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ABBREVATIONS

BCRU	Bone and Cartilage Research Unit
BMC	Bone mineral content
BMD	Bone mineral density
CLA	Conjugated linoleic acid
DXA	Dual energy x-ray absorptiometry
EPIC	European Prospective Investigation into Cancer and Nutrition
FFQ	Food Frequency Questionnaire
HRT	Hormone replacement therapy
OC	Oral contraceptives
OSTPRE	Kuopio Osteoporosis Risk Factor and Prevention Study
PTH	Parathyroid hormone
SD	Standard Deviation
WHI	Women's Health Initiative

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1 INTRODUCTION AND OBJECTIVES

Osteoporosis imposes an enormous financial burden to the health service worldwide and is a major cause of mortality and morbidity in the elderly population [PRENTICE, 2004]. It is clinically diagnosed by reduced bone mineral density (BMD) or the occurrence of fracture [MELTON III and COOPER, 1998]. Public health strategies to maintain bone health and to prevent osteoporosis in later life are urgently needed. Nutritional factors are of considerable importance for osteoporosis prevention. There is general agreement that calcium and vitamin D are important nutrients for bone health, however, little is known about the effect of other nutrients on BMD [TUCKER et al., 2001]. Effects have been hypothesized for fatty acids, protein, magnesium, vitamin C, potassium and vitamin K. Furthermore, there continues to be a substantial debate about the role of acid and basic components of the diet and bone health.

The importance of the skeleton in maintaining the acid-base homoeostasis has been known for 4 decades. Wachman and Bernstein suggested that the skeleton functions as a buffer base and that the increased incidence of osteoporosis with age might be the result of a lifelong buffering of the acid load of the diet [WACHMAN and BERNSTEIN, 1968]. It is hypothesized that fruit and vegetables could be favourable for bone health, because they may balance this excess acidity by providing alkaline salts of potassium. A few recent observational studies supported the positive association between fruit and vegetable intake and markers of bone metabolism and BMD [LANHAM-NEW, 2008]. In contrast, a high protein diet has been associated with increased calcium loss and bone resorption and might result in osteoporosis [ITOH et al., 1998]. However, results of observational studies are somewhat contradictory and further research is needed to examine the associations between several nutrients and food groups and BMD in different population groups. If the beneficial effect of fruit and vegetables on bone health will be confirmed, a diet rich in fruit and vegetables and could provide a natural alternative for osteoporosis prevention and treatment in the future and goes along with several other health-related benefits [LANHAM-NEW, 2008].

The first aim of the present study was to assess the daily intake of foods, energy and 13 nutrients in postmenopausal Finnish women using food frequency questionnaire (FFQ). The second aim was to examine the associations between nutrient intake and BMD at the lumbar spine and femoral neck and the associations between fruit and vegetable intake and bone measurements in this specific population.

2 REVIEW OF THE LITERATURE

2.1 Osteoporosis

2.1.1 Definition and classification

According to the World Health Organisation (WHO) osteoporosis in postmenopausal Caucasian women can be defined as BMD of 2.5 standard deviation or more below the young normal mean at lumbar spine, femoral neck or forearm, whereas low bone mass is defined as bone mineral density of more than 1 but less than 2.5 SD below the mean [WHO, 1994].

Osteoporosis can be divided into type 1 and type 2 osteoporosis. Type 1 osteoporosis, also called postmenopausal osteoporosis, is caused by oestrogen deficiency and is characterized by trabecular bone loss in women within 15 to 20 years after menopause. Type 2 osteoporosis is related to the aging process and affects both cortical and trabecular bone in men and women over 75 years of age [RIGGS, 1990].

2.1.2 Epidemiology of osteoporosis and osteoporotic fractures

Osteoporosis is clinically recognized by reduced BMD or the occurrence of fracture [MELTON III and COOPER, 1998]. Low BMD is associated with increased fracture risk [HEANEY, 2005]. However, other factors are also associated with increased fracture risk. A recently published study among postmenopausal women found that women with fractures had nonosteoporotic bone mineral density values, i.e. did not have a bone mineral density of 2.5 SD below the mean [CRANNEY et al., 2007].

Age is associated with increased fracture risk. It is known that the incidence rate for hip fracture rises exponentially with age. Furthermore, other factors that are not yet known may be associated with increased fracture risk because there are locations in the world where the elderly population has a low BMD, but osteoporotic fractures hardly occur

[PRENTICE, 2004]. The most common osteoporotic fractures are fractures of the distal forearm, vertebral fractures and hip fractures, but also many other fractures have an increased incidence in postmenopausal women [ALLEN, 1998]. According to Melton III et al. every third women over the age of 50 experiences at least one fragility fracture during her residual life [MELTON III et al 1992]. Besides, there is strong evidence that patients with previous fractures have an increased risk of new fractures [KLOTZBUECHER et al, 2000].

Osteoporosis is a major cause of mortality and morbidity in the elderly population. Occurrence of hip fracture is associated with an increased risk of mortality [SAARENPÄÄ et al., 2006; TOSTESON et al., 2007]. More precisely, women with hip fractures have two times higher mortality rate than women without hip fractures [EMPANA, 2004.]. Many hip fracture patients become lastingly disabled and vertebral fractures are associated with chronic pain and limitations in mobility [ETTINGER et al., 1992]. Furthermore, social implications of the fracture can cause depression and reduce life quality [KING and LI, 2005].

It is difficult to compare incidence and prevalence rates of osteoporosis because several definitions exist and it is difficult to diagnose osteoporosis. For that reason, fracture rates are often compared among the elderly population. When comparing races, it is useful to compare hip fractures because many countries have hip fracture registers [PRENTICE, 2004]. A more detailed description of fracture risk in different ethnicities is given in chapter 2.2.2.

Osteoporosis imposes an enormous financial burden to the health service globally. Worldwide approximately 1.66 million hip fractures occur each year and the incidence is expected to quadruple until 2050 because the number of elderly people is on the raise [PRENTICE, 2004]. In Europe, the number of hip fractures was approximately 0.89 million in 2000 and the number of total osteoporotic fractures about 3.79 million [KANIS and JOHNELL, 2005]. Annually 611000 women are expected to experience hip fractures. The total cost of fractures attributable to osteoporosis is 25 billion Euros [MELTON III et al., 2003]. In the USA, the costs of osteoporotic fractures were estimated to be 13.8 billion dollar in 1995 [RAY et al., 1997]. In a recently released population based study in Finland it was pointed out that the number of hip fractures increased by 70% between 1992 and 2002 [LÖNNROOS et al., 2006].

2.1.3 Measurement of bone mineral density

Commonly used methods include single x-ray absorptiometry and dual x-ray absorptiometry (DXA). DXA is the most widely used central measurement technique and measures the spine, hip and total body BMD. Single x-ray absorptiometry can be used at sites without much overlying fat and muscle tissue, such as wrist and heel. Studies of the radiation dose have confirmed that the patient radiation is rather low [NJEH et al., 1999]. Peripheral dual x-ray absorptiometry (P-DXA) is a type of DXA test and is used to measure the density of bones in the arms or legs, such as the wrist. Other methods include dual photon absorptiometry (DPA), ultrasound, quantitative computed tomography (QCT) and digital x-ray radiogrammetry (DXR) [BONNICK, 2004].

2.2 Non-dietary factors associated with bone mineral density

2.2.1 Gender

Women are four times more likely to develop osteoporosis than men [LEITZMANN et al., 2003]. However, even if the prevalence of osteoporosis and risk of fractures are lower in men, there is some evidence that the disease is more severe in men. It has been observed that men have a 2-fold higher risk of dying after a hip fracture than women [WEHREN et al., 2003].

2.2.2 Ethnicity

Hip fracture prevalence is higher in Caucasian women than in Asian women. Eventhough that the number of hip fractures in Asia varies in different Asian countries, the prevalence is lower in all of them as compared to the Caucasian populations [XU et al., 1996; LAU et al., 2001]. Despite the current situation, Copper et al. suggest that with economic growth and aging of the Asian populations more than half of all hip fractures will occur in Asia by the year 2050 and therefore hip fracture will be a major public health concern in Asia [COPPER et al., 1992].

2.2.3 Genetics

Studies in twins and families have shown that BMD is under strong genetic control [RALSTON, 2007]. It is estimated that the heritability of BMD is about 60% to 80%. In addition, osteoporotic fractures are believed to be genetically determined, although the heritability is estimated to be lower than for BMD [DENG et al., 2000a; DENG et al., 2000b].

Currently, research is focusing on identifying the specific genes that are responsible for bone loss and fracture. The associations between polymorphisms of the vitamin D receptor (VDR) gene and BMD and the occurence of fractures are examined [GRUNDBERG et al, 2007; HORST-SIKORSKA et al., 2007]. Genes coding for the estrogen and androgen receptors (AR) are also held responsible for part of the genetic background [RETORNAZ et al.; 2006; SILVESTRI et al., 2006]. Over the past years, there has been an increasing recognition of polymorphisms in regulatory regions of the collagen type Iα1 (COLIA1) gene and of several enzymes involved in the biosynthesis of estrone and estradiol [THIJSSEN, 2006]. On the other hand, there are studies that could not find any effect of polymorphism of the VDR, estrogen or COLIA 1 genes on BMD [BANDRES, 2005; VIDEMAN et al., 2007].

It is generally agreed that BMD and fractures are to a large degree genetically determined, but it is not yet clear what genes are responsible. The mechanisms and genes involved in the pathogenesis of osteoporosis are believed to be rather complex and most probably there are interactions between nutritional and genetic factors [CUSACK and CASHMAN, 2003].

2.2.4 Menopause

In industrialized societies the average age when menopause occurs is about 51. Smoking has been associated with earlier and use of oral contraceptives with later menopause [GOLD et al., 2001]. During growth, bone mass increases until peak bone mass is reached at the age of about 30. Between the achievement of peak bone mass and the onset of menopause the bone mass stays relatively constant. The majority of the bone loss during the life time occurs during menopause. It is maximal in the beginning of menopause and decreases in later menopause [SIROLA et al., 2003]. During the first years of menopause, women may lose as far as 2% of their bone mass per year [POULLES et al., 1995].

2.2.5 Body weight

Body weight is a key determinant of BMD. A low body mass index (BMI) is related to an increased risk of osteoporosis and an increased bone loss has been described in patients with anorexia nervosa when compared to controls [LEGROUX-GEROT et al., 2007]. However, a BMI above 40 kg/m^2 has also been reported to be unfavourable for the bone [NUNEZ et al., 2007]. It is well known that weight loss is related to bone loss. However, physical activity during weight loss leading to increased muscle strength may mitigate the negative effect of weight loss in postmenopausal women [SIROLA et al., 2006]. Mizuma et al. investigated whether the body composition is important for maintaining BMD and has shown that higher lean body mass is associated with a higher BMD in premenopausal women and women up to 10 years after menopause, whereas the body fat mass begins to influence BMD in postmenopausal women over 60 years old [MIZUMA et al., 2006]. The underlying mechanisms are not completely understood yet, but several recently published studies indicate that the hormone leptin, which is produced by the fat tissue, is involved in bone formation [COCK and AUWERX, 2003; HAMRICK and FERRARI, 2007].

2.2.6 Physical activity

There is strong evidence that physical activity can reduce the risk of fractures and improve BMD in postmenopausal women. It has been suggested that physical activity during youth has long lasting beneficial effects on bones in postmenopausal women [RIDEOUT et al., 2006]. However, BMD is not exclusively determined during adolescence. Different intervention programs carried out in postmenopausal women vary in exercise type and duration, but they have overall shown to be favourable for the bones. Englund et al. conducted a trial containing 48 study participants and randomly assigned the participants to either an exercise or a control group. The exercise group accomplished twice a week a weight bearing program lasting for 50 minutes over a period of 1 year. Compared to the control group, the intervention group showed increased BMD as well as improved grip strength and walking ability [ENGLUND et al., 2005]. Similar results were found in the 3-year longitudinal Erlangen Fitness

Osteoporosis Prevention Study among postmenopausal women, where the exercise group with 4 training sessions per week maintained BMD and furthermore showed reduced pain frequency and intensity in the spine [ENGELKE et al., 2006]. Furthermore, results of observational studies have demonstrated that habitual physical activity is associated with reduced fracture risk. In the Nurses Health Study including a sample size of 61,200 postmenopausal women, it was reported that moderate physical activity, walking for more than 4 hours per week is associated with a lower risk of hip fracture during a follow-up of 12 years [FESKANICH et al., 2002]. A significant relationship between muscle strength and a reduced risk of fracture has been reported by Sinaki et al, who demonstrated the long-term protective effect of stronger back muscles on vertebral fractures in postmenopausal women [SINAKI et al., 2002].

2.2.7 Smoking

Cigarette smoking appears to have an independent, dose-dependent negative effect on BMD [WARD and KLESGES, 2001]. The adverse effect of smoking on BMD has been observed in current smokers. Smoking cessation has shown to have a positive influence on bone mass. The results of a study by Gerdhem and Obrant are very promising, since no differences in BMD between non-smokers and former smokers were observed [GERDHEM and OBRANT, 2002]. A recent meta-analysis has shown that current smoking increases a person’s risk for almost any fracture, whereas the highest risk is observed for hip fractures [KANIS et al., 2005]. The association may be partly explained by the fact that smoking decreases intestinal calcium absorption [KRALL et al., 1999]. Other contributing factors might be the lower body weight or earlier menopause observed among smokers [NICKLAS et al., 1999; REYNOLDS et al., 2005].

2.2.8 Lactation

Lactating women have a decreased bone mass compared to nonlactating women. This is because the calcium needed for producing breast milk is derived mainly from the maternal skeleton. However, it has been noticed that the lost bone mass is gained back within a short period after lactating [POLATTI et al., 1999]. The majority of the studies conclude that neither breastfeeding nor the number of pregnancies influences BMD in later life [CARRANZA-LIRA and MERA, 2002; KOJIMA et al., 2002]. These studies, however, did not investigate women at risk, such as adolescent women or women with calcium or vitamin D deficiencies.

Studies suggest that breastfeeding is not lastingly associated with a mother's BMD, but it could be very beneficial for the children's bones. Jones et al. reported that children who were breastfed for more than 3 months showed higher BMD values at age 8 than children who were breastfed for less than 3 months or who were not breastfed at all. Whether this effect persists until the attainment of peak bone mass is not known yet [JONES et al., 2000].

2.2.9 Oral contraceptive use

The use of oral contraceptive (OC) may be associated with lower BMD. One recent cross-sectional study among 248 young Caucasian women demonstrated an unfavourable effect of OC on BMD. As compared to women who had never used OC, women with more than 2 years of OC use and OC initiation within 3 years after menarche showed 10% lower femoral neck BMD and 5% lower spine BMD. Furthermore, women who had sometimes used OC had lower bone mass than those who had never used OC [HARTARD et al., 2007]. A study among 18 to 35 year old athletes found similar results. Study participants were allocated into either the OC group or control group. Subjects younger than 22 years were assigned into the OC group if they reported OC use for more than 3 years. Subjects between 22-35 years were allocated into the OC group if they used OC for more than 50% of the time after menarche. The BMD values in the OC group were 8.8 % lower at the femoral neck and 7.9 % lower at the spine than in the control group. Therefore, introduction of OC at early age may be an important risk factor for low peak bone mass [HARTARD et al., 2004]. These findings were supported by Almstedt Shope and Snow, who found significantly lower bone mass in young women currently using OC than in the controls [ALMSTEDT

SHOPE and SNOW, 2005]. However, some studies failed to demonstrate any effect of OC on BMD or peak bone mass [LYOYD et al., 2000; REED et al., 2003]. In summary, it can be concluded that the more recent studies support the hypothesis that the use of OC is linked with loss of BMD in young women.

