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Clinical Investigations

The Effect of Nutrient Intake on Bone Mineral Status in Young Adults: The Northern Ireland Young Hearts Project

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Abstract. Optimizing peak bone mass in early life may reduce osteoporosis risk in later life. Such optimization may be partly dependent upon diet. In the present study, nutrient intakes and selected lifestyle parameters were assessed in adolescent subjects (238 males, 205 females; aged 15 y) and again, in the same subjects, on one occasion in young adulthood (aged between 20 and 25 y). The extent of the relationships between these parameters and bone mineral density (BMD), dual energy X-ray absorptiometry (DXA), lumbar spine (L2–L4), and femoral neck measured concurrently with diet in young adulthood only, was assessed.

Adjusted linear regression models were constructed. Variables included a measure of pubertal status (at age 15 y), age (at young adulthood), height, weight, physical activity, smoking, and mean daily intakes of energy, calcium, protein, vitamin D, phosphorus, total fat, and alcohol.

In both sexes, body weight at adolescence and young adulthood was the only factor consistently positively associated with BMD at both measurement sites. Effects of nutrient intake on BMD were inconsistent. Vitamin D and calcium intakes reported by female adolescents showed significant positive relationships with BMD measured in young adulthood (vitamin D measured at the lumbar spine; calcium measured at the femoral neck). The positive relationship between vitamin D and BMD remained significant at young adulthood, but at the femoral neck rather than at the lumbar spine. Also in females, intakes of phosphorus and the calcium:phosphorus ratio (Ca:P) at adolescence were strongly negatively related to femoral neck BMD measured at young adulthood. In males, however, Ca:P reported at young adulthood had a significant positive relationship with lumbar spine BMD,

whereas Ca:protein was negatively associated with BMD at the lumbar spine. Intakes of Ca reported by adolescent males also had a strong negative effect on lumbar spine BMD measured at young adulthood.

Key words: Adolescence — Bone mineral density — Nutrients — Young adulthood

Osteoporosis is widely recognized as a major public health problem [1]. Acquiring an optimal bone mineral density (BMD) in early life is thought to be of prime importance in prolonging skeletal adequacy and reducing the risk of osteoporosis in later life. The exact age of peak bone mass attainment is unclear [2, 3], but most investigators agree that, depending on skeletal site, at least 90% is accumulated by the end of early adulthood [4, 5].

Strong genetic influences on bone mass are recognized, but modifiable lifestyle factors, such as diet, are also likely to play an important role. Although dietary Ca and its effects on bone has received most attention, results to date have been inconsistent, with some studies demonstrating a positive association between high Ca intake and BMD [2, 6], and others reporting no association [7, 8].

Although vitamin D has been cited as a prerequisite for bone mineralization and Ca homeostasis [9], there is limited evidence for the effect of dietary vitamin D intake on bone status in young people. It has also been observed that high intakes of protein or phosphorus may be detrimental to the conservation of bone mass with aging. However, existing evidence is inconsistent and to date, much of the research has been carried out on older age groups [6, 10]. In addition, foods rich in Ca are often rich in protein, and although some studies have demonstrated a positive effect of a high Ca:protein ratio on BMD [2, 11], others have found no effect [12].

The aim of the present study was to explore the relationships between BMD measured on one occasion at young adulthood (subjects aged between 20 and 25 y) and diet related factors assessed in the same subjects on two occasions: adolescence (aged 15 y) and young adulthood (concurrent with the measurement of BMD).

Subjects and Methods

Study Population

This study was conducted as part of a longitudinal, observational study entitled The Young Hearts (YH) Project, which initially examined the prevalence of coronary risk factors in young people in Northern Ireland. Sampling procedures and study design have been described fully elsewhere [13]. In brief, the initial study (YH1) began in 1989/90 and involved 1015 randomly selected schoolchildren (12 year-old boys, $n = 251$; 12 year-old girls, $n = 258$; 15 year-old boys, $n = 252$; 15 year-old girls, $n = 254$). In 1992/93, the original 12 year-old population (then aged 15 y) was reassessed (YH2) using identical procedures. All YH1 subjects were invited to participate in the third phase (YH3), which took place between October 1997 and October 1999 when the subjects' ages were between 20 and 25 y. The response rate in YH3 was 48.2%. Of the 251 males and 238 females who agreed to participate in YH3, measurements of BMD were performed on 238 males and 205 females. For the purposes of this study, only complete data relating to subjects at age 15 y (from YH1 and YH2) and early adulthood (YH3) have been included. For each phase of the study, ethical approval was obtained from the Medical Research Ethical Committee of The Queen's University of Belfast, and written informed consent was obtained from all subjects prior to participation.

Dietary Intake Assessment

At all study time points, dietary data were obtained using the diet history method [14]. This consisted of a detailed, open-ended one-to-one interview, the purpose being to ascertain the habitual weekly food intake of each subject. The diet history method was used for two reasons. Firstly, it has been shown in the younger age group to provide more valid estimates of energy intake at the group level than weighed records [15]. Secondly, given that a diet history can be obtained from a subject in approximately 1 hour, it was the most feasible and cost-effective method for obtaining dietary information from a cohort of this size. Subjects were asked to report their usual weekly meal and snack food intake in terms of the frequency, amounts and methods of preparation of foods consumed. A photographic atlas and common household measures (e.g., cups, glasses, bowls, and measuring spoons) were used to assist in portion size estimations. Energy and nutrient intakes from foods, beverages, and alcohol were calculated using a computerized dietary analysis program based on UK food composition tables (WISP, Tinuviel Software, Warrington, UK). Nutrient intakes were standardized for energy intake by dividing the mean daily intake of each nutrient (in g/d, mg/d, or $\mu\text{g/d}$ depending on nutrient) by the mean daily energy intake (MJ/d).

