Haematological, Biochemical and Bone Density Parameters in Vegetarians and Non-vegetarians

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ABSTRACT

Objective: *The objective is to determine any possible differences between haematological, biochemical and bone mineral density in vegetarians (vegans and lacto-ovovegetarians) and non-vegeterians.*
Methods: *The examined group consisted of 100 individuals: 50 non-vegetarians and 50 vegetarians. The vegetarian group was further divided in 2 subgroups: 20 vegans and 30 lacto-ovovegetarians. In all participants, plasma levels of erythrocytes, haemoglobin, haematocrit, iron, low density lipoprotein, (LDL), high density lipoprotein (HDL) total cholesterol, triglycerides and glucose were measured. Quantitative ultrasound parameters of the right calcaneus were determined in all participants.*
Results: *The results showed that lacto-ovovegetarians had statistically significantly higher red blood cell counts and haematocrit values than non-vegetarians. Vegans also had higher haematocrit values than non-vegetarians. Statistically significant differences were found between iron plasma levels in the examined groups. Iron levels were lower in non-vegetarians than in vegans and lacto-ovovegetarians. Non-vegetarians had much higher levels of cholesterol, triglycerides and LDL than the other two groups, but there were no differences found between same values in vegans and lacto-ovovegetarians.*
Conclusion: *A well planned and balanced vegetarian diet, with avoidance of risk factors, does not result in abnormalities in laboratory tests and bone status parameters.*

Parámetros de Densidad Ósea, Hematológica, y Bioquímica en Vegetarianos y no Vegetarianos

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RESUMEN

Objetivo: *El objetivo es determinar las diferencias entre la densidad hematológica, bioquímica y mineral ósea en vegetarianos (veganos y ovolactovegetarianos) y no vegetarianos.*
Métodos: *El grupo examinado consistía en 100 individuos: 50 no vegetarianos y 50 vegetarianos. El grupo vegetariano estaba a su vez dividido en 2 subgrupos: 20 veganos y 30 ovolactovegetarianos. A todos los participantes se les midieron los niveles plasmáticos de eritrocitos, hemoglobina, hematocrito, hierro, lipoproteína de baja densidad (LDL), lipoproteína de alta densidad (HDL), colesterol total, triglicéridos, y glucosa. Se determinaron los parámetros cuantitativos de ultrasonidos del calcáneo derecho en todos los participantes.*
Resultados: *Los resultados mostraron que los ovolactovegetarianos tuvieron conteos de glóbulos rojos y valores de hematocrito significativamente más altos en términos estadísticos, que los no vegetarianos. Los veganos también tuvieron valores de hematocrito más altos que los no vegetarianos. Se halló diferencia estadísticamente significativa entre los niveles séricos de hierro en los grupos examinados. Los niveles de hierro fueron más bajos en los no vegetarianos que en los veganos y los ovolacto-vegetarianos. Los no vegetarianos tuvieron niveles de colesterol, triglicéridos y LDL mucho más altos que los otros dos grupos, pero no se encontraron diferencias entre los mismos valores en los veganos y los ovolactovegetarianos.*
Conclusión: *Podemos concluir que una dieta vegetariana bien planeada y equilibrada, que evite*

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factores de riesgo, no trae consigo anormalidades en las pruebas de laboratorio y los parámetros del estado óseo.

West Indian Med J 2009; 58 (6): 513

INTRODUCTION

It is well-known that everyday diet mainly based on animal fat may cause different diseases leading to death. According to the International Food and Agriculture Organization data from 36 countries, a significant correlation between the amount of consumed milk products and animal fats and higher prevalence of prostate, colon, lung and breast cancer were reported (1). Nutrition based on vegetables was related to a minor risk of cardiovascular disease, diabetes, oesophageal, colon and gastric cancer. It can also help to prevent osteoporosis (2).

Various studies have been reported so far based on vegetarians, people who eat less or no animal products. Vegetarians could be divided in to 3 groups, lacto-vegetarians (consume milk), lacto-ovovegetarians (consume milk and eggs) and vegans, a group of people who do not consume any animal products.

A long-term prospective trial, the Oxford Vegetarian Study, included more than 11 000 healthy vegetarians and omnivorous volunteers in Great Britain. Plasma LDL cholesterol and total cholesterol levels were lower in vegans than in individuals who eat meat (3). Other study showed that vegetarians and people who eat fish had normal LDL cholesterol and total cholesterol plasma levels (4). It was shown that ischaemic heart disease associated mortality was 24% lower in vegetarians than in non-vegetarians (5). Some studies also reported a lower body mass index (BMI), lower total cholesterol and glucose plasma levels in vegetarians than in non-vegetarians. Vegetarians have higher levels of HDL cholesterol, a protective factor for atherosclerosis (6).

