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**FOOD CONSUMPTION PATTERNS AND BONE DENSITY OF THAI
MENOPAUSAL WOMEN: A COMPARISON BETWEEN
VEGETARIANS AND NON-VEGETARIANS**

CHOMJIT WONGRATTANACHAI

อภินันท์นทาการ

จาก

บัณฑิตวิทยาลัย ม.มหิดล

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Finally, I would like to express my grateful appreciation to my parents and my husband for their continuous support, love and cheerfulness throughout my graduate study.

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CHOMJIT WONGRATTANACHAI: FOOD CONSUMPTION PATTERNS AND BONE DENSITY OF THAI MENOPAUSAL WOMEN: A COMPARISON BETWEEN VEGETARIANS AND NON-VEGETARIANS. THESIS ADVISORS: MANDHANA PRADIPASEN, M.D., Dr.P.H. REWADEE CHONGSUWAT, Ph.D. WONGDYAN PANDII, Dr.P.H. WANNA TRIVITAYARATANA, M.D., M.Sc. 131 p. ISBN 974-664-048-8

This cross-sectional comparative study aimed to investigate whether the bone density and osteoporosis were different between vegetarian and non-vegetarian menopausal women. The menopausal women were recruited from Pratom-A-Sok, and Santi-A-Sok religious communities for vegetarian group and the clubs for the elderly at Phramongkutklao, Siriraj hospital non-vegetarian. Each group was matched by years since menopause (± 2 years) and consisted of 30 subjects. Data were collected using questionnaires including demographic information and food consumption. Measurement of undominant distal forearm was performed to assess bone density for indicated biological variability and prediction for fracture risk. The energy and nutrients intakes were calculated with nutrition calculated program. The differences of energy and nutrient intakes and bone density between the two groups were compared by paired t-test.

The findings of this study showed that bone density and risk of osteoporosis were similar among vegetarian and non-vegetarian menopausal women. The vegetarians' top ten most frequently consumed food items were cooked home-pounded rice, bean curd, soybean sauce, home-pounded rice soup, black sesame seeds, ripe banana, orange, soymilk, straw mushroom and cone mushroom whereas the other group's were cooked milled rice, orange, stir-fried pork (lean-meat), soybean sauce, fish sauce, cooked home-pounded rice, boiled pork (lean-meat), ripe banana, soymilk and pineapple. The average energy, animal protein, carbohydrate, calcium, and vitamin B1 intakes were significantly higher in non-vegetarians ($p < 0.05$). On the contrary, protein from plant, fiber, and ferrous intakes were significantly higher in vegetarians ($p < 0.05$). Long-term differences in protein, calcium and phosphorus intake could affect bone mineral mass.

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หุ้มจิตร วงศ์รัตนชัย: รูปแบบอาหารบริโภคและความหนาแน่นของกระดูกในสตรีไทยวัย
หมดประจำเดือนศึกษาเปรียบเทียบระหว่างสตรีที่บริโภคและไม่ได้บริโภคอาหารมังสวิรัตติ
(FOOD CONSUMPTION PATTERNS AND BONE DENSITY OF THAI MENOPAUSAL
WOMEN: A COMPARISON BETWEEN VEGETARIANS AND NON-VEGETARIANS)

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การศึกษากาตดขวางนี้มีวัตถุประสงค์เพื่อศึกษาผลของรูปแบบอาหารบริโภคและความ
หนาแน่นของกระดูกในสตรีไทยวัยหมดประจำเดือนที่บริโภคและไม่ได้บริโภคอาหารมังสวิรัตติ
ทั้งสองกลุ่มจับคู่โดยใช้จำนวนปีที่หมดประจำเดือน (± 2 ปี) โดยกลุ่มที่บริโภคอาหารมังสวิรัตติ
อาศัยอยู่ในชุมชนพุทธสถานสันตือโสภและปฐมอโสภ จำนวน 30 คน กลุ่มที่ไม่ได้บริโภคอาหาร
มังสวิรัตติเป็นสมาชิกชมรมผู้สูงอายุจากโรงพยาบาลพระมงกุฎเกล้าและโรงพยาบาลศิริราช จำนวน
30 คน การเก็บข้อมูลประกอบด้วยการสัมภาษณ์ข้อมูลทั่วไป ความถี่อาหารบริโภคและการวัด
ความหนาแน่นของกระดูกบริเวณข้อมือ เปรียบเทียบค่าเฉลี่ยของพลังงาน สารอาหารและความ
หนาแน่นของกระดูกระหว่างสตรีทั้งสองกลุ่ม โดยใช้สถิติ paired-t-test

ผลการศึกษาพบว่าค่าเฉลี่ยความหนาแน่นของกระดูกและความเสี่ยงของภาวะกระดูก
พรุนในสตรีทั้งสองกลุ่มแตกต่างกันอย่างไม่มีนัยสำคัญทางสถิติ แบบแผนการบริโภคของกลุ่ม
บริโภคอาหารมังสวิรัตติประเมินจากความถี่สูงสุด 10 อันดับแรก ได้แก่ ข้าวสวยกถ้อง เต้าหู้แข็ง
ซีอิ้วขาว ข้าวต้มกถ้อง งาดำ ถั่วขยน้ำว่าสุก ส้มเขียวหวาน น้ำเต้าหู้ เห็ดนางฟ้าและเห็ดฟาง ส่วน
กลุ่มที่ไม่ได้บริโภคอาหารมังสวิรัตติ ได้แก่ ข้าวสวย ส้มเขียวหวาน หมูเนื้อแดงผัด ซีอิ้วขาว น้ำปลา
ข้าวสวยกถ้อง หมูเนื้อแดงต้ม ถั่วขยน้ำว่าสุก น้ำเต้าหู้และสับปะรด กลุ่มบริโภคอาหารมังสวิรัตติมี
ค่าเฉลี่ยของโปรตีนจากพืช เส้นใยอาหารและธาตุเหล็กสูงกว่าอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$)
ส่วนกลุ่มที่ไม่ได้บริโภคอาหารมังสวิรัตติมีค่าเฉลี่ยของพลังงาน โปรตีนจากสัตว์ คาร์โบไฮเดรต
แคลเซียม และวิตามินบี 1 สูงกว่าอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) การบริโภคอาหารที่มีส่วน
ประกอบของโปรตีน แคลเซียม และฟอสฟอรัสในปริมาณที่แตกต่างกันเป็นเวลานาน ทำให้เกิดผล
ต่อความหนาแน่นของกระดูก

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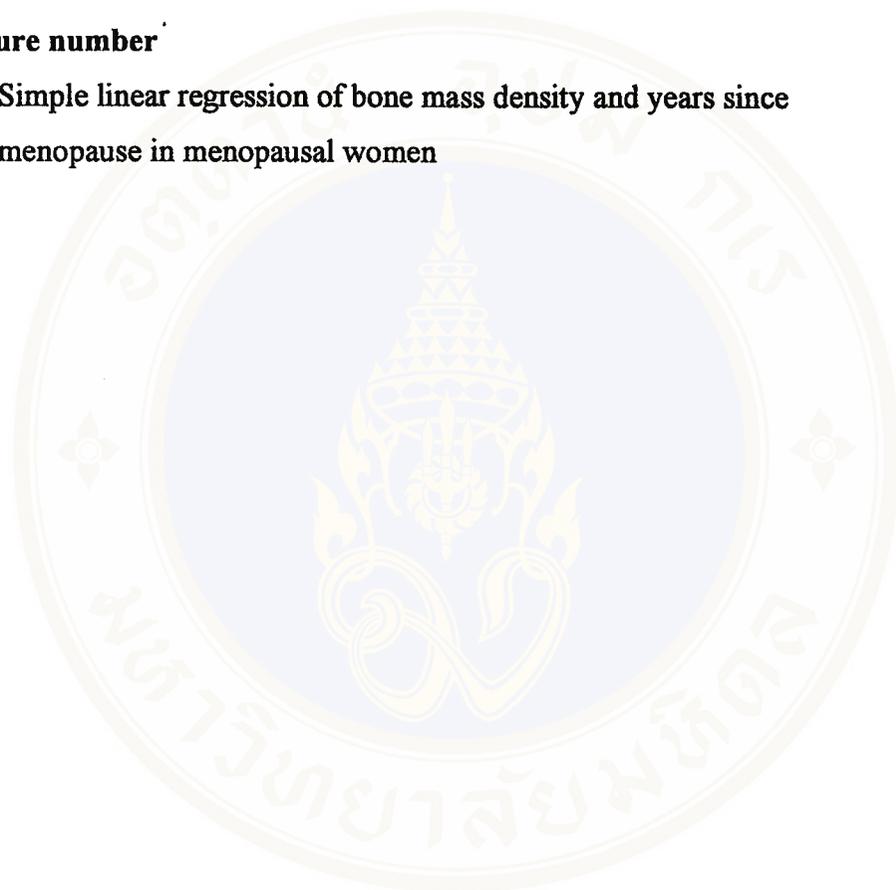
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Chapter I

Introduction

Rationale

The Thai population continue to grow about 20% each decade (1). By the end of 1998 it reached 60 millions (2) and was expected to be 67.3 millions and 71.6 millions in 2010 and 2025 (3).

Both numbers and proportion to population of the Thai elderly people were growing from 1.2 millions to 4.8 millions or 4.6% to 7.4% in the last 30 years, elderly women increased more than elderly men by 0.8-1.0 % (1). This event will continue to grow because of the increasing life expectancy in Thai people. In 2010 the Thai elderly will reach 7.6 millions or 11.4%, elderly women will have more numbers than elderly men by 1.2-1.9% (5). In addition to the growing numbers of elderly people, the older population itself is getting older and elderly women are growing in higher rate because their life expectancy are longer than that of men. In 2010, life expectancy for Thai women will be 73 years and 68.8 years for men (4). There will be 12.3 millions of women aged 65 and older, compared to 10.4 millions men (5). Today women can expect to live nearly one-third of their live after menopause.

The menopause is a significant period in women's life and is a time for education. Certainly preventive health care education is important throughout life, but at the time of the menopause, attention is now being focused on cardiovascular disease and osteoporosis.

Estrogen deficiency is the major cause for osteoporosis and health problem in postmenopausal period. In further aging, bone mass is lost at a relatively constant rate. Postmenopausal women experience an accelerated bone loss in the first few year after menopause. Average rates of bone loss after age 50 are 1-2% per year, bone loss 3% or above per annum is frequently found in the first 5-8 years following menopause (6,7), and it is considered the primary cause of the high incidence of fragility fracture in elderly populations. Age-adjusted mortality during the first year after hip fracture is two to five times higher than for the general population and substantial morbidity decrease quality of life are associated with both hip and vertebral fractures (8). Osteoporosis cause considerably suffering, disability and mortality in these women. The number of osteoporotic patients will increase in the future due to increase life expectancy. Thus, the costs for the care of osteoporosis and its consequences are expected to dramatically increase in the next decades.

Osteoporosis is caused by a reduction in bone mass, but the cause of the osteoporosis fracture is multifactorial. Factors contributing to osteoporosis were identified as genetic or constitutional, life style and nutritional, medical disorders, and certain medications (9). Now estrogen replacement is continued to be the mainstay therapy for prevention of bone loss and osteoporotic fractures. Although in general the risk/benefit ratio is very favorable to the use of estrogen, considering its protective effect on cardiovascular disease and mortality (10,11). The advantages of the hormonal therapy may be substantially offset by increased risks and diminished efficacy for long-term treatment regimens and it has a lot of side effects such as vaginal bleeding, breast enlargement, fluid retention and increase risk of breast cancer with age (12).

Certain foods can prevent or decrease the high rate of bone loss that occurs just after menopause. Many researches suggested that diphenolic compounds, found in phytoestrogen-rich diet and structurally resemble estradiol (E2) were shown to have weak estrogenic activity. When digested in relatively large amounts, dietary phytoestrogens have been shown to have significant biological effects in several animal species and in humans (13,14,15,16,17,18,19). Phytoestrogens are particularly abundant in soybeans, but they were also found, in lower concentrations, in cereal bran, whole wheat and legumes. Previous studies found that diets consisting primarily of meat may differ substantially in composition from the vegetarian diet, based on grains, vegetables, fruits, legumes, and seeds, with respect to protein, calcium, and phosphorus content. Because of such differences, long term ingestion of these diets could affect bone mineral mass (20,21,22,23,24,25,26).

The effect of difference in dietary consumption on bone density can be early searched. This research is aimed to give an indication whether the same measure of bone mass density and risk of osteoporosis are found in vegetarian and nonvegetarian menopausal women and if certain nutrients affect bone loss and osteoporosis in these women. This will enable us to promote healthy food habit to prevent or slow down further osteoporosis and fracture in Thai menopausal women, leading to reduction of their suffering, morbidity from further diminishing bone mass and subsequently fracturing of skeletal part(s).

Research question

Do different food consumption patterns of vegetarian and nonvegetarian Thai menopausal women have a different effect on their bone mass density?

Objective

To determine the effect of food consumption patterns on bone mass density in Thai vegetarian and nonvegetarian menopausal women

Specific-Objectives

1. To compare energy, nutrients and phytoestrogen from foods consumed by vegetarian and nonvegetarian menopausal women
2. To compare bone mass density between vegetarian and nonvegetarian menopausal women
3. To determine risk of osteoporosis in vegetarian and nonvegetarian menopausal women
4. To predict bone mass density loss by nutrients consumption among vegetarian and nonvegetarian menopausal women
5. To predict bone mass density loss by years since menopause among vegetarian and nonvegetarian menopausal women

Hypotheses

1. There is a significant difference in energy, nutrients and phytoestrogen intake between vegetarian and nonvegetarian menopausal women
2. There is a significant difference in bone mass density between vegetarian and nonvegetarian menopausal women
3. There is a significant difference in the risk of osteoporosis between vegetarian and nonvegetarian menopausal women

Limitation of the study

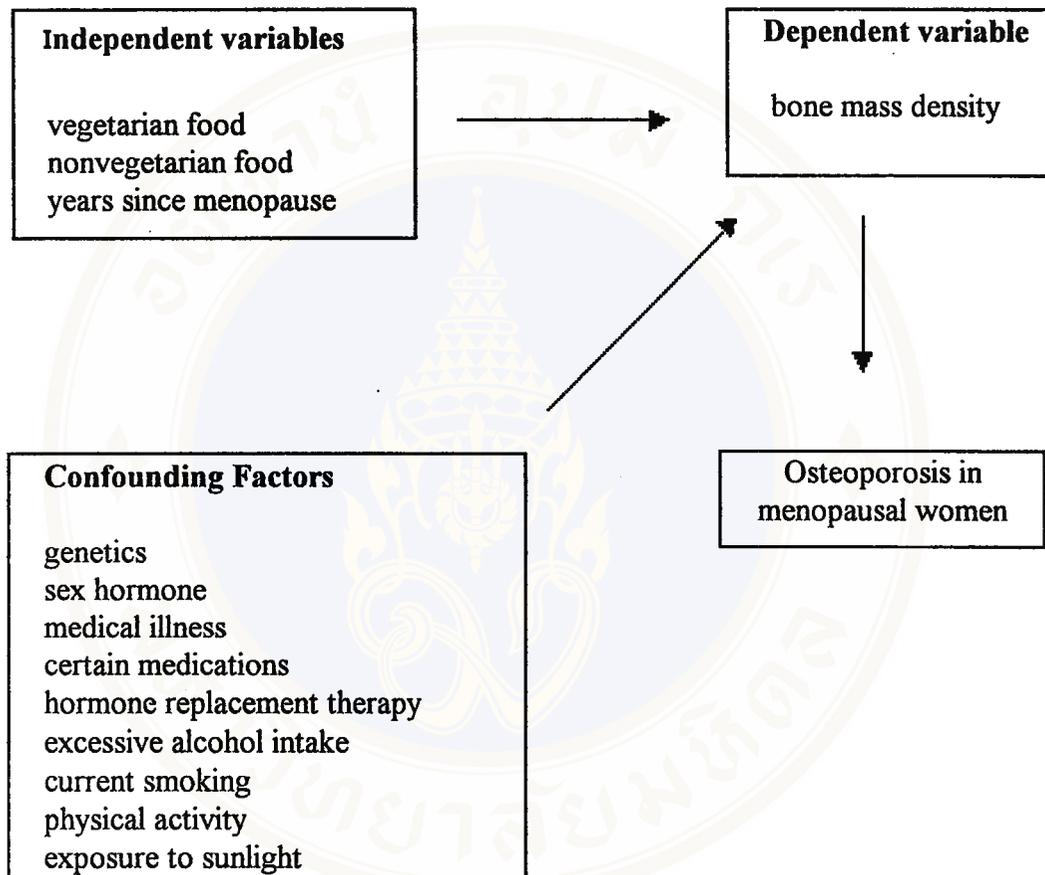
This study is established to give an indication whether the bone density is similar in vegetarian and nonvegetarian menopausal women. Vegetarian and nonvegetarian consumptions classify both sample groups. Since vegetarians with specified inclusion criteria are very rare, subjects in this group are recruited from two specified communities, whereas the control group is comprised of general menopausal women not belonging to that community, for reasons of data collection convenience and limited budget. The vegetarian groups live at Pratom-A-Sok and Santi-A-Sok religious communities and the nonvegetarian groups come from the clubs for the elderly at Phramongkutklao, and Siriraj hospital in Bangkok and lived in the near vicinity. These two groups are considered comparable for both are of mix nativity e.g. Bangkok, Minburi, Nontaburi, Nakorn Pratom, Nakorn Sawan, Pichit, Samut Prakran, Chonburi, Nakorn Ratchasima.

