengths. First, our combined use of *a priori* and *a posteriori* defined dietary patterns provided an opportunity to study both adherence to existing guidelines and population-specific patterns, in relation to frailty. Whereas the first approach provided us insight into the potential of current dietary guidelines to prevent frailty, the latter could provide additional insight to improve these guidelines in the future. Furthermore, the frailty index is a validated measure that includes multiple aspects of health.

Nevertheless, we also recognize some limitations. Foremost, due to the cross-sectional design, we were not able to state if participants became more frail as a consequence of their dietary patterns or if they adapted their dietary patterns due to their frailty status [47]. Future research will be needed to establish longitudinal relations between dietary pattern adherence and trajectories of frailty in different populations. Due to the relatively low age of the participants, participants had relatively low frailty indices, which could result in less pronounced associations. Similar, weaker or frailer elderly may be less able or willing to come to the study center [47, 48], which might have led to selection and could have influenced the external validity of our results in participants that suffer from more extreme frailty. Furthermore, definition and labelling of the *a posteriori* defined patterns involved some arbitrary choices, including the definition of food groups, and the cut-off values of factor loadings and Eigenvalues. Lastly, the dietary patterns identified only explained 20% of the variance of the total diet, reflecting the complexity of reducing the variation in dietary intake data into single components.

CONCLUSION

In conclusion, in this population of middle-aged and elderly participants, we observed no consistent cross-sectional association between dietary pattern adherence and frailty. Future studies with a longitudinal design are needed to study associations with changes in frailty during follow-up.

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SUPPLEMENTS

Supplementary Table 1. Scoring system of The Dutch Healthy Diet Index

Component	Minimum score = 0 points	Maximum score = 10 points
1. Physical activity	0 activities a week	≥ 5 activities a week
2. Vegetables	0 gram a day	≥ 200 grams a day
3. Fruit and fruit juices	0 gram a dag	≥ 200 grams a day
4. Fibers	0 gram per 1000 calories a day	≥ 14 grams per 1000 kcal a day
5. Fish and fish oil capsules	0 milligram EPA and DHA a day	≥ 450 milligrams EPA and DHA a day
6. Saturated fatty acids	≥ 16.6 energy% a day	< 10 energy% a day
7. Trans fatty acids	≥ 1.6 energy % a day	< 1 energy% a day
8. Acidic drinks and foods	> 7 occasions a day	≤ 7 occasions a day
9. Sodium	≥ 2.45 grams a day	< 1.68 grams a day
10. Alcohol (ethanol)	Male: ≥ 60 grams a day Female: ≥ 40 grams a day	Male: ≤ 20 grams a day Female: ≤ 10 grams a day

EPA = eicosapentaenoic acid. DHA = docosahexaenoic acid.

Supplementary Table 2. Food items included in each food group

Food group	Summary of included products
Whole grain products	Muesli, whole wheat bread, multigrain bread, brown rice
Refined grain products	Cereals, white bread, ginger bread, white pasta
Lean dairy products	Skim milk, low fat yoghurt, low-fat cheese, buttermilk
Fat dairy products	Full milk, full-fat cheese, mousse, whipped cream
Fruit	Apple, banana, orange, strawberry etc.
Vegetables	Cauliflower, carrot, lettuce, tomato, etc.
Legumes	Kidney beans, white beans, soup with legumes
Potatoes	Boiled potatoes, mashed potatoes
Fried potatoes	Frites, fried potatoes, baked potatoes
Poultry	Chicken, turkey
Unprocessed red meat	Cooked liver, meatloaf, beef, lamb
Processed meat	Liver products, ham, hamburger, bacon
Meat alternatives	Products based on tofu or tempeh, or other meat alternatives
Eggs	Boiled eggs, baked eggs
Lean fish	Mussels, cod, trout, crab
Fatty fish	Herring, salmon, mackerel
Readymade meals	Pizza, pancakes
Tea	Black tea, green tea, herbal thee
Coffee	Coffee
Water and diet soda	Water, diet soda
Sugar sweetened beverages	Orange juice, other fruit juices, soda, lemonade, water ice
Alcohol	Beer, red wine, white wine, strong alcoholic beverages
Sweet snacks	Sugar, cookies, chocolate, candy bars, candy
Savory snacks	Fried fish, mini snack products, chips, peanuts, crackers
Nuts	Pine nuts, walnuts, mixed nuts, linseed
Vegetable oils and spreads	Low fat margarine, margarine, liquid margarine, olive oil
Animal fats	Butter, solid cooking fat, solid frying fat
Soup, sauce, gravy and dressing	Soups without legumes, mayonnaise, ketchup, tomato sauce

5

GENERAL DISCUSSION

GENERAL DISCUSSION

The overall purpose of this thesis was to unravel the association between dietary intake and bone health in middle-aged and elderly people. We used several approaches to determine dietary intake by zooming into single nutrients from the diet and in plasma and zooming out to overall dietary patterns. Moreover, we have studied a broad spectrum of bone properties including mineral density, trabecular bone integrity, measures of macro geometry and risk of fractures. Lastly, we studied associations between dietary intake in relation to overall frailty, defined as the accumulation of health deficits. In this general discussion, we will summarize our main findings. Also, methodological considerations regarding the assessment of our exposures and outcomes will be addressed. Next, some potential confounders, intermediates and effect modifiers with emphasis on anthropometrics and challenges accompanying the analyses of longitudinal data will be discussed. At last, this discussion will provide suggestions for future research and practical implications.

SUMMARY OF OUR MAIN FINDINGS

Socio-economic indicators and dietary quality

Dietary intake is shaped by cultural habits of and availability in a specific population(1). Within populations, the quality of dietary intake might vary according to socio-economic class. Therefore, prior to investigating associations between dietary intake and bone health, we studied the association between socio-economic indicators and participants' adherence to the Dutch dietary guidelines in chapter 2. We observed that high education was the most pronounced socio-economic indicator of high dietary quality. Our results highlight that different socio-economic indicators influence dietary quality in different manners. Future studies are required to explore potential barriers of adhering to the dietary guidelines in the lowest educated elderly and women. Since high education and income were also associated with BMD in our cohort, socio-economic indicators were included as potential confounders in our analyses on dietary intake and bone outcomes.

Findings of nutrient-analyses on bone outcomes

The aim of chapter 3 was to study associations between nutrients and bone outcomes. We studied single nutrients from the diet (retinol and beta-carotene) as well as dietary acid load that reflects the ratio of nutrients that are metabolized to acids to those metabolized to bases. Lastly, we studied plasma values of uric acid, a metabolic end product of purine breakdown. Also, we were interested in potential nutrient-interactions in relation to bone outcomes. We hypothesized that vitamin A might interact with vitamin D, dietary acid load with dietary fibre and uric acid with vitamin C. In contrast to earlier studies, we observed that intake of dietary

retinol and total vitamin A, but not beta-carotene, was associated with a lower risk of fractures in participants of the first cohort of the Rotterdam Study. However, this favourable association was only observed in participants who were overweight or obese (BMI > 25). Associations between high intake of vitamin A and high BMD were explained to a large extent by differences in BMI and no interaction with vitamin D was observed. Moreover, we found that dietary acid load, expressed as net endogenous acid production (NEAP) the ratio of animal protein to potassium ratio (AnPro/K) or was associated with low trabecular bone score (TBS), but not with BMD. TBS is a measure of bone integrity combining information on connectivity density, trabecular separation and trabecular number. However, we observed that this association was differently shaped for AnPro/K than for the ratio of vegetable proteins to potassium (VegPro/K) as index of dietary acid load. Our data suggested that adverse associations between acid load and bone outcomes might be more prominent in participants with high dietary fibre intake. Lastly, we observed that higher levels of serum uric acid, a breakdown of purines found in meat, fish, grains and pulses, were associated with higher BMD, at expense of thicker cortices and narrower bone diameters. These associations were more prominent in individuals older than 65 years and in participants with high intakes of vitamin C.

Altogether, our findings emphasize the importance of specific single nutrients in relation to measures of bone health. However, nutrient interactions or the food source from which the nutrient is derived, such as animal or plant-based sources, might be important. Over the last decades, several statistical approaches to identify and study associations between overall dietary patterns in relation to health outcomes have been developed. Studying dietary patterns might help us to identify potential additive, synergistic, or antagonistic effects between components of the full diet that may affect bone outcomes (2). Also, cumulative effects of a combination of nutrients on BMD might be easier to identify than the effect of a single nutrient, since the latter might be too small to detect (3).

Findings of dietary pattern analyses on bone health and overall frailty

Therefore, the aim of chapter 4 was to study associations between overall dietary patterns and BMD.

Dietary patterns can be identified using an *a posteriori* or *a priori* approach. *A posteriori* approaches will result in data-driven, population-specific dietary patterns (3). Using this approach, we identified three dietary patterns (table 1) that explained most of the variance in overall dietary intake in our population. Of these patterns, we observed that a "Health conscious" pattern was associated with high BMD, whereas a "Processed" pattern was associated with low BMD, independent of important covariates including body weight and height. Favourable associations between a "Traditional (Dutch)" dietary pattern and BMD were mainly explained by differences

in body weight and height. In contrast, prior knowledge on the association under study is needed for application of the *a priori* approach. Applying this approach and based on a narrative review of studies investigating associations between dietary patterns and BMD, we developed a BMD- Diet Score, reflecting a participants' consumption of eight food groups which were either consistently shown to be associated with high BMD (fruits, vegetables, fish, whole grain products, dairy and legumes & beans) or low BMD (meat and confectionary). We have shown that in our Dutch population, this BMD-Diet Score was significantly associated with high BMD, independent of important confounders and body weight and height. The effect size of this standardized association was approximately three times as strong as that of the association between the Healthy Diet Indicator, a reflection of the current guidelines of the WHO, and BMD.

