A lifecourse study of bone resorption in men age 49-51 years: The Newcastle thousand families cohort study

MS Pearce[1], CL Relton[2], A Groom[2], RT Peaston[3] RM Francis[4].

[1]Institute of Health and Society, Newcastle University, Newcastle upon Tyne, UK

[2]Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, UK

[3]Department of Clinical Biochemistry, Freeman Hospital, Newcastle Hospitals NHS Trust, Newcastle upon Tyne, UK

[4]Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, UK

Correspondence to Dr Mark S Pearce, Institute of Health and Society, Newcastle University, Sir James Spence Institute, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, United Kingdom

Email m.s.pearce@ncl.ac.uk, tel +44 191 2821355, fax +44 191 2824724

Keywords: birth weight, bone resorption, cohort, lifecourse, socio-economic status

Abstract

It has been suggested that bone health in adulthood is programmed by development *in utero*. Most previous investigations addressing this topic have focussed on bone mineral density or content, rather than other indicators of bone health, such as biochemical markers of bone turnover. This study investigated whether potential predictors, from different stages of life, influence bone resorption in men aged 49-51 years in the Newcastle Thousand Families birth cohort. The cohort originally consisted of all 1142 births in the city of Newcastle upon Tyne, UK in May and June 1947. Detailed information was collected prospectively during childhood, including birth weight and socio-economic circumstances. At 49-51 years of age, 574 study members completed a detailed 'Health and Lifestyle' questionnaire, including the European Prospective Investigation of Cancer (EPIC) food frequency questionnaire and 412 study members attended for clinical examination, including 172 men in whom bone resorption was assessed by measurement of serum β C-telopeptide of type 1 collagen (CTX).

A significant trend was seen between increasingly disadvantaged socio-economic status at birth and increased bone resorption (p=0.04, r-squared 2.6%). However, birth weight, standardised for sex and gestational age, was not associated with serum CTX (p=0.77, r-squared 0.05%). Significant trends were also seen between increasing total energy intake (p=0.03, r-squared 2.9%), dietary intake of saturated fat (p=0.02, r-squared 2.6%), protein (p=0.04, r-squared 2.5%) and carbohydrates (p=0.04, r-squared 2.6%) and higher serum CTX. However, on adjustment for total energy intake, none of the other dietary variables significant at the univariate level maintained significance.

Our findings suggest that early socio-economic disadvantage and later dietary factors may be associated with increased bone resorption in middle aged men. However, as little of the variance in serum CTX was explained by the variables included within this investigation, further longitudinal studies, with sufficient statistical power, are required to assess predictors of bone resorption in adulthood and their relative importance.

Introduction

Bone health in adulthood has been proposed to be 'programmed' during development *in utero*, with studies suggesting that poor fetal and infant growth is associated with decreased skeletal growth, low bone mass and an increased risk of osteoporosis and fragility fractures in later life. [1-4] However, adult lifestyle factors which affect the rate of bone loss and fracture risk, may be more important predictors of adult bone health than fetal factors. [5] It is also possible that other factors in childhood, including socio-economic deprivation, may affect future bone health, through programming of endocrine function or bone turnover. [6]

While most previous lifecourse investigations of bone health have concentrated on bone mineral density (BMD) or bone mineral content (BMC), measurement of the biochemical markers of bone turnover, such as serum β C-telopeptide of type I collagen (CTX), may add to the understanding of the way in which bone health may be influenced by a range of factors operating across the lifecourse.

Although most often used in the setting of clinical trials, where they can be used to assess the efficacy of treatments for osteoporosis, markers of bone turnover are becoming of increasing interest, particularly as assay precision improves, as a tool for the assessment of patients with bone disease. [7]. Biochemical markers of bone resorption correlate well with the rate of bone loss, but they also predict fracture risk, which has been shown, at least in part, to be independent of BMD. [8-9]

The Newcastle Thousand Families cohort provides an opportunity to investigate the fetal, childhood and later life determinants of bone resorption, assessed by measurement of CTX, in male cohort members. A previous analysis of the BMD data for this cohort at the age of 50 years showed no significant association with birth weight, although associations were seen for a number of other factors operating at different stages of life. [5] However, strong associations were seen in that study between birth weight and skeletal size, as assessed by scan area. This study aimed to investigate whether a range of factors from fetal life (birth weight, standardised for gestational age, socio-economic position at birth, position in family) ,

infancy (duration breast-fed) and adulthood (smoking, achieved height and weight, diet, alcohol consumption, socio-economic circumstances and physical activity) were related to CTX, as measured at the age of 50 years in this cohort.