Anthropometry and Other Measurements

Height and weight of each subject were measured at each time point. Standing height was measured to the nearest millimeter using a Harpenden portable stadiometer (Holtain, UK). Body weight was measured to the nearest 0.1 kg using an electronic balance (200 kg $\times$ 0.1 kg, SECA, Germany). For both measurements, subjects wore light indoor clothing and no shoes.

Lifestyle data were obtained at each time point by a questionnaire designed to assess socioeconomic status, smoking habits, and oral contraceptive use (in females). Habitual physical activity was assessed by self-report questionnaires. Physical activity scores in adolescence were calculated according to the method of Riddoch et al. [16] which assessed normal daily activity patterns based around the typical school day. Activities were scored from 1–100 according to their frequency, intensity, and duration. As the school-based questionnaire was irrelevant to subjects between 20 and 25 years of age, a modification of the Baecke questionnaire [17] was used in YH3 to quantify habitual work activity, sports activity, and non-sports leisure activity. Indices based on a five-point Likert scale were calculated for each activity and summed to give a physical activity score ranging from 3–15. Socioeconomic status was assessed using four measures: number of cars in household, years of education, highest qualification attained, and occupation of father during the subjects' adolescence. Pubertal status at 15 y was assessed by Tanner's stages of pubic hair development [18].

Assessment of BMD

In YH3, BMD of the lumbar spine region (L2-L4) and femoral neck (hip) were measured by DXA using the Lunar EXPERT-XL bone densitometer (Lunar Corporation, Madison, WI, USA) which has a precision of 1.0% *in vivo* and 0.5% *in vitro*. Prior to each scan, the densitometer was calibrated by a qualified radiographer, according to the manufacturer's recommendations. Separate scans were taken of the L2-L4 lumbar vertebrae and the femoral neck and the results were expressed as BMD (g/cm^2). Scans of females were only undertaken within 10 days of the start of their last menstrual period.

Statistical Analyses

Statistical analyses were performed using SPSS for Windows (version 9.0.0; SPSS Inc.). Independent samples *t*-tests were performed to determine whether the physical characteristics, at adolescence, of those subjects who agreed to participate in YH3 were significantly different from those who did not participate.

Linear regression analyses were then performed to examine the relationships between BMD of the lumbar spine and the femoral neck, and other diet and lifestyle factors. Data for males and females were analyzed separately. Two analyses were performed: unadjusted regression analyses in which relationships between BMD and lifestyle predictor variables were analyzed separately; and adjusted regression analyses in which lifestyle factors were analyzed together. Height, weight, and pubertal status were included in all models as they are well recognized as important for measurement and prediction of BMD. Results from these analyses were expressed as standardized regression coefficients (β), which represent the predicted change (in SD) of the dependent variable corresponding to a change of 1 SD of the explanatory variable, all other variables being held constant.

Results

The physical characteristics, at adolescence, of those participating in the current phase of the Young Hearts study (YH3), and those who did not participate, are presented in Table 1. Males who did not participate in YH3 were slightly heavier ($P = 0.04$) than their counterparts who agreed to take part, whereas females who did not participate in the current study were shorter ($P < 0.01$) at adolescence than participants. However, when weight, body fat, and physical activity at adolescence were categorized into quartiles, there was no dif-

Table 1. Physical characteristics and intakes of selected nutrients of subjects measured at adolescence (YH1 and YH2, age 15 y): comparison of participants and non-participants in YH3^a

	Males			Females		
	Participants (n = 251) Mean (SD)	Non-participants (n = 252) Mean (SD)	<i>P</i> ^b	Participants (n = 238) Mean (SD)	Non-participants (n = 274) Mean (SD)	<i>P</i> ^b
Height (m)	1.59 (0.12)	1.60 (0.13)	0.57	1.57 (0.08)	1.55 (0.80)	<0.01
Weight (kg)	49.5 (11.1)	51.8 (13.4)	0.04	50.7 (11.0)	49.8 (10.5)	0.34
% body fat	6.43 (0.82)	6.59 (0.98)	0.05	5.67 (0.52)	5.60 (0.49)	0.12
Physical activity score ^c	30.8 (15.1)	29.8 (15.7)	0.49	21.0 (13.8)	20.6 (12.6)	0.75
Total energy intake (MJ/d)	12.2 (3.2)	13.1 (3.9)	<0.01	9.5 (2.7)	9.6 (2.7)	0.67
Calcium (mg/d)	1151 (433)	1196 (485)	0.27	866 (320)	862 (362)	0.89
(mg/MJ/d)	94.8 (27.2)	92.3 (27.0)	0.30	92.4 (25.8)	90.5 (27.9)	0.45
Total fat (g/d)	124.6 (36.4)	134.6 (45.1)	<0.01	98.7 (33.4)	100.0 (32.8)	0.67
(g/MJ/d)	10.2 (1.1)	10.2 (1.09)	0.70	10.3 (1.19)	10.3 (1.19)	0.74
Vitamin D (µg/d)	1.75 (1.45)	1.73 (1.44)	0.85	1.55 (1.26)	1.68 (1.41)	0.30
(µg/MJ/d)	0.14 (0.11)	0.14 (0.11)	0.60	0.16 (0.12)	0.18 (0.14)	0.32