However, it was previously shown that characteristics of the bone beyond BMD reflecting its macro geometry might play a key role in susceptibility to fractures (4). Therefore, we identified two dietary patterns that explained most of the variance in parameters of bone geometry (bone width, section modulus that reflects bending strength and buckling ratio that reflects instability) plus BMD using an *a posteriori* approach. We found that a "Fruit, vegetables and dairy" pattern might be associated with fracture risk due to high BMD, high bending strength and more stable bones. A "Sweets, animal fat and low meat" pattern might be associated with increased fracture risk due to widened, unstable bones, independent of BMD (table 1). Bone health is an important aspect during ageing. Ultimately, dietary intake might facilitate the maintenance of healthy bones during ageing as part of preventing frailty in the broader perspective. To conclude this thesis, we therefore studied different dietary patterns in relation to the frailty index; an overall measure of health composed of 45 health deficits covering the following domains: functional status, health conditions, cognition, diseases, nutritional status and mood in middle-aged and elderly participants of the Rotterdam Study. Also in this cohort of younger participants (>45 years) we identified three patterns that we named "Traditional, "Carnivore" and "Health conscious". Moreover, we studied adherence to the national dietary guidelines. We observed no consistent cross-sectional association between any of these dietary pattern adherence and frailty at baseline.

In summary, findings from these overall dietary pattern analyses showed that different population-specific dietary patterns could be identified that might favour bone outcomes. Comparison with patterns associated with BMD identified in other populations showed comparable results in term of food groups. Overall, a diet high in fruits, vegetables, dairy products plus fish and poultry, rather than processed meat, might favour bones in our population (table 1 and BMD-Diet Score).

Table 1. *A posteriori* defined dietary patterns outlined in this thesis which were shown to be associated favourable or unfavourably with bone outcomes

	Favourably associated with bone outcomes			Unfavourably associated with bone outcomes	
	"Traditionally Dutch"	**"Fruit, Vegetable and Dairy"**	**"Health Conscious"**	**"Processed"**	**"Sweets, animal fat and low meat"**
Food groups with high factor loadings	Potatoes Meat (red and processed) Animal fats Oils Eggs	Fruits, Vegetables Milk Yoghurt	Fruit Vegetables Alcohol Fish Poultry	Processed meat Mixed meals Alcohol Eggs	Sweets Animal fats Porridge
Food groups with low factor loadings	Soy products Mixed meals	Sweets Animal fats	Sweets	Fruit Yogurt	Soy Meat (red and processed) Poultry
Chapter	*4.1.*	*4.3*	*4.1*	*4.1*	*4.3.*
Bone outcome	*High BMD (explained by BMI)*	*High BMD, ,bending strength and stability Low fracture risk*	*High BMD*	*Low BMD*	*Wider bones High stability High fracture risk*

METHODOLOGICAL CONSIDERATIONS

However, even the most robust observational evidence is accompanied by methodological challenges. In this chapter, the most important challenges regarding the assessment of our dietary exposures and bone outcomes will be discussed in detail. Moreover, we will point out methodological considerations regarding the use of body weight, height or BMI as potential confounder, effect modifier or mediator in the analyses on dietary intake and bone health. Lastly, methodological challenges related to nutrient interactions, confounding and the use of longitudinal data, including selective participation, faced during the realization of this thesis will be addressed.

Assessment and analysis of dietary intake data

Potential information bias: underreporting and overreporting in Food Frequency Questionnaires

Information on dietary intake was derived from Food Frequency Questionnaires (FFQs). More specifically, we used two different FFQs: one containing 172 food items conducted at the baseline visit of the first cohort of the Rotterdam Study and a more extensive FFQ containing 387 food items at the fifth visit of the first same cohort and the first visit of the third cohort of the Rotterdam Study. FFQs are widely

applied in observational studies to measure habitual dietary intake. The benefits of an FFQ are that it requires minimal training of the participant, and has relatively low cost(5) However, limitations should also be considered. Participants might give socially desirable answers. This might result in overreporting of food items that are perceived to be healthy, such as fruits and vegetables and underreporting of foods that are perceived to be unhealthy, such as sweets or savoury snacks(6, 7). The size of this information bias might depend on factors that affect knowledge on a healthy diet, such as education level, as well as on body weight, since we know that overweight subjects tend to be more likely to underreport their intake of high calorie food items(8).

Different methods for dietary pattern identification

Dietary patterns can be extracted from FFQ data using different methods(3). In this thesis, we used Principal Component Analysis (PCA), Reduced Rank Regression (RRR) and Diet Scores or Indices. These methods have different strengths and limitations, which are schematically shown in table 2.

Studying dietary patterns in general might help us to identify potential additive, synergistic, or antagonistic effects between components of the full diet that may affect bone outcomes or frailty (2). Also, cumulative effects of a combination of nutrients on these outcomes might be easier to identify than the effect of a single nutrient, which might be too small to detect (3). On the other hand, if a potential effect is driven by a single nutrient (such as calcium), studying dietary patterns might dilute this effect too much to detect it.

A second advantage is that results of dietary pattern analyses are easy to translate to practical dietary recommendations. The definition of food groups included in these patterns are often based on prior knowledge regarding associations between either specific foods or nutrients and the outcome of interest or on the basis of similarities in nutrient composition or in culinary use of specific foods. As a result, when comparing to other studies, definitions of food groups might differ, which may affect the generalizability of the respective dietary pattern.

Adjustment for total energy intake

As the intakes of specific nutrients, particularly macronutrients, are correlated with total energy intake, they may be non-causally associated with bone outcomes as a result of confounding by total energy intake. Also, measurement errors in the assessment of nutrient intakes are strongly correlated with the measurement error of total energy intake(9). To adjust for total energy intake, different statistical methods can be applied. A frequently applied method in nutritional epidemiology is the residual method, which assumes a linear relationship between a specific food item or nutrient and total energy intake(9). This method allows a more precise ranking of true low and high intakes and is widely applied in studies on single nutrients or food items in relation to health

Table 2. Comparison of 3 methods for dietary pattern identification: Principal Component Analysis (PCA), Reduced Rank Regression (RRR) and Diet Scores or Indices

	PCA	RRR	Diet Scores or Indices
Nature	*A posteriori* (data driven)	*A posteriori* (data driven)	*A priori* (pre-defined)
Requires pre-specification of	Food groups	Food groups and biologically important risk markers of the outcome under study; the "response variables"	Dietary guidelines (based on literature)
Output reflects	The standardized linear function of foods with the maximal variance in food group intake These values can be interpreted as adherence to naturally occurring dietary patterns in the population which explain variance in overall food group intake.	The standardized linear function of foods with the maximal variance in response variables These values can be interpreted as adherence to dietary patterns in the population which explain variance in risk markers. In this thesis, we used parameters of bone density and geometry as response variables	Adherence to pre-specified guidelines or a reflection of a participants' dietary quality
Examples in this thesis	Adherence to the "Traditional Dutch", "Processed Foods" and "Health Conscious" pattern (*chapter 4.1*).	Adherence to the "fruit, vegetable and dairy" or "Sweets, animal fat and low meat" pattern (*chapter 4.3*).	(a) the Dutch Healthy Diet Index, reflecting adherence to the Dutch Guidelines for Healthy Eating (*chapter 2.1 and 4.4*), (b) the Healthy Diet Indicator, reflecting adherence to the dietary guidelines of the World Health Organisation (*chapter 4.2*) and (c) the BMD-Diet Score, a newly developed score based on results from *a posteriori* defined dietary patterns that were shown to be associated with measured BMD in the literature (*chapter 4.2*).
Strengths	Reflect the sum of non-adjusted single effects of food groups or nutrients, thereby considering their correlation structure.		Could be used to quantify a participant's dietary quality, regardless of its source population, which facilitates comparisons between populations
Limitations	Identification of dietary patterns is limited by the food groups that are consumed in that population. For example, a dietary pattern high in soy products might be very beneficial for the prevention of disease X, but will never be identified in a Traditional Dutch population that hardly consumes soy products		Contain selected aspects of the diet which do not necessarily consider the correlation structure of food and nutrient intakes

Sources: (3, 21)

outcomes(10). Therefore, we applied this method in our analyses on vitamin A and dietary acid load in relation to bone outcomes. Theoretically, the same method could be applied in dietary pattern analyses. However, a downside of this approach is that dietary patterns with high factor loadings for energy-dense products may not be identified(11). Therefore, we did not apply the residual method for our dietary pattern analysis in chapter 4.1, but to investigate the effects of total energy intake by adding it as a covariate to our statistical models. In contrast, in our RRR analysis applied in chapter 4.3 we did use energy-adjusted values of food groups as input variables, since the aim of this study was to identify dietary patterns that explained most of the variance in bone outcomes, independent of anthropometrics and total energy, rather than to explain variance in overall dietary intake.

Thus, zooming into single nutrients and zooming out to overall dietary patterns are accompanied by specific challenges and opportunities. The definition of bone health, the main outcome of this thesis, and challenges regarding the operationalization of this definition for epidemiological analysis are described next.

Assessment of bone outcomes

A definition of bone health

A "healthy" bone might be best described as highly adaptive to physiological challenges(12). Physiological challenges differ across the life course and vary from growing in childhood to hormonal changes during puberty in both boys and girls and menopause in females. Adaptations to changes in mechanical loading are present throughout the life-course, e.g., due to changes in body size during growth, increases in body weight during puberty and adulthood and decreasing body weight during ageing(13). On the one hand, bone must be resistant to deformation in response to loading. On the other hand, bone must be flexible and be able to deform a little in order to absorb energy when compressed without fracturing. Impaired bone health (or fragility) can be the results of insufficient material (reflected by low BMD) or structural adaptations (e.g. expanded bone) to physiological challenges(14). In this thesis, we used the following parameters to characterize "healthy" bones: high quantity reflected by BMD, high cortical thickness, bone width, section modulus (which reflects bending strength) and trabecular bone score (combining information on trabecular connectivity density, trabecular separation and trabecular number), low buckling ratio (which reflects instability) and incidence of fractures.

Anthropometrics (body weight and height, BMI) are the most important determinants of mechanical loading of the weight bearing bones. It can be argued that these might be confounders in the associations between dietary intake and bone outcomes. The methods applied to study these different possible scenarios in the present thesis are discussed in chapter 5.2.3.