Materials and Methods

Study participants

The Newcastle Thousand Families study began as a prospective study of all 1142 children born in May and June 1947 to mothers resident in Newcastle upon Tyne, UK. [10] The health, growth and development of the cohort were followed in great detail up to age 15 years. Throughout the first years of the children's lives, all families were visited both on a routine (up to every six weeks during infancy and at least quarterly until age five years) and *ad hoc* basis by the study team, which consisted of health visitors and paediatricians. Children were formally examined by a paediatrician at the end of the 1st, 3rd and 5th years During the school years, visits were made at least once a year to record height, weight and any health problems up to the age of 15 years in 1962. Additional data were provided from GP and hospital consultations, details of which were provided to the study team. Further follow-ups, of subsets of the original cohort took place in 1969 and 1979.

The cohort underwent a major follow-up at age 49-51 years. Participants were members of the cohort who were either traced through the National Health Service Central Register or contacted the study team in response to media publicity. Between October 1996 and December 1998, health and lifestyle questionnaires were sent out for completion and return and study members invited to attend for clinical examination which took place over the same time period. The previous follow-ups had received ethical approval from appropriate Local Research Ethics Committees, while this study received a favourable ethical opinion from Newcastle and North Tyneside Local Research Ethics Committee.

Investigations at age 49-51 years

Bone resorption was assessed by measurement of serum CTX on samples collected between 9.00 and 10.00 am after an overnight fast. All samples used in this investigation were collected between 1996 and 1998. Analysis of the samples was carried out in 2008, by electrochemiluminescence in a sandwich immunoassay (Elecsys 2010, Roche Diagnostic Ltd, Lewes, UK). Inter assay imprecision at 0.15 ug/L was <5%. [11] BMD, BMC and total bone area of the hip and lumbar spine were measured using a Hologic QDR 2000 machine (Hologic Instruments, Waltham, MA, USA). [5] The scanning machine had a coefficient of variation of 0.5% throughout the study period. Height and weight were also measured at this time using a stadiometer.

Measurement of early life experience

Information on early life was recorded prospectively for all study members. [10] Birth weights, as recorded by the midwife at the time of the child's birth, were standardised for gestational age and gender. [12] Socioeconomic status (SES) at birth (I to V, with I assumed to be the most advantaged and V the least advantaged) was measured by paternal occupational social class at the time of the child's birth. Duration of breast-feeding was defined as the length of time a study member was at least partly breast fed, as recorded by the health visitors. Position in family was calculated from the number of older surviving siblings, including half-siblings at the time of the child's birth.

Measurement of adult socioeconomic position and lifestyle

Social class, the number of pack-years of cigarettes smoked, alcohol consumption and dietary information, were derived from the returned self-completion questionnaire data at age 49-51 years. [10] Occupational details of the main wage earner in the household were coded according to the 1990 UK Registrar General's Standard Occupational Classification and hence social class was derived.

Four categories of alcohol consumption at were derived: No drinking; light drinking (up to ten units /week of alcohol); moderate drinking (11-28 units) and heavy drinking (>28 units). In the UK, one unit is

10ml or 8g of pure alcohol. The number of pack-years of cigarettes smoked (one pack-year = one pack of cigarettes smoked per day for one year) was estimated from the study members' smoking habits at ages 15, 25, 35 and 50, as ascertained at age 49-51 years. Total dietary energy intake and dietary intake of vitamins C and D, protein, carbohydrate, cholesterol, iron, potassium, selenium, thiamine, saturated fats and calcium at age 49-51 years were estimated from responses to the European Prospective Investigation of Cancer and Nutrition (EPIC) food frequency questionnaire (FFQ),[13] included within the self-completion questionnaire. The EPIC FFQ asks how frequently certain food and drink items have been consumed over the previous year, with responses converted to nutrient values. Physical activity assessment at age 49-51 years was based on that used in the Medical Research Council's National Survey of Health and Development.[14] Subjects were classed as physically inactive at age 49-51 years if they never walked more than one mile, did no heavy gardening, 'do-it-yourself', cycling, sports or vigorous leisure activity, and if in work, did no strenuous activity.