YH1, YH2, YH3; first, second, and third measurement phases of the longitudinal Young Hearts Study
^a Data for YH3 participants are for all subjects, including those who did not consent to having BMD measured
^b Differences between participants and non-participants assessed using independent samples *t*-test
^c Possible range of physical activity scores = 1–100

ference in attendance rates in the current study (YH3) between subjects who were in the highest quartile for these variables compared with attendance rates for subjects in the other three quartiles. There did appear to be a slight social class bias in the response rate. Significantly more subjects from the non-manual social classes (assessed at adolescence) participated fully in the current study than those who were from the manual social classes originally (Chi square 18.6, df 1, *P* < 0.01) (data not shown). Mean absolute intakes of energy and total fat in adolescent males were significantly higher in nonparticipants than in participants. However, when standardized by energy intake, no differences were evident in reported total fat intakes between participants and nonparticipants. Calcium and vitamin D intakes did not differ significantly between the two groups in either males or females. Attendance rates in the current study did not differ significantly between subjects who were alcohol consumers (defined as taking alcohol on at least one occasion) and non-alcohol consumers at adolescence (data not shown). When smoking status at adolescence was analyzed, there was no difference in participation in the current study (YH3) between non-smokers and subjects who had smoked on at least one occasion (data not shown).

For those subjects for whom a complete dataset was available, the physical characteristics measured at adolescence (age 15 y) and at young adulthood (one occasion between the ages of 20 y and 25 y) are presented in Table 2 (males) and Table 3 (females). Between adolescence and young adulthood, height and weight increased in both sexes. BMI increased with age in males and decreased in females.

Intakes of selected nutrients reported by males and females during adolescence and young adulthood are also presented in Tables 2 and 3. Sodium and potassium were not included in the analyses as it is not feasible to reliably assess intakes of these nutrients using the diet history method. Mean intakes of energy and total fat decreased in both sexes between adolescence and young adulthood. However, in both males and females, intakes of protein, phosphorus, vitamin D, and alcohol increased between the two time points. For both sexes, mean intakes of calcium, protein, and phosphorus exceeded the current UK reference nutrient intakes (RNI) [19] at both adolescence and young adulthood. In the UK, the RNI is defined as ‘the amount of the nutrient that is enough, or more than enough, for about 97% of people in a group’ [19].

Unadjusted Analysis of Dietary and Lifestyle Factors Affecting BMD

Table 4 presents the unadjusted regression analyses for the associations between BMD, measured on one occasion during young adulthood, and physical characteristics, observed at adolescence and again in the same subjects at young adulthood. For males, at both time points, greater weight and physical activity scores were significantly associated with higher lumbar spine and femoral neck BMD. At both measurement sites, there were significant positive associations between adolescent height and BMD measured at young adulthood. Greater BMI at adolescence was significantly associated with higher BMD at the femoral neck, but not at the lumbar

Table 2. Male physical characteristics and intakes of selected nutrients at 15 y and between 20 and 25 y (n = 238)

	Adolescence Mean (SD)	Young adulthood Mean (SD)
Height (m)	1.70 (0.74)	1.78 (0.07)
Weight (kg)	59.0 (8.9)	75.1 (11.5)
Body mass index (kg/m ²)	19.3 (3.8)	23.7 (3.1)
Physical activity (score) ^{a,b,c}	28.3 (14.5)	7.96 (1.4)
BMD lumbar spine (g/cm ²)	nm	1.25 (0.14)
BMD femoral neck (g/cm ²)	nm	1.14 (0.17)
Total energy intake (MJ/d)	13.6 (3.2)	13.1 (3.6)
Calcium (mg/d)	1263 (504)	1145 (469)
(mg/MJ/d)	92.9 (27.7)	88.0 (30.2)
Protein (g/d)	95.0 (25.5)	101.1 (28.5)
(g/MJ/d)	7.1 (1.1)	7.8 (1.4)
Phosphorus (mg/d)	1591 (483)	1806 (544)
(mg/MJ/d)	118.1 (25.3)	138.8 (26.8)
Calcium:protein ^d	13.2 (3.3)	11.2 (3.0)
Calcium:phosphorus ^e	0.79 (0.20)	0.63 (0.14)
Total fat (g/d)	137.6 (38.8)	113.1 (38.9)
(g/MJ/d)	10.1 (1.2)	8.6 (1.5)
Vitamin D (µg/d)	1.85 (1.59)	2.25 (1.6)
(µg/MJ/d)	0.14 (0.12)	0.17 (0.11)
Alcohol (g/d)	3.83 (9.2)	45.7 (40.4)
(g/MJ/d)	0.26 (0.61)	3.38 (2.69)

nm = not measured
^aPhysical activity: adolescence n = 234, young adulthood n = 236
^bMethod used for calculation of activity scores at age 15 y was different than that used for young adults
^cPossible range of physical activity scores: adolescence = 1–100; young adulthood = 3–15
^dCalcium:protein = ratio of calcium intake (mg/d) to protein intake (g/d)
^eCalcium:phosphorus = ratio of calcium intake (mg/d) to phosphorus intake (mg/d)