Limitations of the DXA measurement

With the exception of the incident fractures, these measures were all derived from femoral or spinal Dual-energy X-ray absorptiometry (DXA) scans. The non-invasive densitometry is an effective, non-invasive and quantitative method(14) which is widely applied in epidemiological research, facilitating comparisons of results between studies. However, one should not disregard the fact that bones are 3D structures and that DXA provides a 2D assessment(4). This way, section modulus is subject to positioning error. Moreover, a fixed ratio of 60% cortical to 40% trabecular bone was used for calculation of the buckling ratio, whereas it has been suggested that fracture cases proportionately loose more cortical than of trabecular bone(15). Since the buckling ratio represents the maximum distance from the center of mass to the medial or lateral surface (dmax) divided by the mean cortical estimate, the buckling ratio might underestimate the true bone instability. However, these measurement errors are unlikely to be different for those with a healthy versus a non-healthy diet and therefore we believe they did not influence our results. Also, people with extreme body size (more than 200 cm in length or 130 kg in weight) cannot be measured in the DXA for practical logistic reasons. Since body weight and height are two of the most important determinants of BMD, this might imply that the most extreme upper values of BMD are by design excluded from our study. Therefore, extrapolation of our findings to (potentially unhealthy) participants with extreme body size should be done with care.

Fracture assessment

Although the incidence of fractures is probably the most hard endpoint in studies on bone health, it might also be argued that it is a more heterogeneous outcome than intermediate measures like BMD. Fractures might have underlying causes beyond bone quality in the ageing population, such as changes in a participants reduced vision which may increase the risk of falls(16). We tried to minimalize the heterogeneity in our outcome measurements by studying osteoporotic fractures separately. Osteoporotic fractures were defined as fractures that were unlikely to be caused by trauma and excluded fractures of the fingers, toes and skull. All events were verified by two trained research physicians who independently reviewed and coded the information according to the International Classification of Primary Care (ICPC) and International Classification of Diseases, 10th edition (ICD-10). Events that were inconsistently coded were reviewed by a medical expert for final classification, thereby reducing the risk of misclassification of reported events. However, misclassification due to non-reported fractures might still be present. It could be argued that some of dietary patterns studied in this thesis are more likely to be associated with non-reporting of fractures than others. For example, adherence

to the Dutch dietary guidelines or a "Health conscious" pattern might reflect an overall health conscious mind-set. Therefore, people with high adherence to this pattern might be more likely to report a suspected fracture than those with a low adherence to such a pattern. This differential misclassification might have led to an underestimation of the true association between adherence to health conscious pattern and low risk of fractures.

Frailty index: a measure of overall frailty beyond measures of bone health?

In addition to a variety of bone outcomes, we studied diet in relation to the frailty index. This frailty index reflects the accumulation of health deficits from different domains important to health. In short, it covers measures of:

- functional status (n=13: dressing & grooming, arising, eating, waking, hygiene, reach, grip, riding a bike, telephone, meal preparation, gardening, doing laundry, doing finances)
- health conditions (n=12: liver enzymes, homocysteine, sex hormone-binding globulin, CRP, creatinine, uric acid, proBNP, systolic blood pressure, hospitalization, falling, mobility, joint complaints)
- cognition (n=6: forgetfulness, aphasia, mini-mental state examination, letter digit substitution test, word fluency test, the Stroop color and word test)
- diseases (n=6: cancer, lung conditions, CVD, stroke, diabetes, vision)
- nutritional status (n=4: vitamin D, hyperlipidemia, HDL, BMI) and mood (n=4: depressed affect, positive affect, somatic and retarded activity, interpersonal)(17) and **Supplemental Table 1**.

Although impaired bone health could be considered an important aspect of frailty in the ageing population, it was not incorporated in the definition of the FI in the Rotterdam Study, due to inconsistent availability of the DXA measurements across the different Rotterdam Study cohorts. However, BMD, TBS and the Frailty Index were available in a subgroup of participants (n=2031) in the third cohort of the RS (data for chapter 4.4). In this subgroup, high FI was significantly associated with low TBS (standardized β (95% CI)= -0.08 (-0.11, -0.05) but not with BMD (β (95% CI)= -0.01 (-0.04, 0.02)) after adjustment for age, sex and BMI. The latter might in part explained by the inclusion of diabetes in the FI since it is known that diabetes patients might have high BMD.

Anthropometrics: confounders, intermediates or modifiers?

In line with the classical definition of a confounder, a participants body weight, height or BMI at the moment of dietary assessment might influence intake of a single nutrient or dietary pattern (and vice versa). For example, someone with a high BMI might habitually consume a high-fat diet. Since preformed vitamin A is found mainly in fatty products of animal origin, these participants will have high intakes of

preformed vitamin A (Fig. 1a). In our analyses we assessed the influence of BMI on the association under study by:

1. Adding body weight and height to the regression model and check the percentage change in regression coefficient
2. Standardizing the outcome measurement (BMD) by body weight and height, using the residual method. This method was applied because it might be a more precise adjustment for body weight and height than presented under (a) when dietary intake was analysed as categorical exposure.
3. Using response variables for our Reduced Rank Regression (BMD and measures of bone geometry) which were standardized for body weight and height.

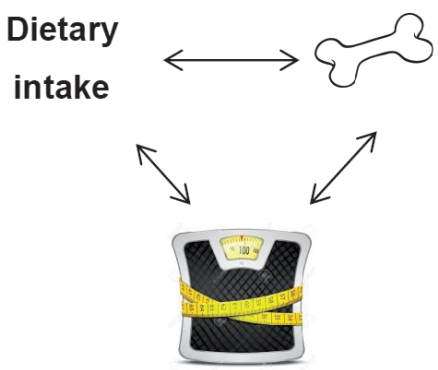

Figure 1a. Body weight and height as potential **confounders**

It could however also be argued that dietary intake in elderly might influence body weight and height directly, thereby affecting bone outcomes. This potential pathway would classify anthropometrics as intermediates (Fig. 1b) rather than confounders. We assessed potential mediation by adding body weight and height to the regression model. Body weight and height were considered to be intermediates if the association strongly diluted (towards a null-effect) after adding them to the model. In this thesis, the association between adherence to the "Tradition Dutch" dietary pattern and high BMD was explained by differences in BMI.

Lastly, anthropometrics could be effect modifiers which implies that associations between nutrients or dietary patterns and bone outcomes are different in e.g. participants with and without obesity (Fig 1c).

We assessed potential effect modification by testing significance of the interaction term in models in which both exposure and the potential modifier (e.g. body weight, height or BMI) were included. In this thesis, data suggested that associations between

dietary vitamin A and fracture risk were mainly observed in participants with high BMI (>25 kg/m^2, P for interaction =0.06).

Figure 1b. Body weight and height as potential **intermediates**

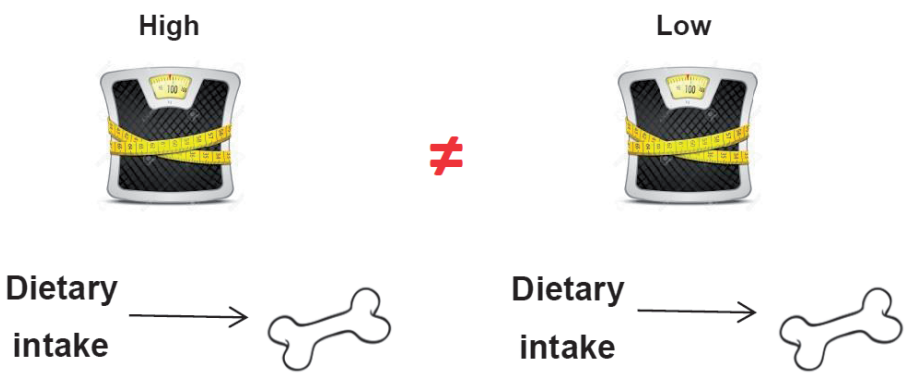

Figure 1c. Body weight and height as potential effect modifiers

Thus, anthropometrics were important covariates in our analyses. Especially in our nutrient-based analyses (chapter 3) other dietary factors might influence associations under study as well.

Residual confounding and limited reporting of dietary supplement use

Associations between single nutrients and bone outcomes are likely to be influenced by other dietary factors, when consumed together. Dietary factors might influence the intestinal absorption or metabolism of calcium, the main mineral constituent of bone. For example, vitamin D is known to enhance calcium absorption(20). In chapter 3.1.we studied associations between dietary vitamin A and BMD. Vitamin A might be associated with vitamin D, as these nutrients co-occur in the diet (e.g. in margarine) or in dietary supplements. Thus, vitamin D might be dietary confounders in the association between vitamin A and BMD. Confounding by dietary factors is especially important in single nutrient analyses, whereas these are taken into account

to some extent in dietary pattern analyses(3). Although we explored a large number of potential confounders in our analyses, residual confounding by factors that were unmeasured or measured sub optimally(18) might be still be present. A potential residual confounder is physical activity, since daily activities such as walking and cycling as well as sports are important determinants of bone health and might influence dietary intake. We did adjust our analyses for total hours spend on total activity (including housekeeping) and vigorous activity or for total metabolic equivalent of task (MET)-hours. However, it could be argued that residual confounding by profession-related physical activity and sedentary behaviour might have been present in the younger participants of our cohort who were still working.

Also, we had limited data on dietary supplement use in our first cohort (data used in chapters 2.1, 3.1 to 3.3 and 4.1 and 4.2). Participants were asked whether they used dietary supplements, how often and of which brand, but no data was available on dosage and on the actual content of these supplements. This might have been an issue for our analyses on dietary vitamin A and bone outcomes, as it could have led to misclassification of the exposure (intake of vitamin A). In addition to the precise dosages, it would have been interesting to have information on (a) whether the supplement contained preformed retinol, the pro-vitamin A carotenoids or a combination of both and (b) if the vitamin A containing supplement did contain vitamin D as well. However, it was shown that dietary supplement use in the Netherlands including the city of Rotterdam was low in the early nineties, when our dietary data were collected; only 16% of people aged 50 years and over used supplements in The Netherlands(19).