Statistical analysis

How representative participants in this study were in relation to the original cohort was tested using chi-squared tests. Physical activity was defined as a binary variable. Social class, position in family and alcohol consumption were defined as ordinal variables and assessed for trend. Other explanatory variables were treated as continuous linear variables. Relationships between bone resorption and potential explanatory variables were estimated using multiple linear regression, with serum CTX as the dependent variable. Dietary variables were included with adjustment for total energy intake within the linear regression models.[15] The level of variation in serum CTX values explained by each of the independent variables was assessed by the r-squared value when included within the model. Study members with missing data were included in all analyses for which they contributed data, although hierarchical models only used study members with complete data on the set of variables included. Regression coefficients (r), denoting the increase in serum CTX for a unit increase in the explanatory variable, are presented with

accompanying 95% confidence intervals (CI). Correlations between dietary variables and total energy intake were assessed using Spearman Rank correlation. Models were checked for validity in terms of the assumptions surrounding linear regression modelling, heteroskedasticity (using the Cook-Weisberg test), omitted variables (using the Ramsey RESET test) and distributions of residuals assessed using normal probability plots. All statistical analyses were done using the statistical software package Stata, version 10 (StataCorp, College Station, TX).

Results

Of the original cohort, 832 (86% of the surviving sample of 967 children whose families remained in Newcastle for at least the first year of the study) were traced at age 49-51 years. Of these, 574 completed the health and lifestyle questionnaire and 412 attended for clinical examination, including assessment of BMD which has been previously reported. [5] Within this group were 172 men for whom bone resorption was estimated. Mean serum CTX was 0.11μg (sd 0.07).

This sample did not differ significantly ($p>0.05$) from the 411 male members of the original cohort not included in this analysis in terms of any of the factors in early life. Descriptive statistics for all continuous variables are given in table 1 and for categorical variables in table 2. UK recommended reference nutrient intakes (RNI) are also given alongside median and inter-quartile ranges for the dietary data included within this study. [16] Serum CTX showed little correlation with BMD, BMC or bone area of either the hip, with all correlations between -0.1 and 0.004 and with p-values ≥ 0.15 (table 3).

Early life

Although standardised birth weight was not associated with bone resorption (unadjusted $p=0.77$, r-squared 0.05%), a significant trend was seen between increasingly disadvantaged socio-economic status

at birth and increasing CTX (p=0.04, r-squared 2.6%) (table 4). No significant associations were seen with position in family or with duration breast-fed.

Adult life

Significant trends were seen between increasing levels of total dietary energy intake (p=0.03, r-squared 2.9%) dietary intake of saturated fat (p=0.02, r-squared 2.6%), carbohydrates (p=0.04, r-squared 2.6%) and protein (p=0.04, r-squared 2.5%) and increasing levels of CTX. No other adult variables, including social class at age 50 showed significant associations at the univariate level. On adjustment for total dietary energy intake, none of the other dietary variables significant at the univariate level maintained significance. Due to the high level of correlation between total dietary energy and fat intakes (r=0.81, p<0.0001), neither was significant when included in the same regression model. Total dietary intake was significantly correlated with each of the dietary variables considered in this investigation (p<0.001). Thirty percent of study members reported dietary energy intakes relative to predicted basal metabolic rate of less than 1.1 (indicative of under-reporting) [17] Over half of the study members included in this analysis reported greater than recommended daily intakes, as defined by RNI, of vitamin C, protein calcium cholesterol and potassium, while over half reported less than recommended daily intakes of total energy, , saturated fat, carbohydrate and selenium.

In a model including both social class at birth and total dietary energy intake at age 49-51 years, only energy intake remained a significant predictor of serum CTX (p=0.02, with p=0.34 for social class at birth). This model did not show significant evidence of heteroskedasticity, non-normality of residuals or omitted variables.

Discussion

Summary of findings

Socio-economic status early in life and dietary saturated fat in adulthood were identified as significant predictors of a marker of bone resorption in a cohort of males at age 49-51 years. However, neither explained more than 3% of the variance in serum CTX, suggesting that more important factors not included within this investigation, including genetic, epigenetic and environmental factors must also have a role.