Table 3. Female physical characteristics and intakes of selected nutrients at 15 y and between 20 and 25 y (n = 205)

	Adolescence Mean (SD)	Young adulthood Mean (SD)
Height (m)	1.62 (0.61)	1.64 (0.06)
Weight (kg)	56.8 (8.9)	64.1 (11.1)
Body mass index (kg/m ²)	24.8 (4.5)	23.7 (3.9)
Physical activity (score) ^{a,b,c}	16.9 (10.8)	7.38 (1.20)
BMD lumbar spine (g/cm ²)	nm	1.19 (0.12)
BMD femoral neck (g/cm ²)	nm	1.05 (0.15)
Total energy intake (MJ/d)	9.46 (2.6)	8.34 (2.25)
Calcium (mg/d)	835 (331)	728 (275)
(mg/MJ/d)	89.4 (25.5)	88.8 (28.1)
Protein (g/d)	63.7 (18.7)	67.7 (19.7)
(g/MJ/d)	6.81 (1.21)	8.26 (1.73)
Phosphorus (mg/d)	1067 (358)	1162 (518)
(mg/MJ/d)	113.2 (22.3)	140.1 (39.2)
Calcium:protein ^d	13.2 (3.0)	10.9 (3.1)
Calcium:phosphorus ^e	0.79 (0.18)	0.64 (0.14)
Total fat (g/d)	97.2 (30.7)	73.8 (25.9)
(g/MJ/d)	10.2 (1.2)	8.8 (1.6)
Vitamin D (µg/d)	1.58 (1.19)	1.67 (1.15)
(µg/MJ/d)	0.17 (0.12)	0.20 (0.12)
Alcohol (g/d)	1.61 (3.65)	15.5 (17.4)
(g/MJ/d)	0.17 (0.39)	1.82 (1.93)

nm = not measured
^aPhysical activity: adolescence n = 200 and young adulthood n = 203
^bMethod used for calculation of activity scores at age 15 y was different than used for young adults
^cPossible range of physical activity scores: adolescence = 1–100 and young adulthood = 3–15
^dCalcium:protein = ratio of calcium intake (mg/d) to protein intake (g/d)
^eCalcium:phosphorus = ratio of calcium intake (mg/d) to phosphorus intake (mg/d)

Table 4. Unadjusted regression analyses for the relationships between physical characteristics of subjects assessed as adolescents (age 15 y) and again as young adults (between 20 and 25 y), and BMD measured when subjects were young adults

		Adolescence				Young adulthood			
		Lumbar spine BMD		Femoral neck BMD		Lumbar spine BMD		Femoral neck BMD	
		β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>
Pubertal status	Males	0.10	0.13	0.09	0.19	na	na	na	na
	Females	0.14	0.04	0.03	0.63	na	na	na	na
Age (y)	Males	na	na	na	na	−0.09	0.17	−0.19	< 0.01
	Females	na	na	na	na	0.09	0.18	−0.06	0.41
Height (m)	Males	0.15	0.02	0.16	0.01	0.07	0.28	0.09	0.19
	Females	0.19	< 0.01	0.19	< 0.01	0.19	< 0.01	0.20	< 0.01
Weight (kg)	Males	0.22	< 0.01	0.28	< 0.01	0.23	< 0.01	0.24	< 0.01
	Females	0.42	< 0.01	0.31	< 0.01	0.42	< 0.01	0.34	< 0.01
BMI (kg/m ²)	Males	0.07	0.29	0.17	0.01	0.23	< 0.01	0.23	< 0.01
	Females	0.32	< 0.01	0.12	0.08	0.37	< 0.01	0.26	< 0.01
Physical activity score	Males	0.18	< 0.01	0.22	< 0.01	0.24	< 0.01	0.19	< 0.01
	Females	0.11	0.11	0.14	0.04	0.06	0.37	0.04	0.60

BMD, bone mineral density (g/cm²): β , standardized regression coefficient; na, not applicable; BMI, body mass index

spine. At young adulthood, BMI had a positive impact on BMD at both measurement sites. Age at young adulthood had a significant negative impact on femoral neck BMD.

In contrast to the males, physical activity scores reported by the females at young adulthood had no significant effect on BMD at either measurement site. Only physical activity reported by the females during adolescence was significantly associated with femoral neck BMD measured in young adulthood. However, height and weight exhibited strong positive associations with BMD at both adolescence and young adulthood. The effect of weight on lumbar spine BMD in the females was almost twice as strong as that observed in males (adolescence: males β = 0.22 vs females β = 0.42; young adulthood: males β = 0.23 vs females β = 0.42). BMI in females at adolescence was also significantly associated with lumbar spine BMD measured in young adulthood. By the time the females reached young adulthood, this positive relationship between BMD and BMI was evident at both measurement sites. Although no significant age effects were apparent in the females, pubertal status assessed at 15 y was positively associated with lumbar spine BMD measured at young adulthood.