Plasma levels of total proteins in vegetarians were not lower than in other people because they consume soybean and leguminous plants which contain essential amino acids (7). Most of the studies did not show statistically significant differences between iron status in vegetarians and in non-vegetarians (8).

The purpose of this study is to establish any possible differences between haematological (erythrocyte, haemoglobin, haematocrit), biochemical (total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, iron and glucose) and bone mineral density (BMD) in vegetarians (vegans and lacto-ovovegetarians) and non-vegeterians.

SUBJECTS AND METHODS

The study group consisted of 100 individuals between 19 and 50-years of age, who were divided as 50 non-vegetarians and 50 vegetarians. The vegetarian group was further divided into 2 subgroups: 20 vegans (who do not consume any animal products) and 30 lacto-ovovegetarians (who do not eat fish and meat but consume eggs, milk and milk products) [Table 1]. The study took place at the Department of Gastroenterology and the Department of Endocrinology, Diabetes and Metabolic diseases, Internal Clinic, University of Rijeka, Croatia. All patients consented to blood tests and urine collection, anthropometric testing measurements and quantitative ultrasound of the heel.

The exclusion criteria were history of genetic, chronic or acute infectious diseases, drug and alcohol abuse, cardiovascular disease or diabetes, premature menopause as well as overweight and obesity. Each of the volunteers was asked about nutritional habits, use of cigarettes and alcohol, physical activity and a family history was obtained.

Nutritional habits were evaluated using the questionnaire on food consumption, and based on that questionnaire patients were divided in 3 groups: vegans, lacto-ovovegetarians and non-vegetarians.

Body mass index was determined for all examined individuals by measuring body weight (kg) and height (cm). Haemoglobin and haematocrit plasma levels and red blood cell counts were detected on automatic blood analyser (Bayer Technicon H.3 RTC, Bayer Diagnostic GmbH, Munchen, Germany) by cianmethaemoglobin-calorimetric method. Low density lipoprotein, high density lipoprotein, total cholesterol and triglyceride levels were determined by using the automatic analyser (Olympus AU 640®, Tokyo, Japan). Using the CHOD-PAP enzyme method LDL, HDL and total plasma cholesterol levels were determine and for triglycerides, the enzymatic GK-PAP method was used (9–11). Iron plasma levels were determined using 2, 4, 6-tri (2 pyridyl)-5-triazin.

Quantitative ultrasound (QUS) parameters of the right calcaneus were determined in all participants using Clinical Bone Sonometar Sahara Hologic ultrasound (manufactured by Hologic, Waltham, MA) which measures the transmission of high frequency sound waves through the heel (12). From the measured signal, three ultrasound parameters were simultaneously determined: speed of sound (SOS), expressed in metres per second (m/s), Broadband Ultrasound Attenuation (BUA), expressed in decibels per megahertz (dB/MHz) and the Quantitative Ultrasound Index (QUI) or 'stiffness'. The Sahara system software estimated BMD (Bone Mineral Density) and T-score according to these determined ultrasound parameters (13). Bone mineral Density was determined in g/cm^2 by simple re-scaling of the QUI value based on its correlation with heel dual-energy absorptiometry (DXA) BMD results ($r = 0.85$). The reported coefficients of variance (CV) for estimated BMD, QUI, SOS and BUA are 3, 2.6, 0.22, and 3.7%, respectively. The same scanning protocol provided by the manufacturer was used in this study. One

measurement of the right foot was obtained on all participants. The second measurement with repositioning of the foot was obtained if the first measurement was technically inadequate. If both of these measurements were technically inadequate or if the right foot was unsuitable for measurement due to deformities, previous fracture *etc* then the subject was excluded from the study. Quantitative ultrasound parameters for healthy young United Arab Emirates (UAE) women were compared with the manufacturer's reference for United States of America (USA) Caucasian, European Caucasian and Chinese Asian healthy young women of the same age range. The instrumental quality control was performed daily, by scanning the manufacturer-provided, temperature-sensitive phantom.