Strengths and limitations

An obvious limitation of this study is the small sample size, although it should be noted that in effectively the same sample of men a number of significant associations have previously been reported in relation to BMD and bone area. [5] Nevertheless, the possibility that a lack of statistical power may have obscured some potentially interesting findings remains. The study sample was comparable for all explanatory variables in early life included in this investigation. In addition, inclusion of cohort members who had moved out of the study region increased the representativeness of the population studied. Only men were included within this study due to the perimenopausal status of many of the female cohort members at the time of sample collection. The possibility of our findings being due to chance rather than a true biological effect remains, as does the possibility that residual confounding due to factors not available for this analysis may play a role in explaining our significant findings.

The samples used in this investigation were collected between 1996 and 1998 and analysed in 2008. Although stability of serum CTX measurements after three years storage as frozen samples has been demonstrated, [18] it is possible that some degradation of the samples may have occurred during this longer period of storage. However, degradation is unlikely to have been related to the potential predictors included in this investigation. Diurnal variation of serum CTX has also been demonstrated previously. [19] However, as all serum samples were fasting samples obtained between 9 and 10am, this is unlikely to have influenced our findings. There is also day to day variation in bone turnover markers, which could

potentially weaken the strength of any association. [20] Reassuringly, inter assay imprecision for serum CTX at 0.15 ug/L was <5%. [11]

The use of serum CTX as a predictor of bone health

CTX is one of a number of biochemical markers for bone turnover to have been developed. No correlation was found between serum CTX and any of BMD, BMC or bone area in this study. However, two studies, in French women, have shown significant positive associations, independent of BMD, between CTX and risk of hip fracture. [21-22] However, a study of French men aged 50 years or over found that, while CTX was positively associated with bone mineral loss, there was no association with fracture risk. [23] Although widely used in clinical trials for assessing the efficacy for treatments of osteoporosis, [24] CTX is becoming of increasing interest for use in the assessment of patients with bone disease. However, it is likely that only with increased assay precision and more consistent evidence as to the association with outcomes, such as bone loss and fracture risk, that CTX will become more widely accepted for use in clinical practice.

Comparison with previous findings

We have previously shown BMD in the same men at the same age to be associated with family position and vitamin C intake in adulthood. Neither was associated with bone resorption in this study.

Future risk of osteoporosis has been suggested to be, at least partly, programmed during early life.[1-3] No association was found in this study between birth weight and bone resorption, nor was one found in our previous study of BMD. [5] Further, in both studies, the amount of variation in bone outcomes explained by birth weight was very small. Experiencing childhood in a post-war environment, including rationing of food, may have resulted in reduced dietary variability than in pre-war birth cohorts. This is of particular relevance for birth weight for which there was no socio-economic gradient at birth in this cohort. [25] However, we did find a relationship between SES at birth and bone resorption in this study,

with increasing resorption being associated with decreasingly advantaged socio-economic status. This was not independent of later total dietary energy intake, but it is likely that SES at different stages of life may influence dietary intake in adulthood. While SES at birth or in early life has, like birth weight, been associated with a range of adverse health outcomes and mortality, [26] we are unaware of previous studies suggesting a link between early SES and later bone resorption.

A small number of studies have demonstrated associations between bone resorption and dietary factors, including vitamin C, [27-28] soy isoflavones [29] and potassium, [27] although conflicting results have also been reported. [30] While univariate associations were seen between serum CTX and dietary intakes of carbohydrates, protein and saturated fats, none of these associations were independent of total dietary energy intake. While such an adjustment may be problematic in terms of multi-collinearity, it is also likely to serve as control for confounding and extraneous variation in dietary nutrient intakes. [15] A previous investigation using data from this cohort suggested that the dietary questionnaire may have resulted in the under-reporting of dietary energy intake. We have previously shown that thirty percent of study members reported dietary energy intakes relative to predicted basal metabolic rate of less than 1.1 (indicative of under-reporting) [17] Other studies have conversely noted an over-reporting of energy intake, [31-32] suggesting that results concerning self-reported dietary intake should be viewed with caution. Further, even if the association between serum CTX and energy intake is a true association, our results suggest that energy intake explains less than 3% of the variation in CTX and a 100Kcal change in energy intake would only lead to a 0.001μg change in CTX. This corresponds to a change of around one seventieth of a standard deviation change. It is possible that dietary factors not included within this investigation may also play a role.