This study measured current bone mass density of subjects and could not control for the similarity in genetics, environmental, and lifestyle factors that determinant of

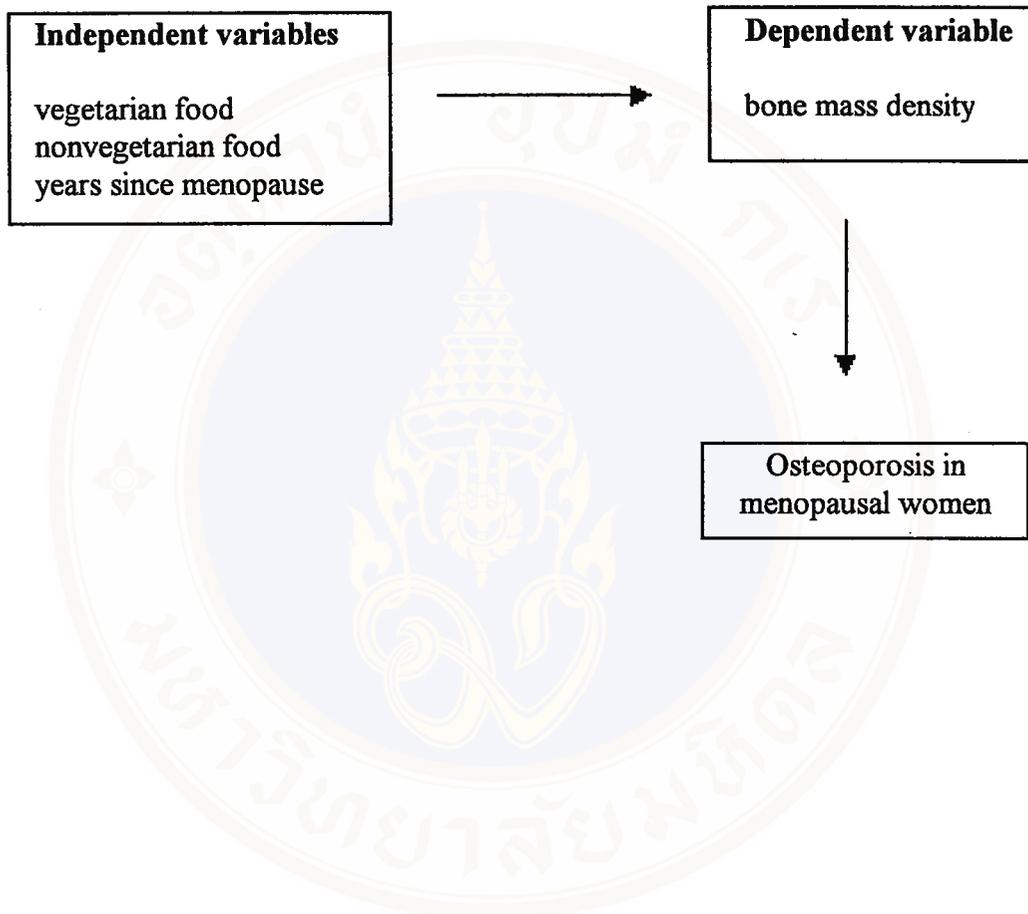
peak adult bone mass in each subjects. Furthermore, it could not control for better self-health monitoring in nonvegetarian group that some of them come from the clubs for the elderly.



Theoretical Conceptual Framework



Research Conceptual Framework



Note

All confounding factors were controlled by excluding persons with these factors from the study

Definition of terms

food consumption pattern is :number of meal, food items that frequently consumed,
amount and adequacy of daily energy and nutrients

consumption

bone mass density is . :bone mineral content (grams per centimeter)
and bone width (centimeters)

vegetarian foods are :types of food that contain no meat, and meat products
including eggs and dairy products

nonvegetarian foods are :types of food containing both plants and meat

vegetarian menopausal woman is
:woman who has been in the stage of permanent ovarian
activity cessation for at least one year, consumed
vegetarian food before menopause until the beginning of
the study

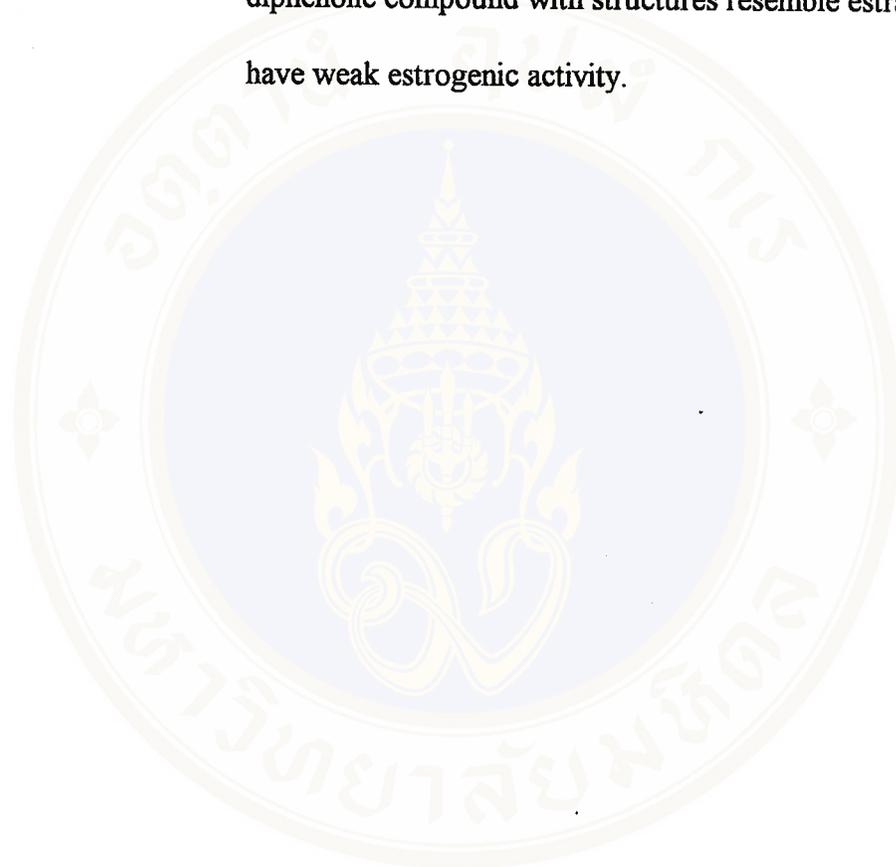
nonvegetarian menopausal woman is
:the woman who has been in the stage of permanent
ovarian activity cessation for at least one year and
consumes nonvegetarian food

years as vegetarian are :number of years from the start of consuming vegetarian
diet until the beginning of study

years since menopause are :number of years from the permanent cessation of
ovarian function until the beginning of study

phytoestrogen-rich food

:certain foods that contain nonsteroidal diphenolic compounds found in plants origin or derived from the in vivo metabolism of precursor present in several plants eaten by humans. They are diphenolic compound with structures resemble estradiol and have weak estrogenic activity.



Chapter II

Theoretical concepts and Literature review

This part were collected, modified and adjusted theoretical concepts and literature reviews related to the rationale, study objectives and scope of the study.

The menopause

The menopause is a wonderful signal, occurring at the right time of life when preventive health care is especially critical. This positive attitude requires an understanding of the epidemiology of the menopause, an understanding that is based on the recognition of this event as a normal stage in development. It incorporates biology, psychology, society, and culture.

The menopause is that point in time when permanent of menstruation cessation occurs following the loss of ovarian activity. Menopause is diagnosed after 12 months of menstruation cessation resulting from the permanent cessation of ovarian function (27). The perimenopause is the period immediately before and after the menopause. The climacteric is the period of time when a woman passes through a transition from the reproductive stage of life to the postmenopausal years.

The age of menopause

The median age for menopause was 51.3 years. Factors that did not affect the age of menopause included the use of oral contraception, socioeconomic status and marital status. A median age of menopause means that only half of the women have

reached menopause at this age. It is more useful clinically to remember the range for the age of menopause, approximately age 48 to 55 (28).

The symptoms of menopause

During the menopausal years, some women will experience severe multiple symptoms, while other will show no reaction, or minimal reaction that go unnoticed. The differences in menopausal reactions in symptoms across different culture is poorly documented. Individual reporting is so conditioned by sociocultural factors that it is hard to determine what it due to biological versus cultural variability.

Women often seek medical assistance for any of the following symptoms (28):

1. Disturbances in menstrual pattern, including an ovulation and reduced fertility, decreased or increase flow, and irregular frequency of menses.
2. Vasomotor instability resulting in the hallmark symptom of the menopause, the hot flush. The physiology of the hot flushes still not understood, but it apparently originates in the hypothalamus and is brought about by a decline in estrogen. However, not all hot flushes are due to estrogen deficiency.
3. Atropic conditions: atrophy of the vaginal epithelium, formation of urethral caruncles, dyspareunia and pruritus due to valvar, introital and vaginal atrophy and urinary difficulties such as stress in continence, urgency and bacterial urethritis and cystitis.
4. Psychological symptoms, including anxiety, mood depression, irritability, insomnia and decreased libido. However, it is by no means certain that these reactions reflect a change in estrogen levels.

Osteoporosis

Osteoporosis defined by The Consensus Development Conference as “ a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk” (29). The more widely utilized definition based on bone mineral density measurements has been provided by the World Health Organization (30). The rationale for using bone mass density is that it is an accurate and reproducible, non-invasive measure which can be used to predict an individual’s future fracture risk. The ability of bone mass to predict fracture is at least as good as, if not better than, the ability of cholesterol to predict heart disease and blood pressure to predict stroke (31). World Health Organization has recently proposed cut-off thresholds of bone mass density to define osteoporosis risk (30). Low bone mass is a bone mass density between 1.0 and 2.5 SD below the mean for young adults. Osteoporosis is a bone mass density more than 2.5 SD below the mean for young adults and severe osteoporosis is a bone mass density more than 2.5 SD below the mean for young adults in the presence of a non-traumatic fracture (30).

Osteopenia is a nonspecific term used to describe a decrease quantity of bone regardless of the cause and as the precursor or early stage of osteoporosis. Osteoporotic fractures cause considerable morbidity, mortality and public health expenditure. Vertebral, hip and wrist fractures are the most common fractures associated with osteoporosis. The risk of a 50-year-old woman to fracture her hip in her remaining lifetime is estimated at 16%; to fracture her wrist at 15%; and sustain a vertebral fracture at 32% (32).

Pattern of bone formation and bone loss

Bone mass increases during childhood and adolescence. At puberty there is an acceleration of mineral accretion in the spine, and to a lesser extent in the hip; but gain in the radius (and perhaps in other long bones) appear to proceed at about the same rate as before puberty (33). In the late teens there is a dramatic slowing of skeletal mineral accumulation. Although a matter of some dispute, recent data demonstrate the potential for further bone mineral accumulation, despite the lack of longitudinal growth, for women in their twenties (34). The accumulation of skeletal mineral ends, at near age 30, bone mass is at its peak, and a period of relative stability probably ensues. Peak bone mass is on average higher in men than women, with larger differences in the cortical skeletal and much smaller differences in trabecular bone (35). Bone loss may begin at differing ages, depending on both the skeletal site and an individual's life style.

Bone loss in women is accelerates by estrogen deficiency, regardless of the cause (e.g., menopause, athletic amenorrhea) (36,37). Women experience a sharp acceleration of bone loss during the 5 years following menopause (29).

Factors influencing peak bone mass

Genetics

Genetic factors are unquestionably the single most important factors in the determination of peak adult skeletal mass (38,39). Familial effects are probably attributable to shared environmental influences on bone loss. Family studies (e.g., parent-offspring) have yielded slightly lower estimates of genetic influences on bone

mass (40). Studies that have examined the assumptions of these genetic models have found evidence suggestive of gene interaction in the determination of bone mass (41).

Daughters of fracture menopausal women had lower bone mass than similarly aged women of mothers without fractures, but these differences are relatively small (40). However, many studies have failed to find family fracture histories valuable in identifying women with low bone mass (42,43). In part this is because fractures in older women reflect not only skeletal fragility but also longevity and likelihood of falling, perhaps due to the loss of protective reflexes and other factors.

Sex hormone

The strong determinant on skeletal mineral in puberty children are probably hormonal, although direct studies of sex steroids, growth hormone, and other similar factors have not been carried out for this age group. However, when there are disturbances in normal hormonal function, clear skeletal deficits appear. Amenorrhea in adolescents, regardless of cause, results in reduced bone mass (36,44). Where as in some circumstances, factors causing the amenorrhea (e.g., the nutritional inadequacies associated with anorexia nervosa) may also contribute to the skeletal deficit (44), and in other circumstances (e.g., athletic amenorrhea) it is probable that the hormonal deficit itself is the sole cause of the bone loss. Thus the prompt correction of menstrual irregularities in young women is probably crucial to allowing this group to achieve their genetic potential.

Environmental factors and lifestyle

Physical activity has been shown to be associated with higher bone mass in children and adolescents (45), which is similar to the findings in adult populations. Those who participate in weight-bearing activities (e.g., basketball, soccer, baseball, softball) had an additional 5% to 7% bone mass for each extra hour per day of weight-bearing activity. Swimming and cycling, in contrast, showed no beneficial effects. These same patterns have also been observed for rate of gain in bone mass (33). Intense physical activity may also be associated with diminished bone mass in young women with amenorrhea (36). These are reports of some elite athletes apparently increasing the negative skeletal consequences of athletic amenorrhea, perhaps as a result of extremely intense training (46,47).

There are several studies indicated that calcium and other factors probably play a major role in developing peak bone mass. Calcium is important for achieving maximum in the accumulation of maximum skeletal mass during the third decade of life (48,49). A recent report found the dietary calcium/protein ratio to be positively associated with the rate of gain in spine bone mass during the third decade of life (34). Dietary protein intake has previously been shown to increase urinary calcium excretion (50,51) and low protein was detrimental effect on bone (52).

The effect of other dietary factors on skeletal growth and mineralization have not been well studied. Caffeine, for example, has been suggested as a potentially negative skeletal influence on increasing urinary calcium excretion (50). However, many studies have been unable to find significant caffeine in bone mass or change in bone mass in the range of usual intakes for coffee, tea, and other caffeine-containing drinks (42,53).