For statistical elaboration, we used programme Statistica 6.1, StatSoft Inc, USA. To compare numeral variables according to determined categories, ANOVA parametric test was used. Kruskal-Wallis median test was used when there were abnormal variable distributions or only a few data available. Pearsons χ^2 test was used to determine a relation between nominal independent variables.

All p values ≤ 0.05 were considered statistically significant.

RESULTS

Haematological and biochemical values of vegans, lacto-ovovegetarians and non-vegetarians are shown in Table 2. Lacto-ovovegetarians had statistically significantly higher red blood cell counts and haematocrit values than non-vegetarians. Vegans also had higher haematocrit values than non-vegetarians. A statistically significant difference was found between iron plasma levels in the examined groups. Iron levels were lower in non-vegetarians than in vegans and lacto-ovovegetarians. Non-vegetarians had much higher levels of total cholesterol, triglycerides and LDL ($p < 0.001$) than the other two groups, but there were no differences found for the same parameters in vegans and lacto-ovovegetarians. Differences in HDL and glucose plasma levels were not statistically different.

Bone status parameters are shown in Table 3.

Results of T-score showed statistically significant higher values in vegans than in non-vegetarians and lacto-ovovegetarians, while BMD values were significantly lower in non-vegetarians than in vegans and lacto-ovovegetarians.

Table 1: Gender age and BMI of examinees M – male, F – female, BMI – body mass index (kg/m^2)

	Gender			Age		BMI	
Groups	**N**	**M**	**F**	**Mean ± SD**	**CI (95%)**	**Mean ± SD**	**CI (95%)**
Omnivores	50	16	34	36.2 ± 9.2	33.5–38.8	23.3 ± 2.7	22.6–24.1
Vegans	20	8	12	34.9 ± 9.2	30.6–39.2	22.2 ± 2.7	20.9–23.5
Lacto-ovovegetarians	30	8	22	34.4 ± 9.7	30.8–38.0	23.1 ± 3.0	21.9–24.2
Total	**100**	**32**	**68**				

Table 2: Haematological and biochemical parameters in our groups of examinees

	Omnivorous (n = 50)		**Vegans (n = 20)**		**Lacto-ovovegetarians (n = 30)**		
	Mean ± SD	**CI (95%)**	**Mean ± SD**	**CI (95%)**	**Mean ± SD**	**CI (95%)**	***p***
Erythrocytes [x10^{12}/L]	4.61 ± 0.67	4.4–4.8	4.97 ±1.31	4.3–5.6	5.11 ± 1.15	4.7 ± 5.5	0.016
Hgb [mmol/L]	134.96 ± 15.4	130.6–139.3	139.1 ± 13.4	132.8–145.4	137.3 ±14.9	131.7–142.8	NS
Hct	0.396 ± 0.05	0.382–0.410	0.418 ± 0.04	0.400–0.435	0.419 ± 0.04	0.405–0.432	0.043
glucose [mmol/L]	4.77 ± 0.6	4.6–4.9	4.73 ± 0.7	4.4–5.0	4.89 ± 0.7	4.6–5.2	NS
cholesterol [mmol/L]	4.89 ± 0.6	4.7–5.1	4.19 ± 0.7	3.8–4.5	4.24 ± 1.0	3.9–4.6	< 0.001
triglycerides [mmol/L]	1.38 ± 0.60	1.22–1.54	0.94 ± 0.4	0.76–1.11	0.93 ± 0.4	0.77–1-08	< 0.001
LDL[mmol/L]	2.97 ± 0.6	2.78–3.16	2.33 ± 0.6	2.01–2.65	2.170.9	1.83–2.50	< 0.001
HDL[mmol/L]	1.15 ± 0.5	1.01–1.31	1.37 ± 0.4	1.17–1.58	1.20 ± 0.7	0.93–1.47	NS
Fe [µmol/L]	14.8 ± 5.4	13.3–16.4	19.0 ± 5.7	16.3–21.6	22.9 ± 23.1	14.3–31.6	0.035

Table 3: Bone status in our groups of examinees

	Omnivorous (n = 50)		**Vegans (n = 20)**		**Lacto-ovovegetarians (n = 30)**		
	Mean ± SD	**CI (95%)**	**Mean ± SD**	**CI (95%)**	**Mean ± SD**	**CI (95%)**	***p***
BMD [g/cm^2]	0.279 ± 0.65	0.094 – 0.465	0.594 ± .18	0.507 – 0.681	0.534 ± 0.12	0.489 – 0.579	0.015
T-Score	-0.66 ±1.1	-0.98 – (-0.34)	0.22 ± 1.6	-0.56 – 0.98	-0.43 – 1.1	-0.84 – (-0.02)	0.031

DISCUSSION

It is important to note that vegetarians have a higher risk of developing megaloblastic or sideropenic anaemia because of nutritional deficiencies of Vitamin B12 and iron. Iron deficiency appears due to lower nutrient intake and impaired absorption, and to inhibitor factors in vegetarian food, while vegetarians, who consume lots of fruit and vegetables containing Vitamin C which helps the absorption, should have adequate iron levels (8, 14).