The unadjusted regression models for the association between BMD and dietary intakes reported at adolescence and young adulthood are presented in Table 5. In these analyses, intakes of calcium, protein, phosphorus, fat, vitamin D, and alcohol have been corrected for energy intake. There were few significant relationships between nutrient intakes reported at adolescence and BMD measured in young adulthood. The only exceptions were vitamin D, for which a significant positive relationship with BMD at the lumbar spine was apparent in females, and total energy intake which was sig-

nificantly positively associated with femoral neck BMD in males. At young adulthood a significant positive relationship between BMD and intakes of vitamin D reported by females was again apparent, this time occurring at the femoral neck. In males, there were no significant relationships between BMD and nutrient intakes reported at young adulthood.

To account for the fact that there were non-consumers of alcohol included in the unadjusted model, BMD measurements for non-consumers and consumers were compared using an independent samples *t*-test. No significant difference was found (data not shown). In addition, when subjects were divided into quartiles based on alcohol consumption reported during young adulthood, no linear trend for BMD was apparent.

Social class determined at adolescence and young adulthood was consistently unrelated to BMD in both sexes (data not shown). Males who reported cigarette smoking at adolescence (*n* = 45) had significantly lower femoral neck BMD at young adulthood than non-smokers (*n* = 187) (1.10 ± 0.17 vs 1.15 ± 0.17 g/cm², respectively, *P* < 0.05). No significant differences were apparent at the lumbar spine. At young adulthood, though male smokers (*n* = 91) still had lower femoral neck BMD than non-smokers (*n* = 152), this did not reach statistical significance. No significant difference was evident at the lumbar spine. BMD measurements did not differ significantly between female smokers and non-smokers during adolescence or young adulthood. Furthermore, in females, BMD measurements in oral contraceptive users (*n* = 88) were not significantly different from non-users (*n* = 123) (lumbar spine BMD = 1.18 ± 0.11 g/cm² vs 1.19 ± 0.13 g/cm², respectively, *P* = 0.42; femoral neck BMD = 1.03 ± 0.14 g/cm² vs 1.07 ± 0.15 g/cm², respectively, *P* = 0.09).

Table 5. Unadjusted regression analyses for the relationships between BMD of subjects measured at young adulthood (between 20 and 25 y), and dietary intakes (adjusted for energy intake) reported by these subjects at adolescence (age 15 y) and again at young adulthood

		Adolescence				Young adulthood			
		Lumbar spine BMD		Femoral neck BMD		Lumbar spine BMD		Femoral neck BMD	
		β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>
Total energy (MJ/d)	Males	0.08	0.20	0.17	<0.01	0.13	0.05	0.11	0.08
	Females	0.06	0.39	0.06	0.38	0.04	0.54	0.08	0.24
Calcium (mg/MJ/d)	Males	−0.06	0.39	0.07	0.30	−0.05	0.42	−0.05	0.41
	Females	0.06	0.39	0.04	0.56	0.02	0.82	−0.01	0.89
Protein (g/MJ/d)	Males	0.05	0.41	0.06	0.38	0.03	0.65	−0.02	0.74
	Females	0.02	0.76	0.06	0.43	0.07	0.33	0.03	0.68
Phosphorus (mg/MJ/d)	Males	0.03	0.69	0.01	0.93	−0.04	0.58	−0.04	0.55
	Females	0.07	0.31	−0.02	0.76	0.03	0.69	−0.02	0.76
Calcium:protein ^a	Males	−0.11	0.11	0.03	0.63	−0.08	0.22	−0.03	0.70
	Females	0.07	0.35	0.01	0.93	−0.03	0.66	−0.04	0.61
Calcium:phosphorus ^b	Males	−0.07	0.26	0.10	0.11	−0.03	0.60	−0.02	0.75
	Females	0.02	0.83	0.08	0.28	−0.01	0.88	−0.01	0.90
Total fat (g/MJ/d)	Males	−0.11	0.09	−0.04	0.57	−0.09	0.15	−0.05	0.44
	Females	0.11	0.12	0.02	0.80	−0.01	0.91	−0.07	0.31
Vitamin D (μg/MJ/d)	Males	−0.09	0.18	−0.08	0.24	0.08	0.22	0.10	0.14
	Females	0.19	<0.01	0.07	0.35	0.09	0.19	0.15	0.02
Alcohol (g/MJ/d)	Males	−0.02	0.71	−0.08	0.25	0.04	0.57	0.11	0.09
	Females	−0.09	0.19	−0.06	0.39	−0.07	0.28	−0.01	0.93

β , standardized regression coefficient; BMD, bone mineral density (g/cm²)
^aCalcium:protein = ratio of calcium intake (mg/d) to protein intake (g/d)
^bCalcium:phosphorus = ratio of calcium intake (mg/d) to phosphorus intake (mg/d)