2.2.10 Hormone replacement therapy

Hormone replacement therapy (HRT) has been shown to be effective in the prevention and treatment of bone loss at the time of menopause [WELLS et al., 2002]. However, attitudes towards HRT use changed when the results of the Women's Health Initiative (WHI) were published [KO, 2008]. In this clinical trial, the health benefits and risks of the most commonly used combined hormone preparation were examined in 16608 healthy postmenopausal U.S women aged 50-79 years. Study participants were followed up over a period of 5.2 years. The study group received a combination of the progestin medroxyprogesterone acetate and conjugated equine estrogens. The major clinical outcomes of the study were significantly more cases of breast cancer, coronary heart disease, strokes and pulmonary emboli and significantly fewer cases of colorectal cancer and hip fractures. The study concluded that the health risks exceeded the health benefits from use of combined estrogen plus progestin [ROSSOUW et al., 2002].

2.2.11 Diseases and medications

If a disease or drug exacerbates bone loss the process is termed as secondary osteoporosis. Most frequently it occurs due to the use of glucocorticoids [MIGLIACCIO et al., 2007]. Several diseases and drugs are associated with an increased risk of osteoporosis, amongst others multiple myeloma, diabetes mellitus, hyperparathyroidism, gastrectomy and celiac disease [NILAS and LISBETH, 1993; STAZI et al., 2008].

2.3 Fruit and vegetable intake and bone health

2.3.1 Fruit and vegetable intake and bone mineral density

Several recent observational studies have examined the association between fruit and vegetable intake and BMD. Except for one study conducted in British women aged 67-79 years, high fruit and vegetable consumption has been associated with greater BMD in various populations and age groups [KAPTOGE et al., 2003]. A study including a large sample of 12- and 15 year-old adolescent boys and girls found a significant positive association between high fruit intake and high heel BMD in 12 year old girls. However, no significant associations were found in boys, or between BMD and intake of vegetables in any of the groups [MCGARTLAND et al., 2004].

Prynne et al. examined the association between fruit and vegetable intake and bone mineral content (BMC) and BMD in a cross-sectional study in 5 age and sex cohorts. There was a positive association between fruit intake and both spine-adjusted BMC and whole-body BMD in adolescent girls and boys aged 16-18 years and in older women aged 60-83 years. The results indicated that doubling the fruit intake in girls could have increased BMD by 2,3%, and in the older women spine BMC by 5%. No associations were observed among young women and older men, or between vegetable intake alone and bone measurements [PRYNNE et al., 2006].

One study has examined the relationship between fruit consumption and BMD in a large Chinese sample of 5848 men and 6207 women aged 25-64. Dietary intake was obtained by a short questionnaire. Higher fruit intake was significantly associated with greater BMD in both sexes. However, there was no indication of a positive association between vegetable intake and BMD [ZALLOUA et al., 2007].

Most of the studies addressing the link between fruit and vegetable consumption and bone health are conducted in peri- and postmenopausal women [NEW et al., 1997; TUCKER et al., 1999; NEW et al., 2000; CHEN et al., 2006]. In a Chinese study among postmenopausal women aged 46-63 years, greater fruit and vegetable intake was associated with increased BMD. The mean daily consumption of fruit and vegetables was 427g, which is a higher amount than consumed in most Western populations [CHEN et al., 2006].

A lot of the research in this field has been done by Dr. New's group. In one of their earlier studies, associations between current and past dietary intake and BMD in 994 premenopausal women aged 45-49 were examined. Current dietary intake was assessed by FFQ. Information on nutrition in early adulthood and childhood was obtained by questionnaires. High current intakes of potassium, magnesium, vitamin C, fiber, and zinc were associated with higher BMD. Additionally, low fruit intake in earlier adulthood was associated with significantly lower BMD [NEW et al., 1997]. A later study by New's group with only 62 subjects aged 45-55 confirmed the previous findings. In the study, the women were grouped as either premenopausal, perimenopausal or postmenopausal. The amount of fruit consumed in the women's childhood was positively associated with femoral neck BMD. Further, key nutrients found in fruit and vegetables, such as potassium, betacarotene, magnesium, and vitamin C were associated with markers of bone metabolism [NEW et al., 2000].

2.3.2 Nutrients found in fruit and vegetables and bone mineral density

Some previous studies have focused on one or more nutrients primarily found in fruit and vegetables, rather than overall fruit and vegetable intake. Vitamin K is now receiving more attention for its function in bone metabolism. Most, but not all earlier studies indicated a positive relationship between vitamin K intake and bone health. A study conducted in a population-based cohort of 2016 perimenopausal women failed to show any association between vitamin K intake and BMD, BMD change and also fracture risk. The main difference between this study and other studies was that median vitamin K intake was rather low, only about one third of the vitamin K intake reported in other studies [REJNMARK et al., 2006]. Several other studies, however, found a significant positive association between dietary vitamin K intake and BMD or fracture risk in both sexes. The longitudinal Framingham Offspring study covered a wide age range (29-86 years) and rather large sample size (n=2591). After controlling for potential confounders, women in the highest dietary vitamin K intake showed significantly higher femoral neck and lumbar spine BMD values [BOOTH et al., 2003]. In both the Nurses´ Health Study and the Framingham Heart Study, an inverse association between vitamin K intake and hip fracture risk has been observed [FESKANICH et al., 1999; BOOTH et al., 2000].

Animal studies have demonstrated the importance of magnesium for bone health [RUDE et al., 1998, CREEDON et al., 1999]. A few population studies have found a significant positive association between dietary magnesium intake and BMD. Most of the studies have been conducted among elderly white women [NEW et al., 1997; MACDONALD et al., 2004]. Ryder et al. conducted a cross-sectional study to examine whether magnesium intake from food and supplements is associated with BMD in older subjects. A further aim of the study was to examine whether the association differs between men and women or white and black subjects. As a result, there was a positive association between total magnesium intake and BMD measured at the whole body in white men and women, but not in black subjects [RYDER et al., 2005].

A study conducted among elderly subjects that were members of the original cohort of the longitudinal Framingham Heart Study examined the associations between the intake of fruit and vegetables and nutrients contributing to an alkaline environment and BMD. The results were similar to those of other studies, showing a significant positive association between fruit and vegetable intake, potassium and magnesium intake and BMD at different sites. Most of the associations were observed for higher potassium intake, which was significantly associated with greater BMD at 4 sites in men and 3 sites in women [TUCKER et al., 1999].

The relation between potassium intake and BMD was also examined in a large population-based investigation including 3226 women [MACDONALD et al., 2005]. Low potassium intake was related to low BMD in premenopausal women, and to increased markers of bone resorption in postmenopausal women. Potassium is important for bone health because it has an influence on calcium homeostasis. Low potassium intake may harm bone health by increasing urinary calcium losses while high intake may reduce it [NIEVES, 2005].

MacDonald et al. examined the relation between dietary factors and BMD and BMD change in 891 women in a longitudinal study. The women were 45-55 year old at baseline and 50-59 years old at follow-up. Calcium was found to be a significant predictor of femoral neck BMD change, monounsaturated and polyunsaturated fatty acids were negatively associated with femoral neck BMD change and modest alcohol intake was found to be positively associated with BMD. In women who were still menstruating and were not affected by the consequences of estrogen withdrawal, vitamin C, magnesium, and potassium were associated with femoral neck BMD and vitamin C and magnesium were also associated with femoral neck BMD change [MACDONALD et al., 2004]. Vitamin C intake and antioxidant vitamin intake in general, may protect the bones from the oxidative stress from smoking [MELHUS et al., 1999; ZHANG et al., 2006].

2.3.3 The acid-base homeostasis

Western diets high in acid forming components and low in base forming components are considered to be a risk factor for osteoporosis and bone fractures. Acid forming components include several amino acids in protein foods, phosphorus and chlorine, whereas base forming components include fruits and vegetables, potassium, calcium, magnesium and vitamin C [TUCKER et al., 2001]. Already in 1968, Wachman and Bernstein suggested that the skeleton functions as a buffer base and that the increased incidence of osteoporosis with age might be the result of a lifelong buffering of the acid load of the diet [WACHMAN and BERNSTEIN, 1968]. The mechanisms for an unfavourable effect of an acid environment on BMD are well established and have already been reported in the late 1980s. Arnett and Dempster demonstrated that a reduction in extracellular pH is followed by a direct increase of osteoclastic activity, independently of the influence of parathyroid hormone [ARNETT and DEMPSTER, 1986; BUSHINSKY et al., 1993]. In later work, Arnett and Spowage and Bushinsky have shown that a small fall in pH, causes a remarkable surge in bone resorption [ARNETT and SPOWAGE, 1996; BUSHINSKY, 1996].

Population-based studies have demonstrated that low protein intake is associated with lower BMD and increased hip fracture risk. Furthermore, protein intake increases urinary calcium loss, because the acid load from dietary protein needs to be buffered by skeletal bone loss [see chapter 2.4.4].

Humans eat foods that both generate and consume protons. As a net result, the consumption of an average Western diet generates about 1 mEq (milliequivalent) protons per kilo body weight every day and thus, is associated with chronic, low-grade metabolic acidosis [NEW, 2008]. If 2mEq/kg body weight of calcium per day is required to buffer this fixed acid every day, an average individual will loose 15% inorganic bone over a period of 10 years [WACHMAN and BERNSTEIN, 1968].

The degree of the metabolic acidosis is determined in part by the net rate of endogenous noncarbonic acid production (NEAP) and varies with the diet consumed [FRASSETTO et al., 1998]. In the Aberdeen studies, New et al. demonstrated that women with the lowest estimate of NEAP have higher lumbar spine and femoral neck BMD and significantly lower urinary pyridinium cross-link excretion, a marker of bone resorption [NEW et al., 2001]. It is hypothesized that fruit and vegetables could be favourable for bone health, because they may balance this excess acidity by providing alkaline salts of potassium. Several observation studies support the positive association between fruit and vegetable intake and BMD (see chapter 2.5.1). However, the positive association between fruit and vegetable intake and BMD may be also partly attributable to some other factors than the effect of alkali excess [LANHAM-NEW, 2008]. In recent years, pharmacologically-active compounds and phytoestrogens have gained considerable attention [MUHLBAUER et al., 2003; see chapter 2.4.5].

2.3.4 Results of intervention studies

The Dietary Approaches to Stopping Hypertension (DASH) trial was the first population based trial showing that an increase in fruit and vegetable intake from 3,6 to 9,5 servings daily decreases urinary calcium excretion [APPEL et al., 1997]. The DASH-sodium trial found that the DASH diet, which is rich in fruits and vegetables, low-fat dairy products and low in red meat, reduces both bone formation and bone resorption compared to a control diet [LIN et al., 2001].

Effect of vitamin K intake on bone was shown in a randomized controlled trial in 181 postmenopausal women aged 50 to 60 years. Vitamin K supplementation of 1000 μg per day for 3 years led to increased hip BMD of 1.3% compared with a controlled group that did not receive any vitamin K supplementation. In this trial, no effect was observed on lumbar spine BMD [BRAAM et al., 2003].

In contrast, a recently conducted double-blind controlled trial in 401 older men and women could not show any beneficial effect of phylloquinone supplementation on BMD over a period of 3 years. In the trial, BMD was measured at the hip and spine. All subjects (intervention and control group) received additional vitamin D and calcium supplements [BOOTH et al., 2008].

In conclusion, the above described investigations highlight the importance of fruit and vegetable intake and its nutrients for bone health, even though many questions remain unanswered. Further research is warranted and should include randomized long-term controlled trials focused specifically on fruit and vegetable intakes to confirm the beneficial association and identify the magnitude of the benefit. Further, experimental studies are needed to determine whether other components are involved and what are the mechanisms [NEW, 2003].

2.4 Other dietary factors associated with bone mineral density

2.4.1 Calcium and calcium metabolism

The average adult body contains about 1000-1300 g calcium, approximately 99% of it resides in the bones. Ingested calcium is absorbed primarily via active transport and requires vitamin D. Consequently, calcium and vitamin D are often used together as dietary supplements. The human body is able to adjust for changes in calcium intake, by increasing calcium absorption when the ingestion is reduced and vice versa. The plasma calcium is under strict homeostatic control, which is achieved through the two hormones: parathyroid hormone (PTH) and 1, 25-dihydroxycholecalciferol (active form of vitamin D).

Calcium and vitamin D are the most important nutrients in osteoporosis prevention and treatment [NIEVES, 2005]. Higher calcium intakes have repeatedly been associated with both higher BMD and reduced bone loss among all age groups and both sexes [ILICH et al., 2003; HEANEY, 2005]. Recent research indicates that dietary calcium may be associated with greater BMD than calcium from supplements [NAPOLI et al., 2007]. A meta-analysis of 15 trials, concluded that calcium supplementation decreases bone loss rates by 1.66% at the lumbar spine and 1.64% at the hip in postmenopausal women [SHEA et al., 2002]. A recent trial showed similar results, reporting that calcium supplementation reduces the rate of bone loss by 1.8% at the lumbar spine and 1.6% at the hip [REID et al., 2006]. There is some evidence that there are only small or even no benefits after discontinuing calcium and vitamin D supplementation in the elderly [DAWSON-HUGHES et al., 2000]. Furthermore, there seems to be a threshold beyond which no further benefit of calcium on bone density can be achieved [LOOKER, 2003]. There is increasing evidence that there are gene-environmental interactions. Studies have found bone-related gene polymorphisms that are interacting with calcium or other nutrients. It is not yet clear whether there are also interactions between calcium intake and physical activity [MURPHY et al., 2003; BONJOUR et al., 2007].

Several studies investigated the effect of calcium intake on the risk of fractures. Some researchers reported that calcium intake has only minor or no effects on fracture risk [SHEA et al., 2002; REID et al., 2006]. However, many other investigators found reduced fracture rates after calcium supplementation, especially when combined with vitamin D (see chapter 2.4.2). Due to these inconsistent findings a definite proof of a beneficial effect of calcium intake on fracture risk is still lacking, but there is strong evidence that calcium and vitamin D together are beneficial in preventing fractures and falls.

2.4.2 Vitamin D

The main biologic function of vitamin D is to maintain calcium, phosphorus and bone homeostasis. More recent studies indicate that vitamin D has functions beyond that. The active form of vitamin D, 1,25-dihydroxycholecalciferol has been shown to inhibit cell proliferation, stimulate cell differentiation and also affect immune system [LIN and WHITE., 2004.; HOLICK, 2004; SHOSHAN-BEN et al., 2007].

Vitamin D is obtained by endogenous production in the skin after sunlight exposure and from the diet. With increasing age the cutaneous synthesis is reduced, therefore the dietary intake of vitamin D gains considerable importance in the elderly. Furthermore, studies in Nordic countries have shown that the vitamin D status is low during winter and that dietary vitamin D intake is a significant predictor of vitamin D status [BRUSTAD et al., 2004; ANDERSON et al., 2005]. After ingestion or sunlight exposure, vitamin D is first metabolized in the liver to 25–hydroxy–vitamin D (calcidiol) and then in the kidney to 1,25-dihydroxycholecalciferol (calcitriol). Calcidiol is the major circulating form of vitamin D and is considered a good biomarker of vitamin D status reflecting an individual's dietary intake and endogenous synthesis [CRANNEY et al., 2007].

Several population-based studies investigated the association between serum 25–hydroxy–vitamin D and BMD in both sexes and different age groups. The vast majority report a positive association between low serum 25–hydroxy–vitamin D and BMD [VÄLIMÄKI et al.,2004; BISCHOFF-FERRARI et al., 2004; ROY et al., 2007]. However, a few investigators failed to show any association between the two variables [SIGURDSSON et al., 2000; HOSSEINPANAH et al., 2008].