Conclusion

Our findings suggest that, while birth weight does not appear to influence bone resorption, early socio-economic disadvantage may be associated with increased resorption in adult life, although this may be

mediated through later dietary influences. As little of the variance in CTX was explained by the variables included within this investigation, further larger longitudinal studies are required to assess the lifecourse predictors of bone resorption in adulthood and their relative impacts.

Acknowledgements

We thank all the Thousand Family Study members for taking part in this study and the study teams past and present, particularly Professors Sir Alan Craft, Sir George Alberti and Louise Parker who led the 50 year follow-up. We thank the previous funding bodies who have contributed to this study since it began. Newcastle Healthcare Charity funded this analysis. EPIC food frequency questionnaires were analysed by the MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge.

References

[1] Cooper C, Fall C, Egger P, Hobbs R, Eastell R, Barker D. Growth in infancy and bone mass in later life. Ann Rheum Dis 1997;56:17-21

[2] Oreffo RO, Lashbrooke B, Roach HI, Clarke NM, Cooper C. Maternal protein deficiency affects mesenchymal stem cell activity in the developing offspring. Bone 2003;33:100-7

[3] Lanham SA, Roberts C, Cooper C, Oreffo ROC. Intrauterine programming of bone. Part 1: Alteration of the osteogenic environment. Osteoporosis Int 2007; 19: 147-156.

[4] Cooper C, Eriksson JG, Forsen T, Osmond C, Tuomilehto J, Barker DJ. Maternal height, childhood growth and risk of hip fracture in later life: a longitudinal study. Osteoporos Int 2001; 12: 623-629.

[5] Pearce MS, Birrell FN, Francis RM, Rawlings D, Tuck SP, Parker L. A lifecourse study of bone health at age 50 years: The Newcastle Thousand Families Cohort Study. Journal of Epidemiology and Community Health 2005; 59: 475-480.

[6] Dennison EM, Syddall HE, Rodriguez S, Voropanov A, Day IN, Cooper C. Polymorphism in the growth hormone gene, weight in infancy, and adult bone mass. J Clin Endocrinol Metab 2004; 89: 4898-4903.

[7] Ross PD, Knowlton W. Rapid bone loss is associated with increased levels of biochemical markers. J Bone Miner Res 1998; 13: 297-302.

[8] Ross PD, Kress BC, Parson RE, Wasnich RD, Armour KA, Mizrahi IA. Serum bone alkaline phosphatase and calcaneus bone density predict fracture risk Osteoporos Int 1998 ; 8 : 76-82.

[9] Civitelli R, Armamento-Villareal R, Napoli N. Bone turnover markers: understanding their value in clinical trials and clinical practice. Osteoporos Int 2009; 20: 843-851.

[10] Pearce MS, Unwin NC, Parker L, Craft AW. Cohort Profile: The Newcastle Thousand Families Study. Int J Epidemiol 2009; 38: 932-937.

[11] Garnero P, Borel O, Delmas PD. Evaluation of a fully automated serum assay for C-terminal cross-linking telopeptide of Type 1 collagen in osteoporosis. Clin Chem 2001;47:694-702.

[12] Freeman JV, Cole TJ, Chinn S, Jones PR, White EM, Preece MA. Cross sectional stature and weight reference curves for the UK, 1990. Arch Dis Child 1995;73:17-24

[13] Bingham SA, Gill C, Welch A, Cassidy A, Runswick SA, Oakes S, Lubin R, Thurnham DI, Key TJ, Roe L, Khaw KT, Day NE. Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers. Int J Epidemiol 1997;26:S137-S51

[14] Kuh DJL, Cooper C. Physical activity at 36 years: patterns and childhood predictors in a longitudinal study. J Epidemiol Comm Health 1992;46:114-9

[15] Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiology studies. Am J Clin Nutr 1997; 65: 1220S-1228S.