Adjusted Analysis of Dietary and Lifestyle Factors Affecting BMD

Table 6 presents the results of the adjusted analysis undertaken to determine the extent of relationships between dietary and lifestyle factors, assessed in males at age 15 y and at young adulthood, and BMD which was measured at young adulthood only. At adolescence, the variables listed in Table 6 accounted for 16.3% and 18.8%, respectively, of the total variation in BMD measured in young adulthood at the lumbar spine and the femoral neck. At young adulthood, 16.7% (lumbar spine) and 18.6% (femoral neck) of the total variation in BMD was accounted for by the model. Of all the variables included in the adjusted model, weight at adolescence and young adulthood was the only factor significantly positively associated with BMD at both measurement sites. Greater physical activity scores at adolescence were associated with higher femoral neck BMD, whereas at young adulthood, physical activity was significantly positively associated with BMD at both measurement sites. Associations between BMD and nutrient intakes were inconsistent. Significant positive relationships with lumbar spine BMD were observed for calcium:phosphorus ratio reported by males at young adulthood. However, relatively strong significant, negative relationships with lumbar spine BMD, measured at young adulthood, were

apparent for Ca intake reported at adolescence, and for calcium:protein ratio at young adulthood.

The results of the adjusted analysis for relationships between BMD measured on one occasion at young adulthood and dietary and lifestyle parameters, reported by females at adolescence and at young adulthood, are presented in Table 7. The factors assessed during adolescence accounted for 26.7% and 17.4% of the total variation in BMD measured at the lumbar spine and femoral neck, respectively. At young adulthood, 24.1% (lumbar spine) and 20.8% (femoral neck) of the total variation was accounted for by the model. Again, weight was consistently positively associated with BMD at both measurement sites and at both time points. In contrast to males, however, there was no relationship between physical activity score and BMD. Age had a significant negative effect on BMD at the femoral neck.

There were few significant associations between reported nutrient intake and BMD in females. Significant positive effects were observed for vitamin D intake reported during both adolescence (lumbar spine) and young adulthood (femoral neck), and also for Ca intake reported during adolescence (femoral neck). Phosphorus intakes and Ca:P ratio reported at adolescence appeared to exert a significant negative effect

Table 6. Males: Adjusted regression analysis for relationships between dietary and lifestyle parameters observed at adolescence (15 y) and again at young adulthood (between 20 and 25 y), and BMD measured concurrently with diet during young adulthood

Variable	Adolescence				Young adulthood			
	Lumbar spine BMD R ² = 0.163		Femoral neck BMD R ² = 0.188		Lumbar spine BMD R ² = 0.167		Femoral neck BMD R ² = 0.186	
	β	P	β	P	β	P	β	P
Pubertal status	0.12	0.10	0.05	0.47	nm	nm	nm	nm
Age (years)	ni	ni	ni	ni	−0.11	0.11	−0.20	< 0.01
Height (m)	−0.03	0.76	−0.08	0.36	−0.09	0.26	−0.08	0.32
Weight (kg)	0.19	0.02	0.32	< 0.01	0.28	< 0.01	0.29	< 0.01
Physical activity (score)	0.11	0.10	0.17	0.01	0.22	< 0.01	0.18	< 0.01
Smoking (current vs non-smoker)	−0.11	0.10	−0.09	0.18	0.00	0.97	−0.10	0.12
Alcohol (g)	−0.08	0.24	−0.14	0.05	0.01	0.93	0.15	0.11
Total energy (MJ/d)	0.26	0.14	0.21	0.24	0.14	0.46	−0.04	0.85
Calcium (mg/d)	−1.03	0.01	−0.12	0.77	−0.41	0.29	−0.23	0.55
Protein (g/d)	0.53	0.13	−0.08	0.83	−0.62	0.13	−0.57	0.16
Phosphorus (mg/d)	0.29	0.41	0.22	0.53	0.95	0.08	0.74	0.17
Calcium:protein ^a	0.43	0.15	−0.10	0.73	−0.83	0.04	−0.66	0.09
Calcium:phosphorus ^b	0.10	0.67	0.22	0.34	0.86	0.03	0.65	0.09
Total fat (g/d)	−0.27	0.08	−0.08	0.59	−0.05	0.72	0.06	0.67
Vitamin D (µg/d)	−0.11	0.10	−0.07	0.27	0.06	0.39	0.11	0.12

R² = explained variation; β = standardized regression coefficient; BMD = bone mineral density (g/cm²); ni = not included in analysis; nm = not measured
^aCalcium:protein = ratio of calcium intake (mg/d) to protein intake (g/d)
^bCalcium:phosphorus = ratio of calcium intake (mg/d) to phosphorus intake (mg/d)

on BMD (femoral neck) measured at young adulthood. Height and intakes of energy, protein, calcium:protein and total fat, showed no significant relationships with BMD.