According to our results, non-vegetarians had lower iron levels than vegans and lacto-ovovegetarians whose plasma levels were normal. Other studies showed similar findings (8, 15). These unexpected results are explained by the fact that vegetarians carefully plan their diet and were well educated about their possible micronutrient deficiencies (16, 17).

Results of this study showed higher red blood cell counts in lacto-ovovegetarians than in non-vegetarians, and also significantlly lower haematocrit levels in non-vegetarians than in vegans and lacto-ovovegetarians while in vegans and lacto-ovovegetarians those results were similar. Haemoglobin levels in all three examined groups were normal. Recent studies that analysed the same haematological parameters showed similar results (18, 19).

There are many studies that indicate the importance of a reduction of dietary animal fat because of their relation to ischaemic heart disease. Recent results showed that serum cholesterol values were 14% lower in lacto-ovovegetarians and 35% lower in vegans than in non-vegetarians (20, 21). Thorgood *et al* reported a significant difference between plasma lipid levels in vegetarians than in non-vegetarians (22). Results of some studies showed low plasma HDL values in vegetarians, indicating an ingestion of fatty dairy products or low intake of alcohol (23, 24). Triglyceride plasma levels of vegetarians and non-vegetarians show a tendency to equalize. Various factors in the vegetarian diet have an influence on plasma cholesterol levels. The vegetarian diet is typically low in saturated fats and dietary cholesterol, but vegans, in particular, have a low intake of saturated fatty acids in their diet. Vegetarians also consume less dairy cholesterol than non-vegetarians, while strict vegans do not consume cholesterol at all since vegetables are cholesterol free (23).

Vegetarians have 50–100% higher intake of dietary fibres than non-vegetarians but lower intake of fibres than vegans. Dietary fibres decrease cholesterol plasma concentrations (25). There are studies that indicate a direct relationship between dietary intake of animal proteins and high cholesterol levels. Lacto-ovovegetarians consume less animal proteins than non-vegetarians while vegans do not consume animal proteins at all. Consumption of 25 g/day of soybean protein, even with consumption of animal fat, reduces plasma cholesterol level in patients with hypercholesterolaemia. Soybean protein also raises the plasma HDL level (26).

Results of the present study showed significantlly higher cholesterol, triglycerides and LDL plasma levels in non-vegetarians than in vegans and lacto-ovovegetarians, but no difference between vegans and lacto-ovovegetarians was found. High density lipoprotein levels in all three groups were normal, slightly higher levels in vegans were not statistical significant. Results of other studies suggested similar findings (21, 23, 27).

According to the results of earlier studies, it was found that vegetarian food, which was rich in dietary fibres, especially soybean and legumes, decreased plasma glucose and lipid concentrations, and helped the lowering of body weight and the prevention of diabetes. Significantly lower glucose and lipid plasma concentrations were found in people who frequently consumed soybean legumes and vegetables that were high in dietary fibres (28, 29). Glucose levels of our examinees were normal.

Analysis of BMI did not show any significant difference among the three groups. Vegans had slightly lower values of BMI than non-vegetarians and lacto-ovovegeterians but this was not statistically significant. Body mass index values of examinees were from 21.0 to 24.3 kg/m^2. Results from the Adventist Healthy Study showed 40% lower values of BMI in vegetarians than non-vegetarians, while the Oxford vegetarian study reported higher levels of BMI in male and female non-vegetarians (3). The English study which included 4000 vegetarians and non-vegetarians also reported the same results (21).

In the present study, we also assesed whether a vegetarian diet affects BMD. By measuring the calcaneus bone density using the quantitative ultrasound, T-score and BMD values of the examinees were determined. Some studies confirmed high correlation of the calcaneus BMD measured by ultrasound densitometry and classic DXA method, so these parameters can be useful in assessing bone density.