At last, the analyses of longitudinal data in an ageing cohort is accompanied by methodological challenges related to potential survival of the healthiest participants. Moreover, the assumption that dietary intake at older age is relatively stable over time, needs further discussion.

Analysis of longitudinal data

Reversed causality

We had repeated measurement of BMD and bone geometry. The availability of longitudinal data as opposed to cross-sectional data reduces the risk of reverse causality. That is, the outcome is influencing the exposure rather than vice versa. For example, participants who are aware of a low BMD at baseline (e.g. those diagnosed with osteoporosis) might have adopted their dietary intake and as a consequence have a higher adherence to the "Fruit, vegetable and dairy" pattern at baseline. Cross-sectional analysis may then result in a negative association between adherence to the pattern and measured BMD. As a result, the direction of this association is likely from low BMD to high dietary pattern adherence whereas our actual aim was to identify dietary patterns that contribute to a higher BMD. The

longitudinal approach (chapter 2.1, 3.1, 3.3, 4.1 and 4.2) provides the opportunity to study whether associations between dietary intake and e.g. BMD change over time (trajectories). In other words, it provides insights on whether the naturally occurring loss of BMD in elderly is faster or slower in participants with a specific diet. However, when no significant interaction between dietary pattern adherence and time in relation to bone outcomes is observed, regression coefficients should be interpreted as cross-sectional analyses between dietary pattern adherence at baseline and bone outcomes (at all time-points included). Therefore, reversed causality might still be present in our observed associations of BMD with adherence to the "Traditional" and "Processed" pattern, but not with the "Health Conscious" pattern. However, it could be argued that a true traditional dietary pattern is more likely to be driven by factors like cultural habits than by the presence or absence of low BMD.

Selection bias

Longitudinal analyses in an ageing population are prone to selective participation and therefore pose a risk for selection bias. The "healthy survivor bias", a subtype of selection bias in which only the healthiest participants visited the study centre(18) and have DXA scans available might be present. It could indeed be argued that DXA scans at follow up are only available for those with the healthiest measures of BMD and geometry of the bones. However, the distribution of our PCA derived dietary patterns did not markedly differ between participants included at baseline and the "survivors" with available BMD data at the fourth visit. For example, the median (+ IQR) Z-scores of adherence to the "Traditional" dietary patterns was -0.02 (-0.54, 0.52) in all participants with BMD at baseline and -0.01 (-0.55, 0.52) in the survivors. Similarly, differences were small for adherence to the "Processed Food" pattern (-0.04 (-0.59, 0.55) versus -0.03 (-0.58, 0.57) and to the "Health Conscious" pattern (-0.14 (-0.67, 0.52) versus -0.11(-0.62, 0.54). This implies that although selective participation might have occurred, selection bias likely did not as participation was unrelated to our exposure. Hence we believe that our conclusions regarding dietary pattern adherence and BMD (chapter 4.1) are still valid.

Dietary stability over time

Also, our exposure was measured only at baseline and the assumption that dietary intake is stable over time could be debated. Dietary intake was found to be stable in a large cohort of Dutch middle-aged and elderly on diet and cancer(20). Although absolute intakes showed small reductions over time, the ability to properly rank participants according to nutrient intake only dropped slightly over time. In our cohort, we observed that higher education was associated with an overall increase in adherence to the Dutch dietary guidelines. Moreover, the most recent FFQ included

more food items than the older one used at baseline. It could therefore be argued that people are likely to report higher intakes of specific food groups, such as vegetables, at follow-up than at baseline. As vegetable intake is incorporated in the Dutch dietary guidelines, this difference in FFQ might lead to an increase in overall dietary quality at the population level. Thus, changes in dietary quality can be caused by within-subject measurement error of the instrument and the true variation that occurred over time. However, the measurement error of the instrument is less likely to be associated with socio-economic indicators. Therefore we believe that our observed associations between education and changes in dietary quality over time are due to true variation and therefore still valid. Nonetheless, we recognise that a lack of repeated measurement of dietary intake using the same FFQ is a limitation of our study.

Considering the challenges faced during the realization of the present thesis, some practical implications of our studies and suggestions for future research will be discussed next.

PRACTICAL IMPLICATIONS OF OUR FINDINGS

A comparison to current Dutch Dietary guidelines

Principal Component Analysis and Reduced Rank Regression are data reduction techniques that provide valuable information on food groups that characterize dietary patterns associated with bone outcomes. However, the factor loadings, Z-scores of dietary pattern adherence and corresponding regression coefficients of the associations with bone outcomes might be difficult to translate into practical recommendations for the general public. To facilitate a more direct comparison between our results and the Dutch dietary guidelines intakes of the most important food groups identified in our dietary pattern analyses were back transformed to grams per day or per week (table 3). From table 3 we can conclude that the findings of this thesis underpin the validity of the dietary guidelines of 2015 for bone health.

Remarkably, we identified one dietary pattern that does not seem to fit well in the current guidelines, that we named the "Sweets, animal fat and low meat" pattern. Although the high intake of sweets and animal fats do meet the guidelines, this pattern was low in unprocessed meat, processed meat and poultry and was significantly associated with adverse bone outcomes. As we know that humans suffering from disturbed glucose metabolism might have unfavourably altered bone geometry, the high intake of sweets rather than the low intake of meat might be important for the association between adherence to this pattern with adverse bone geometry and high fracture risk.

Implications of small effect sizes

The effect sizes of associations between dietary exposures and bone health explored in this thesis are relatively small. For example, findings from table 4 illustrate that independent of each other, age and gender are most strongly associated with BMD in our cohort. Moreover, the association of BMI with BMD is approximately 3 times as strong as of our BMD-Diet Score with BMD. Considering this information and our observation that associations between specific dietary patterns (such as the "Traditional" pattern in chapter 4.1) and BMD are mainly explained by differences in BMI, we believe that it is important to emphasize the role of maintaining a healthy BMI for proper bone health.

In summary, the results of our studies on dietary acid load and dietary patterns on bone outcomes are in line with current dietary guidelines and underpin the importance of maintain a healthy BMI. To further disentangle the role of dietary intake in healthy aging, some additional data might have to be collected. Also, more novel statistical techniques could be applied to strengthen the evidence presented in this thesis in terms of causality.

SUGGESTIONS FOR FUTURE RESEARCH

Collection of additional FFQ data

The most recent FFQ used in the Rotterdam Study included an extensive number of food items, providing detailed insights in the dietary intake of our participants. However, as we observed a significant association between high education and adherence to the dietary guidelines of 2006, it would be interesting to include a number of questions regarding the awareness of the guidelines in future FFQs.

For example, it could be asked if partcipants are familiar with the guidelines and to which extent they believe their dietary intake is aligned with these guidelines. The latter would provide a measure of perceived rather than measured compliance to the guidelines. Lastly, it would be interesting to collect data on potential reasons for non-compliance, such as costs, taste or personal believes or attitudes.

The answers to these questions could be used to further confirm whether associations between SES indicators and adherence to the guidelines are influenced by knowledge on healthy nutrition or specific motivations for noncompliance. Also, taking into account the rising variety in dietary supplements, detailed questions on duration, brand and dosage of supplement use would add valuable information.

Repeated measurements

FFQs are developed to quantify a participant's habitual dietary intake. To properly study habitual dietary intake in relation to repeatedly measured outcomes or in survival analyses, repeated measurements of dietary intake will be needed in the future. Preferably, cohort studies should include repeated measurements of dietary intake, assessed using the same questionnaires.

Table 3. Dietary guidelines from 1986 to 2015 versus findings from this thesis

1986	2006 Basis of the DHDI (chapter 2.1 and 4.4)
Consume a diet with sufficient variety	Be physically active for at least 30 minutes per day at five or more days per week. Activities should be moderately heavy and could include walking, cycling or working in the garden.
	Eat 150-200 grams of vegetables and 200 grams of fruit per day
Consume plenty of complex carbohydrates (starches) and dietary fibres and prevent a high intake of sugars (mono and disaccharides)	Consume 30-40 grams of dietary fibre per day, preferably derived from vegetables, fruits and whole grain products
	Eat fish twice a week (servings of 100 to 150 grams), of which at least one is fatty fish
Limit the intake of fat, especially of saturated fat and consume sufficient amounts of polyunsaturated fatty acids	Limit the use of saturated fatty acid to <10 energy% and of trans fatty acids to <1 energy%
Limit the intake of cholesterol	
	Limit the use of foods and drinks with easily fermentable sugars and acidic drinks to 7 occasions (including main meals) daily
Limit the intake of alcohol	If you would like to consume alcohol beverages, limit the intake to two (males) or one (females) standardized consumptions per day
Limit the intake of table salt	Limit the intake of table salt to 6 grams per day

2015	Findings from this thesis (chapter)
Consume a more plant-based, less animal-based dietary pattern according to the below mentioned guidelines.	Dietary acid load expressed as ratio of animal protein to potassium but not of vegetable protein to potassium was associated with low trabecular bone integrity (2.2)
Eat at least 200 grams of vegetables and at least 200 grams of fruit per day	-Q5 "Health conscious" pattern: 291 g of fruits and 219 g of vegetables per day (3.1) -Q5 "Fruit, Vegetable and Dairy" pattern: 300 g of fruits and 227 g of vegetables per day (4.1) -Q1 "Processed Food" pattern: 377 g fruits per day (3.1) -Q5 BMD-Diet Score: 252 g vegetables and 183 g fruit per day (3.2)
Consume at least 90 grams of brown bread, wholegrain bread or other whole grain products daily	BMD Diet Score: 115 g of whole grain products per day (3.2)
Eat pulses/ legumes weekly	Q5 BMD Diet Score: 27 g pulses per week (3.2)
Eat ≥ 15 grams of unsalted nuts daily	
Consume a number of dairy servings daily, among which milk and yoghurt	Q5 "Health Conscious" pattern: 264g milk per day and 64g yoghurt per day (3.1) Q5 "Fruit, Vegetable and Dairy" pattern: 500 g milk per day and 128 g yoghurt per day (4.1) Q1 "Processed Foods" pattern: 150 g yoghurt per day
Eat fish once a week, preferably fatty fish	Q5 "Health Conscious" pattern: 250 g per week (including battered fish, such as fish fingers)(3.1) Q5 BMD-Diet Score: 50 g per week (3.2)
Drink 3 cups of tea daily	
Replace refined grains by whole grains	High intake of refined grains in the unfavourable "Sweets, animal fat and low meat" pattern (4.3)
Replace butter, hard margarines and cooking fats by soft margarines, soft cooking fats and vegetable oils	Low intake of animal fats in the favourable "Fruit, Vegetable and dairy" pattern High intake of animal fats in the unfavourable "Sweets, animal fat and low meat" pattern (4.3)
Replace unfiltered coffee by filtered coffee	
Minimize the consumption of red meat and especially of processed meat	High intake of of processed meat in the unfavourable "Processed Food" pattern (4.1) Meat is included "Low BMD" component in our BMD Diet Score
Limit the consumption of sugar sweetened beverages	
Do not consume alcoholic beverages or at least limit it to one glass per day	
Limit the intake of table salt to 6 grams per day	
The use of dietary supplements is unnecessary, except for those who belong to specific target groups	*Not studied in detail in this thesis*