Discussion

One limitation of the present study was the relatively low response rate. In the first and second phases of the Young Hearts Project (YH1 and YH2), all of the test protocols were carried out in schools throughout Northern Ireland. In YH1, the response rate was 78%, and in YH2, approximately 90% of the eligible YH1 cohort agreed to be retested. In the current study (YH3), subjects were contacted by letter and asked to attend a single testing center based in a Belfast hospital. Although it could be suggested that the relatively low response rate (48.2%) may have biased the results, it is important to note that physical characteristics at adolescence were generally not a major predictor of whether subjects were likely to return for testing in young adulthood (YH3). However, there did appear to be a slight response bias in terms of social class assessed at adolescence. More subjects from non-manual social classes returned for testing in YH3 than those from manual social classes. The higher mean intakes of energy and total fat in non-participating males may also be associated with social class differences [20]. It is difficult

to ascertain whether this small bias had a major impact on the results from the present study, but as social class, energy (MJ/day), and fat (g/MJ/d) intakes assessed at adolescence were unrelated to BMD, it seems unlikely. In common with findings from previous studies [4, 21], the present observational study has shown sex differences in BMD, with males having significantly higher BMD measurements than females, irrespective of bone site. In both sexes, there was a significant negative association between age and BMD at the femoral neck, but not at the lumbar spine. It is possible that femoral neck peak bone mass is achieved earlier than lumbar spine, and had already been reached in this group. In agreement with previous studies [10, 11], both unadjusted and adjusted analyses confirmed that body weight was a strong determinant of BMD in males and females at both measurement sites. Body weight seems to augment mechanical loading so that the greater the weight, the greater the mechanical forces placed on the bones and consequently the greater the stimulus for osteogenesis [22]. Total physical activity in males, at both adolescence and young adulthood, was also a predictor of BMD in this study, with significant relationships evident at both the femoral neck and lumbar spine. However, physical activity scores were consistently unrelated to BMD in the females. This suggests either that the physical activity undertaken by females was of an insufficient level to create a demonstrable effect on BMD or that the

Table 7. Females: Adjusted regression analysis for relationships between dietary and lifestyle parameters observed at adolescence (15 y) and again at young adulthood (between 20 and 25 y), and BMD measured concurrently with diet during young adulthood

Variable	Adolescence				Young adulthood			
	Lumbar spine BMD R ² =0.267		Femoral neck BMD R ² =0.174		Lumbar spine BMD R ² =0.241		Femoral neck BMD R ² =0.208	
	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>
Pubertal status	0.08	0.27	0.00	0.96	nm	nm	nm	nm
Age (years)	ni	ni	ni	ni	0.01	0.94	-0.15	0.03
Height (m)	-0.06	0.43	-0.02	0.78	0.06	0.44	0.10	0.19
Weight (kg)	0.46	<0.01	0.34	<0.01	0.44	<0.01	0.35	<0.01
Physical activity (score)	0.08	0.25	0.10	0.14	0.02	0.77	0.00	0.97
Smoking (current vs non-smoker)	-0.14	0.06	-0.06	0.43	-0.09	0.21	-0.06	0.39
Alcohol (g)	-0.02	0.74	-0.04	0.61	-0.12	0.13	-0.05	0.53
Total energy (MJ/d)	0.06	0.77	0.25	0.26	0.07	0.71	0.18	0.34
Calcium (mg/d)	0.25	0.49	0.86	0.03	0.35	0.20	0.24	0.37
Protein (g/d)	0.12	0.76	0.47	0.27	-0.11	0.61	-0.04	0.87
Phosphorus (mg/d)	-0.60	0.18	-1.40	<0.01	-0.09	0.49	-0.21	0.14
Calcium:protein ^a	0.24	0.37	0.20	0.47	-0.05	0.84	0.00	0.98
Calcium:phosphorus ^b	-0.35	0.16	-0.64	0.02	-0.19	0.32	-0.15	0.44
Total fat (g/d)	0.22	0.23	-0.03	0.87	-0.11	0.48	-0.22	0.15
Vitamin D	0.23	<0.01	0.09	0.23	0.06	0.47	0.21	<0.01

R² = explained variation; β = standardized regression coefficient; BMD = bone mineral density (g/cm²); nm = not measured; ni = not included in analysis

^a Calcium:protein = ratio of calcium intake (mg/d) to protein intake (g/d)
^b Calcium:phosphorus = ratio of calcium intake (mg/d) to phosphorus intake (mg/d)

questionnaires did not fully capture or characterize physical activity patterns of females.

A further observation that may be worthy of comment is the relationship between alcohol intake and BMD, and smoking status and BMD. Although only approaching statistical significance, our results suggest a possible inverse association between these factors. These observations are, however, limited by the small sample of smokers and alcohol consumers in the current study and as such, probably warrant future investigation.

Among the nutritional factors, vitamin D intake reported by the females, at both adolescence and young adulthood, showed the most consistent positive associations with BMD measured at young adulthood. In the unadjusted analysis, vitamin D intakes reported by the girls at age 15 y were significantly positively related to BMD at the lumbar spine. This relationship remained after controlling for other factors in the adjusted analysis. However, at young adulthood, though vitamin D intakes were still positively associated with BMD, this was at the femoral neck in both the unadjusted and the adjusted regression analyses. In contrast, vitamin D intakes reported by the males, at adolescence and at young adulthood, were not associated with BMD. It is difficult to compare these results directly with other studies, as much of the previous research has concentrated on older age groups. Nevertheless, there are some similarities.