Although the DXA has been widely used for assessing bone mass in children and adults, quantitative ultrasound technique has been suggested as an alternative method. There is no exposure to radiation and, as an added advantage, ultrasound provides information about bone architecture. It measures the properties of bone that contribute to mechanical bone strength. In addition, it is shown that ultrasound parameters depend upon trabecular orientation. The ultrasound bone parameters correlated highly with BMD of the heel itself (r = 0.8–0.9) and also with bone structure and quality.

Determination of a bone mineral density by using the quantitative ultrasound can be used as a screening test in populations at high risk, because it is fast, economical and safe (30, 31).

Results showed higher T-score values in vegans than in non-vegetarians and lacto-ovovegetarians who had similar results. Non-vegetarians had significantly lower values of BMD than vegans and lacto-ovovegetarians; in vegans and lacto-ovovegetarians these values were not different.

Various studies reported that a high animal protein diet increases calcium excretion in urine (32, 33). Sellemeyer reported that the bone mass loss and a risk of femur fracture were greatest in those consuming diets with the highest ratio of animal to vegetable protein content (34). Animal proteins are rich in sulphur that metabolizes to sulphuric acid which causes stimulation of osteoclastic and inhibition of osteoblastic activity. Women who consumed high animal protein diet, including milk and its products, have a greater opportunity for bone mass loss and higher risk of bone fracture, than women who consume only vegetable proteins. Hannan *et al* in the Framingham study correlated bone loss with lower intake of dietary protein, but in elderly men and women (35). According to the results from other studies, no significant difference was identified between BMD in premenopausal vegetarian and non-vegetarian women (36).

There are only a few data that analyzed BMD in vegans, showing that they have a lower BMD than non-vegetarians (37). We found that vegans have higher BMD and significantly better T-score than non-vegetarians which indicate better bone density in vegans.

We can conclude that a well planned and balanced vegetarian diet, with avoidance of risk factors, do not show abnormalities in laboratory tests and bone status parameters.