[16] Department of Health. Report on health and social subjects: 41. Dietary reference values for food energy and nutrients for the United Kingdom. London: HMSO 1991

[17] Lamont D, Parker L, White M, Unwin N, Bennett SM, Cohen M, Richardson D, Dickinson HO, Adamson A, Alberti KG, Craft AW. Risk of cardiovascular disease measured by carotid intima-media thickness at age 49-51: lifecourse study. Br Med J 2000;320:273-8

[18] Qvist P, Munk M, Hoyle N, Christiansen C. Serum and plasma fragments of C-telopeptides of type I collagen (CTX) are stable during storage at low temperatures for 3 years. Clin Chem Acta 2004;350:167-173.

[19] Kong SY, Stabler TV, Criscione LG, Elliott AL, Jordan JM, Kraus VB. Diurnal variation of serum and urine biomarkers in patients with radiographic knee osteoarthritis. Arthrtis Rheum 2006;54:2496-504.

[20] Hannon R, Eastell R. Preanalytical variability of biochemical markers of bone turnover. Osteoporos Int 2000;11(suppl 6):30–44.

[21] Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, Cormier C, Bréart G, Meunier PJ, Delmas PD. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS Prospective Study. J Bone Miner Res 1996; 11: 1531-1538.

[22] Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res 2000; 15: 1526-1536.

[23] Szulc P, Montella A, Delmas PD. High bone turnover is associated with accelerated bone loss but not with increased fracture risk in men aged 50 and over: the prospective MINOS study. Ann Rheum Dis 2008; 67: 1249-1255.

[24] Baim S, Miller PD. Assessing the clinical utility of serum CTX in postmenopausal osteoporosis and its use in predicting risk of osteonecrosis of the jaw. J Bone Miner Res 2009; 24: 561-574.

[25] Wright CM, Parker L. Forty years on: the effect of deprivation on growth in two Newcastle birth cohorts. Int J Epidemiol 2004; 33: 147-152.

[26]Galobardes B, Lynch JW, Davey Smith G. Is the association between childhood socioeconomic circumstances and cause-specific mortality established? Update of a systematic review. J Epidemiol Comm Health 2008; 62: 387-390.

[27] New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, Grubb DA, Lee, SJ, Reid DM. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr 2000; 71:142-151.

[28] Sadideen H, Swaminathan R. Effect of acute oral calcium load on serum PTH and bone resorption in young healthy subjects: an overnight study. Eur J Clin Nutr 2004; 58: 1661-1665.

[29] Yamori Y, Moriguchi EH, Teramoto T, Miura A, Fukui Y, Honda K, Fukui M, Nara Y, Taira K, Moriguchi Y. Soybean isoflavones reduce postmenopausal bone resorption in female Japanese immigrants in Brazil: A ten-week study. J Am Coll Nutr 2002;6:560-563.

[30] Brink E, Coxam V, Robins S, Wahala K, Cassidy A, Branca F, PHYTOS Investigators. Long-term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. Am J Clin Nutr 2008; 87: 761-770.

[31] Pietinen P, Hartman AM, Haapa E, Räsänen L, Haapakoski J, Palmgren J, Albanes D, Virtamo J, Huttunen JK. Reproducibility and validity of dietary assessment instruments. I. A self-administered food use questionnaire with a portion size picture booklet. Am J Epidemiol 1988;128:655-66.

[32] Andersen LF, Nes M, Lillegaard IT, Sandstad B, Bjørneboe GE, Drevon CA. Evaluation of a quantitative food frequency questionnaire used in a group of Norwegian adolescents. Eur J Clin Nutr 1995;49:543-54.

Table 1.Descriptive statistics for continuous variables

Variable	N	Mean (SD)	Range	
Fetal, infancy and childhood life				
Birth weight (kg)[a]	172	3.38 (0.49)	1.92 to 4.65	
Standardised birth weight	172	-0.33 (0.99)	-3.49 to 2.18	
Duration of breast fed (months)	170	3.97 (3.94)	0 to 13.7	
Adulthood (measured at age 49-51 years)				
Lifetime cigarette smoking (pack years) [b]	112	24.7 (18.3)	0.10 to 101	
Height (m)	172	1.73 (0.07)	1.51 to 2.01	
Weight (kg)	172	81.0 (12.6)	52.1 to 130.6	
	N	**Median (IQR)**	**Range**	**RNI**
Total dietary daily energy intake (Kcal)	167	2220 (1699 to 2588)	578.5 to 5044	2500
Total adult dietary daily vitamin C intake (mg)	167	95.9 (65.8 to 131.2)	19.6 to 499.0	40
Total adult dietary daily vitamin D intake(µg)	167	3.04 (2.13 to 5.12)	0.58 to 13.3	-
Total adult dietary daily protein intake (g)	167	87.7 (71.3 to 102.2)	21.7 to 175.8	55
Total adult dietary daily saturated fat intake (g)	167	29.7 (22.8-39.3)	6.62 to 100.8	30