Factors influencing skeletal health after peak bone mass is achieved

Studies examining factors affecting bone mass in older people include both prospective studies of bone loss and, more commonly, cross-sectional or retrospective investigations of factors associated with low bone mass or fractures.

Genetics

To address the question of genetic influences, studies of twin have been completed. Although there are highly significant correlation's between members of twin pairs, these correlation's are similar for identical and fraternal pairs, suggesting that a common environmental, rather than genetic factors, plays the major role in this bone loss (54).

Sex hormone

Although bone loss probably begins before the menopause, there is an acceleration of loss that accompanies the cessation of ovarian hormone production (55, 56). This corresponds to the period of most rapid skeletal loss in women, amounting to several percent per year in the spine and 1% or more per year in predominantly cortical sites (55). These rates of bone loss in the late peri- and early postmenopausal years are primarily determined by endogenous estrogen concentrations. Among the causes of menopausal bone loss, serum estrogen deficiency is the most important, but it is also probably the best understood and the most easily altered. The more rapid rates of skeletal turnover and bone loss of menopausal women are immediately returned to premenopausal levels with estrogen therapy (57,58).

Increased hormonal circulation in obese women and greater conversion of adrenal androgens to estrogen linked to greater mass of adipose tissue. Body weight is needed to confer a reduced risk of osteoporosis is questioned. Generally, a BMI of around 30 is associated with a 4-8% greater lumbar spine BMD, 8-9% greater hip region BMD, and 25% greater radius BMD compared with a BMI of about 20 (59). Previous studies shown that cases of vertebral fractures between otherwise-matched subjects were more likely seen in subjects with a BMI ≤ 24 than in subjects with a BMI ≥ 26 (59). Many literature does not allow setting a distinct BMI cutoff value for osteoporosis risk, but generally a BMI $< 22-24$ is associated with less bone density throughout the body compared with a BMI $> 26-28$ (59,60,61).

Environmental factors and lifestyle

Dietary factors

Calcium

Bone loss slows as time from the menopause increases; and as loss slows, the effects of nonhormonal influences are more easily observed. The influence of calcium intake on rates of bone loss has been the subject of considerable debate. Although there is little to suggest that additional calcium can slow the bone loss driven by estrogen deficiency immediately following the menopause, recent data have shown that supplemental calcium can slow bone loss 3-5 years after menopause (62,63). However, these effects were seen primarily in women who had quite low dietary calcium intake (< 400 mg/day). Whether additional calcium beyond the 800 mg/day RDA figure will be useful remains to be shown. It has been suggested that older women may require as

much as 1500 mg of calcium daily, but clinical trials demonstrating the utility of such intake have not yet been completed.

Phosphorus

Phosphorus is widely available in both animal and plants including meat, poultry, fish, eggs and dairy products and in cereals, nuts and legumes. Dietary phosphorus can be either organic or inorganic in origin, most absorption-taking place in the inorganic form. The RDA for both calcium and phosphorus is 800 mg in men and women.

Low level of calcium-rich foods intake become the major problem because of the lowering of the Ca:P ratio and the development of a persistent elevation of PTH. A diet containing adequate amounts of calcium can overcome this adverse effect of P.

A healthy eating pattern should contain a ratio within the range 0.7:1 to 1:1. Intake ratios at or below 0.5:1 are of concern because of the likelihood of persistently elevated PTH concentrations and the potential loss of bone mass which could lead to fragility fractures (64).

Protein

Some studies have suggested that protein also increase urinary excretion of calcium and may have a detrimental effect on bone mass (51). However, one recent cross-sectional study found a positive association between protein intake and bone density in pre-menopausal women but not post-menopausal women (65). In addition, low protein intake may have a detrimental effect on bone acquisition and on bone loss (51,66) but this may reflect a state of relative malnutrition with more complex effects on bone mass.

Fluoride

Several recent studies had suggested that exposed to fluoride may not be benign. High concentrations of fluoride (about 4 ppm) in drinking watersupplies had been associated with increased hip fracture rates in these communities (67). Similarly, communities with fluoridated water (about 1 ppm) had recently been observed to have higher hip fracture rates than non fluoridated communities (68). The bone produce may not be normal. Its strength may be less than that predicted by its mass because of the presence of fluoride crystals in the mineral structure. The issue of bone quality, in the context of the effects of both therapeutic agents and environmental exposures, remains poorly understood.

Caffeine

Many studies have been unable to find significant caffeine in bone mass or change in bone mass in the range of usual intakes for coffee, tea, and other caffeine-containing drinks (42,53). Caffeine consumption tended to be associated with higher bone mineral at younger ages, but among those aged 60 years and above, there was a small negative relationship between bone mineral and caffeine intake (53). Caffeine has been suggested as on increasing urinary calcium excretion (50,53).

Cigarette Smoking

Women smokers have been shown to have both lower bone mass around the time of menopause (69) and greater rates of bone loss after menopause (70). Women with vertebral crush fractures are also more likely to be smokers than non smokers (71). The mechanism for the detrimental effect of smoking on the skeletal is not known, but several reports have indicated that smoking probably interferes with aspects of estrogen

metabolism, rendering postmenopausal estrogen therapy less effective (72) and altering estrogen metabolism premenopausally (73).

Alcohol consumption

Alcohol abuse has been associated with low bone mass and fracture risk in both women and men (74). The effect of moderate alcohol consumption on the female skeletal is not as clear as that of smoking. Many studies of volunteers have shown no apparent detrimental effect of moderate alcohol consumption (42,75,76). Alcoholism may exert a deleterious effect on bone via a number of mechanisms. These include a direct toxic effect in osteoblasts, or indirectly associated nutritional deficiencies, liver disease and chronic illness and also by predisposing to falls (74).

Medication

A number of medications had been associated with either increase or decrease in bone mass density. Thiazide diuretics had been associated with higher bone densities (77,78) and lowering of rates of bone loss (79). There had been no randomized trials of thiazide use and fractures but a recent meta-analysis found decreased risk of fracture among current and long-term thiazide user (80). Drugs associated with bone loss are long-term corticosteroid use (81,82). Other medications associated with bone loss include anticonvulsants, aluminium-containing antacids and excess thyroid hormone (9,83).

Medical illness

Medical conditions associated with low bone mass and increased fracture risk include endocrine disease e.g. hypercortisolism or Cushing's disease, hyperthyroidism, hyperparathyroidism, osteomalacia (vitamin D deficiency) and osteogenesis imperfecta. Other medical conditions include renal disease, post-gastrectomy, rheumatoid arthritis and possibly diabetes as well as immobilization (9,74).

Physical Activity

Physical activity of occupations was coded as largely sedentary, intermediate, or largely standing, mainly according to the proportion of working day that subjects were likely to have spent sitting. Previous study was found a J-shaped relationship in women between occupational physical activity at age 50 years and risk of hip fracture (84). Both sedentary jobs and mainly weight-bearing jobs appear to be associated with higher risk of hip fracture than jobs with intermediate physical activity levels e.g. housekeeper, housewife, teacher. A possible explanation for the lack of a dose-response relationship between exercise and hip fracture risk is that the threshold level needed to stimulate osteoblastic activity is relatively low, and the protective effects of exercise do not increase beyond this threshold level (85).

The role of exercise in the determination of bone mass remains controversial. It is generally well accepted that more physically active individuals have a higher bone mass than sedentary individuals. Prolonged bed rest cause the skeleton almost completely relieved of loading forces, result in rapid bone loss (86). Exercise probably cause a modest increase in bone mass but varies according to the type of exercise, that disuse results in bone loss and that exercise cannot compensate for the bone loss due to

estrogen withdrawal. Conversely, professional and elite amateur athletes demonstrate markedly increased skeletal densities in the areas stressed by their particular endeavors. Studies such as these, however, do not address the question of how much activity is necessary or useful for the average person, and whether increases in activity should be recommended for the prevention of osteoporosis. It is likely, particularly in the elderly population, that part of the benefit of exercise may be the increase in muscle strength that may not have any effect in bone mass per se but rather on the prevention of falls. Post-menopausal subjects in a high-load/low-repetition strength training regimen had a greater increase in bone mass than those in a low-load/high-repetition (87). Several frequently recommended forms of exercise, including swimming and walking, have not been shown to slow bone loss or to be associated with higher bone mass in menopausal women (88,89) but varied aerobic and high-impact exercise has been shown to have a modest beneficial effects, including a reduction of bone loss (90).

Nonskeletal factors influencing the risk of osteoporosis fractures

Reduced bone mass alone cannot account for all osteoporotic fractures. Although some hip fractures may be spontaneous, certainly more than 90% result from a fall. The trauma associated with a fall from standing high or less (e.g. upon rising from a chair) osteogenic hip, but many falls in older osteoporotic women do not result in fractures. It has been shown that the nature of the fall may be critical to determining whether or not a fracture occurs. Falls to the side and those that result in an impact in the hip, increase the risk of hip fracture by more than 10-fold (56). Falling either forward or backward was associated with a much lower fracture risk.

Measurements of bone mass

There are several methods for measurement of bone mass. These include dual photon and dual X-ray absorptiometry (DPA/DXA), single-photon and single X-ray absorptiometry (SPA/SXA), quantitative computed tomography (QCT), quantitative ultrasound (QUS) and radiographic absorptiometry (RA). Dual and single X-ray absorptiometry have largely replaced dual and single-photon absorptiometry. The principle of the two methods are the same, but in X-ray absorptiometry, an X-ray source is used rather than a radionuclide source resulting in greater accuracy and precision and a shorter examination time (91). DXA is the most widely used technique for axial (spine and hip) measurements (92) and SXA/SPA the most commonly used techniques for appendicular bone measurements. QCT has a role in the assessment of spinal osteoporosis while RA and QUS are newer techniques whose role is yet to be determined.

Most of the data used in the prediction of fracture and in the diagnosis of osteoporosis have involved either SPA/SXA or DPA/DXA. DXA has low radiation expose (<0.1% of the natural yearly radiation) (93) and good precision (1-1.5% for the PA spine and 1.5-3% for the proximal femur) (91). It can be used for monitoring changes in bone density and assessing treatment. The PA measurement of the spine has potential problems, particularly in the elderly population as the value obtained may be artificially elevated due to previous fracture, the presence of degenerative arthritic disease resulting in osteophytes, disc space narrowing, or to a calcified aorta.

Quantitative computed tomography (QCT) has been used mainly for determining trabecular bone density of the vertebral. It has higher radiation dose than DXA and requires a longer scanning time. Its precision error is worse than for spinal PA DXA

and comparable with that of the lateral DXA, at 2-4% (91). However, because QCT can selectively assess the more metabolically active trabecular component, higher rates of bone loss have been reported with QCT than with DXA in cross-sectional studies. Its use is still somewhat limited by its cost, radiation dose and poorer precision.

Radiographic absorptiometry (RA) is a quantitative assessment of bone density from a radiograph of the hand. This method required further investigation, including assessment of response to therapy.

Quantitative ultrasound (QUS) is a lower cost, relatively portable instrument for analysis bone without ionizing radiation that has received considerable interest in recent years. It has been used mainly to assess trabecular bone at the calcaneus, but studies have also examined ultrasound properties at the tibia, patella and the phalanges. The correlation between QUS and BMD measurements at the calcaneal site has range between 0.5 and 0.8 (91).

Bone mineral content assessment with Single photon absorptiometry, Dual photon absorptiometry/Dual X-ray absorptiometry, Quantitative computed tomography, and quantitative ultrasound all seem to have same predictive power for future osteoporotics fractures (94). The correlation between the different techniques is modest (typically between 0.6 and 0.7) (31,94,95,96,97). This precludes prediction of bone mass at one site by bone mass measurement at another site. This is due both to technical differences between the techniques and to differences in measurement site, which have a different composition (ratio cortical/trabecular bone) and which differ in metabolic activity. The various techniques are therefore complementary rather than competitive. For clinical trials, it is advised it measure bone mass at least two different skeletal sites. Priority

should be given to measurement of sites of biological relevance (e.g., the spine for vertebral osteoporosis). For day-to day clinical practice, the choice of technique will also depend on the availability of techniques and specialists.

Dual X-Ray Absorptiometry

Dual X-Ray Absorptiometry (DXA) is the modern, upgrade version of Dual Photon Absorptiometry (DPA). The radionuclide source has been replaced by a stable X-ray tube. A dual energy spectrum is generated by rapid switching of the tube voltage supply or by K-edge filtering. Examination time is reduced and reproducibility of the measurements has improved compared to DPA. DXA method has gained widespread acceptance and distribution (94).

Melton et al. (1993) (96) Bone mass density of human forearm can be measured to determine the amount of bone or bone loss at the scanning site and to predict the risk of forearm fracture (95). The load at fracture was most specially predicted ($R^2 = 0.74$) by bone mass and geometric of the cortex at the shaft of radius (97).

Interpretation of bone measurements

As bone density decreases with aging, and the differences in bone density exists between the sexes and races, bone density measurements should be compared with those of age-, sex-, and race-matched controls. Therefore a normative database is mandatory for interpreting the level of bone mineral content. Usually, the estimated bone density is given as a Z-score. The Z-score gives the patients results as the deviation from the mean of age-matched controls divided by the standard deviation of this mean, which is an indication of the biological variability. In addition to the Z-score, the bone density of a patient is compared to the peak bone mass of young normal

adults. Then the estimation is given as the T-score. The T-score, in similarity to the Z-score, gives the patient's result as a deviation from the mean of young normal adults divided by the standard deviation of the mean. The latter score is predictive for fracture risk (6,94).

Estrogen and bone

An original hypotheses by Heaney suggested that the absence of estrogen rendered the skeleton more sensitive to the bone resorbing effects of parathyroid hormone (98). It is thought that PTH is responsible for recruitment of new remodeling sites, however, and the increased activation in estrogen deficiency could be a manifestation of the greater skeletal sensitivity. For that to occur, it seems reasonable to suggest that cells within the skeleton, or perhaps the marrow, should be responsive to estrogens. In addition to effects on skeletal remodeling, estrogen status may affect calcium homeostasis.

Recently, specific receptors for estrogens have been identified in cells of the osteoblast lineage (99,100). Physiological responses of these cell lines have been demonstrated in response to incubation with biologically meaningful concentration of 17 beta estradiol (101). These include changes in growth, alterations in synthesis and secretion of potential second messenger, as well as changes in collagen and noncollagen protein synthesis.

Heaney (1978) (102) In addition to its effects on skeletal remodeling, estrogen status may affect calcium homeostasis. The elegant work of Heaney has demonstrated that calcium economy deteriorates after menopause. The efficiency with which calcium is absorbed across the intestine declines, and calcium loss from the kidney increases.

The consequence is increased utilization of skeletal calcium to maintain serum calcium within its tightly defined limits. The increase in skeletal remodeling is the histological consequence.

Heaney et al. (1978) (102) The onset of menopause cause a relatively acute change in calcium balance. They summarized the data from balance studies on 207 women with an average calcium intake of 650 mg/day. They found that balance was -20 mg/day in 42-year-old women (premenopausal) compared to -43 mg/day in 47-year-old (postmenopausal) women. The different in balance was caused equally by a reduced intestinal absorption and an increased urinary excretion. The majority of postmenopausal women was also in negative calcium balance by 50 to 70 mg/day. It has been thought for many years that diet influences the incidence of osteoporosis.

Ballamore et al. (1970) (103) demonstrated a pronounced fall in calcium absorption in both men and women after the age of 60 years; absorption was reduced by one-third in 70 to 79 years compared to 20 to 59 years. All subjects more than 80 years of age showed severe malabsorption of calcium.