REFERENCES

1. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 1992; **18:** 1–29.
2. Kunkel HO. Interests and values in the Recommended Dietary Allowances and nutritional guidelines for Americans. J Nutr 1996; **126:** 239–7.
3. Appleby PN, Thorogood M, Mann JI, Key TJ. The Oxford Vegetarian Study. Am J Clin Nutr 1999; **70:** 516–24.
4. Dwyer JT. Vegetarian eating patterns, science, values and food choices-where do from here? Am J Clin Nutr 1994; **59:** 1255S–1262S.
5. Lin CL, Fang TC, Gueng M. Vascular dilatatory functions of ovo-lactovegetarians compare with omnivores. Atherosclerosis 2001; **158:** 247–51.
6. Mann JI, Appleby PN. Dietary determinants of ischemic heart disease in health-conscious individuals. Heart 1997; **78:** 450–5.
7. Bederova A, Kudlackova M, Simoncic R, Magalova T, Grancicova E, Klvanova J. Comparation of nutritient intake and corresponding biochemical parameters in adolescent vegetarians and non-vegetarians. Cas Lek Cesk 2000; **139:** 396–400.
8. Ball MJ, Barlett MA. Dietary intake and iron status of Australian vegetarian women. Am J Clin Nutr 1999; **70:** 353–8.
9. Maier J (Weilheim DE), Gloger M (Weilheim, DE), Drager, B (Tutzing, DE). Method for the determination of low density lipoprotein. United States Boehringer Mannheim GmbH (Mannheim, DE) 1984.
10. Rifai N, Bachorilk PS. Albers lipids, lipoproteins and apolipoproteins. In: Burtis CA Ashwood ER, eds. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: WB Saunders Company; 1999; 809–61.
11. Cole TG, Klotzsch SG, Namara MC. Measurement of triglyceride concentration. In: Rifai N, Warnick GR, Domiminiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press; 1997; 115–26.
12. Grampp S, Genant HK, Mathur A. Comparisons of noninvasive Bone Mineral Measurement in assessing age-related loss, fracture discrimination and diagnostic classification. J Bone and Miner Res 1997; **12:** 697–711.
13. Kanis J. Guide to preventive cardiology for woman. Osteoporosis Int. 1994; **4:** 368–81.
14. Gibson RS, Hotz C. Dietary diversification/ modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. Br J Nutr 2001; **85:** S159–66.
15. Latta D, Liebman M. Iron and zinc status of vegetarian and non-vegetarian males. Nutr Rep Int 1984; **30:** 141–9.
16. Larsson CL, Johansson GK. Dietary intakes and nutritional status of young vegans and omnivores in Sweden: Am J Clin Nutr 2002; **76:** 100–6.
17. Haddad EH. Vegetarian diets and dietary guidelines for chronic disease prevention: How meatless diets conform to current recommendations for healthy eating. In: Sabate J, ed. Vegetarian Nutrition Boca Raton. FL: CRS Press; 2001: 371–409.
18. Alexander D, Ball MJ, Mann J. Nutrient intake and hematological status of vegetarianas and age-seks matched omnivores. Eur J Clin Nutr 1994; **48:** 538–46.
19. Haddad EH, Berk LS, Kettering JD, Hubbard RW, Peters WR. Dietary intake and biochemical, hematological, and immune status of vegans compared with nonvegetarians. Am J Clin Nutr 1999; **70**: 586S–593S.
20. Positions of the American Dietetic Association and Dietitians of Canada: Vegetarian Diets. J Am Diet Assoc 2003; **103:** 7748–65.
21. Key TJ, Fraser GE, Thorogood M, Appleby PN, Beral V, Reeves G et al. Mortality in vegetarians and nonvegetarians: detailed findings from a collaborative analysis of 5 prospective studies. Am J Clin Nutr 1999; **70:** 516–24.
22. Thorogood M, Roe L, McPherson K, Mann J. Dietary intake and plasma lipid levels; lessons from a study of the diet of health conscious groups. BMJ 1990; **300:** 1297–301.
23. Messina MJ, Messina VL. The Dietitians Guide to Vegetarian Diets: Issues and Applications Gaithersburg, MD: Aspen Publishers; 1996.
24. Mosca L, Grundy SM, Judelson D, King K, Limacher M, Oparil S Pasternak R, Pearson TA, Redberg RF, Smith SC Jr, Winston M, Zinberg S. AHA/ACC Scientific Statement Consensus panel statement. Circulation 1999; **99:** 2480–4.
25. Brown L, Rosner B, Willet WW, Sacks FM. Cholesterol-Lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr 1999; **69:** 30–42.
26. Smit E, Nieto FJ, Crespo CJ. Blood cholesterol and apolipoprotein B levels in relation to intakes of animal and plant proteins in US adults. BR J Nutr 1999; **82:** 193–201.
27. Sacks FM, Ornish D, Rosner B, McLanahan S, Castelli WP, Kass EH. Plasma lipoprotein levels in vegetarians. The effect of ingestion of fats from dairy products. JAMA 1985; **254:** 1337–41.
28. Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D, Willett WC. Prospective study of major dietary patterns and risk of coronary heart disease in men. Am J Clin Nutr 2000; **72:** 912–21.
29. Fung TT, Willett WC, Stampfer MJ, Manson JE, Hu FB. Dietary patterns and the risk of coronary heart disease in women. Arch Intern Med 2001; **161:** 1857–62.
30. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K et al. Bone density at various sites for prediction of hip fractures. The study of Osteoporotic Fractures Research Group. Lancet 1993; **341:** 72–5.
31. Peretz A, Tjeka R, Negaban N, Wouters M, Bergmann P. Quantitative ultrasound bone measurements: normal values and comparison with bone mineral density by dual X-ray absorptiometry. Calcif Tissue Int. 1995; **57:** 6–10.
32. Linkswiler HM, Zemel MB, Hegsted M, Schuette S. Protein induced hypercalciuria. Fed Proc 1981; **40:** 2429–33.
33. Kerstetter JE, Allen LH. Dietary protein increases urinary calcium. J Nutr 1990; **120:** 134–6.
34. Sellemeyer DE, Stone KL, Sebastian A, Cummings SR. A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Study of Osteoporotic Fractures Research Group. Am J Clin Nutr 2001; **73:** 118–22.
35. Hannan MT, Tucker KL, Dawson -Hughes B, Cupples LA, Felson DT, Kiel DP. Effect of dietary protein on bone loss in elderly men and women: the Framingham Study. J Bone Miner Res 2000; **15:** 2504–12.

36. Lloyd T, Schaeffer JM, Walker MA, Demers LM. Urinary hormonal concentrations and spinal bone densities of premenopausal vegetarian and non vegetarian women. Am J Clin Nutr 1991; **54:** 1005–10.

37. Marsh AG, Sanchez TV, Michelsen O, Chaffee FL, Fagal SM. Vegetarian lifestyle and bone mineral density. Am J Clin Nutr 1988; **48:** 837–41.