Total adult dietary daily calcium intake (mg)	167	959 (744-1254)	165.6 to 2063	700
Total adult dietary daily carbohydrate intake (g)	167	246 (198-301)	83.0 to 616.2	300
Total adult dietary daily cholesterol (g)	167	284 (226-385)	72.2 to 770.2	250
Total adult dietary daily potassium (mg)	167	3873 (3210-4524)	1102 to 7918	3500
Total adult dietary daily selenium (μg)	167	55.3 (43.2-71.9)	21.0 to 140.2	75

[a]Birth weight standardised for gestational age and sex in all analyses, but unstandardised summary given in this table.

[b]Excluding 58 study members who had never smoked.

SD – Standard deviation

IQR – Inter-quartile range

RNI – Recommended nutrient intake [16]

Table 2. Descriptive statistics for categorical variables

Variable		Number	%
Social class at birth	I,II	21	12.6
	III	96	57.5
	IV,V	50	29.9
Position in family	1st	89	51.7
	2nd	53	30.8
	3rd	15	8.7
	≥4th	15	8.7
Social class at age 49-51 years	I,II	79	48.1
	III	65	39.6
	IV,V	20	12.2
Alcohol consumption at age 49-51 years	None	12	7.1
	Light	68	40.0
	Moderate	70	41.2
	Heavy	20	11.8
Physically inactive at age 49-51 years	Yes	14	8.2
	No	156	91.8

Table 3. Correlations between serum CTX and BMD, BMC and total bone area at age 49-51 years.

	Hip		Spine	
	r	**p**	**r**	**p**
BMD	-0.05	0.53	-0.11	0.15
BMC	-0.06	0.42	-0.08	0.30
Bone area	-0.02	0.83	0.004	0.96

Table 4. Unadjusted regression coefficients (and corresponding 95% confidence intervals) relating lifecourse factors to serum CTX (µg) in men at age 49-51 years

Source	r (95% CI)	p-value	r-squared (%)
Early life			
Standardised birth weight	0.002 (-0.01, 0.01)	0.77	0.1
Social class at birth	0.01 (0.001, 0.03)	0.04	2.6
Duration of breast fed (per 30 days)	-0.001 (-0.004, 0.002)	0.63	0.1
Position in family	-0.004 (-0.02, 0.01)	0.56	0.2
Adulthood (measured at age 49-51 years)			
Social class at age 49-51 years	0.001 (-0.003, 0.01)	0.18	0.5
Lifetime cigarette smoking (pack-years)	-0.0002 (-0.007, 0.001)	0.66	0.1
Alcohol consumption	0.008 (-0.006, 0.02)	0.27	0.7
Physical activity	0.01 (-0.01, 0.02)	0.22	0.2
Total adult dietary daily energy intake (per 100 Kcal)	0.001 (0.0002, 0.003)	0.029	2.9
Dietary daily vitamin C intake (per g)	0.002 (-0.01, 0.02)	0.81	0.03
Dietary daily vitamin D intake (per µg)	0.002 (-0.002, 0.006)	0.30	0.06

Dietary daily protein intake (per 100 g)	0.04 (0.001, 0.1)	0.04	2.5
Dietary daily saturated fat intake (per 10 g)	0.01 (0.001, 0.01)	0.02	2.6
Dietary daily calcium intake (per g)	0.02 (-0.01, 0.05)	0.13	1.4
Total adult dietary daily carbohydrate intake (per 100 g)	0.01 (0.001, 0.02)	0.04	2.6
Total adult dietary daily cholesterol (per 10 g)	0.001 (-0.00003, 0.001)	0.06	2.1
Total adult dietary daily potassium (per g)	0.007 (-0.003, 0.02)	0.18	1.1
Total adult dietary daily selenium (per mg)	0.27 (-0.1, 0.7)	0.21	1.0