There is general agreement on the underlying mechanism responsible for the association between serum calcidiol and BMD. Low serum 25–hydroxy–vitamin D is associated with reduced gut calcium absorption. As a consequence, PTH secretion is stimulated, thus PTH levels increase. Higher concentrations of PTH lead to increased bone turnover and bone loss [DEVINE et al., 2002; WILLETT, 2005].

Only very few studies have examined the association between dietary vitamin D and BMD. Michaelsson et al. observed recently a positive association between dietary vitamin D intake and BMD in older Swedish men [MICHAELSON et al., 2006]. Ilich et al. found a few years earlier only a weak association between dietary vitamin D intake and BMD in postmenopausal women [ILICH et al., 2003]. There is good evidence that intakes from vitamin D fortified foods increase serum calcidiol in children, adults and the elderly [TANGPRICHA et al., 2003; JOHNSON et al., 2005; PIIRAINEN et al., 2007]. However, in Finland the vitamin D fortification of liquid milks and margarines did not significantly change the mean winter 25–hydroxy–vitamin D concentrations in young Finnish men and women [VÄLIMÄKI et al., 2007; LEHTONEN-VEROMAA et al., 2008].

Daily vitamin D3 supplementation of 10 μg, obtained from either fish oil capsules or multivitamin tablets has shown to significantly increase serum 25–hydroxy–vitamin D concentrations [HOLVIK et al., 2007]. Several randomized controlled trials showed a beneficial effect of vitamin D supplementation together with calcium supplementation on BMD [DI DANIELE et al., 2004; ZHU et al., 2007]. A meta-analyzes of randomized controlled trials reported that vitamin D supplementation in doses between 700 to 800 IU (equalling 17.5 to 20 micrograms) per day, with or without calcium, prevent hip and nonvertebral fractures in ambulatory or institutionalized elderly persons [BISCHOFF-FERRARI et al., 2005]. In addition, associations between vitamin D and calcium supplementation and reduced fall rates have been reported in elderly women [LARSEN et al., 2005].

A very comprehensive review by Cranney et al. concluded that there is discordance on how to define the most beneficial threshold of circulating 25–hydroxy–vitamin D for bone health. The reported optimal concentrations differ considerably, ranging from 40 to 120 nmol/l. According to the same review, there is little evidence that vitamin D doses above the reference intakes are harmful [CRANNEY et al., 2007]. However, the authors warn that most trials conducted so far, did not evaluate long-term harms and were not properly designed to measure adverse events [CRANNEY et al., 2007].

In summary, the available data on vitamin D and bone health suggest that vitamin D has favourable effects on BMD, fractures and fall risk and therefore plays a major role in the prevention of osteoporosis.

2.4.3 Fatty acids

Animal studies have suggested that n-3 fatty acids polyunsaturated fatty acids are positively associated with BMD. Supposedly these fatty acids prevent bone loss by modulating bone formation and bone resorption factors [SUN et al., 2003; BHATTACHARYA et al., 2007]. Recent findings indicate that docosahexaenoic acid may be more effective in preventing bone loss than eicosapentaenoic acid [RAHMAN et al., 2008]. In addition, conjugated linoleic acid (CLA) has been investigated in animal studies. It has been shown that CLA fed mice have higher BMD and CLA fed rats have reduced bone loss when compared with control groups. This might be explained by the fact that CLA modulates markers of inflammation and osteoclastogenic factors [KELLY and CASHMAN, 2004].

Only a few observational studies have been performed so far. A recent study conducted in a small sample of Swedish men found that serum concentrations of n-3 fatty acids were positively associated with peak bone mass [HÖGSTRÖM et al., 2007]. Human studies containing a bigger number of subjects include the Third National Health and

Nutrition Examination Survey (NHANES III) and the Rancho Bernardo Study. The association between dietary fat intake and hip BMD was explored in the NHANES III cohort, which includes 14850 subjects. The major finding of the analysis was that dietary saturated fat is negatively associated with hip BMD and the strongest association was observed among men [CORWIN et al., 2006]. The longitudinal Rancho Bernardo Study showed that not only the type of fat in the diet is important for optimal bone health, but also the ratio of different fatty acids to each other. This study found that a high ratio of n-6 to n-3 fatty acids is associated with lower BMD at the hip in both men and women [WEISS et al., 2005].

The association between fat intake and osteoporotic fracture is not yet well established and studies are very rare. One study conducted in a Spanish population suggests that high polyunsaturated fatty acid intake is related to an increased fracture risk, while a high ratio of monounsaturated fatty acids to polyunsaturated fatty acids is associated with a reduced risk of fracture [MARTINEZ-RAMIREZ et al., 2007].

To summarize, there is rising evidence from animal and human studies that n-3 fatty acids and conjugated linoleic acids are positively associated and saturated acids negatively associated with BMD. Further studies should be carried out to proof these hypotheses and to better understand the association between dietary fat intake and the risk of fracture.

2.4.4 Protein

The role of dietary protein intake on bone health is controversial. Central to this debate, has been the endogenous acid hypothesis suggested by Wachman and Bernstein in 1968 [WACHMAN and BERNSTEIN, 1968]. This hypothesis suggests that protein contributes to an acidic environment and as consequence calcium is released from the skeletal bone to neutralise the acid effect. Nowadays studies still support this theory, reporting that a high-protein intake increases urinary calcium excretion [KERSTETTER et al., 2003]. Itoh et al. observed in a Japanese population that subjects consuming a diet high in animal protein loose more calcium in their urine than those with a high vegetable protein intake [ITOH et al., 1998].

Animal protein appears to have a different effect on bone health than vegetable protein. One study examining the ratio of animal to vegetable protein intake in 9704 elderly white women concluded that an increase in vegetable protein intake and a decrease in animal protein intake reduced bone loss [SELLMEYER et al., 2001]. The population-based European Prospective Investigation into Cancer and Nutrition (EPIC) Potsdam Study, that includes a large number of female participants, strengthened the assumption that a high intake of vegetable protein is favourable, whereas animal protein is adversarial for bone health [WEIKERT et al., 2005].

On the contrary, there are data that could not prove the detrimental effect of a high protein diet. Low total and animal protein intake has been linked with bone loss in the longitudinal population-based Framingham Osteoporosis Study. The greatest bone loss was observed in those elderly who had the lowest intake of total protein and animal protein, while protein intake of nonanimal origin was not related to bone loss at all [HANNAN et al., 2000]. Vegetable protein was negatively associated with BMD in an elderly population of the southern California community Rancho Bernando. Furthermore, results of the study are absolutely contradictory to the endogenous acid theory, since the positive association between protein intake and BMD was greatest in elderly women with the lowest calcium intakes [PROMISLOW et al., 2002].

There is strong evidence that low protein intake contributes to the risk of fracture [WENGREEN et al., 2004]. Protein supplementation leads to reduced bone loss in patients with hip fracture [SCHÜRCH et al., 1998]. Consequently, an adequate protein intake according to the recommendations is absolutely essential for elderly persons, whose usual protein intake has been reported to be rather low [HANNAN et al., 2000].

To summarize, the effect of animal protein on bone health should be examined in future studies, because it has been reported to be both positively and negatively associated with BMD in the elderly subjects. Even though the role of protein in osteoporosis has been inconsistent, most of the studies conclude that relatively low protein intake is associated with fractures and bone loss. On the other hand, excessively high protein intake has been shown to increase calcium excretion and therefore, there have been concerns that it is also unfavourable for bone health.

2.4.5 Phytoestrogens

Recently various studies have been published about the potential positive effect of phytoestrogens on bone health. Since the concern about the side effects of HRT is rising, the use of phytoestrogens, especially isoflavones as an alternative to HRT has gained substantial interest [MCKEE and WARBER, 2005]. Phytoestrogens are a type of plant chemical with estrogenic characteristics. They are able to bind the estrogen receptors displaying either estrogen-agonist or estrogen-antagonist effects [MOUTSATSOU, 2007].

Studies among the Asian populations provide evidence on the association between isoflavones and bone health. Researchers have shown that postmenopausal women with a high isoflavone intake have a higher BMD [SOMEKAWA et al., 2001; MEI et al., 2001]. A single-blind randomized, placebo controlled trial has been carried out in China to detect whether there is a dose dependent linear relationship between soy isoflavone intake and femoral neck and lumbar spine BMD. The study population was assigned in 3 treatment groups with daily soy isoflavone supplementation of 0, 84 and 126 mg for 6 months. As a result there was a dose- dependent linear relationship between soy isoflavones supplementation and BMD after 12 weeks but not after 24 weeks after adjusting for confounding factors [YE et al., 2006].

In Western populations, where habitual soy intake is very low, the research on phytoestrogens and bone health is very limited. A study carried out in Italy compared the effect of a soy rich diet and HRT on the main biomarkers of bone turnover and BMD at postmenopausal age. The study population was randomized into a soy rich diet group, a HRT group and a control group. In this study, BMD has significantly decreased in the control group, but not in the 2 intervention groups. The study showed that a soy rich diet is effective but not as effective as HRT in reducing the postmenopausal turnover [CHIECHI et al., 2002].

Before any recommendation for the use of phytoestrogens can be made, further studies are needed to examine the effects of phytoestrogens in Western populations. The mechanisms underlying the effect of phytoestrogens, and the optimal dosage at different age groups and in different populations are not known. The effect of isoflavones in Asian people should also be further explored, because the studies conducted up to now had small sample sizes and did not investigate long term effects.

2.4.6 Alcohol

Light to moderate alcohol intake may have a beneficial effect on BMD in postmenopausal women [WILLIAMS et al., 2005; WOSJE and KALKWARF, 2007]. An alcohol intake of 28.6 g to 57.2 g per week (equalling 0.5-1 unit per day) was found to be the optimal dose for a protective effect of alcohol [RAPURI et al., 2000]. The reason for the positive association could not yet be elucidated. Several mechanisms are currently discussed [JUGDAOHSINGH et al., 2006]. However, there is no association between high intake of alcohol and BMD. Furthermore, alcohol intake above 2 units per day is associated with an increased risk of fracture [KANIS et al., 2005].

2.4.5. Caffeine

A recent large prospective cohort study has suggested that caffeine intake may be associated with increased osteoporotic fracture risk. Swedish women consuming 330 mg of caffeine or 4 cups of coffee or more per day were associated with an increased risk of fractures, especially if their calcium intake was low [HALLSTRÖM et al., 2006]. This could be explained by a negative effect of caffeine on calcium balance by inducing calcium excretion [HEANEY and RAFFERTY, 2001]. The negative effect of caffeine on BMD has been found particularly in women with relatively low calcium intake. It has been shown that this effect can be balanced by increasing calcium intake

[BARGER-LUX and HEANEY, 1995]. In the study by Hallström et al. no association could be found between tea intake and fracture risk. However, in previous studies tea consumers were reported to have a decreased risk of bone loss and fractures [WU et al., 2002; CHEN et al., 2003].

2.5 Food frequency questionnaire

2.5.1. Definition and purpose

The FFQ has been defined by Nelson and Bingham as the following: "A questionnaire in which the respondent is presented with a list of foods and is required to say how often each is eaten in broad terms such as x times per day/per week/per month, etc. Foods listed are usually chosen for the specific purpose of a study and may not assess total diet …." [MARGETTS and NELSON, 1997].

The FFQ belongs to the group of retrospective dietary assessment methods and is designed to assess usual diet rather than measuring the diet of one or a few days. FFQs are developed to ascertain either food consumption or nutrient intake. Due to the fact that FFQ is time-saving, cost-effective and appropriate to assess usual eating habits, it has become the most frequently used dietary assessment method in epidemiology [WILLETT, 1998; CADE et al., 2002]. It is also the method that has been most often used in studies on bone health and nutrition [LAU and LAU, 2004].

2.5.2 Development and administration

The FFQ may either be developed from basic principles for a specific study and population or by modifying an already existing questionnaire. The most common adapted questionnaires are the Block Health Habits and History Questionnaire (Block FFQ) and the Harvard Semiquantitative Food Frequency Questionnaire (Willett FFQ). Questionnaires may be self- or interviewer -administered. Interview-administered include face-to-face interviews and telephone interviews [CADE et al., 2002]. Molag et al. detected no significant differences in correlation coefficients between the two administration methods [MOLAG et al., 2007].

2.5.3 The food item list

For a food item to be informative it must be consumed reasonably often by a considerable number of the population, it must contain a large amount of the target nutrient and the use of the food must vary from person to person to discriminate among subjects. To detect foods that most discriminate between subjects a stepwise linear regression analysis on a recent nutrient dataset from a suitable population can be used. The food list can also be complied by including food items that are known to be associated with a certain disease [WILLETT, 1998; CADE et al., 2002]. Alternatively, specific combinations of foods can be used as predictors for intakes of certain nutrients. Examples include the frequency of consumption of green leafy vegetables and carrots as predictors of carotenoid intake or dairy products as predictors of calcium intake [O`NEILL et al., 2001; BARR et al., 2001].

FFQs vary from very short questionnaires with only five food items to comprehensive food lists numbering 530 items [CADE et al., 2002]. A comprehensive assessment of dietary intake is mostly preferred and is required to calculate total energy intake. However, too long questionnaires with more than 130 questions might lead to boredom and tiredness and may provide either inaccurate or incomplete results. Shorter FFQs can be useful if the primary aim is not to assess the whole diet, but to rank individuals according to one or a few nutrients only [WILLETT, 1998]. The very frequently modified Willett FFQ includes 126 food items, whereas the also widely used Block FFQ asks about the consumption of 106 food items [SUBAR et al., 2001]. In relation to bone health, shorter questionnaires have been developed to measure calcium intake [TAYLOR and GOULDING, 1998; CLOVER et al., 2007].

The food list may contain single food items and grouped food items. Single items are better than grouped items, because when grouped food items are converted to nutrient intakes assumptions must be made about the relative frequencies of intakes and portion sizes. Some grouping is, however, necessary given that many different foods exist and the food list could be continued endlessly. Not only is the grouping of foods important when designing a FFQ, but also the order of foods within the questionnaire. Food groups of special interest should be positioned in the first part of the FFQ but not at the very beginning, because the participants need to get first familiar with the questionnaire design [CADE et al., 2002].

2.5.4 Frequency response section

The frequency response section gathers information on the frequency with which each food item is consumed. Choices on frequency of consumption range usually from 5 to 10. Frequency categories should always be continuous and be more detailed at the high frequency end, because foods consumed less than once per week do not contribute considerably to nutrient intake [WILLETT, 1998]. Questions on frequency of consumption should be closed rather than open, because the closed version improves clearness, reduces errors and coding time [SUBAR et al., 1995; NELSON and BINGHAM, 1997].

2.5.5 Portion size information

A variety of methods may be used to assess portion sizes. It is possible to collect no information on portion sizes. In this case, standard portion sizes need to be assigned in order to be able to calculate gram weights or nutrient intakes [CADE et al., 2002].
A second opportunity is to specify a portion size as part of the question on frequency of consumption. Including portion size information produces semiquantitative food frequency data. This option is clear for foods that have natural units such as slices or cups, however, is less clear for foods that do not exist in natural units such as meat [WILLETT, 1998]. Semiquantitative questionnaires have become a commonly used tool in large epidemiologic studies such as the Nurses` Health Study and the EPIC study [WILLETT et al., 1985; BRANDSTETTER et al., 1999]. A third possibility is to ask subjects to describe the amounts consumed. Subjects may be requested to describe their

usual portion size as “small”, “medium” or “large” [BLOCK et al., 1986]. Alternatively, individuals may be asked to describe portion sizes consumed with the help of food models or photographs [MCBRIDE, 2001; FOSTER et al., 2006].