Phytoestrogen

Phytoestrogen (PE) is a general term used to define classes of compounds that are nonsteroidal and are either of plant origin or derived from the in vivo metabolism of precursors present in several plants eaten by humans. The main classes of these compounds are the isoflavonoids and lignans (104). These diphenolic compounds structurally resemble estradiol (E2) and were shown to have weak estrogenic activity. When digested in relatively large amounts, dietary phytoestrogens have been shown to have significant biological effects in several animal species and in humans

(13,14,15,16,17,18,19). Phytoestrogen given to postmenopausal women cause rapid alleviation of menopausal symptoms, reduce serum luteinizing hormone and follicular stimulating hormone, increase serum sex hormone-binding globulin (SHBG) and enhance maturation of vaginal epithelium (18,19).

Soybeans are particularly abundant sources of phytoestrogen, but they also are found, in lower concentrations, in cereal bran, whole wheat and legumes (19,64).

Arjmandi et al (13) suggest that dietary soybean protein is effective in preventing bone loss due to ovarian hormone deficiency in rat model of osteoporosis. They confirmed that ovariectomy enhances and 17β -estradiol suppresses the rate of bone turnover. Despite the higher rate of bone turnover on the soybean-fed animals, the vertebral and femoral bone densities of these rats were significantly greater than those of rats, in the ovariectomy group, suggesting that formation exceeded resorption.

Adlercreutz et al (14) found that certain diphenolic food components, lignans and isoflavonoids, which are converted to biologically active hormone-like substances by intestinal microflora, might be cancer-protective agents. The urinary excretion of lignans was low but that of the isoflavonoids was very high. The excretion of isoflavonoids correlated with soybean-product intake. The low mortality in breast and prostate cancer of Japanese women and men, respectively, may be due to high intake of soybean products.

Cassidy et al (15) The influence of a diet containing soy protein on the hormonal status and regulation of the menstrual cycle was examined in premenopausal women. Soy protein given daily for 1 month significantly increased follicular phase length and/or delayed menstruation. Midcycle surges of luteinizing hormone and follicle

stimulating hormone were significantly suppressed during dietary intervention with soy protein. These effects are presumed to be due to nonsteroidal estrogens of the isoflavone class, which behave as partial estrogen agonists/antagonists.

Shoff et al (16) Relation between the consumption of phytoestrogen-containing foods and serum sex hormones and sex hormone-binding globulin were studied in a population-based sample of postmenopausal women. Although the magnitude of association between hormone and phytoestrogen-containing foods was small, the data are consistent with the possibility that consumption of some phytoestrogen-containing foods may affect levels of testosterone in postmenopausal women

Murkies et al (17) found that postmenopausal women with at least 14 hot flushes per week, that their daily diet supplemented with soy flour could reduce flushes compared with wheat flour over 12 weeks when randomized and double blind. Menopausal symptom score decreased significantly in both groups. Vaginal cell maturation, plasma lipids and urinary calcium remained unchanged. Serum FSH decreased and urinary hydroxyproline increases in the wheat flour group.

Armstrong et al (18) In comparison with matched nonvegetarian women, were found to have lower urinary levels of estriol and total estrogens, lower plasma prolactin levels, and higher plasma SHBG levels in vegetarians. These hormone differences may explain the lower rates of endometrial and possible breast cancer that have been observed previously in vegetarian women.

Brzezinski et al (19) The serum levels of phytoestrogens significantly increased in most of the subjects assigned a phytoestrogens-rich diet, whereas the concentrations in the control group remained unchanged. The reduction in hot flushes and vaginal

dryness scores were significant in the women assigned a phytoestrogens-rich diet than in the controls. The authors conclude that 12 weeks' partial substituted of omnivorous postmenopausal women with type of food increased their serum levels of SHBG. It may alleviate symptoms such as hot flushes and vaginal dryness.

The nutritional status of vegetarians and bone

Previous study concluded that a vegetarian met the nutritional requirements of all age groups, and that vegan diets comprised unrefined cereal products, legumes, nuts, vegetables and fruits produce no detectable deficiency signs (105). However, they indicated that vegan and vegetarian diets are inadequate if they are low in calories or contain a high proportion of refined cereals or of starchy foods and a deficiency of vitamin B12 may develop after a variable period on an unsupplemented vegan diet.

The proximate analysis of the vegan diets is remarkable normal, and the average nutrient intakes meet recommended daily allowances. However, the pattern of nutrient intake is different from that of people eating a mixed diet. It was found that the intake of total calories, protein, fat, and the percentage of calories from fat were all less in vegetarian than in omnivorous, while intakes of calcium, thiamin and ascorbic acid were greater. As in most human diets, the sulfur amino acids are most limiting and somewhat lower than those for mixed diets. A restricted food intake may have reduced the protein value of the diets in some of our vegans (mean daily calorie intake ranged from 1130 to 4150 kcal), but since the subjects were in positive nitrogen balances the diets were considered adequate.

Ellis et al. (1972) (21) The vegetarians included in this study had bone densities significantly greater than those of individually matched omnivores. These results

support the hypotheses that bone dissolution greater in individuals who utilized diet high in acid ash, e.g. omnivores. The results suggest that vegetarians are less prone to osteoporosis than omnivores. When bone density was related to age, both sets of measurements showed that the bone density of the omnivores decreased with age; this was also seen in the vegetarians group but to a lesser degree. No further decrease in bone density appeared to take place in the vegetarians who were approximately 69 years old, whereas it continued to decrease in the omnivore group. These results suggest that there is less likelihood of vegetarians developing osteoporosis in old age.

Dwyer 1988 (20) Vegans have diets that are lower in Ca than diets of nonvegetarians. Vegetarians, especially vegans, are also a good deal leaner than the general population, sometimes to the point of being underweight. These characteristics increase vegetarians' risks for osteoporosis over those of nonvegetarians. However, in many other respects vegetarians' risk is the same or lowers than those of omnivores. Vegetarians' intakes of protein and P are rarely much higher than those of the general population but vegetarians do not differ greatly from nonvegetarians in their incidence of osteoporosis.

Marsh et al 1980 (22); Marsh et al 1983 (23) shown that the vegetarians had bone densities significantly greater than those of individually matched omnivorous. These may result in bone dissolution grater in individuals who utilize diet high in acid ash, i.e., omnivores. The results also suggest that vegetarians are less prone to osteoporosis than omnivorous.

Two cross-sectional studies (24,25) were conducted in the late 1980s to compare the BMC of vegetarians and omnivores. In these studies, 88 subjects living in North Carolina and 144 subjects living in California were investigated. The mean calcium and



calorie intakes were similar between vegetarians and omnivores, but mean protein intakes were significantly lower in the vegetarians. The BMC at the forearm of vegetarians were found to be similar to omnivores.

Methods used in nutritional assessment

Nutritional assessment systems utilize a variety of methods to characterize each stage in the development of a nutritional deficiency state. The methods are based on a series of dietary, laboratory, anthropometric, and clinical measurement, used either alone, or more effectively, in combination (106).

Dietary methods

The first stage of a nutritional deficiency is identified by dietary assessment methods. During this stage, the dietary intake of one or more nutrients is inadequate, either because of a primary deficiency (low level in the diet), or because of a secondary deficiency. In the latter case, dietary intake may appear to meet nutritional needs, but conditioning factors (such as certain drugs, dietary components, or disease states) interfere with the ingestion, absorption, transport, utilization, or excretion of the nutrient(s).

Food consumption of individuals

Methods used for measuring food consumption of individuals can be classified into two major groups. The first group, known as quantitative daily consumption methods, consists of recalls or records designed to measure the quantity of the individual foods over a one-day period. By increasing the number of measure days for these methods, quantitative estimates of actual recent intakes, or, for longer time

these methods, quantitative estimates of actual recent intakes, or, for longer time periods, usual intakes of individuals, can be obtained. Assessment of usual intake is particularly critical when relationship between diet and biological parameters are assessed.

The second group of methods includes the dietary history and the food frequency questionnaire. Both obtain retrospective information on the patterns of food use during a longer, less precisely defined time period. Such methods are most frequently used to assess intake of foods or specific classes of foods. With modification, they can provide data on usual nutrient intakes.

Twenty-four-hour recall method

In the twenty-four-hour recall method, subjects, their parents, or caretakers recalls food intake of previous twenty-four-hour in an interview. Quantity estimated in household measures using food models as memory aids and/or to assist in quantifying portion sizes. Nutrient intakes calculated using food composition data.

Useful for assessing average usual intakes of a large population provided that the sample is truly representative and that the days of the week are adequately represented. Used for international comparisons of relationship of nutrient intakes to health and susceptibility to chronic disease. Inexpensive, easy, quick, with low respondent burden so that compliance is high. Large coverage possible; can be used with illiterate individuals. Element of surprise so less likely to modify eating pattern. Single twenty-four-hour-recalls likely to omit foods consumed infrequently. Relies on memory and hence unsatisfactory for the elderly and young children. Multiple replicate twenty-four-hour-recalls used to estimate usual intakes of individuals.

Estimated food record

Record of all food and beverages as eaten (including snacks), over periods from one to seven days. Quantities estimated in household measures. Nutrient intakes calculated using food composition data.

Used to assess actual or usual intakes of individuals, depending on number of measurement days. Data on usual intakes used for diet counseling and statistical analysis involving correlation and regression. Accuracy depends on conscientiousness of subject and ability to estimate quantities. Long term frames results in a higher respondent burden and a lower co-operation. Subjects must be literate.

Weighed food record

All food consumed over defined period is weighed by the subject, caretaker, or assistant. Food samples may be saved individually, or as a composite, for nutrient analysis. Alternatively nutrient intakes calculated from food composition data.

Used to assess actual or usual intakes of individuals, depending on the number of measurement days. Accurate but time consuming. Condition must allow weighing. Subjects may change their usual eating pattern to simplify weighing or to impress investigator. Requires literate, motivated, and willing participants and expensive.

Dietary history

Interview method consisting of a twenty-four-hour recall of actual intake, plus information on overall usual eating pattern, followed by a food frequency questionnaire to verify and clarify initial data. Usual portion sized recorded in household measures. Nutrient intakes calculated using food composition data.

Used to describe usual food and/or nutrient intakes over a relatively long time period that can be used to estimate prevalence of inadequate intakes. Such information used for national food policy development, food fortification planning, and to identify food patterns associated with inadequate intakes. Labor intensive, time consuming and results depend on skill of interviewer.

Semi-quantitative food frequency questionnaire

A semi-quantitative food frequency questionnaire is designed to obtain qualitative and quantitative, descriptive information about usual food consumption patterns. The questionnaire consists of three components: (a) a list of foods (b) quantity portion sizes of food items and (c) a set of frequency-of-use response categories. The list of foods may focus on specific groups of foods, particular foods, or foods consumed periodically in association with special events/seasons, when it is designated a focused questionnaire.

The aim of the food frequency questionnaire is to assess the frequency and quantity of certain food items or food groups consumed during a specified time period (e.g. daily, weekly, monthly, yearly). Specific combinations of foods included in a focused questionnaire can be used as predictors for intakes of certain nutrients or non-nutrients, provided that the dietary components are concentrated in a relatively small number of foods or specific food groups.

The data for the food frequency method may be obtained by a standardized interview or self-administered questionnaire, both taking fifteen to thirty minutes to complete. The results generally represent usual intakes over an extended period of time and are easy to collect and process. The food frequency questionnaire imposed fewer

burdens on respondents than most of the other dietary assessment methods. Epidemiological studies for ranking subjects into broad categories of low, medium, and high intakes of specific foods, food components or nutrients, for comparison with the prevalence and/or mortality statistics of a specific disease. The food frequency questionnaire can also be used in combination with more quantitative methods, providing additional or confirmatory data. Food frequency questionnaire can also identify food patterns associated with inadequate intakes of specific nutrients. Method is rapid with low respondent burden and high response rate but accuracy is lower than other methods.

Chapter III

Materials and Methods

Research design

This research was a cross-sectional comparative study aimed to give an indication whether the same measured of bone mass density and osteoporosis were found in vegetarian and nonvegetarian menopausal women and to examine the effects of certain nutrients on bone mass density and osteoporosis in these women.

Sample population

Sample population was the elderly women who had been in the stage of menopause for at least one year. The vegetarian menopausal women who live at Pratom-A-Sok and Santi-A-Sok religious communities and the nonvegetarian menopausal women from the clubs for the elderly at Phramongkutklao, and Siriraj hospital in Bangkok and lived in the vicinity were recruited for the study. Both groups were literate and willing to participate in the study.

Sample size and sampling technique

All Thai elderly women from both of religious communities and the clubs for the elderly were interviewed, screened and invited to participate in the study. Both groups of subjects in this study were recruited by purposively matched and controlled by years since menopause (± 2 years). Each group consisted of 30 postmenopausal women.

Inclusion criteria

The subjects must be of Thai citizenship, an Asian background, and apparently healthy. They must have been in the stage of permanent ovarian cessation for at least one year.

Exclusion criteria

A physician and investigator checked all subjects by interview and physical examination. Study subjects with the following characteristics were excluded from the study:

- had family history of fracture
- had removal of any ovaries (oophorectomy), undergone hysterectomy, matched subject were difference in body mass index between 24 and 26 kg/m²
- had any medical illness e.g. hypercortisolism (Cushing's disease), hyperthyroidism, hyperparathyroidism, hypoparathyroidism, osteomalacia (vitamin D deficiency), Paget's disease of bone (osteitis deformans), osteogenesis imperfecta, renal disease, diabetes, hypertension, rheumatoid arthritis, and post-gastrectomy
- were taking certain medications e.g. drugs influencing bone turnover (an anabolic steroid, glucocorticoid, progestin, fluoride, bisphosphonate, estrogen), drugs altering bone or calcium metabolism (vitamin A or D, anticonvulsant, phosphate-binding antacids), drugs inhibiting gastric acid secretion (H₂-blocker), thiazide diuretic, thyroid drug

- had current smoking, excessive alcohol intake

- hold largely sedentary jobs and mainly weight-bearing jobs

Instrument

The instrument of this study consisted of 2 parts as following

1. Questionnaire consisted of 4 parts (appendix):

- Part 1 Demographic information
- Part 2 General health information
- Part 3 Physical examination and radiological investigation record
- Part 4 Semiquantitative food frequency questionnaire

2. Physical examination and radiological investigation

- Dual-X-ray absorption bone densitometry (BMD-3 bone densitometer
Panasonics®)
- Height measuring instrument
- Weighing machine
- Tape measure

Part 1 Demographic information

This part contained the questions about baseline characteristics of the subjects including ethnicity, nationality, religious, marital status, education, income and occupation. The investigator developed this part considering study objectives and modified from previous studies, especially report of “The quality of life of the elderly

in Thailand” for health and self-monitoring of the elderly in central part of Thailand, Mahidol University (107).

Part 2 General health information

This part included the years since menopause, parity, lactation, physical activities, alcohol, and caffeine consumption and years as vegetarians for vegetarians group. In this part, the investigator adjusted the questions related to the objectives and scope of the study. This part displayed the baseline characteristics and was used to match for similarity of years since menopause of the subjects.

Part 3 Physical examination and radiological investigation record

This part was employed to record physical examination and radiological outcome. Including weight (kg), height (cm), body mass index (kg/m^2), bone mass density (g/cm^2), the indicator of the biological variability (Z-score) and the prediction of fracture risk (T-score).

Part 4 Semiquantitative food frequency questionnaire

This part of questionnaire was designed to obtain qualitative and quantitative data of energy and nutrients, descriptive information about usual food consumption pattern of the study subjects. It included 11 food groups and divided into three components: list of 115 food items, quantity of food items and 11 frequency-of-use response categories.

The 11 food groups include meat, meat products and egg (25 items), milk and products (5 items), cereal and products (15 items), legume and products (15 items),

vegetables (26 items), fruits (9 items), desserts (6 items), fats (2 items), beverages (6 items), condiments (3 items) and miscellaneous (3 items).

Instrument Development

The questionnaires for demographic information, general health information and three-day food records were created and modified from literature review, and previous studies. The questionnaires were developed and edited for rationale and covered the objectives of the study. The semiquantitative food frequency questionnaire (SFFQ) was developed from three-day food records.