Table 4. Standardized associations between the BMD-Diet Score developed in this thesis with BMD in relation to other main determinants of BMD

	β	95% CI
BMI	**0.21**	**(0.19, 0.23)**
BMD- Diet Score	**0.07**	**(0.04, 0.10)**
Total energy intake	0.02	(-0.01, 0.04)
Total physical activity	0.02	(-0.01, 0.05)
Gender	**-0.70**	**(-0.73, -0.63)**
Age	**-0.27**	**(-0.29, -0.24)**

Lastly, repeated assessment of the frailty index would be of added value. In the third cohort of the Rotterdam Study, the frailty index was calculated in participants that were 45 years and older at study entrance. Due to their relatively low age, they had relatively low frailty indices. It would be very interesting to study associations between trajectories of dietary patterns and trajectories of the frailty index to further contribute to the development of dietary guidelines that facilitate healthy ageing.

Additional measurements to validate dietary intake

National dietary guidelines were developed based on the most up-to-date available evidence with respect to dietary intake and chronic diseases(22). The shift of a food-group based or whole diet approached as opposed to a single nutrient approach (table 3) might require an accompanying change in validation studies of the FFQ. Whereas the aim of the validation studies was traditionally to compare the estimation of nutrient intakes derived from the FFQ with food record or 24h recalls, a comparison at the food group level might be of added value.

Also, given the measurement error that is present in self-reported dietary data, important biomarkers related to dietary intake in plasma or urine would be informative to include in future studies. For example, urinary pH and urinary net acid excretion (titratable acids plus ammonium minus bicarbonate) could be measured for analyses on dietary acid load and urinary nitrogen could be measured as an unbiased estimate of protein intake. Metabolomics might be applied to identify novel biomarkers related to food group intake and dietary patterns using metabolomics. For example, a recent study identified stachydrine is associated with the intake of citrus fruits(23) and plasma 14:1 and 17:1 fatty acids and serum 15:0 cholesteryl esters have been suggested to reflect dairy intake(24).

Doubly-labelled water methods could be used as a more precise measure of total energy intake(25).This method could be used as a validation of the energy intake derived from the FFQ and thereby provide an indication of its measurements error

at the individual level. In addition, repeated measures of plasma fat soluble vitamins (e.g. carotenoids, retinol and vitamin D) would be useful to investigate whether these metabolites explain our observed effect modification by BMI in the association between vitamin A and fracture risk. A novel area that deserves further interest is metabolomics.

Residual confounding and potential intermediates

To minimize residual confounding by physical activity in cohorts of younger participants, detailed information on sedentary behaviour, measured as hours of television watching or total time spent sitting in the occupational setting or during leisure time(26) could be collected.

In chapter 4.1 we identified dietary patterns that explained most of the variance in baseline BMD and measures of bone geometry. However, we assumed that these patterns might affect long term fracture risk by influencing trajectories of these potential intermediates. In this future, it might therefore be interesting to use a Reduced Rank Regression approach with the change in BMD and geometry as response variables, rather than their absolute values at baseline.

Also studying the microbiome might help us to study potential mechanistic pathways underlying associations between dietary patterns and bone health. Although this research area is fairly new, it was recently shown that nutrition was of the main determinants of variation in the microbiome(27, 28). Also the potential effects of the microbiome on bone outcomes are largely unexplored but are suggested to involve specific immune cells, and plasma levels of steroid hormones, fatty acids, serotonin and vitamin D ((29)

Establishment of causality

We believe that observational studies are particularly suitable for identification of overall dietary patterns in specific populations. However, to establish causal relationship between dietary patterns and measures of bone health and frailty, different approaches could be considered.

In cohort studies, a Mendelian Randomization Approach could be considered. In brief, the Mendelian Randomization approach exploits the principle that genotypes are not generally associated with confounders in the population and should be therefore immune to reverse causation bias. The basic principles of a Mendelian Randomization are displayed in figure 2. The target exposure (E) is causally associated with the outcome (O) if the following conditions are held:

1. the genetic variant (G) is a valid instrument, meaning that it is reliably associated with the exposure (E)
2. there is no association of the genetic variant with the outcome (O), except through the exposure
3. the genetic variant (instrument variable) is independent of any measured or unmeasured confounding factors (C) (30).

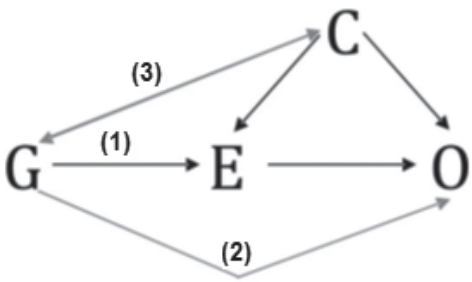

Figure 2. Basic principles of Mendelian randomization (adopted from (30))
E: Target exposure (e.g. plasma uric acid)
O: Outcome (a measure of bone geometry, e.g. bone width)
G: Genetic variant (e.g. SNPs identified in ref (31))
C: Confounding factors (e.g. BMI)

Especially the first assumption is a challenging one if the exposure variable of interest is dietary intake. Whereas it could be speculated that the quantity of dietary intake (total energy intake) might be causaly related to specific genetic variants involved in satiety mechanisms, the quality of dietary intake is rather shaped by a variety of factors. These factors included cultural habits, local food availability(1) and socio-economic indicators (chapter 2.1). However, serum uric acid levels are highly heritable and therefore a Mendelian Randomization approach would be a valuable next step in studying causality of our association between serum uric acid levels and measures of bone geometry (chapter 3.3). In a recent meta-analysis of genome-wide association studies including participants from European descent, 954 single nucleotide polymorphisms (SNPs) were identified to be significantly associated with uric acid levels(31), which might serve as a starting point for further investigation under the assumption that the identified SNPs are not associated with measures of bone geometry.

Lastly, a randomized controlled trial might be the most optimal design to proof causality of the associations observed in this thesis. A trial design might be particularly suitable to manipulate a diet with more extreme values of dietary acid load than observed in observational studies. Outcome measures, next to BMD and incidence of fractures, could include measures of bone microarchitecture, such as the TBS and of macro geometry.

CONCLUSION

Socio-economic indicators are associated with dietary patterns and should be carefully considered when studying diet in relation to bone health. Single nutrients beyond dietary calcium and vitamin D as well as dietary patterns are associated with bone outcomes in the middle aged and elderly. Associations with fracture risk are not always explained by BMD, but other characteristics of the bone such as trabecular microarchitecture, bending strength and cortical instability might play a role in these associations. Longitudinal analyses are needed to further explore associations between dietary patterns and trajectories of overall frailty.

Supplemental table 1. Overview of included deficits in the frailty index and used cut-off values

#	Item	Item(s) – additional information	Cutoff value	Source & reference
1	Dressing and grooming	Able to get clothes from closets or drawers; able to dress; able to shampoo your hair; able to comb your hear or do your make up	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Stanford Health Assessment Questionnaire (32)
2	Arising	Able to stand up from a straight chair without using your arms for support; able to get in and out of bed	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Stanford Health Assessment Questionnaire (32)
3	Eating	Able to cut meat and lift a full cup or glass to your mouth; able to open a new carton of milk	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Stanford Health Assessment Questionnaire (32)
4	Walking	Able to walk outdoors on flat ground; able to climb up five steps	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Stanford Health Assessment Questionnaire (32)
5	Hygiene	Able to wash and dry your entire body; able to take a shower/bath	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Stanford Health Assessment Questionnaire (32)
6	Reach	Able to reach and get down a 1kg object from just above your head; able to bend down to pick up clothing from the floor	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Stanford Health Assessment Questionnaire (32)
7	Grip	Able to open a car door? Able to open jars which have been previously opened	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Stanford Health Assessment Questionnaire (32)
8	Riding a bike	Able to ride a bike	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Lawton Instrumental Activities of Daily Living scale (33)
9	Telephone	Able to use the telephone	Without any difficulty = 0 With some difficulty or using a customized phone = 0.33 With much difficulty = 0.66 Unable to do = 1	Lawton Instrumental Activities of Daily Living scale (33)
10	Meal	Able to prepare meals	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Lawton Instrumental Activities of Daily Living scale(33)

Supplemental table 1. Overview of included deficits in the frailty index and used cut-off values (Continued)