The best method of obtaining information on portion sizes has been a controversial topic. A disadvantage of using standard portions is that interindividual variance decreases [NOETHLINGS et al., 2003]. On the other hand, results of a recent meta-analysis of 40 validation studies showed that FFQs that include portion size questions were not necessarily better than FFQs using standard portion sizes as indicated by the correlation coefficients between nutrient intakes derived from FFQs and validation methods. Ranking of individuals was poorer for protein and vitamin C intakes determined by FFQs using portion-size questions instead of standard portion sizes [MOLAG et al., 2007]. According to some investigators assigning a standard portion size reduces the respondents' burden and increases data completeness. Furthermore, as pointed out by Noethlings et al., portion sizes vary less among subjects than do frequencies of consumption. Therefore, the assignment of standard portions seems to be adequate [NOETHLINGS et al., 2003; SCHLUNDT et al., 2007].

2.5.6 Calculation of nutrient intake

In order to convert reported frequencies of consumption and portion sizes into nutrient values, a nutrient database, portion size database and computer software is needed. The use of national databases is recommended, because they are more likely to include data on the foods available in the country under observation [WEST and VAN STAVEREN, 1997]. Examples for national nutrient databases include the USDA National Nutrient Database for Standard Reference, the United Kingdom National Nutritional Database, the German Nutrient Database BLS and the Finnish Food Composition Database, Fineli. However, for international studies, or for those comparing data between different countries, international food tables or databases can be used. The FAO has published food composition tables for different regions including Africa, East Asia and Latin America [LUPIEN, 1998]. In Europe, there is no agreed comprehensive nutrient database available. However, attempts are made in developing a European Nutrient

Database. During the EPIC Study, a database was established to compare nutrient data of the 10 European countries participating in the EPIC study. The EPIC nutrient database includes 550-1500 foods and 26 components [SILMANI et al., 2007]. After choosing a suitable database, a further challenge is to assign a single nutrient value for a grouped line item consisting of many individual foods. One person may consume all, some or only one food listed in the food group. This step is usually accomplished by considering the relative consumption frequency and portion sizes of the individual foods in the population under investigation [CADE et al., 2002]. It is important to recognize that nutrient databases do not represent exactly the nutrient content of the food eaten by every subject, because the nutrient content of a food is not constant as it varies by e.g. growing and harvesting conditions, processing, storage and cooking methods [WILLETT, 1998]. This is especially problematic for trace elements. It has been shown that the selenium content of foods is not constant because their content depends on local soil, which can vary enormously by region [GIBSON, 2005].

2.5.7 Errors and variation in food frequency questionnaire

Every dietary assessment method includes systematic and random error. Sources of error include the use of nutrient databases or tables, assessment of portion size and frequency of consumption, individual daily variation in food intake, interviewer bias, recall bias and overreporting of energy [NELSON and BINGHAM, 1997]. Energy misreporting in FFQ has gained much attention, because it might distort the association between self-reported dietary intake and health outcomes. Studies have demonstrated that obese subjects underreport to a greater extend than lean people [BRAAM et al., 1998; LIVINGSTONE and BLACK, 2003]. Underreporting does not affect all foods to the same degree. Foods high in sugar, fat and energy are underreported to a larger extend than foods generally considered healthy, such as vegetables and yoghurt [LAFAY et al., 2000; HEITMANN et al., 2000].

Additionally to underrecording of food items, a decline in body mass has been observed among obese subjects during study periods, indicating that they reduce their food intake for the time of the study [GORIS et al., 2000]. Overreporting of foods generally considered as healthy and underreporting of “unhealthy” energy-dense or high-fat food items may arise by the wish to give social desirable answers [HERBET et al., 2008].

Eventhough underreporting exists at the individual level, FFQ have been frequently reported to overestimate energy and nutrient intakes. Some authors have suggested that overestimating in FFQ may occur due the use of long lists, which make it difficult for subjects to choose a frequency category [MOORE et al., 2007]. Furthermore, the frequency of consumption of some foods may vary by season. Foods consumed near the time of FFQ administration may prime the memory and therefore subjects may report mainly recent dietary intake rather than usual dietary intake [SHAHAR et al., 2001; FOWKE et al., 2004].

Despite the methodological limitations of FFQ it is generally agreed that it is valuable for ranking individuals according to food items or nutrients or for comparing the intakes of groups. However, validation studies are necessary to assess the degree to which the questionnaire measures correctly the aspects of the diet it is intended to measure. For validation studies, a more accurate dietary assessment method, often food records, are used [WILLETT, 1998; CADE, 2002].

3 SUBJECTS AND METHODS

3.1 Subjects and data collection

A subgroup of 101 postmenopausal women aged 68 to 74 years was selected from the Kuopio Osteoporosis Risk Factor and Prevention Study (OSTPRE). The population-based OSTPRE cohort (n=11500) was established in 1989 to examine factors affecting the pathogenesis of peri- and postmenopausal osteoporosis as well as falling and fracture risks of women born between 1932 and 1941. Inside the OSTPRE study a randomized population based trial was started in 2002 in order to study the effect of vitamin D and calcium on falls and fractures in postmenopausal women (OSTPRE-FPS -study). The intervention group (n=1200) obtained vitamin D (800 IU/day) and calcium (1000mg/day) supplementation for 3-year period and the control group (n=1200) did not.

Clinical trial inside the OSTPRE-FPS -study comprised 300 women from the intervention group and 300 women from the control group. Comprehensive examinations were carried out among the participants of the clinical trial during their visits to the Bone and Cartilage Research Unit (BCRU) at the University of Kuopio at the baseline and after 3 years intervention. Participants were physically examined, laboratory evaluations and blood samples were taken, anthropometric and bone mineral density measurements were obtained and comprehensive questionnaires were completed. Food-diaries were kept at baseline and after 3 years and food-frequency questionnaires were completed at year 3. This Master's thesis includes subjects with food-frequency questionnaires (n=101) filled in between April and June 2006. 63 subjects were participants of the intervention group, whereas 38 subjects belonged to the control group.

3.2 Background information

Height, weight, waist circumference, grip strength, quadriceps strength, visual sight, and body sway were measured by hospital personnel. Information on health, use of drugs, use of contraceptive pills, HRT history, gynaecological operations, self-rated health, life satisfaction, physical activity, functional/walking ability, smoking and alcohol use was collected by questionnaires. A comprehensive set of clinical and laboratory test were undertaken. Further, blood samples were taken for DNA analyzes.

A categorical variable (yes/no) was created for diseases and medications that affect BMD. The variable included the following diseases: hyperthyroidism, disease of parathyroid gland, chronic liver disease, chronic intestinal disease, celiac disease, chronic kidney disease, lactose intolerance, arthritis, osteoporosis and surgery of stomach or intestines. The drugs included in the variable were the following: loop-diuretics, epilepsy drugs that increase the need of vitamin D, other epilepsy drugs, asthma drugs, inhaled glucocorticoid, tamoxifen, other cancer drugs and glucocorticoids.

3.3 Bone density measurement

BMD was measured from the total body, lumbar spine and femoral neck with DXA and from the calcaneus with peripheral DXA and quantitative ultrasound devices. BMD was obtained at baseline and 3 years after supplementation with calcium and vitamin D. For the present analysis BMD measured at the lumbar spine and femoral neck at year 3 was used. All BMD measurements were conducted in a single study centre. 21 women were excluded from the analyses due to missing lumbar spine BMD values and 2 women due to missing femoral neck BMD values. The final data set comprised 77 women for the lumbar spine analyses and 96 women for the femoral neck analyses.

3.4 Usual dietary intake

Usual dietary intake was assessed by using a 89-item semiquantitative FFQ. The FFQ has been developed to study associations between dietary habits and osteoporosis in postmenopausal women participating in the OSTPRE Study.

Foods and portion sizes in the FFQ were determined based on food records at year 0 from the same population and on previously developed FFQs. More precisely, FFQs used in the Health 2000 survey in Finland, in the Aberdeen Prospective Osteoporosis Screening Study (APOSS) and in the Evaluation of Nutrients Intakes and Bone Ultra Sound study (EVANIBUS) in a Swiss elderly population.

The questionnaire consisted of a list of foods and a frequency response section for subjects to report how often each food was eaten. Several typical dishes commonly eaten in eastern Finland (e.g. Karelian rice pastries, Finnish fish pastries) were included in the food list. The food items were grouped under subheadings: “Cereals”, “Dairy products”, “Potato, rice and pasta”, “Meat and poultry”, Fish and egg”, “Food preparation and sauces”, “Vegetables”, Fruit and berries”, “Drinks” and “Sweets and snacks”. Additional empty lines allowed subjects to report commonly consumed foods that were not included in the food list.

The 9 frequency of use response categories were the following: never or very seldom, 1-3 times a month, once a week, 2-4 times a week, 5-6 times a week, once a day, 2-3 times a day, 4-5 times a day and more than 5 times a day.

Portion sizes were specified as part of the question on frequency. For instance, it was asked how often one glass of milk or one piece of tomato was consumed. The portion size was specified using natural units, grams or decilitres. If a subject’s usual portion was bigger than the specified amount, they were asked to report a higher frequency of use and vice versa. The questionnaires included additional questions regarding usual eating habits, usual food preparation methods, use of bread spreads, vitamin- or mineral supplements and special diets.

The FFQ was given to the subjects at the examination visit. The subjects were asked to fill in the FFQ and mail it back using prepaid envelope. Follow-up telephone calls were made to the participants whose questionnaire was incomplete. Subjects with only a few missing food items in the FFQ were included in the analyses. Three women were excluded from the analyses because they reported an implausibly high daily energy intake of more than 3500 kcal per day. The final data set comprised 98 women for the nutrient and food intake calculation.

3.4.1 Conversion of foods to nutrients

The online version of the computer-based food composition database of the National Public Health Institute Fineli (www.fineli.fi) was used for converting food consumption data to nutrient intakes. The database is the major source of food composition data in Finland. It contains currently over 3000 foods and mixed dishes and 290 nutrient factors. On their web site, 2000 foods and 52 nutrient factors are available. The composition of dishes has been calculated from Finnish cook books. The database takes into account factors for water loss and nutrient loss during food preparation. The values are listed as g, mg or µg per 100 gram of the food.

First, an excel database was established containing all appropriate foods for each FFQ line item. For instance, for the line item Porridge 51 porridges were found in Fineli. The nutrient value for each composite line item was derived weighted by the frequency of consumption of single food items belonging to the group (e.g., the value for porridge consists of a mix of oat flake porridge prepared with water, Semolina gruel with milk, Semolina porridge with lingon berries). As for the line item fish the database included only information for the raw and untreated fish, a factor for calculating the nutrient content of prepared foods from raw fish was used. The method of compiling the values for each line item and food groups was clearly documented in excel tables. Daily intake was computed for energy and 11 nutrients, including energy, protein, fat, carbohydrate, fibre, calcium, iron, phosphorus, potassium, magnesium, vitamin B12, vitamin C, vitamin D and vitamin K.

3.4.2 Conversion of portion sizes into grams

A portion size table needed to be established for converting the portion sizes specified in the FFQ into grams. The specified portion sizes in the FFQ are the following: slice, teaspoon, plate, tablespoon, piece, glass, jar, scoop, cup, portion, bottle and bar. For each line item in the FFQ the stated portion size had to be converted into grams. For instance, it is asked how often one plate of porridge is consumed. Information on a standard portion of 1 plate of porridge was obtained from the Finnish standard portion size table [SÄÄKSJÄRVI and REINIVUO, 2004]. Moreover, portion sizes were compared with the food records that were collected 3 years earlier from the same population. The food portions were adapted when the reported portions in the food records differed markedly from the standard sizes. Accordingly, the portion size was determined for each line item.

3.4.3 Calculating individual's nutrient intake

The food frequency data of 101 study participants was entered to SPSS. The 9 frequency categories were assigned codes ranging from 1 (never or very seldom) to 9 (more than 5 times a day). For calculating the weekly portion consumption the codes that were assigned to the 9 frequency response categories were recoded (Table 1).

TABLE 1: Assigned codes to the 9 frequency response categories and the recoded values for obtaining the weekly portion consumption frequency

Frequency response categories	Codes	Weekly consumption frequency (portions/week)
never or very seldom	1	0
1-3 times per month	2	0.5
once per week	3	1
2-4 times per week	4	3
5-6 times per week	5	5.5
once per day	6	7
2-3 times per day	7	17.5

Continued:

Frequency response categories	Codes	Weekly consumption frequency (portions/week)
4-5 times per day	8	31.5
more than 5 times per day	9	42

Next, the nutrient intake was calculated using the SPSS Syntax window with the following formula: nutrient from food = weekly frequency * portion size * content in food/100 g. The weekly frequency was calculated previously (Table 1) and the portion size and the nutrient content in food was taken from the beforehand established excel tables. Figure 1 shows an example of the nutrient intake calculation for the line item porridge. This calculation scheme was followed when computing the nutrient intakes for the other 88 line items.

FIGURE 1: SPSS Syntax for computing nutrient intake from porridge per week

```
COMPUTE energyporridge = Porridgefw * 220 * 53.5/100.
COMPUTE carbohydrateporridge = Porridgefw * 220 * 8.69/100.
COMPUTE fatporridge = Porridgefw * 220 * 1.07/100.
COMPUTE proteinporridge = Porridgefw * 220 * 2.03/100.
COMPUTE fibreporridge = Porridgefw * 220 * 1.06/100.
COMPUTE potassiumporridge = Porridgefw * 220 * 68.01/100.
COMPUTE magnesiumporridge = Porridgefw * 220 * 14.77/100.
COMPUTE calciumporridge = Porridgefw * 220 * 27.72/100.
COMPUTE phosphorusporridge = Porridgefw * 250 * 56.4/100.
COMPUTE ironporridge = Porridgefw * 220 * 0.61/100.
COMPUTE vitaminDporridge = Porridgefw * 220 * 0.08/100.
COMPUTE vitaminKporridge = Porridgefw * 220 * 0.51/100.
COMPUTE vitaminCporridge = Porridgefw * 220 * 0.23/100.
COMPUTE vitaminB12porridge = Porridgefw * 220 * 0.06/100.
EXECUTE.
```

As the FFQ asks additional questions on bread and spread consumption (Appendix) a different calculation operation had to be used to add nutrient intakes from bread and spreads. To compute the nutrient intake from bread per day the following formula was used: 7* SlicesBread* portion size * content in food/100g. Accordingly, the nutrient intake for other kind of bread (crisp bread, white bread, graham bread) was calculated. Figure 2 displays an example for the calculation of nutrient intake from rye bread.

FIGURE 2: SPSS Syntax for computing the nutrient intake from rye bread per week

```
COMPUTE energyryebread = 7 * SlicesRyeBread * 30 * 205/100.
COMPUTE carbohydrateryebread = 7 * SlicesRyeBread * 30 * 40.7/100.
COMPUTE fatryebread = 7 * SlicesRyeBread * 30 * 1.4/100.
COMPUTE proteinryebread = 7 * SlicesRyeBread * 30 * 6.7/100.
COMPUTE fibreryebread = 7 * SlicesRyeBread * 30 * 11/100.
COMPUTE potassiumryebread = 7 * SlicesRyeBread * 30 * 381.8/100.
COMPUTE magnesiumryebread = 7 * SlicesRyeBread * 30 * 84.6/100.
COMPUTE calciumryebread = 7 * SlicesRyeBread * 30 * 24.1/100.
COMPUTE phosphorusryebread = 7 * SlicesRyeBread * 30 * 269.5/100.
COMPUTE ironryebread = 7 * SlicesRyeBread * 30 * 3.7/100.
COMPUTE vitaminDryebread = 7 * SlicesRyeBread * 30 * 0/100.
COMPUTE vitaminKryebread = 7 * SlicesRyeBread * 30 * 4.33/100.
COMPUTE vitaminCryebread = 7 * SlicesRyeBread * 30 * 0/100.
COMPUTE vitaminB12ryebread = 7 * SlicesRyeBread * 30 * 0/100.
```

The FFQ asked subjects to report the amount of spread per slice of bread and the most commonly consumed spread type (Appendix). To compute the nutrient intake from spread the total number of consumed bread slices per day were summated (tbreadslices = SlicesRyeBread + SlicesCrispBread + SlicesGrahamBread + SlicesWhiteBread.) and codes were assigned to the 5 different amount categories and 4 different types of spread (Table 2 and Table 3).