Step 1 Three-day food records for collecting dietary information to developed SFFQ was designed (appendix).

Step 2 Ten menopausal women who were recruited for the study in each vegetarians and nonvegetarians group were selected by sampling. Objectives of the study were explained to them and the dietary data were collected by an assistant investigator who had been trained on interviewing techniques. Since elderly subjects may have relatively poor short-term memory and interday variations would not be taken into account, the investigator asked them to record all their food items and roughly precise quantity of each food items in a day. The investigator confirmed amount of each food items at the end of each day. The study subjects were asked to record of food intake for 3 days (2 weekdays and one weekend day). Food models and 1-kilogram scale were used to estimate the amount of food intake for accuracy.

Step 3 The 3 days food record data were rechecked. Lists of all 3 days food items that subjects consumed were grouped and then averaged into one day. All of the subjects' diurnal average amounts of all food item were input in nutrition calculated

program, INMUCAL, of Institute of Nutrition, Mahidol University, to compute energy and nutrients consumption for each subject.

Step 4 Most of food items consumption were divided into 11 groups via food groups and main nutritive value that were meat and products, milk and products, cereal and products, legume and products, vegetables, fruits, desserts, fats, beverage, condiments and miscellaneous. Each food item of meat, meat products, and vegetables were sorted out by used oil or not in cooking process, for example stir-fried, deep-fried, boiled, blanched, steamed. Certain vegetables were not separated if they were always eaten raw. The desserts were segregated by containing-coconut milk or not and the amount of sugar in the recipes. All seasoning food items in 3-day food records were deleted from the SFFQ. The usual portion size was determined by mode of each subject's consumption quantity. Three portions sized of food items were ranged and 11 frequency-of-use response categories (108) were set as follow

Portion size of food items were divided into three scales (109)

Less than the quantity of portion size scale means that food item was consumed 0.5 times the portion size of the instrument

Equal to the quantity of portion size scale means that food item was consumed equal to the portion size of the instrument

More than quantity of portion size scale means that food item was consumed 1.5 times the portion size of the instrument

Frequency-of-use response categories of each food items were divided into 11 categories: three times per day, two times per day, one time per day, five to six times per week, three to four times per week, one to two times per week, three times per month, two times per month, one time per month, seldom and never.

Frequency-of-use response in one day

Three times per day means the food item was consumed three times daily.

Two times per day means the food item was consumed twice daily.

One time per day means the food item was consumed once daily.

Frequency-of-use response in one week

Five to six times per week means the food item was consumed 5-6 times per week.

Three to four times per week means the food item was consumed 3-4 times per week.

One to two times per week means the food item was consumed 1-2 times per week.

Frequency-of-use response in one month

Three times per month means the food item was consumed 3 times per month.

Two times per month means the food item was consumed two times per month.

One times per month means the food item was consumed one time per month.

Seldom means the food item was consumed only once within 2-3 months

Never means the food item was never consumed

Step 5 Pilot study of SFFQ was done by sampling the same 10 postmenopausal women in each group. The study subjects self-administered the questionnaires under the investigator supervision. Any food items that were never or rarely consumed were excluded from the SFFQ. Less or more than usual portion size responded by subjects were adjusted into usual one.

Step 6 Trial out for SFFQ was done by sampling another 10 menopausal women from each of the groups. Then food items and portion size was compared and adjusted with 3-day food records.

Calibration of study instrument

The questionnaires were rechecked and edited for content validity and completeness by instructors of nutrition department, Mahidol University. After the first pilot study of SFFQ, all of food items that subjects never or rarely consumed were deleted. Daily energy and nutrients of each subject were computed by nutrient calculated program. Nutritive values of SFFQ and 3-days food records were compared using Pearson's correlation coefficient. The result of the first pilot study showed that there was no significance correlation in energy and nutrients of food intake between SFFQ and 3-day food records at p-value less than 0.05 (appendix).

Food items that subject never or rarely consumed were excluded and less or more than usual portion size reported by subjects was adjusted into usual portion size. After the second pilot study, food items and portion size of SFFQ were readjusted. The result of the second pilot study showed that there was significance correlation in energy and nutrients of food intake between SFFQ and 3-day food records at p-value less than 0.05 (appendix). Finally the SFFQ included 115 food items as follow

Group 1 : meat and products, and egg	25 items
Group 2 : milk and products	5 items
Group 3 : cereal and products	15 items
Group 4 : legume and product	15 items
Group 5 : vegetables	26 items
Group 6 : fruits	9 items
Group 7 : desserts	6 items
Group 8 : fats	2 items
Group 9 : beverages	6 items
Group 10 : condiments	3 items
Group 11 : miscellaneous	3 items.

Data collection

Study subjects in both vegetarian and nonvegetarian groups were interviewed for demographic information, general health information, and food consumption and examined for bone density. Data collection was done as follow

Step 1 The investigator applied for permission letters from faculty of Graduate Studies, Mahidol University to the directors of the clubs for the elderly at Phramongkutklo hospital, and Siriraj hospital, Pratom-A-Sok and Santi-A-Sok religious communities for data collected participation.

Step 2 The investigator contacted to directors and information centers of the clubs for the elderly and religious communities for collaboration and made appointments for data collection.

Step 3 The investigator assistance was trained on interviewing techniques. Data were collected by interviewing subjects through the use of questionnaires. Study subjects were interviewed by an assistant investigator for demographic information and general health information. All study subjects self-administered the SFFQ under the investigator's supervision.

Step 4 The investigator measured and recorded height and weight of study subjects. The physician operated and recorded radiological investigation for bone mass density measurement of the participants. They were asked to take off their rings, bracelets and watches. They were ruled length from wrist to elbow to find out 1/10 distal radius position. Then their undominant forearm bone mass density was measured with bone densitometer. It took around 30 seconds for each run.

Data collection in step 1-4 were held on August 1999 to January 2000

Step 5 The collected data were rechecked and prepared for analysis by the investigator.

Statistical Analysis

1. Descriptive of demographic information, baseline characteristics and nutrition status were analyzed by percent, mean±standard deviation, median, minimum and maximum.
2. The amount of food consumption per day was calculated by multiple quantity of food items with frequency-of-use response score.

The quantity of food items were calculated by

Multiple usual portion size intakes with 1 if they checked the item of equal to quantity of portion size scale

Multiple usual portion size intakes with 0.5 if they checked the item of less than quantity of portion size scale

Multiple usual portion size intakes with 1.5 if they checked the item of more than quantity of portion size scale

The frequency-of-use response score were calculated by

1. Score 3, 2, 1 for the subject that checked the item of 3 times per day, 2 times per day and 1 time per day scales, respectively.
2. Score 0.8, 0.5, 0.2 for the subject that checked the item of 5-6 times per week, 3-4 times per week and 1-2 time per week scales, respectively.
3. Score 0.1, 0.07, 0.03 for the subject that checked the item of 3 times per month, 2 times per month and 1 time per month scales, respectively.
4. Score 0.01 for the subject that checked the item of seldom scale (consumed 2-3 months for once)
5. Score 0 for the subject that checked the item of never scale

3. The average of energy, carbohydrate, protein, fat, minerals and vitamins of one-day consumed in each subject were analysed by nutrition calculates program, INMUCAL, of Institute of Nutrition, Mahidol University. The amount of phytoestrogen one-day consumed in each subject was calculated from previous study data (appendix). The energy and nutrients were compared those of with Thai

recommended daily dietary allowance (RDA) for the elderly to assess the adequacy of food consumption.

4. The amount of energy, protein, fat, carbohydrate, minerals, vitamins and phytoestrogen intake in both groups were compared by paired t-test.

5. The bone mass density, Z-score and T-score in vegetarians and nonvegetarians were compared by paired t-test

6. The risk of osteoporosis between vegetarians and nonvegetarians was analyzed by odd ratio.

7. Simple linear regression was used to predict bone mass density loss by nutrients intake in vegetarians and nonvegetarians menopausal women.

8. Simple linear regression was used to predict bone mass density loss by years since menopause in vegetarians and nonvegetarians menopausal women.

9. The amount of energy, protein, fat, carbohydrate, minerals and vitamins of food intake between 3-day food records and SFFQ were compared using Pearson's correlation coefficient.

All statistics are considered significant if p-value is less than 0.05

Chapter IV

Results

This research was aimed to investigate whether bone mass density and osteoporosis were different between vegetarian and nonvegetarian menopausal women and the effects of certain nutrients on bone mass density and osteoporosis in these women. Study subjects, vegetarian menopausal women and nonvegetarian menopausal women, were interviewed for food consumption and examined for bone density.

Tables and explanation as following presented the research results

Part 1 Description of demographic information, baseline characteristics and nutrition status of vegetarians and nonvegetarians

Part 2 Food consumption patterns of vegetarians and nonvegetarians

Part 3 Description of radiological investigated for bone mass density of vegetarians and nonvegetarians measurement

Part 1 Description of demographic information, baseline characteristics and nutrition status of vegetarians and nonvegetarians

The study subjects were elderly women who had been in the stage of menopause for at least one year, of Thai citizenship, with an Asian background, apparently healthy, and they all were Buddhist. The ages ranged from 46 years to 71 years. The mean age of the vegetarians was 56.7 (SD=5.5) and nonvegetarians 57.9 (SD=6.2) years. Regarding age distribution, vegetarians were younger than nonvegetarians. The vegetarian study subjects usually practiced vegetarianism for religious reasons, and they tended to prefer to be single to being married. Less than one-third of vegetarians were married, whereas two-thirds of nonvegetarians were married. Due to the preference to be single, the average number of living children in vegetarians (1.7 ± 1.8), were lower compared with nonvegetarians, (2.2 ± 1.8). The nulliparous rate in vegetarians was also greater than that of nonvegetarians.

Education levels were different between the two groups. Three-fourths of vegetarians attained grade 10 or less whereas three-fourths of nonvegetarians attended higher than grade 10.

Most of the nonvegetarians came from clubs for the elderly and lived in the near vicinity. Half of them were currently working as government officers or retired government officers. The majority of their occupations were lecturer and registered nurse. In contrast, vegetarians were lay persons who lived by bartering products in the community. One quarter of the nonvegetarians were supported by money from their offspring. Few of them were launderers. One in each group was a gardener. On account of the fact that more than half of vegetarians were bartering products within

the community, the median income among these women was much less than that of the others. This is shown in Table 1.

Table 1 Description of demographic information of vegetarians and nonvegetarians

	vegetarians		nonvegetarians	
	n	%	n	%
Total	30	100.0	30	100.0
Age (year)				
46-50	2	6.7	2	6.7
51-55	13	43.3	11	36.7
56-60	9	30.0	6	20.0
61-65	3	10.0	9	30.0
≥66	3	10.0	2	6.7
mean±SD	56.7±5.5		57.9±6.2	
minimum	48		46	
maximum	71		70	
Marital Status				
Single	11	36.7	4	13.3
Married	7	23.3	21	70.0
Widowed	5	16.7	3	10.0
Divorced	7	23.3	2	6.7
Parity (no.)				
0	12	40.0	7	23.3
1-2	7	23.3	9	30.0
≥3	11	36.7	14	46.7
mean±SD	1.7±1.8		2.2±1.8	
minimum	0		0	
maximum	6		7	

Table 1 (continued)

	vegetarians		nonvegetarians	
	n	%	n	%
Education				
None	1	3.3	0	0
Prathom (grade 4)	14	46.7	3	10.0
Mathayom (grade 10)	8	26.7	5	16.7
Diploma	0	0	7	23.3
Bachelor degree or higher	7	23.3	15	50.0
Occupation				
Labor	0	0	2	6.7
Merchant	10	33.3	3	10.0
Agriculture	1	3.3	1	3.3
Official government	2	6.7	9	30.0
Retired official government	1	3.3	7	23.3
Others	16 ^a	53.3	7 ^b	23.3
Income per month (baht)				
Less than 3000	15	50.0	1	3.3
3001-9000	8	26.7	0	0
more than 9000	7	23.3	21	70.0
median	3250		9500	
QD	5812		10937	
minimum	800		2500	
maximum	25000		30000	

^a included bartering products within the community

^b included supported by money from their offspring

As a result of matching characteristics, it was found that vegetarians and nonvegetarians were similar in respect to distribution and years since menopause. The majority had entered natural menopause less than 4 years previously. Vegetarians had lower parity than nonvegetarians, they breast-fed their children less than the others by twenty percent. This is presented in Table 2. All of the participants who had borne a viable child had breast-fed their child.

More than half of both study groups did not exercise. Fewer than one-third of nonvegetarians had joined aerobics 2 or 3 times weekly at clubs for the elderly. One-third of vegetarians went for a morning walk.

Table 2 Description of baseline characteristics of vegetarians and nonvegetarians

	vegetarians		nonvegetarians	
	n	%	n	%
Total	30	100.0	30	100.0
Years since menopause (year)				
1-4	11	36.7	11	36.7
5-9	10	33.3	9	30.0
≥10	9	30.0	10	33.3
mean±SD	7.6±5.8		7.7±5.7	
minimum	22		20	
maximum	1		1	
Lactation				
Never breast-fed*	13	43.3	7	23.3
Breast-fed	17	56.6	23	76.7

Table 2 (continued)

	vegetarians		nonvegetarians	
	n	%	n	%
Exercise				
No	18	60.0	18	60.0
Walking	10	31.3	0	0
Calisthenics	2	6.7	4	13.3
Aerobic	0	0	8	26.7

* never breast-fed included single and married women who had never borne a viable child

Most vegetarians consumed vegetarian food between 11-20 years before their menopausal period until the beginning of the study, while one-fourth had consumed vegetarian food for up to 20 years, as presented in Table 3.

Table 3 Description of years as vegetarian of vegetarians

	vegetarians (n=30)	
	n	%
Years as vegetarian (year)		
1-10	9	30.0
11-20	15	50.0
≥ 21	6	20.0
mean±SD	14.13±6.16	
minimum	3	
maximum	23	

Overview of nutrition status, defined by body mass index (BMI) (110): More nonvegetarians were able to satisfy normal BMI than the others. The majority of study subjects were normal in weight (BMI 18.5-24.9 kg/m²). Mild thinness (BMI 17.0-18.49 kg/m²) was observed to a greater extent in vegetarians. A few participants in both groups were overweight (BMI 25.0-29.9 kg/m²). Nevertheless, there was no obese subject (BMI \geq 30.0 kg/m²) in this study. Paired differences of BMI were significantly higher in nonvegetarians (p=0.007).

Table 4 Nutrition status defined by body mass index of vegetarians and nonvegetarians

	vegetarians		nonvegetarians	
	n	%	n	%
Total	30	100.0	30	100.0
BMI (kg/m ²)				
<18.5	4	13.3	1	3.3
18.5-24.9	21	70.0	24	80.0
\geq 25.0	5	16.7	5	16.7
mean \pm SD*	21.7 \pm 2.8		22.8 \pm 2.4	
minimum	17.1		17.2	
maximum	27.3		27.3	

* paired differences were significant at p = 0.007

Part 2 Food consumption patterns of vegetarians and nonvegetarians

Most of the study vegetarians took one or two meals, while the nonvegetarians took two or three meals daily. Both of them reported that they seldom used oil, sugar or coconut milk in the cooking process. The vegetarians' and nonvegetarians' top five most frequent food items are shown in Tables 5 and 6.

Vegetarians mainly consumed cooked rice or home-pounded rice soup and whole-seed Job's tear while the other group mainly consumed milled cooked rice alternated with home-pounded rice every other day. The nonvegetarians consumed both stir-fried, and lean boiled pork meat almost every day. They consumed boiled and stir-fried youngwhite-meat chicken and boiled slices of freshwater fish two or three times weekly. Although nonvegetarians consumed various types of milk, they preferred to consume low fat milk to other types.