#	Item	Item(s) – additional information	Cutoff value	Source & reference
11	Gardening	Able to maintain garden	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Lawton Instrumental Activities of Daily Living scale (33)
12	Laundry	Able to do the laundry	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Lawton Instrumental Activities of Daily Living scale (33)
13	Financial	Able to do finances	Without any difficulty = 0 With some difficulty = 0.33 With much difficulty = 0.66 Unable to do = 1	Lawton Instrumental Activities of Daily Living scale(33)
14	Depressed affect	I felt that I could not shake off the blues even with help from family or friends; I felt depressed; I thought my life had been a failure; I felt lonely; I had crying spells; I felt sad	Rarely or none of the time = 0 Some or a little of the time = 0.33 Occasionally or a moderate amount of time = 0.66 Most or all of the time = 1	The CES-D scale: a self-report depression scale (34)
15	Positive affect	I felt that I was just as good as other people; I felt hopeful about the future; I was happy; I enjoyed life	Rarely or none of the time = 1 Some or a little of the time = 0.66 Occasionally or a moderate amount of time = 0.33 Most or all of the time = 0	The CES-D scale: a self-report depression scale (34)
16	Somatic and retarded activity	I did not feel like eating my appetite was poor; I had trouble keeping my mind on what I was doing felt that everything I did was an effort; I felt fearful; my sleep was restless; I talked less than usual; I could not get "going"	Rarely or none of the time = 0 Some or a little of the time = 0.33 Occasionally or a moderate amount of time = 0.66 Most or all of the time = 1	The CES-D scale: a self-report depression scale (34)
17	Interpersonal	I was bothered by things that usually don't bother me; people were unfriendly; I felt that people dislike me	Rarely or none of the time = 0 Some or a little of the time = 0.33 Occasionally or a moderate amount of time = 0.66 Most or all of the time = 1	The CES-D scale: a self-report depression scale (34)
18	Falling	How often did you fell the past 12 months?	No falling = 0 Less than once a month = 0.5 More than once a month = 1	
19	Joint complains	Did you have joint pain or other complains from the knees, hips, back or hand?	No = 0 Yes = 1	

Supplemental table 1. Overview of included deficits in the frailty index and used cut-off values (Continued)

#	Item	Item(s) – additional information	Cutoff value	Source & reference
20	Mobility	Do you use any support to walk?	No = 0 Walking aid = 0.5 Wheelchair = 1	
21	Forgetfulness	Do you sometimes forget what you was about to do?	No = 0 Yes = 1	
22	Aphasia	Do you have difficulties with finding the right words?	No = 0 Yes = 1	
23	Liver enzymes	ALAS, ALAT, Gamma-glutamyl transpeptidase	All values within the range = 0 One or more abnormal values = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
24	Vitamin D		Value > 50 nmol/L = 0 Value 30-50 nmol/L = 0.5 Value < 30 nmol/L = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
25	Sex hormon-binding globulin		Male SHBG 10-70 nmol/L = 0 Other values = 1 Female SHBG 20-120nmol/L = 0 Other values = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
26	CRP		Values < 10 mg/ml = 0 Values ≥ 10 mg/ml = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
27	Creatinine		Male 65-115 umol/L = 0 Other values = 1 Female 55-90 umol/L = 0 Other values = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
28	Uric acid		Male 0.20-0.42 mmol/L = 0 Other values = 1 Females 0.12-0.34mmol/L = 0 Other values = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC

Supplemental table 1. Overview of included deficits in the frailty index and used cut-off values (Continued)

#	Item	Item(s) – additional information	Cutoff value	Source & reference
29	Pro BNP		Values < 15 pmol/L = 0 Values ≥ 16 pmol/L = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
30	Homocysteine		Values 6-19 umol/L = 0 Other values = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
31	Hyperlipidemia	High cholesterol or medication against high cholesterol	Statin use and/or cholesterol >6.5 mmol/L No statin use and cholesterol 2.9-6.5 mmol/L	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
32	HDL		HDL ≥ 1.55 = 0 HDL < 1.55 = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
33	Systolic blood pressure	Measure three times, average is taken	Systolic blood pressure 90-140 = 0 Systolic blood pressure 140-160 = 0.5 Systolic blood pressure < 90 = 0.5 Systolic blood pressure > 160 = 1	Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus MC
34	MMSE	Mini Mental State Examination	Unimpaired >25 = 0 Impaired ≤ 25 =1	
35	LDST	Letter-Digit Substitution Test: the number of correct digits	Above mean or less than 1SD below mean = 0 One SD below mean = 0.5 Two SD below mean = 1	
36	STROOP	Stroop test	Above mean or less than 1SD above mean = 0 One SD above mean = 0.5 Two SD above mean = 1	
37	WFT	Word Fluency test	Above mean or less than 1SD below mean = 0 One SD below mean = 0.5 Two SD below mean = 1	

Supplemental table 1. Overview of included deficits in the frailty index and used cut-off values (Continued)

#	Item	Item(s) – additional information	Cutoff value	Source & reference
38	Cancer		No = 0 Yes = 1	
39	Lung condition (COPD/ Asthma)		No = 0 Yes = 1	
40	Cardiovascular diseases		No = 0 Yes = 1	Prevalent CHD (=MI and/or revascularization) M.J.G. Leening - 29.05.2014 (35)
41	Stroke		No = 0 Yes = 1	Prevalent stroke (35)
42	Diabetes Mellitus		No = 0 High glucose= 0.5 Yes = 1	Prevalent DM
43	BMI		Normal weight = 0 Overweight = 0.5 Obese or underweight = 1	
44	Hospital admission	Last 12 months	No = 0 Yes = 1	
45	Age-related macular degeneration	Fundus photography after pharmacologic mydriasis. The eyes of each participant were graded and classified separately, and the eye with the more severe grade was used to classify the person.	0 = 5-year risk of developing advanced AMD in at least one eye is 0.5% 0.25 = 5-year risk is 3% 0.50 = 5-year risk is 12% 0.75 = 5-year risk is 25% 1= 5-year risk is 50%	(36)

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6

SUMMARY/ SAMENVATTING

6.1. SUMMARY

The main aim of this thesis was to evaluate dietary intake in relation to various aspects of bone health in Dutch middle aged and elderly people. Dietary intake was assessed using Food Frequency Questionnaires (FFQs). These FFQs included a large number of food items and asked if and how often our participants habitually consumed the food items. Bone mineral density (BMD) was used as an important measure of bone health. BMD reflects the degree of mineralisation of the bones. Additionally, more novel measures of bone health that provide information on the structure of the bones were covered. Examples are the Trabecular Bone Score (TBS, a reflection of trabecular integrity) and structural measurements of hip, such as cortical bone width. Moreover, risk of fractures of different types was studied. The main findings from this thesis are summarized next.

Main findings on socio-economic indicators and dietary quality

What we eat is determined by a variety of factors, including cultural habits and local food availability. Our dietary pattern might be influenced by indicators of our socio-economic status. Therefore, we studied associations between socio-economic indicators and dietary quality, defined as adherence to national dietary guidelines in **chapter 2**. We observed that higher education but not income or previous occupation were associated with better dietary quality at baseline, and with more improvement of dietary quality over time. Subsequently, we studied associations between dietary intake and bone health. In these analyses, socio-economic indicators were considered as potential confounders.

Main findings on nutrients and bone health

In **chapter 3**, we studied associations between specific nutrients and bone health. Nutrients included vitamins and minerals derived from the diet, such as vitamin A and potassium, but also included measures of nutrients in plasma, such as uric acid. Some previous studies showed that high intake of dietary vitamin A was associated with low BMD and increased risk of fractures. Studies in animals showed that retinol, the biologically active form of vitamin A, inhibited activity of bone forming cells and stimulated the formation of the bone resorbing cells. Our results did not confirm these findings. In contrast, we observed a favorable relationship between high vitamin A dietary intake and fracture risk in overweight subjects where the association between vitamin A and BMD was mainly explained by BMI. We studied vitamin A derived from dietary sources. Based on our results, we cannot draw any conclusion regarding the effect of (extremely) high intakes of supplemental vitamin A (**chapter 3.1**).

Additionally, we studied dietary acid load (DAL) in relation to bone outcomes. A high DAL reflects a diet that is rich in nutrients that are metabolized to non-carbonic acids (e.g. sulfuric acid from the metabolism of protein) in amounts that exceed the amount of alkali bicarbonate produced from combustion of organic salts (such as potassium chloride in vegetable foods). It has been suggested that long-term consumption of such a diet might cause mild but chronic systemic acidosis (i.e. an altered pH of the blood). Under these circumstances, the bone might serve as the primary buffering system for alkali components such as calcium and potassium. We studied DAL in relation to the Trabecular Bone Score (TBS), a novel measure of trabecular bone integrity, and BMD. DAL was calculated using three indices. The first was the net endogenous acid production (NEAP) based on the ratio of total protein to potassium. Additionally, two indices based on the ratios of animal or vegetable protein to potassium (AnPro/K and VegPro/K) were studied. NEAP was associated with low TBS. Associations of AnPro/K and VegPro/K and TBS were non-linear and differently shaped. No significant associations of DAL with BMD were observed, nor was any significant interaction between DAL and renal function. Mainly in participants with high intake of dietary fibre, DAL might be detrimental to bone (**chapter 3.2**).

In addition to nutrient intakes based on FFQ data, we studied plasma values of uric acid. Uric acid is the final product of purine metabolism and, therefore, it has been viewed as a metabolic byproduct. In excess, uric acid may cause gouty arthritis. The effect of uric acid in skeletal metabolism remains to be unraveled. In our population, higher levels of serum uric acid were associated with higher BMD (at expense of thicker cortices and narrower bone diameters) and may be a protective factor in bone metabolism. These associations were more prominent in older individuals (65+) and in participants with intakes of vitamin C above the median (**chapter 3.3**). Purines are present in a variety of food products, including meat, fish and grains. However, it is important to realize that the contribution of diet to the overall variation in uric acid concentrations is limited. The breakdown of purines from DNA and RNA is a main endogenous contributor to variation in uric acid.

Altogether, our findings emphasize the importance of specific single nutrients in relation to measures of bone health. Other factors may be important too, including nutrient interactions or the animal or plant-based source from which the nutrient is derived. Over the last decades, several statistical approaches to identify and study associations between overall dietary patterns in relation to health outcomes have been developed. Studying dietary patterns might help us to identify potential additive, synergistic, or antagonistic effects between components of the full diet that may affect bone outcomes (2). Also, cumulative effects of a combination of nutrients on BMD might be easier to identify than the effect of a single nutrient, which might be too small to detect (3).