TABLE 2: Assigned codes to the various amount categories of spread

Amount of spread per slice of bread	Codes
About 2 teaspoons	1
About 1 ½ teaspoons	2
About 1 teaspoon	3
About ½ teaspoon	4
I don’t use spreads	5

TABLE 3: Assigned codes to the different types of bread spread

Type of bread spread	Codes
Butter	1
Margarine, 60-80% fat	2
Margarine, less than 60% fat	3
Spreadable cheese, low fat	4

Next the amount of spread in gram consumed per each subject could be calculated using the “DO IF” command. If the subject reported to use about 2 teaspoons of spread per bread slice, then the spread amount per slice of bread obtained from the portion size table was 9 gram. In a similar manner the amount of different types of spreads in grams was calculated for the other amount categories (Figure 3).

FIGURE 3: SPSS Syntax for computing the amount of spread in grams per slice of bread

```
do if (UseOfMargarine eq 1).
compute spreadamount = 9.
else.
if (UseOfMargarine eq 2) spreadamount = 6.75.
if (UseOfMargarine eq 3) spreadamount = 4.5.
if (UseOfMargarine eq 4) spreadamount = 2.25.
if (UseofMargarine eq 5) spreadamount = 0.
end if.
```

Then the nutrient intake from spread per 100g was computed using the “DO IF” command. Figure 4 shows an example for the calculation of energy from spreads. The same format was used to calculate the intake of other nutrients from spreads. Subsequently, the weekly nutrient intake from spreads could be calculated (Figure 5).

FIGURE 4: SPSS Syntax for computing the energy intake from spread

```
do if (margarinehabits2 eq 1).
compute energyspread = 719.
else.
if (margarinehabits2 eq 2) energyspread = 619.
if (margarinehabits2 eq 3) energyspread = 368.
if (margarinehabits2 eq 4) energyspread = 179.
end if.
```

FIGURE 5: SPSS Syntax for computing the nutrient intake from spreads per week

```
energyspreadw = 7 * tbreadslices * spreadamount * energyspread/100.
carbohydratespreadw = 7 * tbreadslices * spreadamount * carbohydratespread/100.
fatspreadw = 7 * tbreadslices * spreadamount * fatspread/100.
proteinspreadw = 7 * tbreadslices * spreadamount * proteinspread/100.
potassiumspreadw = 7 * tbreadslices * spreadamount * potassiumspread/100.
magnesiumspreadw = 7 * tbreadslices * spreadamount * magnesiumspread/100.
calciumspreadw = 7 * tbreadslices * spreadamount * calciumspread/100.
phosphorusspreadw = 7 * tbreadslices * spreadamount * phosphorusspread/100.
ironspreadw = 7 * tbreadslices * spreadamount * ironspread/100.
vitaminDspreadw = 7 * tbreadslices * spreadamount * vitaminDspread/100.
vitaminKspreadw = 7 * tbreadslices * spreadamount * vitaminKspread/100.
vitaminB12spreadw = 7 * tbreadslices * spreadamount * vitaminB12spread/100.
```

In a final step the total nutrient intake was computed for 14 nutrients by summing the previously calculated weekly nutrient intakes and then dividing with 7. The example given in Figure 6 demonstrates the accounting operation for energy. Thus, an estimate of each subject’s total daily nutrient intake was obtained.

FIGURE 6: SPSS Syntax for computing total energy intake per day

```
COMPUTE tenergy (kcal) = (energyporridge + energybcereals + energybran +
energymuesli + energyricepastry + energypastry + energyfritter + energywbun +
energypancake + energyscake +  energybiscuit + energyskimmedmilk +
energylowfatmilk + energywholemilk + energylowfatjogurt + energyflavouredyoghurt
+ energyplainyoghurt + energylowfatcheese + energycheese + energyquark +
energycream + energyicecream + energypotato + energypotatofried + energyrice +
energypasta + energymincedmeat + energyporklowfat + energyporkhighfat +
energyveal + energyreindeer + energyliver + energysausagedish + energysausage +
energycoldcut + energyliverpate + energychicken + energylowfatfish + energyfattyfish
+ energyherring + energysaltfish + energytuna + energyshrimps + energyfishpastry +
energyegg + energyfcream + energyvegetableoil + energybrownsauce + energyoil +
energytomato + energycucumber + energygreensalad + energymsalad +
energyvegetables + energycarrot + energypeas + energycabbage + energyvegsteacks +
energyvegsoups + energypickles + energymushroom + energyjuice + energycitrusfruits
+ energyapple + energybanana + energycfruit + energydfruit + energybsoup +
energyberries + energywater + energycoffee + energytea + energycreamcoffee +
energymilkcoffee +  energysugaradded + energysugaradded + energybjuice +
energysugardrinks + energyhbeer + energybeer + energywine + energyspirits +
energychocolate + energyosweets + energynuts + energychips + energyryebread +
energycrispbread + energygrahambread + energywhitebread + energyspreadw)/7.
EXECUTE.
```

3.4.4. Calculating individual's food intake

Each study participant's daily intake of foods in gram was calculated by multiplying the weekly consumption frequency of all listed food items with the serving size and dividing it by 7. Daily consumption of fruit in grams was derived by summing up the daily intakes of citrus fruits, apples, bananas, canned fruit, dried fruit, berry soups and berries. Daily vegetable intake in grams was computed by summing up the daily intakes of tomatoes, cucumbers, green salad, mayonnaise based salad, cooked vegetables, carrots, peas, cooked cabbage, vegetable steaks, vegetable soups, pickles and mushrooms. For the purpose of this study, fruit juice was excluded from fruit intake, and potatoes were excluded from the vegetable group.

Vegetables were further divided into fresh and cooked vegetables. Fresh vegetables included tomatoes, cucumbers, and green salad. Cooked vegetables included cooked vegetables as a side dish, cooked cabbage, vegetable steaks and vegetable soups.

3.9 Statistical analyses

All analyses were performed by using the SPSS statistical software package (version 14.0; SPSS Inc., Chicago). Normality was tested by Kolmogorov-Smirnov test. BMD values were found to be normally distributed. Descriptive statistics (means, medians, standard deviations and ranges) were ascertained for all variables.

The participants were stratified into two groups below and above the median for each dietary variable and the mean BMD at the lumbar spine and femoral neck were calculated. Univariate analysis was carried out to assess whether mean BMD measured in the low and high intake group differed significantly. To analyse the association between dietary variables and BMD a series of models were established. In the crude model nutrient, vegetable and fruit intake were expressed in terms of crude intake (g/d). The first model examined the association between nutrient variables and BMD, adjusting for age, weight, total energy intake and the current use of HRT. Model 2 used Model 1 with the addition of the use of calcium and/or vitamin D supplements, disease and medication and study group (intervention or control group). Age, weight and total energy intake were expressed as continuous variables. For current HRT use, the subcategories were nonusers, use of one hormone, use of two hormones and use of 3 hormones. The subcategories for calcium and vitamin D supplements were yes and no. The associations between categorized potential confounders and bone mineral density were tested by t-test and correlation coefficient was used for estimating associations with age. A p-value less than 0.05 was considered significant.

4 RESULTS

4.1 Characteristics of the study subjects

Anthropometric characteristics and age of the 98 subjects are presented in Table 4. The subjects had a mean age of 71 years, ranging from 68 to 74 years. The average BMI was 28.5 kg/m^2. 45 % of the women were overweight and 32 % were obese, demonstrating a rather high prevalence of overweight and obesity in this elderly population. The BMI categories were defined as BMI 25-29.99 kg/m^2 for overweight and BMI $\geq$ 30 for obese [WHO, 2000].

TABLE 4: General characteristics of the women included in the study (n = 98)

Characteristics	Mean	SD	Range
Age (years)	70.8	1.85	68-74
Height (cm)	158.4	5.29	147-172
Weight (kg)	71.3	11.66	47-104
BMI (kg/m2)	28.5	4.82	18-43

Table 5 shows the summary statistics for BMD at the femoral neck and lumbar spine. These values were normally distributed. BMD at the lumbar spine was positively correlated with weight (r = 0.245, p = 0.032) and BMI (r = 0.232, p = 0.042). BMD at the femoral neck was also positively correlated with weight (r = 0.348, p<0.001) and BMI (r = 0.258, p = 0.011). Use of HRT, voluntary calcium and vitamin D supplementation, alcohol, smoking and age were not significantly associated with lumbar spine or femoral neck BMD. Femoral neck BMD was significantly higher in subjects without any disease or medication affecting BMD (p<0.01). There was also an indication of higher femoral neck BMD in the intervention group (use of calcium and vitamin D supplementation) compared to the control group (p=0.05).

TABLE 5: Lumbar spine and femoral neck bone mineral density (n=98)

BMD	Mean	SD	Range
Femoral neck (n=96)	0.83	0.12	0.62-1.14
Lumbar spine (n=77)	1.08	0.18	0.73-1.51

Other basic characteristics of the women are shown in Table 6. 39.8 % of the women used voluntary calcium and vitamin D supplementation. A high percentage of the women reported never smoking cigarettes (95.4%) and never consuming alcohol (70%), indicating low alcohol consumption and low smoking prevalence in this elderly population. Only 10.3% used HRT at the time of the study. 21.4% of the women had a disease or used a medication that is known to influence BMD. 61.2% were part of the intervention group that received vitamin d and calcium supplementation.

TABLE 6: Percentage distribution of selected characteristics of the study population

Characteristics	N	No (%)	Yes (%)
Current voluntary use of calcium or vitamin D supplementation	98	60.2	39.8
Current smoker	87	95.4	4.6
Use of alcohol	90	70	30
Current use of hormone therapy (HRT)	97	89.7	10.3
Disease or medication affecting BMD	98	78.6	21.4
Intervention group (vitamin D and calcium supplementation)	98	38.8	61.2

4.2 Intake of foods and food groups

4.2.1 Fruits and vegetable consumption

For each food or food group in the FFQ the daily intakes were calculated. Table 7 presents the arithmetic mean, standard deviation and range of all the fruit and vegetable items that were included in the fruit and vegetable group. Potatoes were not included in the vegetable group and juices were not included in the fruit group. The average daily intakes of fruit and vegetables were 346 and 310 g, respectively. Around one third of the vegetable intake was derived from tomatoes, followed by cucumbers and green salad. Of the mean 346 g fruit intake, 108 g were derived from the group “Apple, pear, nectarine, apricot”, followed by citrus fruits and berry soups. 63.5% of the women had mean daily intakes above the recommended 400 g.

TABLE 7: Estimated daily intake of fruits and vegetables g/d (n=98)

Fruit or vegetable	Mean	SD	Range
Tomato	105	88	0-312
Cucumber	49	48	0-225
Green salads	42	46.	0-270
Mayonnaise based salads	11	28	0-188
Cooked vegetables as a side dish	24	24	0-175
Carrot (as such, cooked)	24	28	0-163
Peas, corn, beans	4.1	8.0	0-51.1
Cooked cabbage in dishes	4.6	6.3	0-43.2
Vegetable steaks / casseroles	3.2	4.7	0-23.6
Vegetable soups	39	51	0-275
Pickles	3.8	5.1	0-18.9
Mushrooms	4.2	5.9	0-250
Citrus fruits or kiwi	68	142	0-475
Apple, pear, nectarine, apricot	108	165	0-400
Banana	31	81	0-299
Canned fruit	4.3	18.5	0-175
Dried fruit and raisins	5.1	21.7	0-150
Berry soup	85	58	0-248
Berries (fresh or frozen)	46	58	0-248
Vegetables, total	310	188	36-944
Fruit, total	346	321	0-1280
Combined fruit and vegetables	649	435	67-1947

4.2.2 Food and food group consumption excluding fruits and vegetables

Results of the calculated daily intakes of foods and drinks that were not included in the fruit and vegetable group are presented in Table 8. Only 4% of this elderly population consumed full fat milk, versus 30% low fat and 66% fat free milk. Potatoes are the most frequently consumed side dish with a consumption of about 105g per day equalling almost 2 pieces per day. Minced meat is the most used meat, followed by chicken and turkey. The most frequently eaten fishes are low fat fishes such as pike and birch. Total fish and seafood consumption in this population is 47g per day.

TABLE 8 : Estimated daily intake of foods and drinks (g) (n=98)

Daily intake of foods and drinks (g)	Mean	SD	Range
Porridge	154	113	16-550
Breakfast cereals	1.3	5.5	0-30
Bran	0.4	1.2	0-6
Muesli	1.5	7.0	0-66
Carelian rice pasty	12	18	0-150
Pastry, salty pies	2.7	9.0	0-75
Fritter, short bread, sweet pastry	11	27	0-225
Sweet wheat bun	45	40	0-125
Pancake	5.3	8.6	0-40
Sponge cake with cream	10.2	14.7	0-70
Biscuit	9.2	12	0-48
Skimmed milk or fat free butter milk	277	231	0-1020
Milk, low fat	124	161	0-425
Whole milk	16	66	0-425
Fat free or low fat yogurt/cultured milk	82	111	0-500
Flavoured yoghurt (> 2 % fat)	18	44	0-200
Plain yogurt or cultured milk (> 2 % fat)	23	66	0-500
Low fat cheese	15	18	0-96

Continued:

Daily intake of foods and drinks (g)	Mean	SD	Range
Cheese (medium fat content)	5.2	8.7	0-40
Cottage cheese, Quark	12	18	0-85
Cream cheese, cream	1.3	3.0	0-15
Ice cream	4.5	7.3	0-45
Potato (baked, smashed, in soups)	99	76	0-540
Potato (fried)	5.5	14	0-540
Rice	20	29	0-160
Pasta, pasta in foods (like lasagne)	14	19	0-130
Minced meat	26	20	0-100
Pork, low fat	10.8	12	0-79
Pork, high fat (ribs, bacon etc.)	6.5	13	0-100
Veal, mutton	8.8	19	0-100
Reindeer, game	4.2	13	0-79
Liver	2.7	4.3	0-14
Sausage (for main dishes)	11	11	0-34
Sausage (as cold cut)	17	29	0-180
Cold cut	6.1	9.4	0-60
Liver pate	0.7	2.4	0-15
Chicken or turkey	23	47	0-375
Low fat fish (pike, birch, etc.)	20	20	0-100
Fatty fish (rainbow trout, salmon, etc.)	9.9	12	0-100
Baltic herring, whitefish, vendace	9.9	26	0-250
Salt preserved fish	1.2	2.2	0-12
Tuna or other canned fish	0.5	1.1	0-3.9
Shrimps, crabs	0.2	0.7	0-3.6
Finnish fish pastry	5.5	12	0-64
Egg (boiled, fried, omelette)	15	24	0-138
Food prepared with cream	6.4	9.0	0-50
Foods with vegetable-oil based cream	10	23	0-125
Brown sauce	21	34	0-250

Continued:

Daily intake of foods and drinks (g)	Mean	SD	Range
Oil, oil based salad dressing of mayonnaise	3.9	6.1	0-34
Juice	22	44	0-250
Water	565	296	0-1020
Coffee	410	203	0-1020
Tea	114	145	0-425
Milk in coffee or tea	16	25	0-90
Cream in coffee or tea	4.9	12.0	0-38
Sugar added to drinks or foods	4.9	6.3	0-24
Hot Chocolate	14	66	0-625
Berry Juice	129	183	0-765
Sugar containing carbonated drinks	4.2	14	0-73
Home made beer	16	65	0-425
Beer, cider	9.1	29	0-141
Red or white wine	3.9	14	0-120
Spirits	0.6	1.8	0-15
Chocolate	5.0	8.9	0-50
Nuts and seeds	1.0	5.4	0-51
Chips and pop corn	0.1	0.4	0-2.1
Amount of slices of rye bread	3.7	1.6	0-10
Amount of slices of crisp bread	0.7	0.9	0-4
Amount of slices of graham bread	1.4	1.2	0-6
Amount of slices of white bread	0.1	0.4	0-2

4.3 Intake of nutrients

The arithmetic mean, standard deviation and range for energy and 13 nutrients are shown in Table 9. The mean daily energy intake was 2173 kcal (± 554 kcal). Table 10 presents the macronutrient intake expressed as percentage of total energy. 17% of total energy intake is derived from protein intake, 31% from fat intake and 51% from carbohydrate intake. The remaining 0.49% is derived from alcohol intake (Table 11).