As a result of abstaining entirely from meat and meat products, vegetarians mostly consumed soybeans and products as an alternative to meat. They consumed bean curd almost every day while soymilk and cooked soybeans were consumed every alternate day. The nonvegetarians did not avoid meats; they also consumed legumes and products such as soymilk and boiled peanuts. In the vegetables group, nonvegetarians consumed cucumber every second day while stir-fried cabbage, straw mushroom, stir-fried chinese white cabbage, and raw long green bean were consumed once or twice weekly. Vegetarians usually consumed both straw mushroom and cone mushroom. They consumed stir-fried chinese swamp cabbage, stir-fried and blanched chinese cabbage twice or three times weekly. Besides that, they likely to consume a variety of fruit. Oranges and ripe bananas (nam-wa) were chiefly consumed by both

study groups. Furthermore, ripe papaya, guava, apple, pineapple, rose-apple, watermelon were consumed to a lesser extent by the study subjects. Vegetarians consumed a smaller amount of sugar than nonvegetarians. Both of them rarely consumed coconut milk in dessert or curry. Nonvegetarians reported that they consumed cake with coffee or fruit juice for refreshments weekly, whereas the other group added honey to fruit juices almost weekly. Vegetarians often added light soybean sauce and black sesame seeds to their dishes, while the other group added both light soybean sauce and fish sauce to their dishes. They also occasionally added black sesame seeds to their cups of milk.

Table 5 Top five most frequently consumed food items defined by food groups of vegetarians

Food items	Average frequency score (time/day/person)
Cereal and products	
Home-pounded rice, cooked	1.44
Home-pounded rice, soup	0.79
Whole-seeds Job's tear	0.44
Pumpkin, steam	0.25
Whole wheat bread	0.24
Legume and products	
Bean curd	0.95
Soymilk	0.52
Soybeans, cooked	0.33
Bean threads, blanched	0.19
Peanuts, boiled	0.18
Vegetables	
Straw mushroom	0.50
Cone mushroom	0.49
Chinese swamp cabbage, stir-fried	0.39
Chinese cabbage, stir-fried	0.38
Chinese cabbage, blanched	0.37
Fruits	
Banana (nam-wa), ripe	0.67
Orange	0.60
Papaya, ripe	0.43
Guava	0.42
Apple	0.38

Table 5 (continued)

Food items	Average frequency score (time/day/person)
Desserts	
Syrup in coconut milk	0.08
Syrup	0.07
Boiled in syrup	0.06
Boiled in coconut milk	0.04
Fat	
Curry with coconut milk	0.07
Curry with condiments and coconut milk	0.01
Beverages	
Honey	0.15
Fruit juice	0.09
Condiments	
Soybean sauce, light	0.82
Sugar	0.05
Miscellaneous	
Sesame seeds, black	0.75
Sunflower seeds	0.23
Sesame seeds, white	0.14

Table 6 Top five most frequently consumed food items defined by food groups of nonvegetarians

Food items	Average frequency score (time/day/person)
Meat and products, egg	
Lean pork meat, stir-fried	0.99
Lean pork meat, boiled	0.70
Youngwhite-meat chicken, boiled	0.35
Youngwhite-meat chicken, stir-fried	0.34
Slices of freshwater fish, boiled	0.33
Milk and products	
Low fat milk	0.34
Sterilized milk, plain	0.22
Fortified calcium low fat milk	0.17
Drinking yogurt	0.06
Yogurt cream	0.03
Cereal and products	
Milled rice, cooked	1.43
Home-pounded rice, cooked	0.77
Milled rice, soup	0.28
White bread	0.27
Whole wheat bread	0.18
Legume and products	
Soymilk	0.60
Peanuts, boiled	0.35
Bean threads, stir-fried	0.22
White soybean curd	0.21
Bean curd	0.19

Table 6 (continued)

Food items	Average frequency score (time/day/person)
Vegetables	
Cucumber	0.43
Cabbage, stir-fried	0.23
Straw mushroom	0.22
Chinese white cabbage, stir-fried	0.21
Long green bean, raw	0.20
Fruits	
Orange	1.05
Banana (nam-wa), ripe	0.68
Pineapple	0.54
Rose-apple	0.40
Watermelon	0.36
Desserts	
Cake	0.13
Syrup	0.12
Boiled in syrup	0.06
Cookie	0.05
Boiled in coconut milk	0.04
Fat	
Curry with coconut milk	0.04
Curry with condiments and coconut milk	0.02
Beverages	
Coffee with sugar and cream	0.46
Fruit juice	0.09
Coffee with condensed milk	0.08
Chocolate drink with sugar and cream	0.06
Chocolate drink with condensed milk	0.05



Table 6 (continued)

Food items	Average frequency score (time/day/person)
Condiments	
Soybean sauce, light	0.97
Fish sauce	0.96
Sugar	0.51
Miscellaneous	
Sesame seeds, black	0.10
Sunflower seeds	0.05

The study vegetarians' food consumption pattern was based on cooked rice and home-pounded rice soup, bean curd and soymilk. They usually added light soybean sauce and black sesame seeds to their dishes. They liked to consume straw mushrooms and cone mushrooms in the vegetable group while ripe bananas (nam-wa) and oranges were the fruits most frequently consumed in the fruit group, as presented in Table 7.

Table 7 The overview of top ten most frequently consumed food items of vegetarians

Food items	Average frequency score (time/day/person)
Home-pounded rice, cooked	1.44
Bean curd	0.95
Soybean sauce, light	0.82
Home-pounded rice, soup	0.79
Sesame seeds, black	0.75
Banana (nam-wa), ripe	0.67
Orange	0.60
Soymilk	0.52
Straw mushroom	0.50
Cone mushroom	0.49

The food consumption pattern of the nonvegetarians was based on cooked milled rice and this was sometimes substituted with cooked home-pounded rice, as shown in Table 8. They primarily consumed stir-fried and boiled lean pork meat. They also drank soymilk every second day. They liked to consume oranges, ripe bananas (nam-wa) and pineapples. They usually put light soybean sauce and fish sauce in their dishes.

Table 8 The overview of top ten most frequently consumed food items of nonvegetarians

Food items	Average frequency score (time/day/person)
Milled rice, cooked	1.43
Orange	1.05
Lean pork meat, stir-fried	0.99
Soybean sauce, light	0.97
Fish sauce	0.96
Home-pounded rice, cooked	0.77
Lean pork meat, boiled	0.70
Banana (nam-wa), ripe	0.68
Soymilk	0.60
Pineapple	0.54

The food consumption pattern energy and nutrient intakes of the participants is presented in Table 9. The average energy, mixed protein diet, carbohydrate, calcium and vitamin B1 were significantly lower in vegetarian women compared with nonvegetarians ($p < 0.05$). On the contrary, protein from vegetables, fiber, and ferrous were significantly higher in vegetarians ($p < 0.05$).

Table 9 Comparison of energy, nutrients intake of vegetarians and nonvegetarians

	vegetarians		nonvegetarians		t	df	p-value
	n	$\bar{X} \pm SD$	n	$\bar{X} \pm SD$			
Energy (kcal)	30	1235.61±353.61	30	1459.04±310.17	2.65	29	0.013
Protein (g)	29 ^a	34.93±12.83	29 ^a	45.58±11.18	3.27	28	0.003
Protein-animal(g)	30	0	30	20.92±7.70		NA	
Protein-veg (g)	29 ^a	34.93±12.83	29 ^a	24.67±7.16	-4.00	28	<0.001
Fat (g)	30	27.26±19.89	30	35.22±13.30	2.00	29	0.054
Carbohydrate(g)	29 ^a	210.97±51.55	29 ^a	243.66±52.01	2.49	28	0.019
Fiber (g)	30	14.60±8.77	30	10.20±5.94	-2.48	29	0.019
Calcium (mg)	30	379.56±209.20	30	566.87±245.64	2.92	29	0.007
Phosphorus(mg)	27 ^a	537.74±141.31	27 ^a	618.33±172.77	1.98	26	0.058
Ferrous (mg)	26 ^a	14.34±4.51	26 ^a	10.89±3.94	-3.17	25	0.004
Vitamin A (RE)	28 ^a	505.11±238.74	28 ^a	480.99±226.74	-0.48	27	0.635
Vitamin B1 (mg)	30	0.60±0.25	30	0.80±0.21	3.27	29	0.003
Vitamin B2 (mg)	30	0.95±0.53	30	1.06±0.43	0.81	29	0.423
Niacin (mg)	30	9.08±3.97	30	9.47±3.69	0.41	29	0.682
Vitamin C (mg)	24 ^a	158.57±99.02	24 ^a	148.54±73.89	-0.41	23	0.685
Phytoestrogen (g)	28 ^a	12.08±5.00	28 ^a	10.44±4.59	-1.65	27	0.109

^a excluded the outlier ; NA = non available

Distribution of subjects by nutrient adequacy level of vegetarians and nonvegetarians, consumption defined by the Thai RDA for the elderly, is shown in Table 10. Regardless of calcium intake, most of the nonvegetarians met 67% nutrient adequacy. More than half of the vegetarians met a calcium, phosphorus and vitamin B1 level below the RDA requirement. In general, the nutrient intake of nonvegetarians was better able to satisfy the requirements than was that of the vegetarians.

Table 10 Distribution of subjects by nutrients adequacy level of average food consumption in vegetarians and nonvegetarians

	vegetarians (n=30)				nonvegetarians (n=30)			
	< 67% RDA		≥67% RDA		<67% RDA		≥67% RDA	
	n	%	n	%	n	%	n	%
Protein (g)	12	40.0	18	60.0	1	3.3	29	96.7
Calcium (mg)	25	83.3	5	16.7	17	56.7	13	43.3
Phosphorus(mg)	18	60.0	12	40.0	8	26.7	22	73.3
Ferrous (mg)	0	0	30	100.0	5	16.7	25	83.3
Vitamin A (RE)	11	36.7	19	63.3	1	3.3	17	97.7
Vitamin B1 (mg)	22	73.3	8	26.7	6	20.0	24	80.0
Vitamin B2 (mg)	15	50.0	15	50.0	10	33.3	20	66.7
Niacin (mg)	15	50.0	15	50.0	14	46.7	16	53.3
Vitamin C (mg)	0	0	30	100.0	3	10.0	27	90.0

The main source of energy intake of vegetarians derived from carbohydrate, smaller percentage from fat and protein, while the nonvegetarians obtained main source of energy from carbohydrate, fat and protein.

Table 11 Average percent energy distribution of vegetarians and nonvegetarians' food intake

	vegetarians (n=30)	nonvegetarians (n=30)
Total	100.0	100.0
Carbohydrate	68.63	66.50
Protein	11.51	12.19
Fat	19.86	21.31

Part 3 Description of radiological investigated for bone mass density of vegetarians and nonvegetarians measurement

The results of measurements of undominant 1/10 distal radius were summarized in Table 12. Nonvegetarians had small relatively bone mass density compared with vegetarians but there were no appreciable differences between vegetarians and nonvegetarians in means of bone mass density, Z-score and T-score.

Table 12 Comparison of bone mass density defined by site of undominant 1/10 distal radius of vegetarians and nonvegetarians

	vegetarians (n=30) ($\bar{X}\pm SD$)	nonvegetarians (n=30) ($\bar{X}\pm SD$)	t	df	p-value
BMD (g/cm^2)	0.47 \pm 0.06	0.46 \pm 0.05	-0.92	29	0.367
Z-score	-0.35 \pm 0.97	0.02 \pm 0.81	1.65	29	0.110
T-score	-2.67 \pm 1.68	-2.66 \pm 1.27	0.02	29	0.984

The bone mass status of participants using the WHO diagnostic criteria (6) was shown in Table 13. The majority of study subjects were osteoporosis whereas low bone mass and normal bone mass of both groups were similar.

Table 13 Description of bone mass density status of vegetarians and nonvegetarians

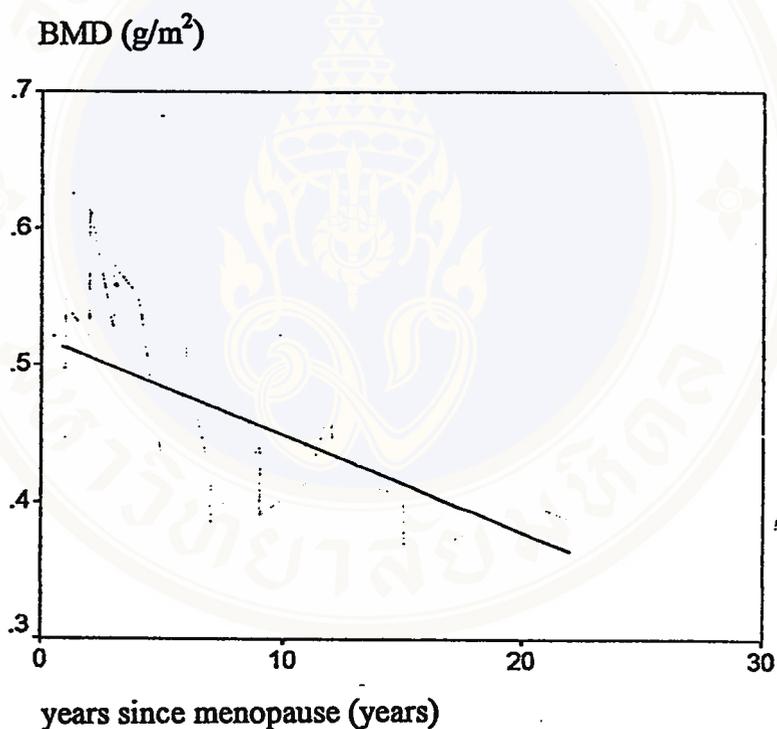
	vegetarians (n=30)		nonvegetarians (n=30)	
	n	%	n	%
Total	30	100.0	30	100.0
Normal bone mass	6	20.0	6	20.0
Low bone mass	6	20.0	5	16.7
Osteoporosis	18	60.0	19	63.3

The osteoporosis odd ratio in vegetarians and nonvegetarians equal to 0.95 (95% CI = 0.64 and 1.40). It means that the odd in favor of osteoporosis were 0.95 time as high among vegetarians as they are among nonvegetarians. There is no significant difference in the risk of osteoporosis between vegetarians and nonvegetarians. As shown in Table 14

Table 14 Risk of osteoporosis in vegetarians and nonvegetarians

	osteoporosis		Total
	+	-	
vegetarian	18	12	30
	19	11	30
Total	37	23	60

A linear regression existed between bone mass density and years since menopause in the vegetarians ($R^2 = 0.400$) at p -value < 0.001 (figure 1), but no linear regression existed in the other group. When added up the others' years since menopause into the model, the correlation was reduced ($R^2 = 0.252$) at p -value < 0.001 . No linear regression existed between bone mass density and years as vegetarian, nutrients intake or the amount of phytoestrogen in this study.



$$\text{BMD (g/m}^2\text{)} = 0.52 - 0.07 \text{ years since menopause (years)}$$

Figure 1 Simple linear regression of bone mass density and years since menopause in menopausal women

Chapter V

Discussion

The study was investigated bone mass density and osteoporosis in vegetarian and nonvegetarian menopausal women and effects of certain nutrients on bone mass density and osteoporosis in these study subjects. Thirty vegetarians and nonvegetarians were the elderly women that in the stage of menopause for at least one years. The ages were ranged from 46 to 71 years and years since menopause were ranged from 1 to 22 years.

There are 2 important topics for discussion as follows

Part 1 Demographic information, baseline characteristics and nutritional status of study subjects

Part 2 Food consumption patterns and bone mass density of study subjects

Part 1 Demographic information, baseline characteristics and nutritional status of the study subjects

The mean age of the vegetarians was 56.7 and of the nonvegetarians 57.9 years. The youngest study subject was 46 years old, whereas the oldest was 71 years. Despite the fact that age distribution in vegetarians was younger than that of the other group, similarity of natural menopause year was observed. Although bone loss probably begins before menopause, there is an acceleration of loss that accompanies the cessation of ovarian hormone production. Among the causes of menopausal bone loss, serum estrogen deficiency is the most important. Thus both groups were comparable in terms of loss of ovarian activity.