Findings from a whole diet approach

Therefore, in **chapter 4,** we used a number of different approaches to identify dietary patterns. We studied associations between adherence to these patterns and BMD in **chapter 4.1 and 4.2.** In **chapter 4.1** we studied dietary patterns that naturally occurred (e.g. explained most of the variance in overall dietary intake) in the Dutch elderly of the Rotterdam Study and which are associated with BMD against a background of relatively high dairy intake. Independent of anthropometrics, a "Health conscious" dietary pattern, high in fruits, vegetables, yoghurt, fish, poultry, alcohol and low in sweets may have benefits for BMD, whereas a "Processed" dietary pattern high in processed meat, ready to eat meals and alcohol and low in yoghurt may pose a risk for low BMD (**chapter 4.1**).

Similar dietary pattern analyses on BMD have been performed in elderly from different countries in Europe, Asia, Australia and the Americas. However, no diet score exists that summarizes the features of a diet that is optimal for BMD. Our aims were (a) to develop a BMD-Diet Score reflecting a diet that may be beneficial for BMD based on the existing literature, and (b) to examine the association of the BMD-Diet Score and the Healthy Diet Indicator. The BMD-Diet Score is based on guidelines of the World Health Organization, including measures of BMD in Dutch elderly participating in the Rotterdam Study, a prospective cohort study. After adjustment, the BMD-Diet Score was positively associated with BMD. This effect size was approximately three times as large as has been observed for the Healthy Diet Indicator.

Whereas dietary patterns are widely studied in relation to BMD, less evidence is available on their relationship to parameters of bone geometry. We identified two dietary patterns that explained most of the variance in measures of bone geometry in our population. Based on our findings, a "fruit, vegetables and dairy" pattern might increase BMD, cortical thickness, bone stability and fracture risk, whereas a "Low Meat" pattern low in meat, meat substitutes and poultry and high in porridge and sweets might affect bone health unfavorably with increased bone width and reduction of bending strength (**chapter 4.3**).

To conclude, impaired bone health is only one aspect of the functional loss that accompanies ageing. The frailty index is an instrument based on the accumulation of health deficits. Therefore, studying diet in relation to this index provides insights on the association with frailty during ageing in the broader perspective. In this population of middle-aged and elderly participants, we observed no consistent cross-sectional association between dietary pattern adherence and frailty. Future studies with a longitudinal design are needed to study the associations with changes in frailty during follow-up (**chapter 4.4**).In summary, findings from these overall dietary pattern analyses showed that different population-specific dietary patterns could be identified that might favour bone outcomes. Comparison with dietary patterns identified in

other populations and their association with BMD showed comparable results in terms of food groups. Overall, a diet high in fruits, vegetables, dairy products and animal sources of protein other processed meat, like fish and poultry, might favour bones in our population.

In conclusion, socioeconomic indicators are associated with dietary patterns and should be carefully considered when studying diet in relation to health. Single nutrients beyond dietary calcium and vitamin D as well as dietary patterns are associated with bone outcomes in the middle aged and elderly. Associations with fracture risk are not always explained by BMD, but other characteristics of the bone such as trabecular microarchitecture, bending strength and cortical instability might play a role in these associations.

6.2. NEDERLANDSE SAMENVATTING

Het belangrijkste doel van dit proefschrift was het evalueren van de relatie tussen voeding en botgezondheid en bij Nederlandse mensen van middelbare en oudere leeftijd. De voeding is nagevraagd met behulp van voedselvragenlijsten. In deze vragenlijsten hebben we nagevraagd welke voedingsmiddelen de deelnemers in onze studie consumeren en hoe vaak. Botgezondheid werd onder andere bepaald door het meten van de dichtheid van het bot, een maat voor mineralisatie. Naast botdichtheid werden in dit proefschrift ook nieuwere metingen van botgezondheid bestudeerd, die informatie geven van de structuur van het bot. Ook is er gekeken naar het risico op botbreuken op de langere termijn. De belangrijkste bevindingen van dit proefschrift zijn samengevat in de volgende paragrafen.

Bevindingen met betrekking tot sociaaleconomische positie en kwaliteit van de voeding

We weten dat onze voedselkeuze wordt beïnvloed door verschillende factoren, zoals culturele gewoontes en de beschikbaarheid van voeding in onze omgeving. De mate waarin we kiezen voor bepaalde voedingsmiddelen kan worden beïnvloed door onze sociaaleconomische positie in de maatschappij. Daarom hebben wij in **hoofdstuk 2** gekeken of er een verband was tussen verschillende indicatoren van de sociaaleconomische status (inkomen, opleidingsniveau en laatste beroep) en de mate waarin ouderen zich houden aan de Nederlandse Richtlijnen Goede Voeding. Wij hebben gevonden dat ouderen met een hoger opleidingsniveau zich beter aan de richtlijnen houden. Vervolgens hebben we de relatie voeding en botgezondheid op verschillende manieren bestudeerd.

Belangrijkste bevindingen met betrekking tot voedingsstoffen en botgezondheid

In **hoofdstuk 3** is gekeken naar losse voedingsstoffen, ook wel nutriënten genoemd. Daarbij valt te denken aan vitamines en mineralen uit de voeding, zoals vitamine A en kalium, maar ook aan stoffen die in het bloed kunnen worden gemeten, zoals urinezuur. Uit eerdere studies is gebleken dat een hoge inname van vitamine A gerelateerd zou zijn aan lagere botdichtheid en een hoger risico op botbreuken. Studies bij dieren hebben aangetoond dat retinol, de biologisch actieve vorm van vitamine A, de activiteit van botvormende cellen kan verminderen en de vorming van cellen die het bot afbreken kan stimuleren. Echter, onze resultaten hebben deze eerdere resultaten niet bevestigd. Wij vonden een verband tussen een hogere vitamine A inname en een lager risico op botbreuken. Verdere analyses toonden aan dat dit verband vooral aanwezig was in mensen met overgewicht. Het verband tussen hoge vitamine A inname en botbreuken werd echter niet volledig verklaard doordat de

botdichtheid hoger was bij de mensen met een hoge vitamine A inname. Mogelijk spelen andere factoren zoals balans of slecht zicht een rol in deze relatie. Deze studie richtte zich op de effecten van vitamine A uit de voeding. Over de effecten van extreem hoge innames van vitamine A uit supplementen kunnen geen uitspraken worden gedaan op basis van deze studie (**hoofdstuk 3.1**).

Daarnaast hebben we gekeken het verband tussen de zuurvormende potentieel (Engels term "dietary acid load") van de voeding en botdichtheid. Ook hebben we in deze studie een relatief nieuwe maat voor botgezondheid bekeken: de Trabeculaire Bot Score (TBS). Deze maat zegt iets over de structuur van het sponsachtige, zachte heup bot. Deze potentieel van de voeding om zuur te vormen is gebaseerd op de verhouding tussen de inname van zuurvormende en basevormende nutriënten. Zo worden eiwitten bijvoorbeeld afgebroken tot zuren en kalium to basen tijdens de vertering. Sommige studies hebben aangetoond dat een eetpatroon met een hoge zuurvormende potentieel het risico op botbreuken kan verhogen omdat het calcium uit het bot als buffer wordt gebruikt om de zuurvorming in het bloed te neutraliseren. We vonden een verband tussen een hoge zuurvormende potentieel en een lage TBS. Dit verband was anders wanneer de zuurvormende potentieel werd berekend op basis van eiwitten uit dierlijke producten dan op basis van plantaardige producten. Ook vonden we aanwijzingen dat dit negatieve verband mogelijk alleen geldt voor mensen met een verminderde nierfunctie en een hoge vezelinname **(hoofdstuk 3.2**).

Naast directe kwantificatie van nutriënten uit de voeding van onze deelnemers hebben we ook gekeken naar urinezuur in het bloed. Deze stof komt onder andere vrij bij de afbraak van purines uit de voeding. In onze studie hadden mensen met hogere urinezuurspiegels in het bloed botten met een hogere dichtheid, dikkere wanden en een kleinere diameter. Deze relatie was het sterkst in mensen ouder dan 65 jaar en in mensen met een hoge vitamine C inname (**hoofdstuk 3.3**). Purines komen voor in verschillende voedingsmiddelen, waaronder vlees, vis en graanproducten. Het is echter belangrijk om ons te realiseren dat de afbraak van purines uit de voeding slechts een kleine bijdrage leveren aan onze urinezuurspiegels. De meerderheid van het urinezuur wordt gevormd in het lichaam zelf tijdens de afbraak van purines afkomstig van DNA en RNA.

Samengevat laten onze bevindingen uit hoofdstuk 3 zien dat losse voedingsstoffen belangrijk zijn voor botgezondheid. Ze benadrukken echter ook dat verbanden verschillend kunnen zijn wanneer deze voedingsstoffen afkomstig zijn uit verschillende voedingsmiddelen en dat interactie tussen voedingsstoffen kan optreden.

Belangrijkste bevindingen op het gebied van complete eetpatronen

Het bestuderen van complete eetpatronen als toevoeging op het bestuderen van losse nutriënten heeft een aantal belangrijke voordelen. Ten eerste stelt het ons in staat om potentiele interacties tussen nutriënten mee te nemen. We weten

bijvoorbeeld uit eerdere studies dat calcium beter wordt opgenomen wanneer het wordt geconsumeerd samen met vitamine D maar juist slechter wordt opgenomen wanneer het wordt geconsumeerd samen met vezels. Daarnaast zijn de resultaten van studies over complete eetpatronen makkelijker te vertalen naar voedingsrichtlijnen voor het algemene publiek. Daarom hebben we verschillende methoden toegepast om eetpatronen te identificeren. Vervolgens hebben we de verbanden tussen deze eetpatronen en botdichtheid bestudeerd (**hoofdstuk 4.1 en 4.2**).

Eetpatronen kunnen op verschillende manier worden vastgesteld, bijvoorbeeld door te kijken welke combinaties van voedselgroepen de meeste variantie van hun totale inname verklaren. Op deze manier hebben we twee eetpatronen vastgesteld die onafhankelijk van onder andere lichaamsgewicht en lengte gerelateerd waren aan botdichtheid. Een "Gezondheidsbewust" eetpatroon rijk aan groente, fruit, yoghurt, vis en kip en laag in zoetwaren was gerelateerd aan een hoge botdicht. Dit in tegenstelling tot een "bewerkt" eetpatroon rijk aan kant- en klaar maaltijden en alcoholische dranken en laag in yoghurt, dat juist gerelateerd was aan een lagere botdichtheid (**hoofdstuk 4.1**).