TABLE 9: Estimated nutrient intakes per day (n=98)

Nutrients	Mean	SD	Range
Energy (kcal)	2173	554	1140-3498
Protein (g)	92	28	45-204
Fat (g)	73	26	26-136
Carbohydrate (g)	271	68	155-461
Fibre (g)	32.8	10.5	12.3-66.2
Calcium (mg)	1324	440	530-2574
Iron (mg)	16.3	4.7	7.4-31.6
Phosphorus (mg)	1958	489	1073-3554
Potassium (mg)	4856	1285	2750-8690
Magnesium (mg)	463	109	246-800
Vitamin B12 (μg)	8.6	5.0	1.1-22.9
Vitamin C (mg)	130	96	32-444
Vitamin D (μg)	11.5	5.0	1.9-30.7
Vitamin K (μg)	159	83	25-460

TABLE 10: Estimated macronutrient intake as percentage of total energy (%)

Macronutrients	Mean	SD	Range
Protein	17.3	2.7	11.0-25.0.
Fat	31.0	5.8	18.0-46.9
Carbohydrate	51.3	5.6	37.7-65.4

Table 11 shows the Finnish nutrition recommendations for 12 nutrients and the percentage of subjects not reaching them [NATIONAL NUTRITION COUNCIL, 2005]. 76.5% of the elderly did not reach the recommended 55% of total energy from carbohydrate. 43.9% of the women did not meet the vitamin D recommendation of 10µg per day. 41.8% had a fibre intake below 30g per day. 34.7% consumed less than the recommended Vitamin C intake. The calcium recommendation could be achieved by 87.8% and the iron, magnesium and potassium recommendation by 96.9% of the women.

TABLE 11 Finnish nutrition recommendations and percentage of elderly women with intakes falling below the Finnish nutrition recommendations (National Nutrition Council, 2005)

Nutrients	Finnish nutrition recommendations	%
Protein	15 % of total energy intake	14.3
Fat	30% of total energy intake	44.9
Carbohydrate	55% of total energy intake	76.5
Fibre	25-35 g/d	8 (< 30g)
Calcium	800 mg/d	12
Iron	9 mg/d	3.1
Phosphorus	600 mg/d	0
Potassium	3.1 g/d	3.1
Magnesium	280 mg/d	3.1
Vitamin B12	2 µg/d	1
Vitamin C	75 mg/d	34.7
Vitamin D	10 µg/d	43.9

4.4 Associations between fruit and vegetable intake and bone mineral density

Univariate analysis was carried out to investigate associations between BMD and fruit and vegetable intake. Therefore, participants were grouped below or equal and above the median according to their intakes. Table 12 shows the descriptive statistics of the 2 groups.

TABLE 12: Fruit and vegetable intake below and above the median

Food group	N	Mean	SD	Minimum	Maximum
Fresh vegetables (g)					
≤ Median	49	91	45	13	172
> Median	49	230	151	174	808
Cooked vegetables (g)					
≤ Median	48	30	19	0	59
> Median	48	112	65	63	352
Vegetables (g)					
≤ Median	48	173	72	36	282
> Median	48	446	168	287	944
Fruit (g)					
≤ Median	49	94	71	0	279
> Median	49	598	271	281	1280
Fruit and vegetables (g)					
≤ Median	48	304	138	67	577
> Median	48	994	345	581	1947

4.4.1 Association between lumbar spine bone mineral density and fruit and vegetable intake

There was no evidence of a positive association between fruit and vegetable intake and lumbar spine BMD (Table 13). Mean lumbar spine BMD was found to be lower in the higher fruit intake group compared to the lower fruit intake group (p=0.07). Fruit consumption was positively correlated with total energy intake (p=0.007, r=0.272), which might explain this finding.

TABLE 13: Associations between lumbar spine BMD and fruit and vegetable intake (n=72)

	Lumbar spine BMD (g/cm^2)		p-values		
	≤ Median	>Median	Crude	Model 1[1]	Model 2[2]
Fresh vegetables	1.066 ± 0.18	1.088 ± 0.18	0.605	0.406	0.356
Cooked vegetables	1.065 ± 0.17	1.091 ± 0.20	0.541	0.967	0.942
Vegetables	1.066 ± 0.18	1.092 ± 0.19	0.551	0.758	0.763
Fruit	1.092 ± 0.21	1.064 ± 0.15	0.505	0.074	0.072
Vegetable and fruit	1.065 ± 0.20	1.094 ± 0.17	0.492	0.528	0.554

[1] Adjusted for age, weight, total energy intake and current use of hormone replacement therapy

[2] Adjusted for age, weight, total energy intake, current use of hormone replacement therapy, use of calcium and/or vitamin D supplement, diseases and medication affecting BMD, and study group

4.4.2 Association between femoral neck bone mineral density and fruit and vegetable intake

Table 14 presents the results of the univariate analysis of fruit and vegetable intake and femoral neck BMD. Higher vegetable intake was found to be significantly associated with femoral neck BMD in the crude model. Femoral neck BMD was 0.053g/cm^2 greater in the high vegetable intake group compared to the low vegetable intake group (p=0.026). Similarly, femoral neck BMD was significantly greater in the group with higher fresh vegetable intake (p=0.048). However, after adjusting for all the confounding variables the observed association did not remain significant.

TABLE 14: Associations between femoral neck BMD and fruit and vegetable intake (n=86)

	Femoral neck BMD (g/cm$^{2)}$		p-values		
	≤ Median	>Median	Crude	Model 1[1]	Model 2[2]
Fresh vegetables	0.808 ±0.11	0.854 ± 0.11	0.048*	0.396	0.597
Cooked vegetables	0.827 ± 0.13	0.833 ± 0.12	0.794	0.955	0.848
Fruit	0.821 ± 0.12	0.840 ± 0.11	0.429	0.547	0.394
Vegetable	0.804 ± 0.11	0.857 ± 0.12	0.026*	0.320	0.401
Vegetable and fruit	0.817 ± 0.12	0.843 ± 0.11	0.283	0.657	0.495

[1] Adjusted for age, weight, total energy intake and current use of hormone replacement therapy

[2] Adjusted for age, weight, total energy intake, current use of hormone replacement therapy, use of calcium and/or vitamin D supplement, diseases and medication affecting BMD and study group

4.5 Associations between nutrient intake and bone mineral density

Table 15 shows energy and nutrient intakes below or equal the median (n=49) and above (n=49) the median used in univariate analysis.

TABLE 15: Energy and nutrient intake in groups of below and above the median intakes

Food group	Mean	SD	Minimum	Maximum
Energy (kcal)				
≤ Median	1721	233	1140	2057
> Median	2626	383	2059	3498
Protein (g)				
≤ Median	71	11	45	85
> Median	112	24	86	204
Fat (g)				
≤ Median	53	12	26	71
> Median	93	19	71	136
Carbohydrate (g)				
≤ Median	214	30	155	273
> Median	328	43	274	461

Continued: Food group	Mean	SD	Minimum	Maximum
Fibre (g)				
≤ Median	24.5	5.5	12.3	33.1
> Median	41.1	7.1	33.1	66.2
Calcium (mg)				
≤ Median	972	223	530	1325
> Median	1675	300	1337	2574
Iron				
≤ Median	12.7	2.0	7.4	15.3
> Median	19.8	3.8	15.4	31.6
Phosphorus (mg)				
≤ Median	1569	222	1073	1902
> Median	2347	354	1931	3554
Potassium (mg)				
≤ Median	3807	536	2749	4679
> Median	5905	895	4689	8690
Magnesium (mg)				
≤ Median	377	52	246	454
> Median	550	78	456	800
Vitamin B12 (μg)				
≤ Median	4.7	1.3	1.1	7.0
> Median	12.4	4.3	7.1	22.9
Vitamin C (mg)				
≤ Median	65	20	32	100
> Median	195	98	101	444
Vitamin D (μg)				
≤ Median	7.8	1.9	1.9	10.3
> Median	15.1	4.5	10.9	30.7
Vitamin K (μg)				
≤ Median	99	28	25	138
> Median	218	78	138	460

4.5.1 Association between lumbar spine bone mineral density and nutrient intakes

Associations between lumbar spine BMD and total energy as well as 13 nutrients were examined (Table 16). Lumbar spine BMD was positively associated with energy intake. After adjusting for confounding variables the trend was still observed (p= 0.058). Higher magnesium intake was associated with higher lumbar spine BMD in the women (p=0.018). After adding age, weight, total energy intake and current use of hormone replacement therapy, use of calcium and/or vitamin D supplement, disease and medication to the model the relation did not remain significant (p=0.326). There was a significant positive association between dietary vitamin D intake and lumbar spine BMD (p=0.012). Mean vitamin D intakes below or equal and above the median were 7.8µg, and 15.1µg, respectively. After adjusting for age, weight, total energy intake and current use of hormone replacement therapy the association remained significant (p=0.041). The significant association persisted even after a further adjustment for the use of calcium and/or vitamin D supplements, disease and medication (p=0.035). Subjects below the median had a mean lumbar spine BMD of 1.027g/cm^2, while the mean BMD above the median was 1.131g/cm^2. Thus, women in the higher vitamin D intake group had 0.104g/cm^2 higher lumbar spine BMD compared to women in the lower intake group (Figure 7).

TABLE 16: Associations between lumbar spine BMD and nutrient intakes (n=73)

	Lumbar spine BMD (g/cm^2)		p-values		
	≤ Median	>Median	Crude	Model 1[1]	Model 2[2]
Energy	1.031 ± 0.17	1.117 ± 0.19	0.039	0.056	0.058
Protein	1.062 ± 0.17	1.094 ± 0.20	0.447	0.498	0.380
Fat	1.042 ± 0.18	1.110 ± 0.19	0.102	0.874	0.817
Carbohydrate	1.039 ± 0.18	1.111 ± 0.18	0.086	0.684	0.524
Fibre	1.043 ± 0.17	1.121 ± 0.19	0.060	0.457	0.542
Calcium	1.048 ± 0.16	1.106 ± 0.20	0.169	0.379	0.416
Iron	1.045 ± 0.18	1.109 ± 0.18	0.127	0.831	0.752
Phosphorus	1.035 ± 0.17	1.116 ± 0.19	0.051	0.452	0.559

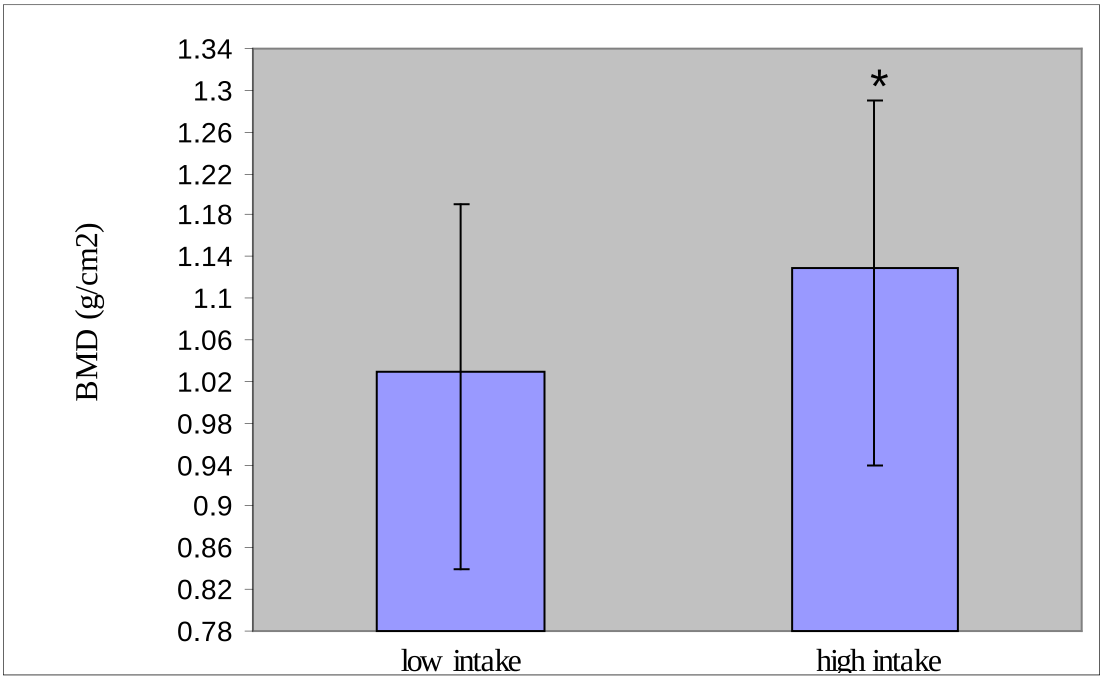
Continued:

	Lumbar spine BMD (g/cm^2)		p-values		
	≤ Median	>Median	Crude	Model 1[1]	Model 2[2]
Potassium	1.047 ± 0.17	1.109 ± 0.11	0.133	0.644	0.565
Magnesium	1.027 ± 0.16	1.125 ± 0.19	0.018*	0.252	0.326
Vitamin B12	1.071 ± 0.19	1.084 ± 0.18	0.764	0.559	0.618
Vitamin C	1.063 ± 0.19	1.095 ± 0.18	0.444	0.526	0.466
Vitamin D	1.027 ± 0.16	1.131 ± 0.19	0.012*	0.041*	0.035*
Vitamin K	1.062 ± 0.16	1.093 ± 0.20	0.462	0.466	0.459

[1] Adjusted for age, weight, total energy intake and current use of hormone replacement therapy

[2] Adjusted for age, weight, total energy intake, current use of hormone replacement therapy, use of calcium and/or vitamin D supplement, diseases and medication affecting BMD and study group

FIGURE 7: Mean (± SD) lumbar spine BMD in groups of low and high dietary vitamin D intake (< 10.35 and > 10.35 μg/d, respectively) in postmenopausal women



Adjusted for age, weight, total energy intake, current use of hormone replacement therapy, use of calcium and/or vitamin D supplement, diseases and medication affecting BMD and study group

* p<0.05, significant difference between the low and high vitamin D intake group

n= 73 (39 subjects have vitamin D intake below the median and 38 subjects above the median)

4.5.2 Association between femoral neck bone mineral density and nutrient intakes

Increased energy or nutrient intake did not show a statistically significant effect on femoral neck BMD in the postmenopausal women (Table 17).