Vitamin D intake has previously been found to have a significant positive effect on BMD in postmenopausal

women aged 60 y and older, although no effect could be found on fracture rate [23]. Picard et al. [24] also reported that vitamin D intake was an independent predictor of bone mass or bone loss. The findings of the current study suggest that dietary vitamin D may play an important and beneficial role in promoting bone health, particularly in young females. These data are in conflict with current guidelines, which suggest that dietary vitamin D is not necessary for the majority of the population [9]. In the United Kingdom, there is no RNI for vitamin D [19] between the ages of 4 and 64 y. Rather, it is assumed that ample production occurs through exposure of the skin to ultraviolet light. This, however, depends on regular exposure. Although most people can attain a sufficient vitamin D status if their skin is exposed for about half an hour per day between April and October [9], this may depend on latitude [25]. In Northern Ireland, it is likely that exposure to ultraviolet light is relatively low, suggesting that the amount of vitamin D obtained through the skin could be low. Moreover, other factors including season, skin pigmentation, and ozone air pollution have also been reported as determinants of vitamin D production [25]. Furthermore, with the risk of skin cancer increasing [26], more people may attempt to reduce their exposure to ultraviolet light with additional clothing and/or the application of sunscreen creams. This may have an adverse impact on vitamin D status [27] and ultimately, may increase reliance on the diet as a source of vitamin D.

The main food sources of vitamin D in Northern Ireland are oily fish, eggs, fortified margarine/fat spreads, and fortified breakfast cereals (North-South Ireland Food Consumption Survey 2000, unpublished observation), all of which were accounted for in the dietary analysis. The results from the present observational study are slightly constrained by the lack of BMD measurements during adolescence and cannot prove causality. However, the positive relationships between BMD and vitamin D intake reported by the females clearly demonstrate the need for further study in younger people.

Of the other nutrients routinely associated with bone health, calcium has received most attention. Yet, despite extensive research, the calcium-bone relationship remains controversial. Calcium intake has been cited by many authors as a major contributor to increased bone mineral measures [6, 28], whereas others have reported no association [7, 8]. In the current study, Ca intakes reported by the females at adolescence showed a significant positive association with BMD (femoral neck) measured at young adulthood. In contrast, Ca intakes reported by the adolescent males had a strong significant negative effect on BMD (lumbar spine). However, at young adulthood, Ca intake showed no relationship with BMD in either sex, at either measurement site. These data add weight to the hypothesis that findings from epidemiological studies on associations between Ca and bone health in adolescence may depend on the time of measurement. In a French cross-sectional study [29], Ca intake was found to be a determinant of vertebral BMD in prepubertal children, but not in older children. Another interventional trial by Johnston et al. [30] suggested that additional dietary Ca would only affect BMD in prepubertal children. Similar findings have recently been reported by Kardinaal et al. [8]. Failure to find consistent associations between calcium and bone may also be attributed to high mean Ca intakes within this cohort. Although mean Ca intakes were significantly higher in males than in females, intakes for both sexes were above the current UK RNI [9]. A meta-analysis has suggested that, in populations with relatively high Ca intakes, the positive effects on bone status may be difficult to observe [31]. It is possible that the reported Ca intakes were above a threshold level to exert an effect on bone. However, when the BMD measurements of subjects whose Ca intakes were < 800 mg/d were compared with the BMD of subjects with higher intakes, no significant differences were apparent (data not shown).

It is also possible that the effects of dietary calcium on bone are confounded by other factors affecting calcium balance. For example, dietary phosphate is known to bind Ca in the gut to produce a non-absorbable salt. On the basis of this evidence, it has been recommended that intakes of Ca should exceed phosphorus on a milligram-to-milligram basis [32]. Contrary to these recommendations, in the present study mean phosphorus intakes exceeded

mean Ca intakes in both males and females, at adolescence and young adulthood. Consistent with the hypothesis that phosphorus may have a detrimental effect on bone, intakes reported by the adolescent females demonstrated a significant inverse effect on femoral neck BMD measured at young adulthood. Further sex differences and longitudinal changes were apparent when associations between the Ca:P ratio and BMD were explored. In females, the Ca:P ratio reported at adolescence had a significant inverse effect on BMD (femoral neck), whereas no effect was apparent at young adulthood. In contrast, in young adult males, the Ca:P ratio showed a positive impact on BMD (lumbar spine), whereas no relationship was evident at adolescence. No relationship between BMD and phosphorus intakes was apparent in young adulthood in either sex, at either measurement site.

Although the influence of dietary protein on bone health has been cited as being either adverse [33] or beneficial [34], results from the present observational study found no evidence to suggest that protein intake was negatively associated with BMD in either males or females. However, in the young adult males, the calcium:protein ratio did have an independent significant inverse effect on BMD (lumbar spine). It may be that the influence of protein on calcium balance depends on the dietary protein source [35]. In addition, protein intake may be, in some way, linked with muscle mass or body weight [36]. This is an issue requiring further study. Some controversy also surrounds the relationship between dietary fat and bone mineral status. Fat may have a negative effect on bone because it reduces net Ca absorption [37]. Alternatively, it has been suggested that the process of fat digestion and absorption in the small intestine occurs at a faster rate than Ca, thereby minimizing the potential negative effect [38]. In the present study, intakes of total fat had no significant impact on BMD at either measurement site in either sex.

This study has provided further insight into the relationships between various nutrients and bone mineral status in young adults. In our population, vitamin D intake reported by females at both adolescence and young adulthood showed a positive association with BMD measured on one occasion during young adulthood. However, no consistent associations were evident between calcium, protein, phosphorus, and fat intakes and BMD in males or females. Further investigation is required to understand the complex nature of the association between bone health and intakes of nutrients and food.

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