Vergelijkbaar met onze studie in hoofdstuk 4.1, is ook in verschillende andere landen en bevolkingsgroepen gekeken welke eetpatronen een verband hadden met botdichtheid. We hebben de resultaten van verschillende gepubliceerde studies over eetpatronen en botdichtheid samengevat in één score, die we de "BMD-Diet Score" hebben genoemd. Voor de ontwikkeling van deze score zijn studies gebruikt uit andere Europese landen, zoals het Verenigd Koninkrijk en Griekenland, maar ook uit landen als Azië, Amerika en Australië. Deze score is gebaseerd op de consumptie van 6 voedselgroepen die zouden kunnen bijdragen aan een hoge botdichtheid, namelijk groente, fruit, volkoren granen, zuivel, vis en peulvruchten en van 2 voedselgroepen die zouden kunnen bijdragen aan een lage botdichtheid, namelijk vlees en zoetwaren. Wij vonden inderdaad een verband tussen deze "BMD-Diet Score" en hoge botdichtheid. Dit verband met botdichtheid was 3 keer zo sterk als het verband tussen een score die de voedingsrichtlijnen van de Wereldgezondheidsraad reflecteert met botdichtheid (**hoofdstuk 4.2**).

Botgezondheid is breder dan botdichtheid alleen. Daarom hebben we ook twee eetpatronen vastgesteld die de meeste variantie in botdichtheid en in geometrie van het bot verklaren. Onze studie liet een verband zien tussen een eetpatroon gekenmerk door hoge inname van groente, fruit en zuivel met een hogere botdichtheid, stabielere compacte botten met dikkere wanden en een lager risico op breuken van het (heup) bot. Dit patroon had overeenkomsten met het "Gezondheidsbewuste" patroon, maar zuivelproducten hadden er een prominentere rol in. Tot slot hebben we een verband gevonden tussen een eetpatroon gekenmerkt door hoge innames van zoetwaren en dierlijk vet en lage innames van vlees, kip en vleesvervangers met een grotere diameter

van het compacte bot, een verminderde buigkracht en een hoger risico op breuken van het (heup)bot. De relatie tussen dit patroon en botbreuken was dus onafhankelijk van de botdichtheid (**hoofdstuk 4.3**).

Een verminderde botgezondheid is een belangrijk aspect van de kwetsbaarheid die ouderen kunnen ervaren. Om dit proefschrift mee te eindigen hebben wij daarom gekeken naar de relatie tussen voeding en de "kwetsbaarheidsindex". De index is een brede maat waarin verschillende aspecten van kwetsbaarheid worden meegenomen, waaronder functionele status, gemoedstoestand en bloedwaarden. Wanneer verschillende eetpatronen, waaronder een mate voor het eten volgens de Richtlijnen Goede Voeding en de kwetsbaarheidsindex op hetzelfde moment werden bepaald, leek er geen consistent verband tussenbeide te zijn. Echter, in deze jongste groep deelnemers van de Rotterdam Studie zullen lange termijn studies moeten uitwijzen of er een verband is tussen bepaalde eetpatronen en veranderingen in kwetsbaarheid tijdens het ouder worden (**hoofdstuk 4.4**).

CONCLUSIE

We vonden een verband tussen opleidingsniveau en de kwaliteit van de voeding in mensen van middelbare en oudere leeftijd. Daarnaast hebben we verbanden gevonden tussen verschillende losse voedingsstoffen of eetpatronen en verschillende maten van botgezondheid. Verbanden tussen voeding en het risico op botbreuken werden niet altijd verklaard door botdichtheid, maar ook andere maten die iets zeggen over de structuur van bot, lijken een rol te spelen. Lange termijn studies zijn nodig om de relatie tussen eetpatronen en algehele kwetsbaarheid verder te bestuderen.

7

APPENDICES

APPENDIX 1: ABBREVIATIONS

AHEI:	Alternate Healthy Eating Index
AIC:	Akaike Information Criterium
AnPro/K	Animal protein to potassium ratio
BMD	Bone Mineral Density
BMI	Body Mass Index
BR:	Buckling Ratio; a hip structural measure reflecting bone instability
CI	Confidence Interval
CRP:	C - reactive protein
CVD:	Cardiovascular Disease
DAL	Dietary Acid Load
DHDI	Dutch Healthy Diet Index
dPRAL	Dietary Potential Renal Acid Load
DXA	Dual X-ray Absorptiometry
eGFR	Estimated Glomerular Filtration Rate
ERGO	Erasmus Rotterdam Gezondheid Onderzoek
FFQ	Food Frequency Questionnaire
FI:	Frailty Index
FN-BMD:	Femoral Neck BMD
GEE	Generalized Estimation Equations
HBGP	Hip Bone Geometry Parameters
HDI:	Healthy Diet Indicator
HDL	High-Density Lipoproteins
HR:	Hazard Ratio
HRT	Hormone Replacement Therapy
ICP	International *classification* of functioning, disability and health
ICD-10	International Classification of Diseases (10th edition)
IQR	Interquartile Range
MDS:	Mediterranean Diet Score
MET	Metabolic Equivalent of Task
MMSE	Mini Mental State Examination
NEAP	Net Endogenous Acid Production
NEVO	Nederlandse Voedingsmiddelentabel (Dutch Food Composition Table)
PCA:	Principal Component Analysis
PUFA:	Polyunsaturated Fatty Acids
RAE:	Retinol Activity Equivalents
RE	Retinol Equivalents
RFS:	Recommended Food Score
RRR:	Reduced Rank Regression

RS	Rotterdam Study
SD	Standard Deviation
SES	Socio-Economic Status
SFA	Saturated Fatty Acids
SM:	Section Modulus; a hip structural measurement reflecting bending strength
SNP:	Single Nucleotide Polymorphism
TBS	Trabecular Bone Score
TFA	Trans Fatty Acids
T2DM	Type 2 Diabetes Mellitus
UA:	Uric Acid
VegPro/K	Vegetable protein to potassium ratio
VIF	Variance inflation Factor
WHO	World Health Organization

APPENDIX 2: PHD PORTFOLIO

Name PhD student: Ester de Jonge
Erasmus MC department: Epidemiology/ Internal Medicine
Research school: NIHES/ Molmed

PhD training	Year	Workload (ECTs)
Netherlands Institute of Health Sciences (NIHES)		
Msc Health Sciences, specialization Epidemiology	2013-2015	70
General courses		
Molmed Basic Introduction Course on SPSS	2013	1.0
Molmed Introductory Course on Statistics & Survival Analysis for Research master/ PhD students & MDs	2012	0.5
Specific courses		
English Biomedical writing	2014	1.5
Basic course on R	2015	1.5
Didactische vaardigheden	2015	0.5
Seminars and workshops		
Vena Workshop "Onderhandelen"	2013	0.1
Total energy in nutritional research/ PCA	2012	0.2
NWO Nutrition Science days, Deurne	2013	0.2
Symposium "Gezonde voeding, gezond ouder worden", Wageningen University	2014	0.2
Course scientific integrity	2015	0.2
Mediawoordvoering (Nederlandse Academie van Voedingswetenschappen)	2016	0.2

	Year	Workload (ECTs)
Oral presentations		
Annual meeting Nederlandse Vereniging voor Calcium en Botstofwisseling	2013	0.5
NWO Nutrition Science days, Deurne	2014	0.5
Healthy Living, EC of Epidemiology, Maastricht	2015	1.0
Conferences/ poster presentations		
CHARGE Meeting Rotterdam	2013	0.5
Annual Meeting of the European Society for Calcified Tissues (ECTS), Rotterdam (poster)	2015	0.2
Collaborations		
UMC Utrecht (J. Praagman)	2014-2015	2.0
Chinese Academy of Sciences and University of Chinese Academy of Sciences (F. Wang)	2015	1.5

Teaching	**Year**	**Workload (ECTs)**
Assistant Erasmus Summer Programme, couse Basic principles of Epidemiology	2015	0.5
Supervising Master's theses		
Medical student (T. Aboudoulaye)	2014	2.0
Medical student (S. Nunn)	2015	1.0
Student Health Sciences (S. Haas)	2015	0.5
TOTAL		**86**

1 ECT represents a study load of 28 hours.

APPENDIX 3: PUBLICATION LIST

Publications based on this thesis

de Jonge E.A.L., Kiefte-de Jong J.C., Campos-Obando N, Booij L., Oscar H. Franco, Hofman A., Uitterlinden A.G., Rivadeneira F., Zillikens M.C., Vitamin A, bone mineral density and fracture risk in the elderly, Eur J Clin Nutr. 2015 Sep16

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APPENDIX 5: ABOUT THE AUTHOR

Ester de Jonge was born and raised in a family of four in Vlissingen, the Netherlands. She obtained her bachelor degree in Food Technology (HAS University of Applied Sciences, Den Bosch) in 2004. After studying the chemistry and physics of food manufacturing, she became curious to learn more about the physiological effects of these foods on human health. Therefore, she continued her studies with a program in nutritional sciences and obtained her master degree in Nutrition and Health (Wageningen University) in 2007.

During her master studies, she spent 5 months as an intern in Cambridge, UK at the department of Human Nutrition Research. After graduating, she worked for five years as a scientist in the area of Nutrition, Health and Wellbeing at Unilever Research and Development. To further develop her scientific and analytical skills, she started a PhD-trajectory at the Erasmus University Medical Centre in 2013 at the departments of Epidemiology and Internal Medicine. As a nutritional epidemiologist, she studied associations between nutrients and overall dietary patterns in relation to bone health, of which the main results are outlined in this thesis. In 2016, Ester worked as a scientist at IVO Institute of Addiction Research in Rotterdam, where she was involved in projects on addiction and lifestyle in vulnerable populations. In her leisure time, she loves to take a walk on the beach, to travel to sunny countries and to enjoy drinks and bites with good friends and family.