During pregnancy and lactation the growing fetal and neonatal skeletons make major demands for calcium. There is good evidence now that during lactation a substantial part of this calcium demand is mobilized from the maternal skeleton even despite high dietary calcium. This effect could be especially important with multiple pregnancies and extended lactation. However, during pregnancy, there is little if any bone loss, presumably because of the greatly increased efficiency of intestinal calcium absorption (111). These changes in calcium homeostasis during lactation seem to be determined by the combined effects of low estrogen concentration and the high concentration of parathyroid hormone-related receptor from the breast, possibly driven by prolactin (112). Breast-feeding may result in a reversible decline in BMD; any long-term effects are likely to be small or nonsignificant (75,83). On the other hand, parity and lactation have consistently not been associated with low bone density or osteoporosis fracture risk in epidemiological or case-control cohort studies, even among women with the highest parity and longest total duration of lactation (113).

Higher socio-economic status (SES), defined in terms of occupation, education or income, is associated with lower hip fracture risk (114). Possible explanations for a protective effect of higher SES against the risk of hip fracture are that people in these groups receive better treatment for medical conditions that predispose to falls, have a better diet, indulge less in unhealthy lifestyles such as smoking or alcohol drinking. The homes of people of higher SES might also have fewer environmental hazards because they can better afford home maintenance, leading to higher bone mass. The same explanation should be applicable to the nonvegetarian group. Any unhealthy women, those who had medical illness, took certain medicines, currently smoked or took excessive alcohol were all excluded from the study. Therefore, these detrimental effects from unhealthy lifestyles, regardless of occupation, education or income, were controlled.

It is generally well accepted that more physically active individuals have a higher bone mass than sedentary individuals. Both sedentary jobs and mainly weight-bearing jobs at age 50 years appear to be associated with a higher risk of hip fracture than jobs with intermediate physical activity levels (84). This also accords with the findings in a published paper (115), which reported no significant difference in bone mineral density between sedentary office workers and letter or newspaper carriers. The same explanation may apply to the participants with intermediate physical activity occupations in this study, who were teachers, registered nurses, merchants, launderers and gardeners. Long periods of repetitive loading, such as an habitual daily morning walk, calisthenics that was observed in these study subjects appeared to be insufficient exercise stimulus to produce an anabolic effect on bone mineralization. In menopausal women, aerobic and high-impact exercise, involving jumping, has been

shown to have a modest beneficial effect, including a reduction in bone loss (90). There might be a higher bone mass in nonvegetarians.

The bone mineralization of vegetarian and omnivore women below the age of forty-nine years was the same. The similarity in bone mineralization of the younger women in both groups and the differences among the older women had been suggested as an explanation for the effects of a diet containing meat on bone mineralization. The postulated action of meat in increasing demineralization of bone appears to be important only among older women, i.e., those sixty-plus years of age, since among women twenty to forty-nine years of age, there appears to be no difference in the degree of bone mineralization (22).

Currently no value is agreed on for weight-to-height versus osteoporosis and related fracture risk, but some extra fat mass, yielding a BMI >26-28 does confer limited protection, whereas a slender figure yielding a BMI < 22-24 increases risk (59). Although subject matching of BMI were not in the range mentioned above, there must be caution that a BMI value is likely to be more appropriate for younger and perimenopausal women, because the loss in height associated with vertebral fractures and spinal deterioration in elderly women independently affects the calculation. A greater mass of adipose tissue increasing conversion of androstenedione to estrone is the principal source of this estrogen in menopausal women (116,117).

For clinical trials, it is advised to measure bone mass in at least two different skeletal sites. Priority should be given to measurement of sites of biological relevance e.g. the spine for vertebral osteoporosis. A previous study has (96) shown that the bone mass density of the human forearm can be measured to determine the amount of

bone or bone loss at the scanning site, with convenience to subjects, taking a short time and at low cost.



Part 2 Food consumption pattern and bone mass density of study

subjects

The study vegetarians' food consumption pattern was based on grains, vegetables, fruits, legumes, and seeds and they abstained entirely from animal products. Most of them restricted food consumption to one or two meals per day and reported that they usually consumed vegetables and occasionally consumed oil, sugar and coconut milk. The main source of protein in vegetarians was bean curd, soymilk, soybeans, bean threads and peanuts. These food items provided calcium, phosphorus, vitamin B2, and ferrous. The primary carbohydrate originated from home-pounded rice, whole-seed Job's tear, pumpkin and whole-wheat bread. These foods were the main sources of carbohydrate, they are also rich in fiber, vitamin B1, vitamin B2, niacin and ferrous. The chief source of animal protein, calcium, phosphorus, ferrous, vitamin B1 and vitamin B2 in nonvegetarians was lean pork meat, youngwhite-meat chicken, and slices of freshwater fish. Vegetable protein came from soymilk, peanuts, bean threads, white soybean curd and bean curd. The major carbohydrate of the nonvegetarian group derived from milled rice, home-pounded rice, white bread and whole-wheat bread. They derived fat from oil used in the cooking process, considering both groups preferred boiled, blanched or stir-fried to deep-fried foods and rarely consumed coconut milk in dessert or curry. Considering the average percent energy distribution of vegetarian and nonvegetarian food intake of carbohydrate, protein and fat, they were nearly equal in both groups. The chief source of calcium in vegetarians came from soybeans and soybean products, chinese cabbage and black sesame seeds, whereas other sources were milk and milk products, meat and soybean products. The non-heme iron in vegetarians came from soybean products, chinese

cabbage, chinese swamp cabbage, black and white sesame seeds, while the other, both heme and non-heme, was derived from meat and soybean products. Fruit and fruit juices were main source of vitamin C in both groups.

The bone mass of vegetarians has been compared to omnivores in Caucasian populations. The results of earlier studies suggested that vegetarians might have higher BMC than omnivores (21,22,23). However, in the more recent studies, the BMC and BMD of vegetarians and omnivores were found not to be different (24,25). The mean calcium and calorie intakes were similar between vegetarians and omnivores, but mean protein intakes were significantly lower in the vegetarians. The BMC at the forearm of vegetarians was found to be similar to omnivores.

This current research studied the BMD of elderly Thai menopausal vegetarian women, and compared these with the value from nonvegetarian controls. The study vegetarians had lower SES, with little exercise to increase bone loss, limited food consumption of to 1-2 meals per day, and they abstained entirely from animal foods. In general, the energy and nutrient intakes of nonvegetarians were better able to meet a normal requirement than that of vegetarians. The average protein from plants, fiber, and ferrous intakes were significantly higher in vegetarians. On the contrary, energy, animal protein, carbohydrate, calcium and vitamin B1 intakes were significantly higher in nonvegetarians. The BMD at the forearm and the risk of osteoporosis were found to be similar in both groups.

Calcium is a very important mineral in the human body. The bones contain large amounts of calcium that helps to make them firm and rigid. Calcium is also needed for many other tasks, including nerve and muscle function and blood clotting. These other tasks are so important for survival, that, when dietary calcium is too low,

calcium will be lost from bone and used for other critical functions. The calcium intake in vegetarians was significantly lower than in nonvegetarians, which was also seen in a previous study (118).

The level of dietary protein intake and the amino acid composition of dietary proteins has a profound effect on calcium utilization in humans. Additionally, the levels of phosphorus intake that generally comes with dietary proteins exert independent effects on calcium utilization and might modify the effects of proteins. The source of dietary protein for vegetarians, especially strict vegetarians for whom sources of dietary calcium are limited, is likely to be a major factor in determining adequacy of nutritive calcium.

As reviewed by a previous study (119) the effect on calcium utilization and hypercalciuria was mainly attributable to dietary protein. At a given level of calcium intake, there was an increase in urinary calcium. Data collected from several studies displayed that at constant levels of calcium and phosphorus intake a twofold increase in dietary protein caused a 50% increase in urinary calcium (120). An increased level of dietary protein, from 48 to 95 g/day in young adult males, caused urinary calcium to increase from 4.6 to 6.0 mmol/d and a further increase in dietary protein, to 142 g/day, caused a further increase in urinary calcium, to 7.5 mmol/day. Similarly, increasing dietary protein from 48 to 95 to 142 g/day increased calcium excretion from 5.4 to 7.6 to 10.6 mmol/day and at calcium intakes of 800 and 1400 mg/day from 5.1 to 8.8 to 9.4 mmol/day. (121). These data clearly showed that dietary protein exerted a quantitatively greater effect on urinary calcium than did dietary calcium intake.

This protein-induced calciuria was unaccompanied by significant changes in calcium absorption at low levels of calcium (500 mg/d) and was only minimally

attributable to increase in calcium absorption at higher levels of calcium intake (119). Consequently, the calciuria effect of protein caused a reduction in calcium balance. As reported (119), young adults were consistently in calcium balance when consuming a moderately low (48 g/day) protein diet, as seen in nonvegetarians in this study, regardless of whether calcium intake was 500, 800 or 1400 mg/day (121). Further increasing dietary calcium while protein was maintained at 48 g/day had no significant effect on calcium retention. In contrast, none of these subjects were able to maintain calcium balance at a calcium intake of 500 mg/day when dietary protein was increased to 95 g/day; at this level of protein intake, 800-mg calcium/day was required to achieve calcium balance. When protein was further increased to 142 g/day, all subjects were in negative calcium balance at calcium intakes of 500 or 800 mg/day and only 3 of 15 subjects were able to maintain balance even at a calcium intake of 1400 mg/day.

Total protein consumption in nonvegetarians was significantly higher than in vegetarians. Nonvegetarians' protein was derived from both plant and animal proteins whereas that of the other group derived from plants only. The mechanism whereby an increase in the level of protein intake causes a decrease in fractional tubular reabsorption of calcium may be related, in part, to the acid ash of a meat-based diet. Animal food contains 39 mg/g of sulfur-containing amino acids whereas legumes have 25, fruit 38 and cereals 28 mg/g. (122). Although sulfate is a significant contributor to the acid load of a meat-based diet, it is obviously not the only contributor. Therefore, the vegetarian diet tends to have an alkaline ash residue, despite the presence of sulfur containing amino acids, that would be predicted to produce lower urinary calcium losses (122).

Protein in the diet is generally accompanied by phosphorus. This phosphorus exerts a hypocalciuretic effect (121) that may modify the hypercalciuretic effect of protein. Increasing the level of phosphorus increases renal tubular reabsorption of calcium and thereby exerts a hypocalciuretic effect. Although phosphates have been thought to interfere with calcium absorption, this does not appear to be the case in humans consuming a wide range of calcium and phosphorus intakes, and phosphorus causes a small but significant increase in calcium absorption at low (< 500 mg/day) level of calcium intake, as seen in vegetarians. Consequently, the hypocalciuretic effect of phosphorus produced an improvement in calcium balance (121).

This hypocalciuretic effect of phosphorus served to offset, in part, the hypercalciuretic effect of protein. Increasing dietary protein from 50 to 150 g/day at calcium and phosphorus intakes of 500 and 1000 mg/day, respectively, caused an increase in urinary calcium from 3.9 to 8.3 mmol/day and thereby reduced calcium balance from 0.6 to -2.9 mmol/day. However, simultaneously increasing protein and phosphorus to 150 g and 2525 mg/day, respectively, caused a significantly smaller increase in urinary calcium from 3.9 to 5.0 mmol and consequently caused a substantially smaller decrement in calcium balance than when dietary protein was raised without a concomitant increase in phosphorus (119,120,121). In contrast, increasing dietary phosphorus at low (50-g/d)-protein intake caused no significant change in calcium balance although the hypocalciuretic effect of phosphate persisted. Thus, the phosphorus that accompanies the protein blunts the hypercalciuretic effect of dietary protein. Cumings et al (123) found that increasing the dietary protein from 63 to 163 g/d using meat cause only a 24% increase in urinary calcium rather than the 80% that would be predicted (120) from the 2.6-fold increase in protein intake. The

blunted calciuretic response to the proteins in this case appears to result from the phosphorus that accompanied these proteins.

Several dietary constituents decrease the bioavailability of calcium in food. Likewise, the fiber in fruits and vegetables can cause negative calcium balance. In cereals, phytic acid is the main constituent of fiber that binds calcium making it unavailable for absorption. As is the oxalic acid present in high concentrations in food spinach. In contrast, calcium absorption from low oxalate vegetables such as kale, chinese cabbage flower leaves, chinese mustard greens, collard green is as good as it is from milk (104).

These data collectively suggest that the effects of dietary protein on calcium excretion and retention are, in large part, dependent on both the sulfur amino acid content of the protein and the quantity of phosphorus that is associated with the protein. Linkswiler et al (119) showed that a daily calcium intake of 500 mg/d is adequate to maintain calcium equilibrium when daily protein and phosphorus intakes were 48 g and 800 mg, respectively; however, increasing protein intakes to 95 g/d without a concomitant increase in dietary calcium and phosphorus caused a significant calciuresis and a negative calcium balance. In contrast, when soy protein sources i.e. soy milk, tofu, were used as the primarily sources of protein in a diet especially in vegetarians, calcium equilibrium was maintained even at protein and calcium intakes of 90 g and 457 mg/d, respectively (121). In addition, it appears that soy protein, even at high levels does not increase calcium excretion the same way that protein from animal sources does (121). This is presumably due both to the lower sulfur amino acid content of soybean and soybean product leads to an increase in phosphorus intake associated with soy proteins.

Diets that are too low in protein, as seen in these vegetarians (around 35 g/day), appear to have a negative effect on calcium status (52), and might result in lower predicate BMD. Besides, a previous study (26) had contended that an adequate intake of calories and protein may be needed to optimize bone mass. Previous studies demonstrated that a low dietary calcium intake was associated with an increased risk of both hip fracture (124) and vertebral fracture (125) in elderly Chinese women. The average dietary calcium intake of 380 mg per day in vegetarians was low, but there was no significant difference in BMD of both study subject groups.

Iron intake was significantly higher in vegetarians than in nonvegetarians. Iron is found in food in two forms, heme and non-heme iron. Heme iron, which makes up 40 percent of the iron in meat, poultry and fish, is well absorbed. Non-heme iron, 60 percent of the iron in animal tissue and all the iron in plants (fruits, vegetables, grains, and nuts) are less well absorbed. However, recent surveys of vegetarians (126,127) have shown that iron deficiency anemia is no more common among vegetarians than among the general population. This might lead to the expectation that a high vitamin C, such as intake, as 159 mg/d in a vegetarian diet acts to enhance the absorption of non-heme iron.

Phytoestrogen (PE) structurally resembles estradiol (E_2) and has been shown to have weak estrogenic activity when digested in relatively large amounts; dietary PE has been shown to have significant biological effects in humans. Although the protective effect of PE on bone mass has been shown in a previous study, this beneficial effect could not be determined, because of the small difference in PE intake in vegetarians and nonvegetarians in this study. The phytoestrogen content of foods

was calculated only from soybean and products that were derived from an other domicile database and might be different from amounts in Thai foods.

No linear regression existed between BMD loss by nutrient intake on this study. The effect of nutrients on BMD might be masked by interference of nutrient composition in food intake. The other reason was that this study measured only dietary intake of study subjects, but other important functional nutrients absorbable and retained in the body, were not included in the study.

BMD loss by number of years since menopause was better linearly predictable in vegetarians than in the other group. This implied that the pattern of BMD loss in vegetarians and nonvegetarians were not similar.

Since the vegetarians spent sometime on outdoor activities, this led to a substantial improvement in vitamin D status. Those vegetarians with improved 1,25-Dihydroxy vitamin D had increased intestinal calcium absorption and seemed to have lower calcium need than some nonvegetarians who seldom ventured outdoors or those who lived in shaded areas. This effect might cause a minor effect on 25-hydroxyvitamin D levels because of there being plenty of sunlight in a sunny climate.