TABLE 17: Associations between femoral neck BMD and nutrient intakes (n=87)

	Femoral neck BMD (g/cm^2)		p-values		
	≤ Median	>Median	Crude	Model 1[1]	Model 2[2]
Energy	0.819 ± 0.13	0.841± 0.10	0.363	0.145	0.090
Protein	0.836 ± 0.13	0.825 ± 0.01	0.639	0.576	0.294
Fat	0.839 ± 0.12	0.822 ± 0.81	0.47	0.158	0.393
Carbohydrate	0.816 ± 0.13	0.844 ± 0.98	0.233	0.525	0.874
Fibre	0.813 ± 0.12	0.849 ± 0.10	0.121	0.299	0.647
Calcium	0.823 ± 0.11	0.838 ± 0.12	0.502	0.343	0.373
Iron	0.810 ± 0.12	0.850 ± 0.11	0.090	0.089	0.138
Phosphorus	0.823 ± 0.12	0.838 ± 0.11	0.537	0.428	0.683
Potassium	0.817 ± 0.12	0.844 ± 0.12	0.259	0.820	0.866
Magnesium	0.816 ± 0.12	0.845 ± 0.11	0.215	0.318	0.481
Vitamin B12	0.850 ± 0.13	0.811 ± 0.09	0.097	0.246	0.395
Vitamin C	0.809 ± 0.12	0.852 ± 0.11	0.069	0.823	0.876
Vitamin D	0.818 ± 0.12	0.843 ± 0.11	0.282	0.327	0.291
Vitamin K	0.816 ± 0.13	0.845 ± 0.11	0.224	0.516	0.583

[1] Adjusted for age, weight, total energy intake and current use of hormone replacement therapy

[2] Adjusted for age, weight, total energy intake, current use of hormone replacement therapy, use of calcium and/or vitamin D supplement, diseases and medication affecting BMD and study group

5 DISCUSSION

5.1 Food group intake in postmenopausal Finnish women

The average intake of fruit in postmenopausal women living in the Kuopio region was 346 gram per day. The most frequent consumed fruits in postmenopausal women in the Kuopio region were the group “Apple, pear, nectarine, apricot”, followed by citrus fruits and berry soups. This is similar to the results of the FINDIET 2002 study, were the average daily fruit and berry consumption was estimated to be 290 gram per day. Average fruit consumption in Finland has been shown to be highest in middle-aged and older women. Furthermore, the diet of middle-aged and older women has the best nutritional quality compared to other groups in Finland [MÄNNISTÖ et al., 2003].

In the national survey, average vegetable intake in 55-64 year old women living in Pohjois-Savo was calculated to be around 178 gram per day. In the present study vegetable consumption in the elderly women was 310 gram per day and thus, is likely to be an overestimation of the true consumption. Tomatoes, cucumber and green salads were the most frequently consumed vegetables which finding is in accordance with the results of the national survey [MÄNNISTÖ et al., 2003].

Fruit and vegetable intake in Finland has been shown to be among the lowest in the European Union [ELMADFA et al., 2005]. However, our results demonstrate well, that even though average fruit and vegetable consumption in Finland is low, older women might have the highest intakes and thus, are more likely to achieve the dietary recommendations than other population groups in Finland. This is in accordance with other studies conducted in Finland [MANNISTÖ et al., 2003].

Even though fruit and vegetable intake in this population group seems to be high and possibly overestimated on average, it is worth mentioning that 36.5% of the study population could not reach the recommendation for fruit and vegetable consumption of 400g per day [NATIONAL NUTRITION COUNCIL, 2005].

Similarly to the European Nutrition and Health Report [ELMADFA et al., 2005], milk consumption was found to be very high among this elderly Finnish population. Interestingly, the use of fat-free and low-fat milk is very common, only 4% of this elderly population consumed full fat milk.

5.2 Nutrient intake in postmenopausal Finnish women

This study reports energy and nutrient intake data in the elderly Finnish population. It has been recommended that the minimum daily energy intake level for a sufficient micronutrient intake for the elderly is 1500 kcal [LOWENSTEIN, 1982]. In this study, 92% of the subjects reached this minimum level. Although energy needs of the elderly are lower, the requirements for most micronutrients remain the same as in adulthood. An adequate intake of dietary calcium is of particular importance in order to prevent osteoporosis. Furthermore, the dietary intake of vitamin D gains importance with increasing age, because with increasing age the body shows reduced ability to synthesize vitamin D and many elderly avoid sunlight exposure [ELMADFA and MEYER, 2008]. In the present study, 43.9% of the subjects had intakes below the Finnish nutrition recommendations [NATIONAL NUTRITION COUNCIL, 2005] for vitamin D intake, but 88% reached the recommendation for calcium intake. The Finnish Nutrition recommendation for fruit and vegetable intake of 400g was not reached by 36.5% of the individuals, which is almost similar to the proportion who did not reach the vitamin C intake recommendation (34.7%). Mean magnesium intake was 463 mg and mean potassium intake 4856 mg per day and thus, much higher than the recommended 280 mg and 3100 mg per day. Both, magnesium and potassium recommendations were reached by 97% of the subjects.

The calculated mean nutrient intakes of the women in this study were compared with the mean nutrient intakes that were assessed in the National FINDIET 2002 Study. This study measured the average food and nutrient intake in different age groups in 5 different areas of Finland. Mean energy intake in the OSTPRE study was 38.5%, mean protein intake 43 %, mean carbohydrate intake 42 %, mean fat intake 28 % and mean fibre 56 % higher than in 55-64 year old women living in Northern Savo as assessed by the national FINDIET 2002 Study. When compared with the national data, micronutrient intake (excluding vitamin D) was on average 46 % higher. The calculated value for vitamin D intake was 145 % higher. However, this could be explained due to the fact that fluid milks and margarines were fortified after the FINDIET 2002 study was carried out [MÄNNISTÖ et al., 2003]. The differences in results may reflect methodological differences between the two studies. Whereas the FINDIET 2002 study assessed dietary intake with 48h recalls, the present study used FFQs to measure usual dietary habits.

As mentioned previously, vitamin D intake was 145 % higher in this study, compared to the FINDIET 2002 study. One recently conducted study in Finland showed the vitamin D fortification was inadequate to prevent vitamin D insufficiency among adolescent girls and women and new innovative ways of improving vitamin D nutrition are needed [LEHTONEN- VEROMAA et al., 2008]. In contrast, an earlier study among Finnish men, demonstrated that the national vitamin D fortification considerably improved the vitamin D status of young Finnish men [LAAKSI et al., 2006]. The effect of the national vitamin D fortification on vitamin D status in postmenopausal women has not been reported. The findings of this study suggest that the vitamin D intake in postmenopausal women has substantially increased due to the national fortification.

In conclusion, the majority of the women reached the nutrient recommendations for most nutrients. However, despite the fortification, the recommended dietary vitamin D intake level was not reached in almost half of the subjects (see Table 11).

5.2 Fruit and vegetable intake and bone mineral density

Evidence for an association between fruit and vegetable intake and BMD from earlier studies remains inconclusive. While studies in premenopausal, perimenopausal or postmenopausal and postmenopausal women generally support a positive association between fruit and vegetable intake and BMD, other studies reported no such association in older men, younger women and boys [NEW et al., 2000; MCGARTLAND et al., 2004, PRYNNE et al., 2006, CHEN et al., 2006].

In this study, there was no positive association between fruit and vegetable intake and BMD in postmenopausal Finnish women. The lack of association may be attributable to over-reporting of vegetable and fruit intake in some of the subjects or because of the rather small sample size.

Even though the results of other studies are somewhat conflicting, there is rising evidence that fruit and vegetables may be favourable for bone health, especially in elderly women. The positive association between fruit and vegetable intake and BMD is not yet confirmed and further research is clearly warranted. However, recommending postmenopausal women to increase their fruit and vegetable intake may be advisable, because of a positive effect of fruit and vegetables on health in general, and possibly also a positive effect on bones.

5.3 Nutrient intake and bone mineral density

The main finding of this study is that dietary vitamin D intake is significantly associated with BMD. Lumbar spine BMD in women in the high vitamin D intake group was 0.104 g/cm2 higher than in the low intake group. The association was significant after adjusting for all potential confounders.

The study differs somewhat from most of the previous studies investigating the association between vitamin D and BMD, because we used dietary vitamin D intake. Many earlier studies explored the link between serum vitamin D and BMD [[ROY et al., 2007; VÄLIMÄKI et al.,2004; BISCHOFF-FERRARI et al., 2004].

One epidemiological study explored the relation between vitamin D intake only from foods and BMD in Swedish men aged 41-76 years and the results of the present study are consistent with its findings. The study reported increased BMD among persons with higher dietary vitamin D intake. Further, the association was especially apparent in subjects with the L/Lpolyadenosine (A) VDR genotype. Similarly to the present study, dietary calcium intake was not associated with BMD measurements. Likewise in Sweden, the main dietary sources of vitamin D in Finland are fatty fish such as salmon and herring and fortified foods such as milk and margarine [MICHAELSON et al., 2006].

The results of the current study suggest that habitual dietary vitamin D intake is associated with BMD in postmenopausal women. This may be of particular importance in the elderly Finnish population, or other populations in Northern latitudes, where sunlight exposure is low during the winter. Our findings were supported by a recently published study by Macdonald et al., who investigated the association between vitamin D status (25(OH)D) and dietary vitamin D intake and bone health in postmenopausal UK women living in higher latitudes. The study found that dietary vitamin D intake was associated with serum vitamin D in winter and spring only, whereas sunlight exposure was associated with serum vitamin D in summer and autumn. Low vitamin D status was associated with increased bone resorption and bone loss. The study clearly shows that dietary vitamin D intake is associated with higher 25(OH)D in winter and spring, and that vitamin D from foods may attenuate the seasonal variation of vitamin D status in postmenopausal women living in northern latitudes [MACDONALD et al., 2008].

Magnesium intake was significantly associated with increased lumbar spine BMD in the crude model. Subjects in the higher magnesium intake group showed 0.098 g/cm2 higher BMD values than subjects in the lower intake group. However, after adjusting for confounders, the association diminished. This is in contrast to other population studies, which found a positive association between dietary magnesium intake and BMD in elderly white women after adjusting for main confounders [NEW et al., 1997; MACDONALD et al., 2004].

5.4 Methodological aspects

This study has several strengths. First, the sample has a narrow age distribution and provides good representativeness of the target population. Secondly, the current study collected extensive information on other factors known to affect bone health and thus, allowed to adjust for several potential confounding factors, e.g. current use of hormone replacement therapy, use of vitamin D and calcium supplements, diseases and medications affecting BMD.

The study has also limitations. The sample size in this study was rather small, and a larger sample size might have been necessary to detect any statistical significant association between fruit and vegetable intake and BMD.

FFQs rely on self-reported intakes and may not be valid measures of absolute intakes, but they are valuable for ranking individuals or for comparing the intakes of groups [WILLETT, 1998]. Misreporting of energy and certain foods is a major methodological problem in studies using FFQ for dietary assessment. Individuals with low education or high BMI have been shown to underreport energy and dietary intake [MAURER et al., 2006]. Obese subjects may both eat less and underrecord more during the study period than non-obese subjects [GORIS et al., 2000]. Underreporting is shown to be food-item selective, fat and other high-energy foods being underreported to larger extent than more “neutral” or healthy food items [HEITMANN et al., 2000]. The underreporting of obese subjects may be macronutrient and food-specific. Fat and carbohydrates have been found to be more likely underreported than protein [LISSNER et al., 2002]. Selective underreporting of both fatty and sweet foods has previously been observed in several populations [MACDIARMID et al., 1998].

In contrast, healthy food items have often shown to be overreported. Some investigators have developed correction factors to correct for overreporting in a FFQ. However, for the purpose of ranking people according to their intakes, correcting individual items with a correction factor based on frequencies does not seem to be advisable [BOGERS et a., 2003]. In the present study we obtained rather high values for nutrient and food intakes similarly to other studies using comprehensive FFQ [ERKKOLA et al. 2001; SHU et al., 2004]. It has been suggested that FFQs generally overestimate the intakes of energy and most foods. This may be attributed to their typically long list of food items [MOORE et al., 2007].

The overestimation of fruit and vegetables in FFQs is also in accordance with other studies. Tucker et al studied the association between potassium, magnesium and fruit and vegetable intake and BMD and has calculated an average fruit and vegetable intake of 4.7 servings per day for men and 5.3 servings for women, which is higher than the data derived from the national survey in the United Stated (3.1-3.4 servings per day) [TUCKER et al., 1999]. In a review of validation studies of FFQs, Cade et al. found that mean correlations between FFQ and reference methods are usually lowest for vegetables. Misreporting of vegetables and fruits can occur due to double counting of items and social desirability bias [CADE et al., 2004]. In contrast to previous studies, no association between fruit and vegetable intake and BMD could be observed and misreporting of those foods by some individuals might partly explain the inconsistent findings.

5.5 Implications for further research

Calcium and vitamin D intakes have been studied mostly in relation to bone health and fracture risk, and there is general agreement on the importance of those nutrients. Other nutrients, such as fatty acids, protein, magnesium and potassium have been less studied and future research should focus on the effect of those nutrients on BMD. There is rising evidence that high fruit and vegetable intake is favourable for bone health. However, most evidence comes from observational studies. Intervention studies and clinical trials have not sufficiently addressed these issues. Furthermore, the mechanisms by which fruit and vegetable intake may affect bones are not yet understood and future investigations are necessary to explain the mechanisms.

6 CONCLUSIONS

The nutritional quality of the diet in postmenopausal Finnish women appears to be adequate or close to adequate with regard to intakes of most nutrients. However, dietary vitamin D intake, an important nutrient for bone health, was found to be low in almost half of the subjects as compared to Finnish nutrition recommendations. The present findings showed that higher dietary vitamin D intake was significantly associated with greater femoral neck BMD, even after controlling for potential confounders. The consumption of foods naturally containing vitamin D or fortified with vitamin D should be encouraged in the elderly Finnish population. In the present study, there was no evidence for a positive association between fruit and vegetables and BMD. Further observational studies with bigger sample sizes and intervention studies should clarify the relation before evidence-based public health recommendations can be made.

7 SUMMARY

Background: Nutritional factors are of considerable importance for osteoporosis prevention. There is general agreement that calcium and vitamin D are important nutrients for bone health, however, little is known about the effect of other nutrients on bone mineral density (BMD). There continues to be a substantial debate about the potential beneficial effect of fruit and vegetables on BMD and bone metabolism and the underlying mechanisms.

Objectives: The aim of this study was to assess the energy and nutrient intake in postmenopausal Finnish women. The second aim was to examine the association between nutrient and fruit and vegetable intake and BMD at the lumbar spine and femoral neck in the same population.

Subjects and Methods: The subjects were 101 postmenopausal women aged 68 to 74 years participating the Kuopio Osteoporosis Risk Factor and Prevention Study. BMD was measured at the lumbar spine and femoral neck by dual-energy X-ray absorptiometry. Usual dietary intake was assessed by using a 89-item semiquantitative food frequency questionnaire. The online version of the food composition database of the National Public Health Institute, Fineli, was used for converting food consumption data to nutrient intakes. The nutrient intake calculation and all analyses were performed by using the SPSS statistical software. The participants were stratified into two groups below and above the median for each dietary variable and the mean BMD at the lumbar spine and femoral neck were calculated. Univariate analysis with adjustment for confounders was carried out to assess whether mean BMD in the low and high intake groups differed significantly.

Results: Femoral neck BMD was 0.83 ± 0.12 g/cm^2 and lumbar spine BMD 1.08 ± 0.18 g/cm^2. Daily energy intake was 2173 ± 554 kcal. Macronutrient intake expressed as percentage of energy intake was 17% for protein, 31% for fat and 51% for carbohydrate. Daily fruit and vegetable intake was 649 ± 435 g. Higher dietary magnesium intake was positively associated with lumbar spine BMD and higher vegetable intake with femoral neck BMD in crude models, but not in the adjusted models. Higher dietary vitamin D intake was associated with higher femoral neck BMD even after adjusting for all the potential confounders.