The peak bone mass achieved is under strong genetic influence. An association between common allelic variants in the gene encoding the vitamin D receptor (VDR) and bone mineral density and turnover in adults has been reported (128,129). This genetic marker defined a very small subgroup (homozygote BB) with a high probability of net bone loss and another larger group (homozygote bb) unlikely to suffer bone loss. Although, in menopausal women, the rate of change of bone mineral density may be related to VDR allelic polymorphism, the larger homozygote bb in

Thai people might cause no significant association between VDR gene polymorphism and bone mass density in Thai people as it was found in Koreans and Japanese (131).



Chapter VI

Conclusion and Recommendations

Conclusion

In this study, the bone mass density and risk of osteoporosis of vegetarians were not found to be significantly different from nonvegetarians. Although the average energy, total protein and calcium intakes were higher in nonvegetarian menopausal women, the average vegetable protein, fiber, and ferrous intakes were lower. As a result, the vegetarians' diet was lower in energy, protein and calcium intakes, and their socio-economic status and little exercise to increased bone loss compared with nonvegetarians, and this might have a negative effect on bone. Higher urinary calcium losses from the presence of a meat-based diet in nonvegetarians may be balanced out by the intake of higher energy, protein and calcium than that of the other group. The advantage of using soy protein as the primary source of protein in a vegetarian diet, was due to the lower sulfur amino acid content of soybeans and products and to an increase in phosphate intake associated with soy proteins, leading to calcium equilibrium being maintained.

Iron deficiency anemia is no more common among vegetarians than it is among the general population. Thus it may be expected that a high vitamin C intake in a vegetarian diet acts to enhance non-heme absorption. The difference in intake of PE between vegetarians and nonvegetarians could mask the helpful effect of the weak estrogenic activity on bone mass density.

Linear regression existed between bone mass density and years since menopause only in vegetarians, and no linear regression existed between bone mass density and nutrient intake. This implied that patterns of bone mass density loss were not similar in both groups and the effect of nutrients on bone mass density might be masked by interference of dietary nutrient composition.

Recommendations

As the result of study, it was found that the daily consumption of food low in calcium, low in animal protein and high in phosphorus results in increased calcium retention and normal in bone mass density. An increased meat-based diet with a concomitant increase in dietary calcium and phosphorus might balance out higher urinary calcium losses. It should be noted that the advantage from mainly consuming dietary protein from plants, especially soy protein due both to lower sulfur-amino acid content and to an increase in phosphorus intake, was shown to have a calcium-sparing effect and lead to lower calcium needs.

This study was cross-sectional, and was the first indication of the effect of food consumption patterns on bone density. Only current bone density was measured and study subjects were limited to specified groups. The long-term effect of certain nutrients on the bone density of the Thai population remains to be determined.

Postmenopausal women experience a sharply accelerated bone loss in the first few years after menopause. In further aging, bone loss probably continues at a relatively constant rate as at pre-menopause. Bone density should be matched by number of years since menopause and age to compare the bone density of subjects.

This study measured dietary intake of study subjects by semiquantitative food frequency questionnaire, developed only for these study subjects. There should be further study to include those absorbed and extended nutrients that play a significant role in the body, which were not detected in this study.

Tissue concentrations of phytoestrogen are known and the complete role of these naturally occurring compounds in human health and disease have been reported. Quantitative phytoestrogen in Thai foods should be elucidated and prove helpful in determining the effect of dietary amounts of phytoestrogen on the health of Thai people.

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Appendix

The correlation of energy and nutrients intake between semiquantitative food frequency questionnaire and three-day food records were determined by Pearson's correlation coefficient. Ten menopausal women were sampling for three-day food records and SFFQ. The correlation coefficients were ranged from 0.01 to 0.67.

Table 1 Correlation of energy and nutrients intake between semiquantitative food frequency questionnaire and three-days food records from the first pilot study

Energy and nutrients	r	p-value
Energy (kcal)	0.452	0.190
Protein (g)	0.217	0.547
Protein animal (g)	0.677	0.209
Protein plant (g)	0.009	0.980
Fat (g)	0.297	0.405
Carbohydrate (g)	0.214	0.553
Fiber (g)	0.335	0.345
Calcium (mg)	0.414	0.308
Phosphorus (mg)	0.142	0.695
Ferrous (mg)	0.359	0.309
Vitamin A (RE)	0.297	0.405
Vitamin B1 (mg)	0.380	0.279
Vitamin B2 (mg)	0.063	0.863
Niacin (mg)	0.469	0.171
Vitamin C (mg)	0.070	0.858

After adjusted food items and portion size of the SFFQ from the first pilot study, sampling other ten subjects did the second pilot study of SFFQ. There were significant in energy and nutrients intake correlation between SFFQ and three-day food records at p-value less than 0.05. The highest correlation coefficient was calcium ($r=0.97$) and the lowest was niacin ($r=0.58$).

Table 2 Correlation of energy and nutrients intake between semiquantitative food frequency questionnaire and three-day food records from the second pilot study

Energy and nutrients	r	p-value
Energy (kcal)	0.71	0.020
Protein (g)	0.79	0.006
Protein animal (g)	0.81	0.069
Protein plant (g)	0.89	0.016
Fat (g)	0.70	0.001
Carbohydrate (g)	0.63	0.052
Fiber (g)	0.75	0.032
Calcium (mg)	0.97	<0.001
Phosphorus (mg)	0.90	<0.001
Ferrous (mg)	0.80	0.001
Vitamin A (RE)	0.69	0.048
Vitamin B1 (mg)	0.89	0.001
Vitamin B2 (mg)	0.87	0.002
Niacin (mg)	0.58	0.078
Vitamin C (mg)	0.72	0.019

Table 3 Thai recommended daily dietary allowances for elderly Thai women

Nutrients	Thai RDA*
Protein (g/kg body weight)	0.88
Calcium (mg)	800
Phosphorus (mg)	800
Magnesium (mg)	300
Ferrous (mg)	10
Iodine (μg)	150
Zinc (μg)	15
Vitamin A (μRE)	600
Vitamin D (μg)	5
Vitamin E (mg α -TE)	8
Vitamin B1 (mg)	1.0
Vitamin B2 (mg)	1.2
Vitamin B6 (mg)	2.0
Folate (μg)	150
Vitamin B12 (μg)	2.0
Niacin (mg)	13
Vitamin C (mg)	60

* Recommended daily dietary allowances for healthy Thais: The committee on recommended daily dietary allowances, department of health, Ministry of Public Health, 1989

Table 4 Amount of phytoestrogens in soybean and soybean products

Soybean and products	Genistein* (mg/g)	Daidzein* (mg/g)
Dry textured vegetable protein	0.6	2.1
Dry whole soybean	0.2	0.7
Soy flakes	0.3	0.2
Soy split	0.7	1.8
Tofu	200	75
Soy milk	20	7
Miso	35	40

* The amounts of isoflavones were derived from previous study (17,19)

แบบสัมภาษณ์และตรวจสอบสุขภาพ

การวิจัยเรื่อง รูปแบบอาหารบริโภคและความหนาแน่นของกระดูกในสตรีไทยวัยหมดประจำเดือน

- คำชี้แจง** แบบสัมภาษณ์นี้ ประกอบด้วย ข้อมูลด้านต่างๆ 4 ส่วน คือ
- ส่วนที่ 1 ข้อมูลทั่วไปของผู้เข้าร่วมงานวิจัย
 - ส่วนที่ 2 ข้อมูลสุขภาพทั่วไปของผู้เข้าร่วมงานวิจัย
 - ส่วนที่ 3 ข้อมูลการตรวจร่างกายของผู้เข้าร่วมงานวิจัย
 - ส่วนที่ 4 แบบสัมภาษณ์ความถี่อาหารบริโภคทั้งปริมาณ

1 2

เลขที่แบบสอบถาม.....

ชื่อผู้เข้าร่วมงานวิจัย (นาง/นางสาว).....นามสกุล.....อายุ.....ปี
 อยู่บ้านเลขที่.....ซอย.....ถนน.....ตำบล.....
 อำเภอ.....จังหวัด.....รหัสไปรษณีย์.....โทรศัพท์.....
 วันที่สัมภาษณ์.....

ส่วนที่ 1 ข้อมูลทั่วไปของผู้เข้าร่วมงานวิจัย

คำชี้แจง ให้ทำเครื่องหมาย / ลงใน () ที่ตรงกับคำตอบของผู้เข้าร่วมงานวิจัย และเติมข้อความลงในช่องว่างตามคำตอบที่ได้

- | | |
|---|---|
| <p>1. สัญชาติ/เชื้อชาติ
 (1) ไทย/ไทย (2) อื่นๆ ระบุ.....</p> <p>2. ศาสนา
 (1) พุทธ (2) คริสต์ (3) อิสลาม (4) อื่นๆ ระบุ.....</p> <p>3. สถานภาพสมรส
 (1) โสด (2) สมรสอยู่ด้วยกัน (3) หม้าย (4) หย่า/แยก</p> <p>4. ระดับการศึกษาของท่านอยู่ระดับใด
 (1) ไม่ได้เรียน (2) ประถมศึกษา (3) มัธยมศึกษา
 (4) อนุปริญญาหรือเทียบเท่า (5) ปริญญาตรีขึ้นไป</p> | <p>สำหรับผู้วิจัย</p> <p>3
 <input type="checkbox"/></p> <p>4
 <input type="checkbox"/></p> <p>5
 <input type="checkbox"/></p> <p>6
 <input type="checkbox"/></p> |
|---|---|

5. ปัจจุบันท่านมีรายได้โดยประมาณเดือนละ.....บาท 7
6. รายได้จากข้อ 5 ส่วนใหญ่ท่านได้จาก
- (1) รับจ้าง (2) ค้าขาย (3) เกษตรกรรม (4) รับราชการ 8
- (5) เงินบำนาญ (6) บุครหลาน (7) อื่นๆระบุ.....

ส่วนที่ 2 ข้อมูลด้านสุขภาพทั่วไปของผู้เข้าร่วมงานวิจัย
คำชี้แจง ให้ทำเครื่องหมาย / ลงใน () ที่ตรงกับคำตอบของผู้เข้าร่วมงานวิจัย และเติมข้อความลงในช่องว่างตามคำตอบที่ได้

1. ท่านดื่มกาแฟ เครื่องดื่มหรืออาหารที่มีส่วนผสมของคาเฟอีนเป็นประจำ 9
- (1) ไม่ใช่
- (2) ใช่ ระบุชนิด.....ปริมาณ.....แก้วต่อวัน
2. ท่านดื่มเครื่องดื่ม หรือยาที่มีส่วนผสมของอัลกอฮอล์เป็นประจำ 10
- (1) ไม่ใช่
- (2) ใช่ ระบุชนิด.....ปริมาณ.....แก้วต่อวัน
3. ท่านออกกำลังกายหรือไม่ 11
- (1) ไม่ใช่
- (2) ใช่ ระบุชนิด.....นาทีต่อวัน.....วันต่อสัปดาห์
- ถ้าไม่ใช่ ท่านทำงานบ้านหรือไม่ 12
- (3) ไม่ได้ทำ (4) ทำบางอย่าง.....วันต่อสัปดาห์ (5) ทำทุกวัน
3. ท่านมีบุตรหรือไม่ 13
- (1) ไม่มี (2) มี จำนวน.....คน
- ถ้ามี ให้นมบุตรทุกคนหรือไม่ 14
- (1) ใช่ (2) ถ้าไม่ใช่ ท่านให้นมบุตร.....คน
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5. ท่านมีประจำเดือนไม่สม่ำเสมอก่อนหมดประจำเดือนหรือไม่ 17
- (1) ไม่ใช่ (2) ใช่ เป็นระยะเวลา.....เดือน/ปี
6. ท่านรับประทานอาหารมังสวิรัตหรืออาหารเจเป็นประจำหรือไม่ 18
- (1) ไม่ใช่ (2) ใช่ รับประทานติดต่อกันเป็นระยะเวลา.....ปี

ส่วนที่ 3 ข้อมูลการตรวจร่างกายของผู้เข้าร่วมงานวิจัย

1. ภาวะโภชนาการจากการชั่งน้ำหนัก/วัดส่วนสูง
 น้ำหนัก.....กิโลกรัม
 ส่วนสูง.....เซนติเมตร
 ดัชนีมวลกาย (BMI).....กิโลกรัม/ลูกบาศก์เมตร

2. ผลการวัดความหนาแน่นของกระดูกด้วยเครื่อง BMD-3 Bone Densitometer
 1/10 Distal Radiusg/m²
 Z-Score.....of standard deviation (SD)
 T-Score.....of standard deviation (SD)

3. ภาวะกระดูกพรุน
 (1) น้อยกว่า -2.5 SD (2) ตั้งแต่ -2.5 SD ขึ้นไป

19	20	21	
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22	23	24	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
25	26	27	28
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สมุดบันทึกอาหารบริโภค

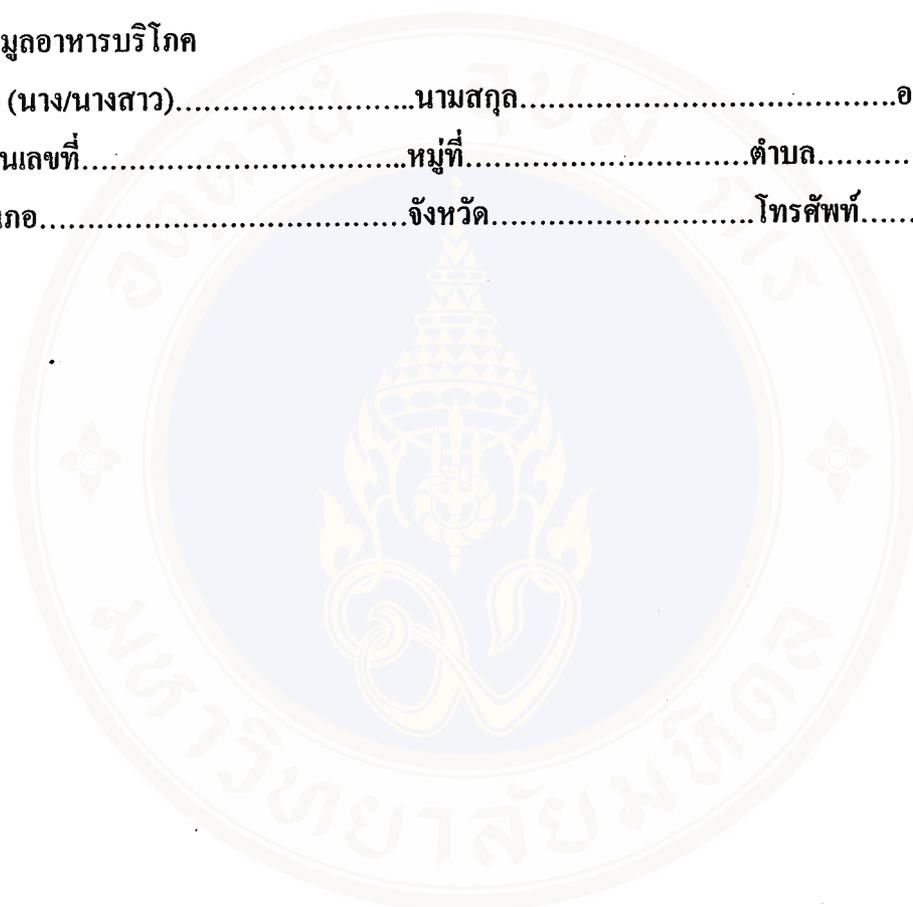
เลขที่ตัวอย่าง.....

ข้อมูลอาหารบริโภค

ชื่อ (นาง/นางสาว).....นามสกุล.....อายุ.....ปี

บ้านเลขที่.....หมู่ที่.....ตำบล.....

อำเภอ.....จังหวัด.....โทรศัพท์.....



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