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FOOD CONSUMPTION QUESTIONAIRE

Name:____

Filling date:____

Telephone number:_______________________________________

We ask you kindly to fill this questionnaire on your food consumption.
Please, read the attached advices carefully before filling the forms.
When answering, think about your usual eating habits.

In the beginning of the questionnaire there are questions on you usual meal pattern and bread consumption and in the end on your potential use of dietary supplements. You will find instructions for answering these questions on the respective sheets.

If you have any question concerning the filling of the questionnaire you may contact registered dietician Henna Karvonen tel. 044-366 1917.

The following instructions are for filling the tables beginning from page 5. At page 2 you will find an example that may also help you in filling the questionnaire. Select on each raw one box that describes you eating habits the best and draw a cross in the box. Note also the presented portion sizes, when you are answering the questions.

If you are typically eating much smaller portions than presented in portion column, mark the cross in a box with less frequent consumption pattern For example, if you drink typically only half a class of milk at the time but twice a day mark that you are drinking one glass milk a day.

Respectively, if you are typically using much bigger portions than given in the tables, mark higher than true consumption frequency. For example, if you use 3 slices of cold cuts once a day, mark that you are eating 1 slice of cold cut 2-3 times/day.

If you are often using food products that can not be found from the food list, write down the food and your typical portion size on the empty space at the end of the list. For example foods for particular nutritional use can be written in this space. If you are you are not at all consuming a certain food product on the list so not leave the raw empty but tick the “Never or very seldom box.”

EXAMPLE PAGE

This example person eats potatoes or smashed potatoes 2-4 times/ week, but most often three potatoes at time. She ticks eating potatoes 5-6 times/week, because the typical portion she eat is bigger than the portion size given in the column.

The person does not eat fried potatoes at all. Therefore she ticks ” Never or very seldom”-box.

The person eats rice typically rice twice a week, but only half of the presented portion size. Therefore she ticks ”Once a week” ¨-box.

The person eats 2 dl of pasta of foods made of pasta 2-4 times/ week. She marks the frequency of 2-4 times/week..

		Average consumption								
Potato, rice and pasta	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Potato (baked, smashed, in soups)	2 small potatoes					X				
Potato (fried)	2 dl	X								
Rice	2 dl			X						
Pasta, pasta in foods (like lasagne)	2 dl				X					

What meals do you usually eat (Mark x according your usual eating habits)

1. Breakfast

☐ Coffee or tea ☐ Sandwich ☐ Porridge ☐ Other, what ________________________________

☐ I don’t eat breakfast

2. Before noon

☐ Coffee or tea ☐ Sandwich or sweet pastry ☐ Other, what________________________________

☐ I don’t eat before noon snacks

3. Lunch

☐ Warm meal, drink, bread ☐ Salad ☐ Desert ☐ Other, what________________________________

☐ I don’t eat lunch

4. In the afternoon

☐ Coffee or tea ☐ Sandwich or sweet pastry ☐ Other, what________________________________

☐ I don’t eat in the afternoons

5. Dinner

☐ Warm meal, drink, bread ☐ Sandwich, drink, no warm meal ☐ Salad ☐ Desert ☐ Other, what____________________

☐ I don’t eat dinner

6. Supper

☐ Coffee or tea ☐ Other, what ____

☐ I don’t eat supper

7. At night

☐ What____________________________________ ☐ I don’t eat at night

Bread and margarine consumption

How many slices of bread do you usually eat?

_____ _ slices of rye bread

_______ slices of crisp bread

_______ slices of graham bread

_______ slices of white bread

How much margarine or other spread do you use on one bread slice? (Mark X according your usual habits)

☐ about 2 teaspoons ☐ about 1 ½ teaspoons ☐ about 1 teaspoon ☐ about ½ a teaspoons ☐ I don’t use spreads

If you use spreads of margarine on your bread, what do you consume most often (mark with X according your typical habits)

☐ Butter

☐ Margarine, 60-80 % fat

☐ Margarine, less than 60 % fat

☐ Other, what____________________________________

		Average consumption								
Cereals	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Porridge	1 plate									
Breakfast cereals	1 plate									
Bran	1 tablespoon									
Muesli	3-4 tablespoons									
Karelian rice pastry	1 piece									
Pastry, salty pies	1 piece									
Fritter, short bread, sweet pastry	1 piece									
Sweet wheat bun	1 piece									
Pancake	1 piece									
Sponge cake with cream	1 slice									
Biscuit	1 piece									
Other___________________	__________									

		Average consumption								
Dairy products	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Skimmed milk or fat-free butter milk	1 glass									
Milk, low fat	1 glass									
Whole milk	1 glass									
Fat free of low fat yogurt or cultured milk	1 jar									
Flavoured yogurt (more than 2 % fat)	1 jar/ glass									
Plain yogurt or cultured milk (more than 2 % fat)	1 jar									
Low fat cheese	2 slices/ 2 tablespoons									
Cheese (edam type ect, medium fat content)	2 slices/ 2 tablespoons									
Cottage cheese, Quark	1 dl									
Cream cheese, cream	1 tablespoon									
Ice cream	1 scoop									
Other________________________	__________									

		Average consumption								
Potato, rice and pasta	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Potato (baked, smashed, in soups)	1 piece									
Potato (fried)	2 dl									
Rice	2 dl									
Pasta, pasta in foods (like lasagne)	2 dl									

NOTE! When filling the table consider also the meat and poultry you eat in dishes like soups and casseroles.

		Average consumption								
Meat and poultry	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Minced meat	100 g*									
Pork, low fat	100 g*									
Pork, high fat (ribs, bacon etc.)	100 g*									
Veal, mutton	100 g*									
Reindeer, game	100 g*									
Liver	100 g*									
Sausage (for main dishes)	1 piece tai 3 hotdogs									
Sausage (as cold cut)	2 slices									
Cold cut	1 slice									
Liver pate	1 cm piece									
Chicken or turkey	1 Portion									
Other________________________	__________									

* 100 g of meat equals 3 medium sized meat balls or one small steak

NOTE! When filling the table consider also fish and eggs you eat in dishes like soups and casseroles.

		Average consumption								
Fish, egg	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Low fat fish (pike, birch etc.)	100 g*									
Fatty fish (rainbow trout, salmon etc.)	100 g *									
Baltic herring, whitefish, vendace	100 g									
Salt preserved fish	3 pieces									
Tuna or other canned fish	½ dl									
Shrimps, crabs	1 dl									
Finnish fish pastry	150 g**									
Egg (boiled, fried, omelette)	1 piece									
Other___________________________________	__________									

* 100 g of fish equals ¼ packet of frozen fish or about 6 x 6 cm piece of salmon or 3 middle size baltic herring or vendace

** 150 g Finnish fish pastry equals about 2 cm thick slices of the pastry

		How often do you eat								
Food preparation and sauces	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Foods prepared with cream	½ dl									
Foods prepared with vegetable-oil based cream	½ dl									
Brown sauce	1 dl									
Oil, oil based salad dressing of mayonnaise	1 tablespoon									
Foods fried in butter of hard margarine										
Foods fried in soft margarine or oil										
Foods that have been coated with bread crumbs of flour before frying										
Other________________________	__________									

		Average consumption								
Vegetables	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Tomato	1 piece									
Cucumber	3 cm piece									
Green salads (lettuce, cabbage, etc.)	1,5 dl									
Mayonnaise based salads	1 dl									
Cooked vegetables as a side dish	1,5 dl									
Carrot (as such, cooked)	1 piece									
Peas, corn, beans	1 dl									
Cooked cabbage in dishes	1 dl									
Vegetable steaks / casseroles	1 piece/ 1 dl									
Vegetable soups	1 plate									
Pickles	4 slices									
Mushrooms	1 dl									
Other___________________	__________									

		Average consumption								
Fruit and berries	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Juice	1 dl									
Citrus fruits or kiwi	1 piece									
Apple, pear, nectarine, apricot	1 piece									
Banana	1 piece									
Canned fruit	1 dl									
Dried fruit and raisins	1 dl									
Berry soup	1 dl									
Berries (fresh or frozen)	1 dl									
Other__________________	__________									

		Average consumption								
Drinks	**Portion**	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Water	glass									
Coffee	1 cup									
Tea	1 cup									
Cream in coffee or tea	1 tablespoon									
Milk in coffee or tea	1 tablespoon									
Sugar added to drinks or foods	1 teaspoon/ piece									
Hot chocolate	1 cup									
Berry juice	1 glass									
Sugar containing carbonated drinks	1 glass									
Home made beer	1 glass									
Beer, cider	1 bottle									
Red or white wine	12 cl									
Spirits	4 cl									
Other___________________	__________									

		Average consumption								
Sweets and snacks	Portion	Never or very seldom	Per month	Per week			Per day			
			1-3 times	Once	2-4 times	5-6 times	Once	2-3 times	4-5 times	More than 5 times
Chocolate	1 bar									
Other sweets	1 dl									
Nuts and seeds	1 dl									
Chips and pop corn	2 dl									
Other____________________	________									

Do you use vitamin- or mineral supplements like calcium preparation or oil supplements like fish oil capsules? (circle yes or no)

No Yes

If you answered yes, what preparations and how much

Name of the supplement Portion How often

____

____

Mark here the use of any special diets

☐ No special diets

☐ Yes, what____________________________

Please, check once more that you have answered all the questions.

Thank you for participating the study!

Zusammenfassung auf Deutsch

Einleitung: Die Ernährung spielt bei der Prävention von Osteoporose eine große Rolle. Während die Bedeutung einer ausreichenden Versorgung mit Calcium und Vitamin D generell akzeptiert ist, stehen viele andere Ernährungsfaktoren, wie die Aufnahme von Obst und Gemüse in der Literatur zur Diskussion.

Ziel der Arbeit: Ziel der vorliegenden Arbeit war es, die Nährstoffaufnahme postmenopausaler finnischer Frauen mittels eines semiquantitativen Häufigkeitsfragebogens (Food Frequency Questionnaire; FFQ) zu ermitteln. Ein weiteres Ziel dieser Arbeit war es den Zusammenhang zwischen der Nährstoffaufnahme, sowie zwischen dem Obst- und Gemüseverzehr und der Knochendichte am Schenkelhals und an der Lendenwirbelsäule zu untersuchen.

Material und Methoden: Die Studienteilnehmer waren 101 postmenopausale Frauen im Alter zwischen 68 und 74 Jahren. Die Knochendichte wurde mittels Energien- Röntgen- Absorptiometrie (Dual Energy X-ray Absorptiometry; DXA) am Schenkelhals und an der Lendenwirbelsäule gemessen. Die Nährstoffzufuhr wurde mit einem aus 89 Die Nährstoffzufuhr wurde mittels eines 89 Fragen beinhaltenden, semiquantitatien FFQs ermittelt. Die finnische online Nährwertdatenbank Fineli (www.fineli.fi) wurde verwendet um aufgenommen Lebensmittel in Nährwerte umzurechnen. Die Nährstoffberechung und alle statistischen Auswertungen wurden mit dem Statistikprogramm SPSS für Windows durchgeführt. Alle Studienteilnehmer wurden für jeden Nährstoff in 2 gleich große Gruppen (über und unter dem Median) eingeteilt. Die Gruppenunterteilung wurde durchgeführt, um eventuelle Unterschiede in der mittleren Knochendichte zwischen der Gruppe mit hoher Nährstoffaufnahme (bzw. hohem Obst- und Gemüsekonsum) und niedriger Nährstoffaufnahme (bzw. niedrigem Obst- und Gemüsekonsum) festzustellen. Unterschiede zwischen den Gruppen wurden unter Berücksichtigung der konfundierenden Variablen mittels ANOVA (univariate Varianzanalyse) getestet.

Ergebnisse: Die DEXA Messwerte der Knochendichte am Schenkelhals ergaben 0.83 ± 0.12 g/cm2 und an der Lendenwirbelsäule 1.08 ± 0.18 g/cm2. Die Erfassung der Energiezufuhr mittels FFQ ergab eine mittlere tägliche Energieaufnahme von 2173 ± 554 kcal. Die Aufnahme von Protein lag bei 17%, die von Fett bei 31% und die von Kohlenhydraten bei 51% der gesamten täglichen Energieaufnahme. Die Aufnahme von Obst und Gemüse betrug im Durchschnitt 649 ± 435 gram täglich.

Frauen mit der höchsten Aufnahme von Magnesium wiesen eine signifikant höhere Knochendichte am Oberschenkelhals auf, jedoch war das Ergebnis nach statistischer Kontrolle konfundierender Variablen nicht mehr signifikant. Eine niedrige Vitamin D Aufnahme mit der Nahrung stand mit einer geringeren Knochendichte am Oberschenkelhals in Zusammenhang und das Ergebnis blieb auch nach Berücksichtigung aller konfundierenden Variablen signifikant ($p<0.05$).

Leonie-Helen Bogl

PERSONAL

Nationality: Austrian

Date of Birth: 04.07.1982

Place of Birth: Linz, Upper Austria

Sex: Female

Marital status: Single

EDUCATION

08/2008	Research Training in Quantitative Medical Research at the Erasmus University in Rotterdam, The Netherlands
09/2007-05/2008	University of Kuopio, Finland, Department of Public Health (Master thesis)
01/2005-06/2005	University of Manchester, Great Britain (Exchange Student)
Since 2001	Sports and Health Sciences, University of Vienna, Austria
Since 2000	Nutritional Sciences, University of Vienna. Expected graduation: December 2008
1996 - 2000	High School with focus on mathematics and science in Wels, Upper Austria (A-level Certificate)
1992 -1996	Boarding school of the Austrian National Ballet School: Secondary school with focus on mathematics and science in combination with dancing education at the Vienna State Opera

RESEARCH EXPERIENCE

Since 01/2008	Research assistant at the Department of Public Health, University of Helsinki, Finland

09/2007-05/2008	Master thesis: Nutrient intake and its association with bone mineral density in postmenopausal Finnish women
07/2005-09/2005	Traineeship at the Department of Public Health, Celal Bayar University, Manisa, Turkey. Project: Malnutrition prevalence and risk factors among children in a rural area.

OTHER WORK EXPERIENCE

Summer 2006, 2007	Life saver, City of Espoo, Finland
05/2002 - 10/2002	Windsurfing instructor, Bodrum, Turkey
Since 2000	Several student jobs including exhibition hostess, salesperson, swimming instructor
1992-1996	Over 50 performances at the Vienna State Opera, e.g. Carmen, The Nutcracker, Tosca, The Knight of the Rose, Samson and Delilah

FELLOWSHIPS	Nihes/Erasmus Summer Programme Fellowship (2008)
	Scholarship for scientific research abroad KWA (2007)
	Leonardo da Vinci grant (2005)
	Sokrates Erasmus grant (2005)
COMPUTER SKILLS	Microsoft Office, SPSS, STATA, Photoshop, Dreamweaver, EPI-Info
LANGUAGES	German (Native), English (Fluent), Turkish (Fluent), Italian (Fair), Finnish (Fair)

LANGUAGE COURSES

07/1999	English language Course at the California State University, Los Angeles
06/2003-08/2003	Language and Culture Course at the Bosphorus University, Istanbul
07/2004-08/2004	Turkish Language Certificate, Istanbul
Since 2004	Finnish language courses at the Universities of Vienna, Kuopio and Helsinki (16 ECTS)
HOBBIES	Competition swimmer until 2002, jogging, diving, windsurfing, (belly) dancing, yoga